J Cell Signal. 2024;5(4):176-182.

Journal of Cellular Signaling Research Article

Elevated Interleukin-6 (IL-6) Levels with Ig (G & M) Antibodies in the Recovery Phase of Patients with COVID-19: Indication of Cytokine Storm and Re-infection

John Bolodeoku[1,*], Terry Gbaa[1]

[1]JB Consulting Limited, Building 3, Level 1, Regus, 111 Concorde Road, Maidenhead, Berkshire. SL6 4BY, UK

[*]Correspondence should be addressed to John Bolodeoku, john.bolodeoku@jbconsultingmdp.com

Received date: August 30, 2024, **Accepted date:** September 18, 2024

Citation: Bolodeoku J, Gbaa T. Elevated Interleukin-6 (IL-6) Levels with Ig (G & M) Antibodies in the Recovery Phase of Patients with COVID-19: Indication of Cytokine Storm and Re-infection. J Cell Signal. 2024;5(4):176-182.

Abstract

The innate immune response to coronavirus disease (COVID-19) by inflammatory cytokines, such as interleukin 6 (IL-6), has been described as an early response, followed by an adaptive immune response with the production of antibodies. IL-6 is produced in response to viral infections and is crucial for the activation of T cells and the differentiation of B cells, which produce antibodies. Immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies are produced during the initial acute period of infection and the recovery period from the period of onset of symptoms. There has been some relationship between the innate response of the cytokine and the adaptive response of the antibodies in patients with COVID-19 infection. There has been a positive correlation between IL-6 levels and the presence of COVID-19 antibodies, suggesting that higher IL-6 levels may be associated with a stronger immune response and studies have confirmed that patients with higher IL-6 levels have a higher antibody response. High IL-6 levels on admission observed within 2 weeks after onset of symptoms were positively correlated with high concentrations of IgG seen 5 weeks after onset of symptoms. We have previously described elevated IL-6 levels in the sera of 68% of COVID-19 convalescent patients (40–93 days) post-onset of symptoms (recovery phase). In this study we decided to investigate whether there was some relationship between the IL-6 levels in patients 40–93 days post-onset of symptom in the recovery phase and their antibody response using a wide range of antibody assays (iCHROMA IgG, GOLD IgG & IgM, Abbott SARS-CoV-2 IgG II Quant-test (Abbott S IgG), Diasorin Liaison SARS-CoV-2 S1/2 (Diasorin S1/2 IgG). The patients were divided into two groups, those with normal IL-6 levels (<7 pg/ml) and those with elevated IL-6 levels (>7 pg/ml). All patients (100%) had positive IgG antibodies, only 8 out the 28 patients (29%) were positive for IgM antibodies. The mean antibodies concentrations of the patients (n = 9) with normal IL-6 levels (<7 pg/ml) for iCHROMA IgG (20.0 Units), GOLD IgG (34.4 AU/mL), Abbott IgG (4175 AU/mL), Diasorin Liaison IgG (75.8 AU/mL) and GOLD IgM (4.7 AU/mL), respectively. The mean antibodies concentrations of the patients (n = 19) with elevated IL-6 levels (>7 pg/ml) for iCHROMA IgG (20.9 Units), GOLD IgG, (34.9 AU/mL), Abbott IgG (2287 AU/mL), Diasorin Liaison IgG (121.7 AU/mL) and GOLD IgM (11.2 AU/mL), respectively. The antibodies of the patients with the elevated IL-6 levels were significantly higher for Diasorin Liaison IgG (p = 0.02) and GOLD IgM (p = 0.003) but lower for Abbott IgG (p = 0.02). In conclusion, this study using a panel of antibody assays highlights that increased levels of IL-6 in the convalescent phase (recovery phase) indicate an active immune response leading to higher concentrations of antibodies especially IgM, suggesting that cytokine storms and re-infection occur in the recovery phase.

Keywords: IL-6, COVID-19, IgG, IgM, Recovery period

Introduction

A variety of cells including macrophages, fibroblasts, and lymphocytes, produce the proinflammatory cytokine interleukin 6 (IL-6). The infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to a direct increase of IL-6 in a dose-dependent increase in IL-6. The rise in antibodies after IL-6 activation is an adaptive immune response; immunoglobulin M (IgM) is the first antibody produced in response to an infection. IgM antibodies are typically detectable in the blood within a few days to a week after the onset of symptoms. In the case of COVID-19, IgM antibodies against the SARS-CoV-2 virus can provide an early indication of an ongoing infection. There has been a positive correlation between IL-6 levels and the presence of COVID-19 antibodies, suggesting that higher IL-6 levels may be associated with a stronger immune response and in COVID-19 patients, elevated serum IL-6 expression have been linked to adverse clinical outcomes and mortality. A prospective cohort study investigated the association between IL-6 serum levels and disease severity in COVID-19 patients demonstrated that elevated IL-6 levels is related to prolongation of hospital stay and higher mortality [1]. At the initial onset of infection, SARS CoV-2 stimulates the production of IgM and immunoglobulin G (IgG), as well as neutralising antibodies [2]. However, IgG antibodies usually persist for weeks or months after the clearance of SARS-CoV-2 [3]. The innate immune response to COVID-19 by inflammatory cytokines such as IL-6 has been described as beginning on day 1 post-onset of symptoms, followed by an adaptive immune response with the production of antibodies such as IgG on day 10 and IgM on day 11 post-onset of symptoms [4]. The 2–7-day period showed the highest median IL-6 concentrations [4,5]. Furthermore, another study investigating the relationship between IL-6 levels and antibody response in COVID-19 patients demonstrated that patients with higher IL-6 levels had a higher antibody response, indicating a more robust immune response to the virus [6]. High IL-6 levels on admission observed within 2 weeks after onset of symptoms were positively correlated with high concentrations of IgG seen 5 weeks after onset of symptoms [7]. Whilst IL-6 levels are elevated in the early stages of COVID-19 infection, we recently described elevated levels of IL-6 in the sera of 68% of COVID-19 convalescent patients (40–93 days) post-onset of symptoms (recovery) indicating continuous activation of the immune system [8]. Despite this knowledge, it remains unclear to date whether elevated serum levels of cytokines such as IL-6 in the convalescent (recovery) phase are associated with anti-SARS-CoV-2 antibody responses. In this study, we undertook to study the relationship between serum IL-6 concentrations and the antibody response using a range of COVID-19 antibody assays.

Materials and Methods

Study cohort

There were twenty-eight (28) SARS-CoV-2 patient donors. These were supplied by Research Donors, an HTA- licensed and ISO 9001-2015-certified company with Research Ethics (REC) approval as a research tissue bank, as well as participating in the UK NEQAS QA scheme.

Sample collection and analysis

Cambridge Bioscience, which procures fresh human blood services in partnership with London-based research donors, provided the samples. The COVID-19 positivity of the donors was confirmed through a positive PCR or MD diagnosis and collected serum samples on average 69.5 days (40–93 days) after the onset of symptoms.

IL-6 analysis was carried out using the iCHROMA method previously described [6]. IgG and IgM analysis and quantification: IgG was quantified using different quantification platforms which included the iCHROMA IgG, GOLD IgG & IgM (> 10 AU/mL positive), Abbott SARS-CoV-2 IgG II Quant-test (Abbott S IgG), Diasorin Liaison SARS-CoV-2 S1/2 (Diasorin S1/2 IgG).

Ethical consideration

The donors accepted to be part of this study, giving their consent for their samples to be taken and analyzed for the given results.

Statistical analysis

F-Test were used for the statistical analysis. Statistical significance was presented as $p<0.05$.

Results

A cohort of 28 convalescent patients (40–93 days) post-onset of symptoms blood samples were collected , the demographic representation (age and gender) of the study participants is seen in **(Table 1)** showing more females [20(71.4%)] than males [8(28.6%)] in this presentation, all patients (100%) had positive IgG antibodies, only 8 out the 27 patients (30%) were positive for IgM antibodies. Six of the 8 (75%) patients with elevated IgM (> 10 AU/mL) antibodies had elevated IL-6 levels.

The 9 patients with normal IL-6 concentrations (<7 pg/mL) consisted of 7 females and 2 males, with a mean age of 49.3 years with IgG positive in all (100%) patients and IgM positive in 2 (22%) patients. The mean IL-6 levels were 4.9 pg/mL and the mean antibodies concentrations for ICHROMA IgG, GOLD IgG, Abbott IgG, Diasorin Liaison IgG and GOLD IgM were 20.0 units, 34.4 AU/mL, 4175 AU/mL, 75.9 AU/mL and 4.9 AU/mL, respectively **(Table 2)**.

The 19 patients with elevated IL-6 concentrations (>7 pg/mL) consisted of 13 females and 6 males, with a mean age of 49.3 years with IgG positive in all (100%) patients and IgM positive in 6 (32%) patients. The mean IL-6 levels were 11.20 pg/mL and

Table 1. Showing demographics of the patient cohort and their IL-6 concentrations, IgG (iCHROMA, GOLD, Abbott, Diasorin Liaison) and IgM (GOLD) (N=28).

Patient #	Age (yrs)	Gender	IL-6 (pg/mL)	iCHROMA IgG (unit)	GOLD IgG (AU/mL)	Abbott IgG (AU/mL)	Diasorin Liaison (AU/mL)	GOLD IgM (AU/mL)
1	38	F	12.24	19.8	27.7	5009.5	254.00	9.1
2	45	F	7.30	19.7	21.8	264.7	23.80	1.3
3	44	F	11.46	20.1	22.3	1106.5	79.30	3.1
4	48	F	6.43	20.3	45	6604.3	121.00	2.6
5	55	F	3.59	20	20	471	29.50	1.2
6	65	F	2.65	21.2	30.5	1359.7	116.00	2.5
7	40	F	5.22	21.6	50.8	1406	108.00	14.8
8	39	M	3.13	20.7	39.2	3784.9	109.00	10.7
9	34	F	12.06	20.6	19.5	689.7	56.70	1.3
10	46	F	9.69	20.4	49.3	332.9	133.00	41
11	30	M	4.77	21.9	37.4	1718.9	18.20	2.6
12	47	F	10.46	20.2	20.4	941.4	57.80	48.6
13	57	F	6.75	21.5	15.6	230.1	24.20	4.5
14	36	M	15.97	23	51.1	1846.9	147.00	28
15	30	F	11.75	22.8	43.5	1440.3	346.00	2.3
16	60	F	5.65	17.4	46.8	20062.7	94.00	1.6
17	30	M	10.00	18.6	17.5	831.6	59.60	1.4
18	50	F	6.09	15.4	24	1938.5	62.90	2.4
19	35	F	29.01	17.8	52	2528	36.30	11.2
20	47	F	9.83	22.6	36.2	455.6	120.00	2.9
21	45	M	8.67	23.5	43.5	4106	114.00	2.9
22	42	F	8.52	15.9	41.5	699.7	38.70	5.1
23	66	M	8.60	14	26.1	305.4	25.60	4.8
24	53	F	11.43	21.5	36	1014.4	83.90	2.5
25	50	M	7.23	25.4	35.3	2812.5	111.00	4.6
26	25	F	8.29	32.6	24.9	1322.7	116.00	4.6
27	63	M	9.10	19.6	58.5	14942.2	355.00	25.4
28	30	F	11.26	19.5	36.1	2802.8	154.00	13

Table 2. Showing individual demographics and IL-6, iCHROMA IgG, GOLD IgG, Abbott IgG, Diasorin Liaison IgG, GOLD IgM concentrations of patients with normal IL-6 concentrations.

Patient	Age	Gender	IL-6 (pg/ml)	iCHROMA (Units)	GOLD IgG (AU/mL)	Abbott IgG (AU/mL)	Diasorin IgG (AU/mL)	GOLD IgM (AU/mL)
6	65	F	2.65	21.2	30.5	1359.7	116	2.5
8	39	M	3.13	20.7	39.2	3784.9	109	10.7
5	55	F	3.59	20	20	471	29.5	1.2
11	30	M	4.77	21.9	37.4	1718.9	18.2	2.6
7	40	F	5.22	21.6	50.8	1406	108	14.8
16	60	F	5.65	17.4	46.8	20062.7	94	1.6
18	50	F	6.09	15.4	24	1938.5	62.9	2.4
4	48	F	6.43	20.3	45	6604.3	121	2.6
13	57	F	6.75	21.5	15.6	230.1	24.2	4.5

the mean antibodies concentrations for ICHROMA IgG, GOLD IgG, Abbott IgG, Diasorin Liaison IgG and GOLD IgM were 20.9 units, 34.9 AU/mL, 2287 AU/mL, 121.7 AU/mL and 11.2 AU/mL, respectively **(Table 3)**.

There was no difference between the mean antibodies detected by the iCHROMA IgG ($p = 0.10$) and GOLD IgG ($p = 0.9$) assays. However, there was a significant difference observed between the mean antibodies detected by the Abbott IgG ($p = 0.02$), Diasorin IgG ($p = 0.02$) and GOLD IgM ($p = 0.003$) antibodies assays of the patients with normal and elevated IL-6 concentrations **(Table 4, Figures 1** and **2)**. Although the significant increase of the antibodies was mainly in the group with elevated Il-6 levels, the Abbott IgG levels in the elevated IL-6 levels were lower, this result appeared to be skewed this way due to the significant level detected in the 60-year-old female **(No. 16, Table 1)**.

Table 3. Showing individual demographics and IL-6, iCHROMA IgG, GOLD IgG, Abbott IgG, Diasorin Liaison IgG, GOLD IgM concentrations of patients with elevated IL-6 concentrations.

Patient	Age	Gender	IL-6 (pg/ml)	iCHROMA (Units)	GOLD IgG (AU/mL)	Abbott IgG (AU/mL)	Diasorin IgG (AU/mL)	GOLD IgM (AU/mL)
25	50	M	7.23	25.4	35.3	2812.5	111	4.6
2	45	F	7.3	19.7	21.8	264.7	23.8	1.3
26	25	F	8.29	32.6	24.9	1322.7	116	4.6
22	42	F	8.52	15.9	41.5	699.7	38.7	5.1
23	66	M	8.6	14	26.1	305.4	25.6	4.8
21	45	M	8.67	23.5	43.5	4106	114	2.9
27	63	M	9.1	19.6	58.5	14942.2	355	25.4
10	46	F	9.69	20.4	49.3	332.9	133	41
20	47	F	9.83	22.6	36.2	455.6	120	2.9
17	30	M	10	18.6	17.5	831.6	59.6	1.4
12	47	F	10.46	20.2	20.4	941.4	57.8	48.6

28	30	F	11.26	19.5	36.1	2802.8	154	13
24	53	F	11.43	21.5	36	1014.4	83.9	2.5
3	44	F	11.46	20.1	22.3	1106.5	79.3	3.1
15	30	F	11.75	22.8	43.5	1440.3	346	2.3
9	34	F	12.06	20.6	19.5	689.7	56.7	1.3
1	38	F	12.24	19.8	27.7	5009.5	254	9.1
14	36	M	15.97	23	51.1	1846.9	147	28
19	35	F	29.01	17.8	52	2528	36.3	11.2

Table 4. Showing F-test result of IL-6, iCHROMA IgG, GOLD IgG, Abbott IgG, Liaison Diasorin IgG and GOLD IgM of patients with normal IL-6 (<7 pg/mL) and elevated IL-6 (>7 pg/mL) concentrations.

	IL-6 (pg/ml)	iCHROMA (Units)	GOLD IgG (AU/mL)	Abbott IgG (AU/mL)	Diasorin IgG (AU/mL)	GOLD IgM (AU/mL)
F-Test	0.0022	0.1036	0.9423	0.02501	0.0211	0.0037

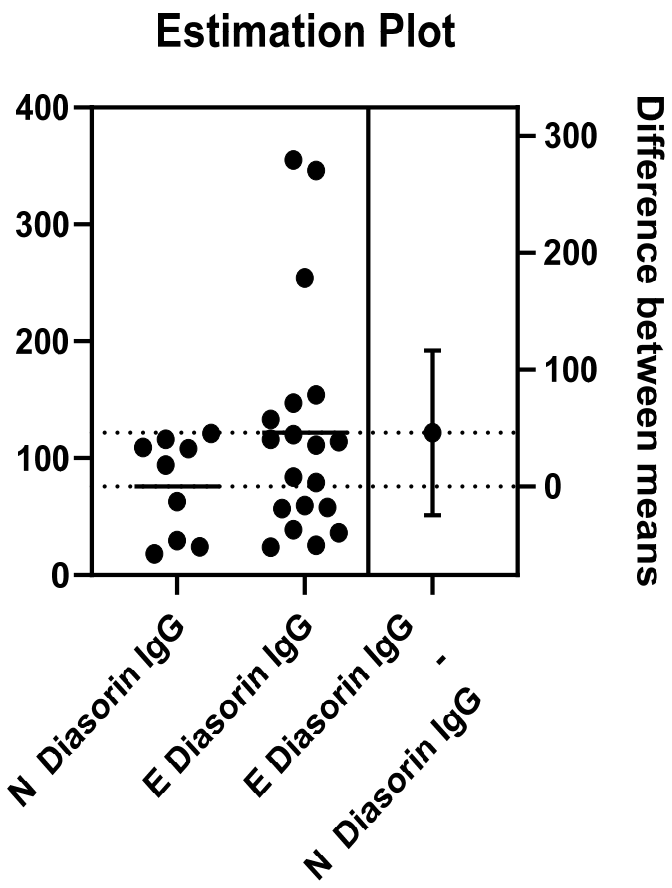

Figure 1. Showing estimation plots Diasorin IgG of patients with normal IL-6 (N) and elevated IL-6 (E) concentrations.

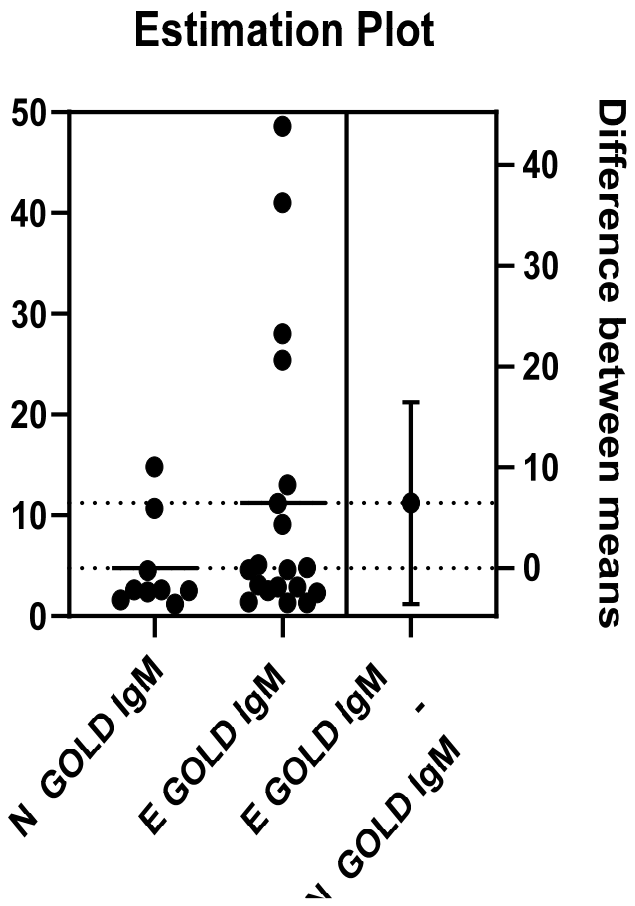

Figure 2. Showing estimation plots GOLD IgM of patients with normal IL-6 (N) and elevated IL-6 (E) concentrations.

Discussion

The infection of COVID-19 produces IL-6, a major cytokine, and stimulates the production of antibodies. The most produced antibodies are IgM and IgG. There is a study by Assaid *et al.* [9] that demonstrated an increase in IgM and IgG, however, the SARS-CoV-2-specific IgG were present after 3 months, and the IgM started to fall. The IgG remained stable at day 90, whereas the IgM waned to lower initial concentrations [10]. The elevation of IL-6 in the early stage of COVID-19 infection correlates positively with late-stage IgG responses [7]. In this study, we have described that the elevation of IL-6 levels in the convalescent phase (recovery) of patients with COVID-19 infection was significantly lower with the Abbott IgG assays ($p = 0.02$), higher but not significant with the iCHROMA IgG ($p = 0.10$) and GOLD IgG ($p = 0.9$) assays, and significantly higher with the Diasorin Liaison IgG assays ($p = 0.02$) and GOLD IgM assays ($p = 0.003$). Immunoglobulin M (IgM) plays a critical role as the first antibody produced in response to infection. IgM is a pentameric antibody that is primarily produced by B cells upon initial exposure to an antigen. It serves as an early defense mechanism, helping to neutralize pathogens and activate the complement system. In the context of viral infections, including COVID-19, the presence of IgM indicates recent exposure to the virus and can be a crucial marker for diagnosing acute infections [10,11]. Unlike, Immunoglobulin G (IgG) which is the most abundant antibody in the bloodstream and is critical for the immune response against pathogens also produced by B cells in response to infection and is essential for neutralizing viruses, opsonizing pathogens for phagocytosis, and activating the complement system [10]. In most studies looking at the antibody responses in COVID-19 infection, the IgG antibody was the most studied, in this study we found that with panel of IgG antibodies studied the results were consistent with an increase seen in all the IgG antibody assays apart from the Abbott IgG assay in which the result appeared to be skewed by one patient's extremely high result, although an inverse relationship between IL-6 and antibodies has been described [12]. This study confirms the patients with elevated IL-6 levels in the recovery phase, in which there was a greater tendency to have an increase in IgG and IgM antibodies. The most interesting observation was that the increase in IgM

antibodies using the GOLD IgM antibody assay was the most significant differentiator between the two groups of patients with normal and elevated IL-6 levels. This is probably not quite a surprise as IgM serves as an indicator of recent infection and IL-6 is the first to rise in an infection prior to the antibodies. Therefore, this increase of IL-6 and IgM in the recovery phase of patients continues to demonstrate that these markers will be good indicators of reactivation of the immune system in response to another attack of the infection.

Conclusion

In conclusion, this study using a panel of antibody assays highlights that increases of IL-6 levels in the recovery phase indicate an active immune response leading to higher concentrations of antibodies especially IgM, suggesting that the combination of elevation of both IL-6 and IgM could be significant indicators of cytokine storms linked to re-infection.

Declaration of Interest

JB and TG carried out this investigation on behalf of JB Consulting MDP Limited which manages the distribution of the iCHROMA device and reagents in the UK for Boditech Med Inc, the manufacturers of the iCHROMA device and reagents.

References

1. Nikkhoo B, Mohammadi M, Hasani S, Sigari N, Borhani A, Ramezani C, et al. Elevated interleukin (IL)-6 as a predictor of disease severity among Covid-19 patients: a prospective cohort study. BMC Infect Dis. 2023 May 9;23(1):311.

2. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clin Infect Dis. 2020 Nov 19;71(16):2027-34.

3. Zhu L, Xu X, Zhu B, Guo X, Xu K, Song C, et al. Kinetics of SARS-CoV-2 Specific and Neutralizing Antibodies over Seven Months after Symptom Onset in COVID-19 Patients. Microbiol Spectr. 2021 Oct 31;9(2):e0059021.

4. Bolodeoku J, Bass M, Anyaeche C, Retnasingham V. A mild case of COVID-19 infection: An observational longitudinal study 27 days post symptom of antigen, antibodies (IgM & IgG), IL-6 and D-Dimer. A review. Am J Biomed Sci & Res. 2021;11(6):519-23.

5. Schultze JL, Aschenbrenner AC. COVID-19 and the human innate immune system. Cell. 2021 Apr 1;184(7):1671-92.

6. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020 Aug;26(8):1200-4.

7. Jing X, Xu M, Song D, Yue T, Wang Y, Zhang P, et al. Association between inflammatory cytokines and anti-SARS-CoV-2 antibodies in hospitalized patients with COVID-19. Immun Ageing. 2022 Mar 5;19(1):12.

8. Bolodeoku J, Anyaeche C, Bass M, Kim TK. Presence of Elevated Interleukin–6 (IL-6) in the Blood of COVID-19 Convalescent Patients (40–93 Days) Post Onset of Symptoms Could be an Indicator of Ongoing Activation of the Immune System. Journal of Cellular Signaling. 2023 Oct 24;4(4):163-8.

9. Assaid N, Arich S, Charoute H, Akarid K, Anouar Sadat M, Maaroufi A, et al. Kinetics of SARS-CoV-2 IgM and IgG Antibodies 3 Months after COVID-19 Onset in Moroccan Patients. Am J Trop Med Hyg. 2022 Dec 12;108(1):145-54.

10. Haslund-Gourley BS, Woloszczuk K, Hou J, Connors J, Cusimano G, Bell M, et al. IgM N-glycosylation correlates with COVID-19 severity and rate of complement deposition. Nat Commun. 2024 Jan 9;15(1):404.

11. Zhou W, Xu X, Chang Z, Wang H, Zhong X, Tong X, et al. The dynamic changes of serum IgM and IgG against SARS-CoV-2 in patients with COVID-19. J Med Virol. 2021 Feb;93(2):924-33.

12. Mollazadeh S, Mahmoudi M, Mohammadi M, Kamal Kheder R, Nasiri Mahallati H, Sarbaz Haghighi S, et al. Investigation of IL-6 serum level in COVID-19 patients with positive COVID-19 IgG/IgM antibody titers to check inflammation and disease progression. Cytokine. 2024 May;177:156564.