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Immunoglobulin G antibody immune response profile following infection with SARS-CoV-2 in COVID-19 Egyptian patients

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Abstract

This study aimed to report the dynamic profile of IgG-specific antibodies to SARS-CoV-2 infection for 6 months after infection. We conducted a prospective study, recruited 33 recently confirmed covid -19 patients and collected 6 samples from each patient. The first samples were collected one month from the start of symptoms and subsequent samples collected at 30 days interval. We measured the IgG by chemiluminescent immunoassay (CLIA). According to the disease severity, patients were categorized as asymptomatic 4 (12.1%), mild 14 (42,4%), moderate 9 (27.3%), and severe 6 (18.2%). Patients were 12 (35.3%) females and 21 (64.7%) males. The mean IgG levels maintained a high level till the second month (92.81 ± 110.15 AU/ml) from the onset of symptoms followed by a gradual decrease till the sixth month after infection (17.42 ± 22.61 AU/ml). The patients with severe symptoms significantly exhibited the highest IgG levels, reached the highest level (mean=237.44 ± 164.13 AU/ml) at the second month. While the lowest levels were detected among the asymptomatic patients (mean= 3.04 ± 2.94 AU/ml) at the second month. Older age correlated with higher IgG antibody level (r= 0.350 *p*=0.046); however, sex was not related to IgG level. In conclusion, Symptomatic COVID-19 disease is followed by protective immunity for more than 6 months. Immunity in asymptomatic patients is low and fades rapidly than symptomatic cases. Patients with severe disease had significantly higher IgG levels compared to mild, moderate, or asymptomatic patients.

Keywords: Covid -19; SARS-CoV-2 IgG; Chemiluminescence.

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Introduction

At the end of 2019, a novel coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan, a city in the Hubei Province of China.[1] It rapidly spread, resulting in an epidemic throughout China, followed by a global pandemic. In February of 2020, the World Health Organization named it COVID-19, which stands for coronavirus disease 2019.[2] COVID-19 is caused by a virus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is an enveloped, single-stranded positive-sense RNA (+ssRNA) virus belong to Beta coronaviruses, family *Coronaviridae*.[3] COVID-19 disease severity ranges from asymptomatic to fatal disease.[4] The human immune response to this novel pathogen has both innate and adaptive arms. One aspect of adaptive immunity is the humoral immune response that features the production of antibodies recognizing specific antigenic epitopes. The humoral immune response to COVID-19 include IgM, IgA, and IgG antibodies. The IgG is the neutralizing antibody carrying the anti-viral infection, helps virus neutralization, and clearance, and protects against subsequent reinfection providing long-term immunity.[5]

Previous studies reported rapid antibody response to SARS-CoV-2 in the first two to three weeks, IgM antibodies appear rapidly and last up to 10 weeks post infection.[6,5] The duration of specific IgG antibodies against COVID-19 is controversial reaching 3 to 6 months, even seroconversion may not occur in some patients as reported previously.[7–10]

Natural infection provokes a protective immune response that can protect from subsequent reinfection and can provide herd immunity, The duration of protective immunity in convalescent COVID-19 patients can affect people mobilization, international travel, social work, and the frequency of vaccination [9–11]. In the current work, we conducted a prospective study to measure the IgG antibody response after COVID-19 infection (convalescence period), and to assess the dynamics of IgG level over 6 months after COVID-19 infection.

Subjects and Methods

Study design

This is a prospective hospital-based study. The study included 33 COVID-19 patients and was conducted in the central research laboratory at Sohag University Hospital, during the period between (June 2020 to January 2021). The inclusion criteria were patients with recently confirmed infection with SARS-CoV-2 clinically and/or based on laboratory findings, by the presence of SARS-CoV-2 RNA, using the qualitative reverse transcription polymerase chain reaction (RT-PCR) nasal and pharyngeal swab specimens. Exclusion criteria included age less than 16 or more than 60 years. Pregnant females and patients with autoimmune diseases were excluded from the study.

Ethical consideration

The study protocol was reviewed and approved by the Medical Research Ethics Committee, Faculty of Medicine, Sohag University (Reference: IBR#S20-133, dated July 2020).

Data collection

The following data were collected from all study subjects, age, sex, occupation, associated chronic disease, symptoms, and signs of COVID-19 infection. In addition, routine laboratory, and radiology data during infection were obtained from hospital records. The symptomatic patients were classified into mild, moderate, and severe according to the National Institutes of Health (NIH) published guidelines.[12] Asymptomatic patients were RT-PCR tested positive contacts to COVID-19 cases.

Sample collection

Venous blood samples (5 ml) were withdrawn from each subject under aseptic conditions and immediately delivered into EDTA tubes and plasma was separated after centrifugation. A total of 6 blood samples were collected from each patient. The first blood sample was collected about one month after the onset of symptoms for symptomatic patients. For asymptomatic cases, blood samples were collected two weeks after their nasal and pharyngeal swab specimens RT-PCR assay

tested negative. One serum sample from each study subject was tested every month (30-day interval) 5 times.

Detection of SARS-CoV-2 IgG Antibody by chemiluminescent immunoassay (CLIA)

SARS-CoV-2 IgG antibody levels in plasma specimens were detected by a commercial kit (YHLO-CLIA-SARS-CoV-2 IgG kits supplied by YHLO, China), using an automated chemiluminescence immunoassay analyzer (iFlash 3000 CLIA analyzer, YHLO, China), according to the manufacturer's instructions. The resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship exists between the amount of anti-SARS-CoV-2 IgG in the sample and the RLUs detected by the analyzer optical system. The results were determined through a calibration curve, which was instrument-specifically generated by 2-point calibration, and a master curve provided via the reagent quick response (QR) code. Antibody levels were expressed as antibody unit (AU)/ml. A value of ≥ 10.0 AU/mL was considered positive, and a value of < 10.0 AU/mL considered negative.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows version 20. Quantitative data were expressed as means ± standard deviation, median, and range. Qualitative data were expressed as numbers and percentages. The data were tested for normality using Kolmogrov–the Smirnov test and the Shapiro-Wilk test. Mann-Whitney U test, Wilcoxon Signed Ranks test, Kruskal Wallis test, Spearman correlation, and Friedman test with multiple pairwise comparisons tests were used for comparison between repeated measurements of the studied patients as data were not normally distributed. A level of >5% was chosen as a level of significance in all statistical tests used in the study.

Results

Patient characteristics

In this study a total of 33 COVID-19 patients were followed. The median age of these patients was 38 years (range, 32-47 years) and 21 were males (63.6%). Of the study subjects, 21 (63.6 %) were health care providers of which 51% probably caught the infection from occupational exposure. The patients were classified according to severity of symptoms into asymptomatic 12.1% (n=4), mild 42.4 (n=14), moderate 27.3% (n=9), severe18.2% (n=6). The mean duration from the onset of symptoms to the first sample collection was 35 days (34 – 36.5), then 5 samples were collected at 30-day interval from the first sample collection (Table 1). Signes and symptoms of COVID-19 in study patients are shown in Table 2.

Table 1. Demographics and severity classification of the 33 study subjects.

Characteristics	Summary statistics No (%)
Gender	
Female	12 (36.4%)
Male	21 (63.6%)
Age (years)	
Mean± S.D.	38.7 ± 9.76
Median (Range)	38 (32 – 47)
The time lag from symptom to zero sample	
Mean± S.D.	35.42 ± 2.02
Median (Range)	35 (34 – 36.5)
Degree of symptoms	
Asymptomatic	4 (12.1 %)
Mild	14 (42.4%)
Moderate	9 (27.3 %)
Severe	6 (18.2 %)
Occupation	
Non-medical	12 (36.4%)
Health care providers	21 (63.6%)
The probable place to catch the infection	
Social	16 (48.5%)
Work	17 (51.5%)
Use of PPE before symptom onset.	
No	19 (57.6%)
Yes	14 (42.4%)

PPE :Personal Protective Equipment as masks, face shields.

Table 2. Clinical presentation of the 33 study subjects.

Characteristics	Summary statistics No (%)
Fever	
No	8 (24.2%)
Yes	25 (75.8%)
Dry cough	
No	15 (45.5%)
Yes	18 (54.5%)
Tiredness	
No	6 (18.2%)
Yes	27 (81.8%)
Sore throat	
No	18 (54.5%)
Yes	15 (45.5%)
Aches and pains	
No	5 (15.2%)
Yes	28 (84.8%)
Headache	
No	14 (42.4%)
Yes	19 (57.6%)
Loss of smell and taste	
No	20 (60.6%)
Yes	13 (39.4%)
Sinusitis	
No	32 (97%)
Yes	1 (3%)
Diarrhea	
No	21 (63.6%)
Yes	12 (36.4%)
Skin rash	
No	32 (97%)
Yes	1 (3%)
Discoloration	
No	32 (97 %)
Yes	1 (3%)
Serious symptoms	
No	24 (72.7%)
Yes	9 (27.3 %)
Breathing difficulty	
No	24 (72.7%)
Yes	9 (27.3 %)
Chest pain	
No	25 (75.8%)
Yes	8 (24.2 %)
Loss of speech or movement	
No	33 (100%)
Yes	0 (0.0%)
Complications	
No	30 (90.9%)
Yes	3 (9.1%)

The profile of anti-SARS-CoV-2 IgG in the 33 study subjects

At the time of the first sample collection, 30 days from the onset of symptoms all the patients were IgG positive. The level of IgG maintained a high level (mean± SD, 92.42 ± 98.79) till the second month from the onset of symptoms followed by a gradual decrease till the sixth month (17.42 ± 22.61), Figure 1, Table 3. The reduction in IgG levels between the mean baseline measure and the third month mean measure was about 44 % and by the sixth month reached about 79% (Table 4).

Comparison between IgG levels in asymptomatic, mild, moderate-severe cases

There were statistically significant differences between IgG profile of patients with mild, moderate, and severe presentation and asymptomatic cases. Patients with severe symptoms had significantly higher mean IgG levels than the mean measures of the other groups, over the study period (6 months) and maintained the highest level till the last sample (Figure 2).

Comparison of the percent decline of IgG level after the third and sixth month between asymptomatic, mild, moderate-severe cases

For the group with severe symptoms, the mean percent of IgG decline by the third and sixth month (17.78 ± 51.76 and 58.97 ± 34.11, respectively) were the least compared to other groups. However, there was no difference between the percent decrease of IgG levels in the third and sixth months for the asymptomatic subjects (Table 4).

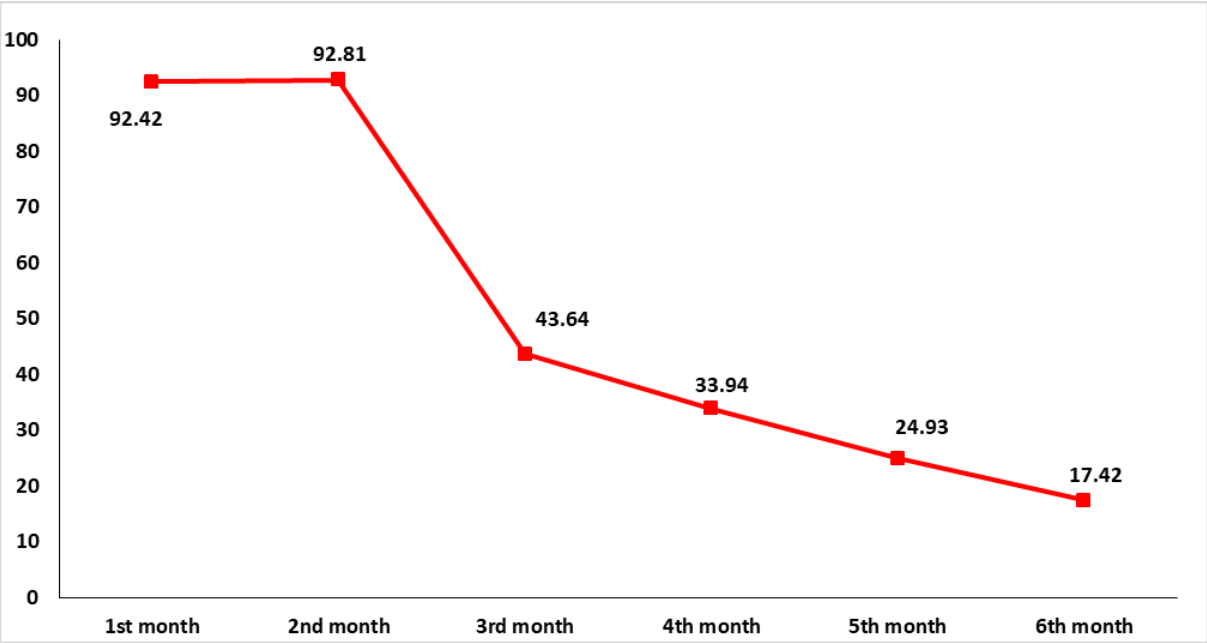

Figure 1. Comparison between the mean anti-SARS-CoV-2 IgG measures.

Table 3. Comparison between the percent decrease of IgG levels in the 33 study subjects after the third and sixth month.

Percent reduction of IgG level	Third month	Sixth month	*p-value
Mean± S.D.	44.26 ± 34.74	79.21 ± 19.58	<0.001
Median (IQ range)	44.5 (25.32 – 74.9)	85.19 (68.64 – 93.72)	

p-value was calculated by Wilcoxon Signed Ranks Test. *$p \leq 0.05$ is significant.

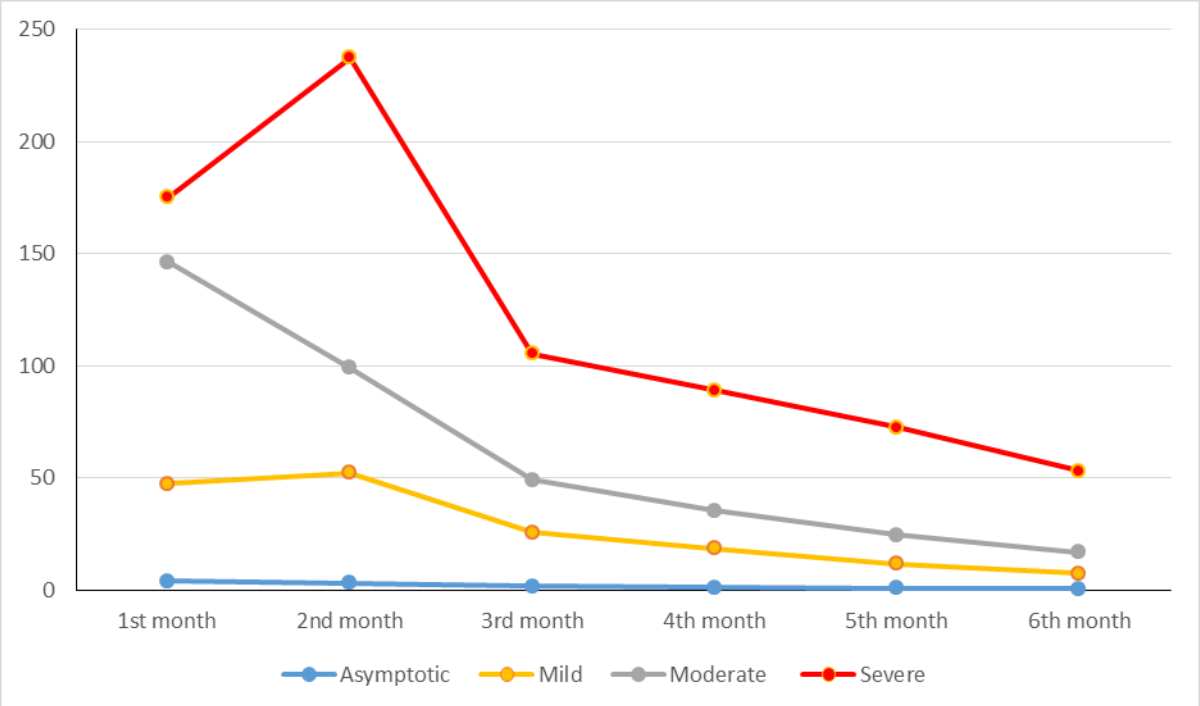

Figure 2. Anti-COVID-19 IgG antibody profile in response to disease severity.

Table 4. The dynamic change of IgG levels in the 33 study subjects after the third and sixth month in relation to disease severity.

Percentage decrease of IgG levels	Third month	Sixth month	*p*-value
Asymptomatic			
Mean± S.D.	61.35 ± 31.7	83.07± 13.86	NS
Median (IQ range)	60.99 (32.01 – 91.06)	85.50 (68.73 – 94.96)	
Mild			
Mean± S.D.	41.95 ± 28.59	84.1 ± 10.73	0.001
Median (IQ range)	38.39 (21.02 – 61.82)	85.31 (77.87 – 94.46)	
Moderate			
Mean± S.D.	57.92 ± 23.57	83.38 ± 13.08	0.008
Median (IQ range)	70.07 (40.19 – 78.19)	91.50 (67.45– 94.05)	
Severe			
Mean± S.D.	17.78 ± 51.76	58.97 ± 34.11	0.028
Median (IQ range)	23.17 (-30.88 – 68.45)	70.26 (26.16 – 87.77)	

p-value was calculated by Wilcoxon Signed Ranks Test. *$p \leq 0.05$ is significant.

Relation between age and gender and IgG level

There was no relation between gender of the study group and IgG levels (Table 5). However, Spearman correlation coefficient indicated that increasing age was correlated to higher IgG levels (r=0.350, *p*=0.046).

Table 5. Relation between gender and IgG levels in the 33 study subjects.

IgG level	Female	Male	*p*-value
Mean± S.D.	102.13 ± 82.18	86.87 ± 108.68	NS
Median (IQ range)	88.38 (51.12 – 110.01)	47.04 (19.68 – 98.3)	

p- value was calculated by Mann-Whitney U Test. *$p \leq 0.05$ is significant.

Discussion

In the current prospective study, we studied the IgG antibodies response after COVID-19 infection (convalescence period), over 6 months after COVID-19 infection. Our study observed the COVID-19 IgG titer for 6 months (180 days) in 33 convalescent COVID-19 patients. All patients were IgG antibody positive, by the fifth week of infection, as 100 % of study subjects were IgG positive at that time. In a recent study by *Sherina et al.,* 2021, 85% of study subjects were positive by the fourth week after onset of symptoms.[9]

In the current study, the curve of the mean IgG titer from all study groups increased, reached the maximum in the second month then declined over the next following 4 months. The mean IgG titer decreased by 44% at the third month and 79% at the sixth month. In the same line, a study by Zhou et al, 2021 reported a decline of anti-SARS-CoV-2 IgG levels after the second month, then the IgG level decreased to its half.[6]

In our study, the curve of IgG levels in severe cases was significantly higher than that in other groups over 6 months after infection and the percentage of decline of IgG levels at the 6 months titer was significantly lower in severely symptomatic patients compared to other groups. Many previous studies showed that severe COVID-19 cases showed higher antibody responses.[13,14]

The severity of the COVID-19 disease significantly affected the level of antibodies all over the period of follow up. Patients with severe COVID-19 diseases had higher IgG compared to mild, moderate, and asymptomatic patients. The more severe disease the higher antibody levels and the

prolonged duration of antibodies persistence. This can be explained by considering that the more severe disease the more virus replication, leading to more expression of virus antigen which stimulates a stronger humoral immune response.[7,15] However, some other studies reported no significant difference between disease severity and antibody levels.[6,9]

In our study, the percent reduction of antibody levels in all patients at the third month was 44% and by the sixth month 79% from the first reported level. However, we noted that the level of reduction was lower in the severe patient's group, as after 6 months, as the mean reduction was about 60% of their starting level. While the mean reduction in other groups; mild and moderate patients' groups, was about 83%. It is expected that patients with severe disease would have a prolonged time of antibody persistence than other groups.

The role of antibodies in COVID-19 severe cases is controversial whether the antibodies are involved in the immunopathology rather than protective function. Other studies showed that antibodies in the plasma of convalescent patients have neutralizing activity.[5] Convalescence plasma was used in trials to treat severely ill COVID-19 patients, although their benefits is controversial.[16]

Asymptomatic patients in our work developed an antibody response that is significantly lower than symptomatic patients which faded rapidly. This can be explained by less virus replication and less stimulation of the immune system. Asymptomatic patients are hence not protected making them liable to reinfection. Several previous studies reported data in support to this findings.[15,17]

In our study, another factor, age of study subjects was correlated to the IgG levels. This observation could be because that subjects with old age had frequently severe disease. However, a recent study by *Moradi et al., 2021* found no relation between age and antibody levels [7]. Our study had one limitation, the small sample size. However, our results agreed with other studies with larger sample sizes.[13,16,18,19]

In conclusion, findings of our study may help in understanding humoral immune response patterns after COVID-19 disease. Such data highlighted that severity of disease and age of the patient can affect IgG levels and post covid immunity.

Author Contributions

AMG; reviewed the protocol, laboratory work, wrote and submitted the manuscript, the corresponding author. HMH; AHA; reviewed the protocol, laboratory work, shared in writing and reviewing the manuscript. HME; reviewed the protocol, collecting data, clinical assessment of the patients, shared in writing and reviewing the manuscript. MF; reviewed the protocol, laboratory work, shared in writing and reviewing the manuscript. EMA; EMM; AKN; approved the protocol, clinical assessment of the patients, reviewed the manuscript. NAM; approved the protocol, statistical analysis, reviewed the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Medical Research Ethics Committee, Faculty of Medicine, Sohag University (Reference: IBR#S20-133, dated July 2020. Clinical Trials.gov ID: NCT04483622.

Informed consent

A signed consent form was obtained from each study participant.

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References

1. Chen N, Zhou M, Dong X, et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*.;395(10223):507-513.

2. Lee CYP, Lin RTP, Renia L, et al. (2020). Serological Approaches for COVID-19: Epidemiologic Perspective

on Surveillance and Control. *Front Immunol*. 11(April):1-7. doi:10.3389/fimmu.2020.00879

3. V'kovski P, Kratzel A, Steiner S, et al. (2021). Coronavirus biology and replication: implications for SARS-CoV-2. *Nat Rev Microbiol*. 19(3):155-170. doi:10.1038/s41579-020-00468-6

4. Solomon M, Liang C. (2022). Human coronaviruses: The emergence of SARS-CoV-2 and management of COVID-19. *Virus Res*.;319:198882. doi:https://doi.org/10.1016/j.virusres.2022.198882

5. Ni L, Ye F, Cheng M-L, et al. (2020). Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. *Immunity*. 52(6):971-977.e3. doi:10.1016/j.immuni.2020.04.023

6. Zhou W, Xu X, Chang Z, et al. (2021). The dynamic changes of serum IgM and IgG against SARS-CoV-2 in patients with COVID-19. *J Med Virol*. 93(2):924-933. doi:https://doi.org/10.1002/jmv.26353

7. Moradi G, Mohamadi Bolbanabad A, Ahmadi S, et al. (2021). Persistence assessment of SARS-CoV-2-specific IgG antibody in recovered COVID-19 individuals and its association with clinical symptoms and disease severity: A prospective longitudinal cohort study. *Int Immunopharmacol*. 98(March):107893. doi:10.1016/j.intimp.2021.107893

8. Siracusano G, Pastori C, Lopalco L. (2020). Humoral Immune Responses in COVID-19 Patients: A Window on the State of the Art. *Front Immunol*. 2020;11. doi:10.3389/fimmu.01049

9. Sherina N, Piralla A, Du L, et al. (2021). Persistence of SARS-CoV-2-specific B and T cell responses in convalescent COVID-19 patients 6–8 months after the infection. *Med*. 2(3):281-295.e4. doi:10.1016/j.medj.2021.02.001

10. Anand SP, Prévost J, Nayrac M, et al. (2021). Longitudinal analysis of humoral immunity against SARS-CoV-2 Spike in convalescent individuals up to 8 months post-symptom onset. *Cell Reports Med*. 2(6):16. doi:10.1016/j.xcrm.2021.100290

11. Gudbjartsson DF, Norddahl GL, Melsted P, et al. (2020). Humoral Immune Response to SARS-CoV-2 in Iceland. *N Engl J Med*. 383(18):1724-1734. doi:10.1056/NEJMOA2026116/SUPPL_FILE/NEJMOA2026116_DISCLOSURES.PDF

12. Clinical Spectrum COVID-19 Treatment Guidelines. Accessed December 4, 2021. https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/

13. Gerhards C, Thiaucourt M, Kittel M, et al. (2021). Longitudinal assessment of anti-SARS-CoV-2 antibody dynamics and clinical features following convalescence from a COVID-19 infection. *Int J Infect Dis*. 107:221-227. doi:https://doi.org/10.1016/j.ijid.2021.04.080

14. Liu X, Wang J, Xu X, et al. (2020). Patterns of IgG and IgM antibody response in COVID-19 patients. *Emerg Microbes Infect*. 9(1):1269-1274. doi:10.1080/22221751.2020.1773324

15. Carsetti R, Zaffina S, Piano Mortari E, et al. (2020). Different Innate and Adaptive Immune Responses to SARS-CoV-2 Infection of Asymptomatic, Mild, and Severe Cases. *Front Immunol*. 11:610300. doi:10.3389/fimmu.2020.610300

16. Snow TACC, Saleem N, Ambler G, et al. (2021). Convalescent plasma for COVID-19: a meta-analysis, trial sequential analysis, and meta-regression. *Br J Anaesth*. 127(6):834-844. doi:https://doi.org/10.1016/j.bja.2021.07.033

17. Long QX, Tang XJ, Shi QL, e et al. (2020).. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med 2020 268*. 26(8):1200-1204. doi:10.1038/s41591-020-0965-6

18. Koutsakos M, Rowntree LC, Hensen L, et al. (2021). Integrated immune dynamics define correlates of COVID-19 severity and antibody responses. *Cell Reports Med*. 2(3). doi:10.1016/j.xcrm.2021.100208

19. Zhou Y, Zhang J, Wang DDD, et al. (2021). Profiling of the immune repertoire in COVID-19 patients with mild, severe, convalescent, or retesting-positive status. *J Autoimmun*. 118(October 2020):102596. doi:https://doi.org/10.1016/j.jaut.2021.102596