Interferon Gamma Levels in Relation to COVID-19 Vaccine

Tanya S. Salih[1], Ameena S. M. Juma[1], Muhsin H. Ubeid[1], Shams M. Saadoon[1], Lusya L. Yousif[1], Saz F. Hassan[1], Krestin P. Azeez[1], Maryana Y. Bakoss[1], Lailan N. Ismael[1], Sara R. Omer[1]

Department of General Biology, Cihan University-Erbil, Kurdistan Region, Iraq.

***Abstract*- One-hundred and twenty cases studied in this study for whom they take vaccine dose or not, for infected and non-infected with coronavirus. They are classified to four groups (Covid-19 – Non-Vaccinated = CN); (Covid-19 – Vaccinated = CV); (Non Covid-19 – Non-Vaccinated = NN); (Non-Covid-19 – Vaccinated = NV) each group involved 30 cases. After withdrawing the blood from each person, the blood poured into two tube (5 ml to Gel tube and 2 ml to EDTA tube) and immunological parameters directly performed by hematological analyzer, while the serum, after centrifugation of blood and storing them in special tubes, used to estimation of IFN- γ by Enzyme Linked Immunosorbent Assay (ELIZA) and Lactate Dehydrogenase (LDH) by chemical analyzer. The study showed that there is a slight increasing in IFN- γ as pro-inflammatory cytokine and LDH concentration which related to the severity of the disease, while there is rising in WBC number both vaccinated groups and cases with COVID-19 infection.**

Key Wards: COVID-19, Coronavirus, Vaccinated or Not-Vaccinated.

I. INTRODUCTION

Coronaviruses (CoVs) are a family of covered viruses, with an enormous range of properties, including positive-sense, single-stranded RNA viruses, they set many types of disease in human and large number of animals in different organs and systems as respiratory, gastrointestinal, hepatic, and neurological systems [1]. And have been divided into five genera: α-coronavirus (α-CoV), β-coronavirus (β-CoV), δ-coronavirus (δ-CoV), γ-coronavirus (γ-CoV), Omicron with 17 subtypes [2,3].

White blood cells, particularly lymphocytes move and circulate throughout the body, producing proteins (cytokines and antibodies) that are help the immune system to protect the body from fighting germs, T cells kill infected abnormal cells in the body that have been harmed by viruses or cancer, whereas B cells fight as viruses and bacteria extracellularly, lymphocytes, in particular, are white blood cells, T cells kill cells in the body that have been injured by viruses or cancer, whereas B cells attack viruses and bacteria. Lymphocytes move throughout the body, creating antibodies and proteins to help the immune system resist illness by battling pathogens, it seems natural that those who have a large number of lymphocytes with no signs and symptoms of coronavirus, this shows that their immune systems are successfully combatting the coronavirus by creating antibodies and attacking infected cells [4].

Interferon gamma is a cytokine effector group of cell mediated immune response, can go through many of anti-microbial functions, it helps cell to increase ability of presenting antigens of antigen presenting cells (APCs) by improving antigen recognition by T-cells, induce antiviral responses by up-regulating of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNIs); in addition, IFN-γ activity kills cancer cells by inducing an anti-proliferative state, and IFN- γ activity is largely responsible for pathogen immunity [5].

Lactate dehydrogenase (LDH) enzyme has a role in converting of lactate to pyruvate in a lot of cells of body, and its activity rises during tissue breakdown [6]. This enzyme is an intracellular enzyme that trigger the oxidation of pyruvate to lactate in anaerobic glycolysis. Clinically, serum LDH is regularly measured in numerous diseases, Elevation in serum LDH concentration is a bad indicate in many diseases, such as malignancies and inflammation; and this case also approached by some researches that acute COVID-19 affects on rising amount of LDH, proving have not be done by any studies of its impact on COVID-19 severity and mortality, in conclusion the blood LDH levels can help determine of severity and mortality of COVID-19 [7].

Multiple investigations, since the current COVID-19 epidemic, have found relationship between falling down of lymphocyte number and severity of the disease, this condition is uncommon among infected children, and the fatality rate is very rare, while it repeats more in old ages, especially in severe cases, also death rate is higher [8].

The most considerable cells of the non-specific line of immune response in immune system, Monocytes and Macrophages, play a crucial role in the body's defense to combat viral infections, they primarily respond to microbial antigens by releasing inflammatory mediators in order to eliminate infectious agents and heal tissue damage; nonetheless, abnormal changes in their activity, such as cytokine storm, can be extremely damaging to the host in corona virus instances of acute respiratory distress

DOI: http://doi.org/10.24086/biohs2022/paper.691

syndrome. These cells may be engaged in hypersensitive and aggravated reactions during infection, contributing to tissue destruction, particularly lung hurt, which results in lung dysfunction and respiratory disease [9].

The pathology and physiology of severe COVID-19 is characterized by changes in neutrophil numbers, phenotype, and function. After infection with SARS-CoV-2, increased of neutrophils count have been found in the nasopharyngeal epithelium and afterwards in the more distant parts of the lung [10]. In a number of researches, eosinopenia has been seen in individuals with moderate-to-severe COVID-19, and there is a relationship between eosinopenia and illness severity [11].

Basophils play a major role in the immune response to the Corona virus. According to earlier studies on viral infections, a drop in basophil counts may reduce the efficiency of IgG responses to Corona virus. According to the researchers, the decrease in basophils occurred within the first three days of hospitalization and was quickly reversed [12].

There is a link betweenCOVID-19 and PLT parameters, a relationship has been established between severe COVID-19 patients and a specific coagulation index, such as a high D-dimer level, a prolonged prothrombin time, and a low PLT count, according to several investigations [13].

II. Materials and Methods

Methods

One Hundred twenty persons participated in this study as four groups The study includes four groups; (Covid-19 – Non-Vaccinated = CN); (Covid-19 – Vaccinated = CV); (Non Covid-19 – Non-Vaccinated = NN); (Non-Covid-19 – Vaccinated = NV) each group involved 30 cases. For Evaluation of Interferon gamma (IF-γ), Lactate Dehydrogenase (LDH) and immunological parameters.

Collection of sample

The total collection of 120 blood samples was aseptically done from all four groups. Seven ml of blood with drawed from all cases in each group via sterile disposable syringes. Then the blood samples distributed and poured into Gel tube (5ml) then centrifuged at 2500 rpm for fifteen minutes for serum collection; the serum of each patient was stored in Eppendorf tubes at -20°C until use for performing IF-γ and LDH, and EDTA tube (2ml) for performing hematological parameters.

IF-γ ELISA Test

Interferon gamma was performed for each sample following the procedure that was conducted according to kit instructions of ELISA test by (Biotek "ELx800"/ USA) after thawing the serum sample and bringing it to room temperature:

The concentrated standard diluted into small Eppendorf's first to get concentrations as (1/ 72 pg/ml, 2/ 48 pg/ml, 3/ 24 pg/ml, 4/ 12 pg/ml, 5/ 6 pg/ml).

Prepare Eliza plate according to the numbers of samples, the first well leaved empty as blank control. Add 50µl to standard wells respectively, then added 40µl Sample dilution buffer with 10µl sample, shaked gentle to be mix well. Then sealed the plate and incubate for 30 min at 37°C.

Washed the wells 5 times (aspirated and refilled with the wash solution and discarded after 30 seconds of filling). Diluted the concentrated (30X) washing buffer before using with deionized water.

Later added 50µl HRP-Conjugate reagent to each well without blank well. Incubated and washed as explained in Step 2 and 3 respectively.

Then added 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mixed well and incubated in a dark place at 37°C for 15 minutes.

Finally added 50 µl stop solution to each well to stop the reaction. The color changed from blue to yellow as reaction termination indicator.

Read optical density (absorbance) at 450nm using Eliza Reader within 15 minutes.

Lactate Dehydrogenase Test

Perform in the LDH test were done by using a full automated chemical analyzer (Accent 200) (made in Switzerland and Poland) for determining of their concentration within serum.

Hematological Tests

Determination of complete blood count (CBC) was done by using the auto analyzer three parameter Convergys x3 Hematology Analyzer for each case in all groups within one hour of sample collection.

Statistical Analysis

Computer program software Statistical package for Social Sciences (SPSS) version 20 was used for statistical evaluation analyzing and organizing data in tables. Quantitative variables were compared accordingly via using the ANOVA (F-test). Results were considered significant if the P value was less than 0.05 and highly significant of 0.01.

III. Results

Mean serum levels of Interferon Gamma.

The Below table represents the mean serum level of IF-γ that significantly upregulated in both vaccinated groups when compared with other non-vaccinated groups ($P<0.05$).

DOI: http://doi.org/10.24086/biohs2022/paper.691

Table: Mean serum levels of IF-γ.

Immunological Parameter	Mean ± SE				F-Test P Value Probability
	CN (No.=30)	CV (No.=30)	NN (No.=30)	NV (No.=30)	
IFN-γ (pg/ml)	7.25 ± 0.46 [a]	11.74 ± 3.60 [b]	6.25 ± 1.51 [a]	13.51 ± 4.82 [b]	0.013 S*
P value ≥0.05: Non significant; *P value <0.05: Significant; **P <0.01: Highly Significant [a,b] Different letters: There is Significant difference between them (Duncan)					

Mean serum levels of Lactate Dehydrogenase.

After running the samples for evaluation of LDH, the result reveals that there is significant difference between NN group with other groups (P<0.05), presented in table below.

Table: Mean serum levels of LDH.

Parameter	Mean ± SE				F-Test P Value Probability
	CN (No.=30)	CV (No.=30)	NN (No.=30)	NV (No.=30)	
LDH (ng/ml)	415.83 ± 10.08 [b]	413.86 ± 10.72 [b]	387.06 ± 17.56 [a]	414.23 ± 7.51 [b]	0.012 S*
P value ≥0.05: Non significant; *P value <0.05: Significant; **P <0.01: Highly Significant [a,b,c] Different letters: There is Significant difference between them (Duncan)					

Table: Mean levels of immunological and hematological parameters

Immunological Parameters	Mean ± SE				F-Test P Value Probability
	CN (No.=30)	CV (No.=30)	NN (No.=30)	NV (No.=30)	
WBC (10^3/uL)	7.51 ± 0.49 [b]	6.43 ± 0.22 [a]	7.41 ± 0.26 [b]	6.44 ± 0.31 [a]	0.029 S*
Lymphocyte (10^3/uL)	2.08 ± 0.10 [a]	2.04 ± 0.10 [a]	2.18 ± 0.10 [a]	1.96 ± 0.13 [a]	0.202 N.S
Monocyte (10^3/uL)	1.68 ± 1.11 [a]	0.57 ± 0.04 [b]	0.58 ± 0.04 [b]	0.49 ± 0.04 [b]	0.017 S*
Granulocyte (10^3/uL)	4.89 ± 0.47 [b]	3.82 ± 0.19 [a]	4.51 ± 0.20 [b]	3.97 ± 0.22 [a]	0.046 S*
Plt (10^3/uL)	270.63 ± 14.01 [a]	290.64 ± 9.91 [a]	275.67 ± 8.45 [a]	272.30 ± 9.79 [a]	0.236 N.S
P value ≥0.05: Non significant; *P value <0.05: Significant; **P <0.01: Highly Significant [a,b] Different letters: There is Significant difference between them (Duncan)					

Mean levels of immunological and hematological parameters

The following table shows all means of immunological and hematological parameters. When the mean of total WBC count in all groups shows significant decrease in both vaccinated groups (P<0.05). While, the mean level of lymphocytes counts reveals non-significant decrease in NV group according to other groups (P≥0.05). But the mean of monocyte counts of CV group reveals significant rise in comparison to other groups (P<0.05). However, the mean counts of granulocyte showed significant increase in both non-vaccinated groups with vaccinated groups (P<0.05).

Platelet mean count presents non-significant difference when between all groups (P<0.05).

IV. Discussion

In the present study, the mean serum level of IFN-γ shows significant upregulated in both vaccinated groups (CV and NV). Gadotti and his researchers demonstrate the higher levels of IFN-γ were related to a poorer indicator; however, these levels were not sustained beyond ten days of symptoms, and mortality increase in individuals with persistent IFN-γ levels [14]. In corona virus recovered patients, the correlation was very strong between identification of IFN-γ expressing cells by intracellular cytokine labeling and measurement of soluble IFN-γ by ELISA [15].

Evaluation of serum LDH concentration reveals that there is significant rising in all three groups (CN, CV, and NV). It is similar to a study display after the First Dose of COVID-19 vaccination there is residue of component of intravascular hemolysis coupled with a major process of extravascular as demonstrated by the presence of very high LDH level [16]. Similar to other independent risk factors increased [17]. The main conclusion of Li and his colleagues' study is that one of the risk factors of COVID-19 is increasing of serum LDH; as well as low lymphocyte, beside of their activity as risk factor they become also risk indicators for death among COVID-19 patients [18].

In the first few days of disease, the total WBC count is normal or low, with low lymphocytes and developing lymphocytopenia in severe instances [19]. But in many other infection total WBC count shows an increasing [20,21]. As in the present study, the mean of total WBC count in all groups shows significant decrease in both vaccinated groups. While, the mean level of lymphocytes counts reveals non-significant decrease in NV group according to other groups. In severe patients, however, lymphocyte number consistently tended to fall [22]. Dynamic alterations in WBCs and differential count could be helpful for the prognosis of COVID-19 patients, the patient's condition should improve

DOI: http://doi.org/10.24086/biohs2022/paper.691

if these parameters return following continued reduction; otherwise, the indications may be bad [19]. The majority vaccinated COVID-19 patients had a low absolute lymphocyte count, many of them had a lymphocyte count less than 10^3 cells/mm3—which could be the reason for their inability to establish an immunological response [22]. The inactivated vaccine recipients also had significantly higher white blood cell counts and lactate dehydrogenase levels at admission [18,23].

In severely and critically COVID-19 disease patients, monocytes were dramatically modified, with an increased percentage of monocytes and significantly reduced variety [24]. Vaccination resulted in major alterations in inflammatory responses, which were found to be mostly occurring in classical monocytes; vaccination also raised classical monocyte content, which has been linked to the development of severe symptoms in monocytes [25]. While the mean of monocyte counts of CV group reveals significant rise in comparison to other groups.

In this study, the mean of granulocyte count showed significant increase in both non-vaccinated groups with vaccinated groups. Non-severe patients had significantly greater leukocyte and neutrophil counts, as well as a higher neutrophil-lymphocyte ratio [26]. Histology revealed neutrophilic pustular dermatitis in various stages of progression, sometimes with necrotizing neutrophilic infiltrate containing nuclear residues ("nuclear dust"), but no evidence of inflammation of blood vessels, IHC revealed a high prevalence of neutrophil's myeloperoxidase and histiocytes (CD68+), with rare T (CD3+) and B (CD20+) cells but no plasma cells [27]. The eosinophil count is particularly decrease [28]. The combination of the maximum virus protection and the lowest risk of eosinophilic immunopathology yields the best results [29]. Another study suggests that corona virus infection might be associated with suppression of T helper 2 (Th2)-polarized immune responses and decreased chemotaxis of CRTH2+ cells [30]. Immediate or type I immunological reaction that occurs after vaccination, it is the worst type of hypersensitivity and is triggered by the activation of mast cells and basophils triggered after binding of IgE to their specific receptor of cell membrane, the subsequent secretion of inflammatory mediators (histamine, tryptase, cytokines, and chemokines) causes a fast indicator from mild symptoms (like pruritus, urticaria, headache, metallic taste, and disorientation) to life-threatening symptoms like mucosal swelling, tachycardia, bronchoconstriction, vomiting and diarrhea, seizures, vasodilation, and shock, to confirm the diagnosis, at least two organs must be involved. It need prompt treatment to avoid disease-specific death [31].

The results of the blood tests were separated into two groups: severe and non-severe, and Mann–Whitney U nonparametric tests were used to compare them. In the severe sample group, Lym percent and HGB were much lower, while lymphocyte percent and Hb were significantly greater, as compared to the non-severe sample group, this meant that as the disease progressed, the number of lymphocytes and the concentration of Hb both dropped, as a result, Lym percent, HGB, and Lym percent and Hb may all be used to distinguish between severe and mild cases. COVID-19 [32].

Patients with COVID-19 who have confirmed lower range platelet counts (but are not thrombocytopenic) [28]. However, normal platelet counts were found in all four groups in this investigation, whether they were vaccinated or not. The risk of thrombocytopenia and venous thromboembolism following corona virus vaccination does not appear to be larger than the risk situation in the general population, which is consistent with the syndrome's rare and sporadic nature [33].

V. Conclusion

There are many immunological changes during infection with coronavirus and administration of its vaccine, IFN-y is one of the pro-inflammatory cytokines rise during the mentioned cases, as well as LDH is a factor can be used as an index of COVID-19 severity. The number of WBCs increase during vaccination and it was an indicator of stimulating immune response.

VI. References

1. He, F., Deng, Y., and Li, W. (2020). Coronavirus disease 2019: What we know? *Journal of medical virology*, *92*(7), 719-725.
2. Qiu, T., Mao, T., Wang, Y., Zhou, M., Qiu, J., Wang, J., ... and Cao, Z. (2020). Identification of a potential cross-protective epitope between a new type of coronavirus (2019-nCoV) and severe acute respiratory syndrome virus. *Of Genetics and Journal Unit Genomics*, *47* (2), 115.
3. Cascella, M., Rajnik, M., Aleem, A., Dulebohn, S., and Di Napoli, R. (2021). Features, evaluation, and treatment of coronavirus (COVID-19). StatPearls.
4. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020, 395, 497–506.
5. Kak, G., Raza, M., & Tiwari, B. K. (2018). Interferon-gamma (IFN-γ): exploring its implications in infectious diseases. Biomolecular concepts, 9(1), 64-79.
6. Wu, M. Y., Yao, L., Wang, Y., Zhu, X. Y., Wang, X. F., Tang, P. J., & Chen, C. (2020). Clinical evaluation of potential usefulness of serum lactate dehydrogenase (LDH) in 2019 novel coronavirus (COVID-19) pneumonia. Respiratory research, 21(1), 1-6.

DOI: http://doi.org/10.24086/biohs2022/paper.691

7. Li, C., Ye, J., Chen, Q., Hu, W., Wang, L., Fan, Y., ... and Lu, H. (2020). Elevated lactate dehydrogenase (LDH) level as an independent risk factor for the severity and mortality of COVID-19. Aging (Albany NY), 12(15), 15670.
8. Karmouty-Quintana H, Thandavarayan RA, Keller SP, Sahay S, Pandit LM, Akkanti B. Emerging Mechanisms of Pulmonary Vasoconstriction in SARS-CoV-2-Induced Acute Respiratory Distress Syndrome (ARDS) and Potential Therapeutic Targets. Int J Mol Sci (2020) 21:8081. doi: 10.3390/ijms21218081
9. Serebrovska ZO, Chong EY, Serebrovska TV, Tumanovska LV, Xi L. Hypoxia, HIF1α, and COVID-19: from pathogenic factors to potential therapeutic targets. Acta Pharmacol Sin (2020) 41:1539–46. doi: 10.1038/s41401-020-00554-8
10. Chua RL, Lukassen S, Trump S, Hennig BP, Wendisch D, Pott F, et al. COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis. Nat Biotechnol (2020) 38:970–9. doi: 10.1038/s41587-020-0602-4
11. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. Nature (2020) 584:463–9. doi: 10.1038/s41586-020-2588-y
12. Archer SL, Sharp WW, Weir EK. Differentiating COVID-19 Pneumonia from Acute Respiratory Distress Syndrome and High Altitude Pulmonary Edema: Therapeutic Implications. Circulation (2020) 142:101–4. doi: 10.1161/CIRCULATIONAHA.120.047915
13. Salamanna, F., Maglio, M., Landini, M. P., & Fini, M. (2020). Platelet functions and activities as potential hematologic parameters related to Coronavirus Disease 2019 (Covid-19). Platelets, 31(5), 627-
14. Gadotti, A. C., de Castro Deus, M., Telles, J. P., Wind, R., Goes, M., Ossoski, R. G. C., ... & Tuon, F. F. (2020). IFN-γ is an independent risk factor associated with mortality in patients with moderate and severe COVID-19 infection. Virus Research, 289, 198171.
15. Crespo, M., Barrilado-Jackson, A., Padilla, E., Eguía, J., Echeverria-Esnal, D., Cao, H., ... & Días, P. (2021). Negative immune responses to two-dose mRNA COVID-19 vaccines in renal allograft recipients assessed with simple antibody and interferon gamma release assay cellular monitoring. American Journal of Transplantation.
16. Jaydev, F., Kumar, V., Khatri, J., Shahani, S., & Beganovic, S. (2022). A Case of Autoimmune Hemolytic Anemia after the First Dose of COVID-19 mRNA-1273 Vaccine with Undetected Pernicious Anemia. Case Reports in Hematology, 2022.
17. Hussein, A. M., Taha, Z. B., Malek, A. G., Rasul, K. A., Hazim, D. Q., Ahmed, R. J., & Mohamed, U. B. (2021). D-dimer and serum ferritin as an independent risk factor for severity in COVID-19 patients. Materials Today: Proceedings.
18. Li, X., Chan, J. M. C., Lam, B., Lung, D. C., Lung, K. C., Chow, C. K. Y., ... and Yuen, K. Y. (2022). COVID-19 mRNA vaccines delay the onset of breakthrough infections with less radiographic abnormalities. Clinical Infectious Diseases.
19. Lu, G., & Wang, J. (2020). Dynamic changes in routine blood parameters of a severe COVID-19 case. Clinica Chimica Acta, 508, 98-102.
20. Salih, T. S., Juma, A. S., and Ubeid, M. H. (2021). Occupational Exposures and Associated Health Effects among Dumpsite Workers. Cihan University-Erbil Scientific Journal, 5(2), 1-5.
21. Juma, A. S., Ubeid, M. H., Haydar, S. S., & Salih, T. S. (2019). Some Immunological and Hematological Parameters among Refugees in Kawergosk Camp–Erbil Governorate. Cihan University-Erbil Scientific Journal, 3(1), 80-84.
22. Achiron, A., Mandel, M., Dreyer-Alster, S., Harari, G., Magalashvili, D., Sonis, P., ... & Gurevich, M. (2021). Humoral immune response to COVID-19 mRNA vaccine in patients with multiple sclerosis treated with high-efficacy disease-modifying therapies. Therapeutic advances in neurological disorders, 14, 17562864211012835.
23. Ubeid, M. H. (2020). Prevalence of leukocytes in type 2 diabetic patients in Erbil City. Medical Journal of Babylon, 17(1), 19.
24. Zhang, W., Zhang, Z., Ye, Y., Luo, Y., Pan, S., Qi, H., ... & Qu, J. (2020). Lymphocyte percentage and hemoglobin as a joint parameter for the prediction of severe and nonsevere COVID-19: a preliminary study. Annals of Translational Medicine, 8(19).
25. Liu, J., Wang, J., Xu, J., Xia, H., Wang, Y., Zhang, C., ... & Liu, Z. (2021). Comprehensive investigations revealed consistent pathophysiological alterations after vaccination with COVID-19 vaccines. Cell discovery, 7(1), 1-15.
26. Seyit, M., Avci, E., Nar, R., Senol, H., Yilmaz, A., Ozen, M., ... & Aybek, H. (2021). Neutrophil to lymphocyte ratio, lymphocyte to monocyte ratio and platelet to lymphocyte ratio to predict the severity of COVID-19. The American journal of emergency medicine, 40, 110-114.
27. Capassoni, M., Ketabchi, S., Cassisa, A., Caramelli, R., Molinu, A. A., Galluccio, F., & Guiducci, S. (2021). AstraZeneca (AZD1222) COVID-19 vaccine-associated adverse drug event: A case report. Journal of Medical Virology, 93(10), 5718.
28. Honda-Okubo, Y., Barnard, D., Ong, C. H., Peng, B. H., Tseng, C. T. K., & Petrovsky, N. (2015). Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic

DOI: http://doi.org/10.24086/biohs2022/paper.691

immunopathology. Journal of virology, 89(6), 2995-3007.
29. Vitte, J., Diallo, A. B., Boumaza, A., Lopez, A., Michel, M., Allardet-Servent, J., ... & Mege, J. L. (2020). A granulocytic signature identifies COVID-19 and its severity. The Journal of infectious diseases, 222(12), 1985-1996.
30. Wollina, U., Chiriac, A., Kocic, H., Koch, A., & Brzezinski, P. (2021). Cutaneous and hypersensitivity reactions associated with COVID-19 vaccination—A narrative review. Wiener Medizinische Wochenschrift, 1-7.
31. Zhang, Y., Wang, S., Xia, H., Guo, J., He, K., Huang, C., ... & Li, L. (2021). Identification of monocytes associated with severe COVID-19 in the PBMCs of severely infected patients through single-cell transcriptome sequencing. Engineering.
32. Zaid, Y., Puhm, F., Allaeys, I., Naya, A., Oudghiri, M., Khalki, L., ... & Boilard, E. (2020). Platelets can associate with SARS-Cov-2 RNA and are hyperactivated in COVID-19. Circulation research, 127(11), 1404-1418.
33. Scully, M., Singh, D., Lown, R., Poles, A., Solomon, T., Levi, M., ... & Lester, W. (2021). Pathologic antibodies to platelet factor 4 after ChAdOx1 nCoV-19 vaccination. New England Journal of Medicine, 384(23), 2202-2211.

DOI: http://doi.org/10.24086/biohs2022/paper.691