Longitudinal observation of antibody responses after SARS-CoV-2 infection at 14 months

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TITLE PAGE

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Longitudinal observation of antibody responses after SARS-CoV-2 infection at 14 months

Introduction

As the worldwide vaccination implementation programs against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causing Coronavirus disease 2019 (COVID-19) are progressing in full swing, information regarding the kinetics and longevity of acquired immunity post-natural infection necessitates analysis as well as documentation. The SARS-CoV-2 shares approximately 79.5% genomic homology with SARS-CoV-1 with a similar receptor-binding domain (RBD) structure. [1] Therefore, much understanding of the immunity offered post-SARS-CoV-2 infection is derived from previous experiences with SARS-CoV-1, where protective antibodies were found to persist for at least 2 years in addition to real-time emerging data. [2-4] A “robust adaptive immune response” with positive S-specific neutralizing antibodies (n Abs), memory B cells, and circulating follicular helper T cells have been demonstrated in recovered patients after SARS-CoV-2 infection. [5-7]

In this study, we aimed to assess the dynamics of IgG antibody titers against SARS-CoV-2 in recovered COVID-19 patients over 14 months after mild and moderately-severe infection. The demographics and clinical profile, that might be associated with the magnitude and longevity of antibody response was also analyzed. To our knowledge, the current study provides the longest follow-up reported in the literature till date.

Materials and methods

Patient cohort

A monocentric pilot observational study, that longitudinally analyzed the presence of antibodies against SARS-CoV-2 was conducted in patients based in Umbria region, Italy

who had tested positive for SARS-CoV-2 in March 2020 by Reverse Transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR). The RT-qPCR tests were performed by the Local health regulatory authorities according to the national guidelines and standard operating protocols. The patients were managed as per the set protocols by treating the doctor, prescribing home isolation for mild and moderate cases, hospitalization for cases with increased severity. On recovery, all subjects were informed about the seroprevalence study and were invited for voluntary participation. After written informed consent, serological samples were collected and antibody titers were analyzed using the MAGLUMI® 2019-nCoV IgM/IgG chemiluminescent analytical system (CLIA) assay and the MAGLUMI® SARS-CoV-2 S-RBD IgG CLIA. (New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China). Both these immunoassays; anti-nucleocapsid (anti NCP) and the anti-Spike-RBD (anti-S-RBD) were granted Emergency Use Authorization by the US Food and Drug Administration. [8] At the first serum sample collection, the participants were asked to provide information about their COVID-19 clinical history along with symptoms and treatment undertaken using a standardized questionnaire. They were then invited for voluntary follow up periodically for sequential serum sample antibody assessment. The study participants did not receive any compensation or any other benefit, but were informed individually about their antibody status.

Patient selection

From May 2020 to January 2021, anti-NCP antibodies developed against of SARS-CoV-2 were analysed using the MAGLUMI® 2019-nCoV IgM/IgG CLIA assay through sequential serum samples. We treated time as a factor and defined six different time points (TPs); (T0-T5). The first blood sample was collected in the month of May 2020, 2 months after the month of infection-March and was defined as T0. Consecutive serological samples were analysed at different TPs; three months (T1), five months (T2), seven months (T3), eight months (T4) and ten months (T5) post infection in June, August, October, November

of 2020 and January 2021 respectively. At this point, a more specific immunoassay; MAGLUMI® SARS-CoV-2 S-RBD IgG CLIA was adopted for future assessments.

From late February 2021, an additional n=12 patients (8 female and 4 male), who met the eligibility criteria for participation, were enrolled in the study and added to the original cohort (n=30). These patients (n=12), similar to the original cohort, had a history of testing positive for SARS-CoV-2 by RT-qPCR in March 2020, updating the sample size to n=42.

Since the legal provisions adopted by the Italian Ministry of Health advised mandatory vaccination for all Healthcare Workers, irrespective of previous disease status, n=10 patients (4 female and 6 male) were gradually vaccinated from mid-March 2021 and hence excluded from the original cohort, making the revised final sample size as n=32. The study design, study findings and the temporal distribution of sequential serological sampling time points are described in Figure 1. The study group was divided into two groups at each TP based on disease severity; Mild and Moderately-Severe and the antibody assessments were done accordingly. [9]

In brief, antibodies against NCP were analyzed at T0-T5 (over 10 months post infection; March 2020-January 2021) in n=30 patients followed by analysis of antibodies against Spike-RBD from T6-T8 (over 12-14 months post infection) in n=32 patients.

The blood samples were collected after informed consent by the patients and with the approval of the ethics committee of the Associazione Naso Sano (Document number ANS-2020/001) at an accredited lab (Laboratory of Nuclear Lipid BioPathology, *CRABION*, Perugia, Italy). Data collection and analysis was masked from main principal investigator, who was also a part of the study sample in order to avoid observer bias. [10,11] The study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. The STROBE statement checklist can be found in Supplementary Table S.

Analytical system used

As per the Specifications, anti-SARS-CoV-2 S-RBD IgG assay had a sensitivity 100% with CI [99.9%-100.0%] at ≥ 15 days post symptom onset and specificity of 99.6%; CI [98.7%-100.0%]. High concentration samples were diluted automatically by analyzers and the recommended dilution was 1:9 with the diluent in kit. The sample, buffer and magnetic microbeads coated with S-RBD recombinant antigen were mixed thoroughly and incubated, forming immune-complexes. After precipitation, decanting of supernatant, and performing a wash cycle, ABEI labeled with anti-human IgG antibody was added, and incubated to form complexes. Again after precipitation in a magnetic field, decanting of supernatant, and performing another wash cycle, the Starter 1+2 were added to initiate a chemiluminescent reaction. The light signal was measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of SARS-CoV-2 S-RBD IgG presented in the sample. The measurements and interpretation of results were made according to the manufacturer's instructions. The analyzer automatically calculates the concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results were expressed in AU/mL. A result less than 1.00 AU/mL (< 1.00 AU/mL) was considered to be non-reactive while a result greater than or equal to 1.00 AU/mL (≥ 1.00 AU/mL) was considered to be reactive. [12]

STATISTICAL ANALYSIS

The descriptive statistics for the main characteristics of the study group were expressed as Median, [1st -3rd] quartile for continuous variables and as absolute frequency (column percentage) for the categorical variables. The normal distribution of data was tested by One sample Kolmogorov-Smirnov test. The p-values resulted from Mann Whitney U test, Friedman Test, Pearson's Chi-squared test (for cell frequency $n \geq 5$) and Fisher's exact test (for cell frequency $n < 5$). Statistical significance was defined for $p < 0.05$. All analyses and data plotting were performed using SPSS Version 22.

RESULTS

Study group characteristics during infection and at 14 months post infection

N abs against S-RBD of SARS-CoV-2 were analyzed for n=32 at 14 months post infection. Of these, n=21 (66%) were females and n=11 (34%) were males. The disease severity was rated as mild and moderately-severe in n=19 (59.3%) and n=13 (40.7%), respectively. It was noted that n=18 (56.2%) declared one or more comorbidities such as asthma/seasonal allergies, diabetes, hypertension or cardiovascular diseases. Of these, n=5 (38.5%) subjects of the moderately-severe category, had a cardiovascular disease which was a significant finding ($p=0.006$). In terms of clinical symptoms experienced, the subjects in the moderately-severe group (n=13) had significant shortness of breath (n=10, $p=0.029$), fatigue (n=12, $p=0.011$) and headache (n=8, $p=0.046$). The baseline clinical, demographic features and disease characteristics of the study subjects at the time of infection (March 2020) are reported in Table 1. The main characteristics were expressed as Median (q2) with First and Third quartiles i.e., (q1-q3) for continuous variables and as absolute frequency and column percentage for binary variables. The p-values resulted from Mann Whitney U test, Pearson's Chi-squared test (for cell frequencies $n \geq 5$) and Fisher's exact test (for cell frequencies $n < 5$). Table 2 reports the baseline clinical, demographic features and disease characteristics of subjects followed up at 14 months with respect to disease severity.

Role of co-morbidities on antibody titers

It was observed that the subjects with one or more comorbidities (n=18) with respect to disease severity, developed a better antibody titer at 14 months as compared to the group (n=14) without any comorbidity. The p value was significant. ($p=0.033$). A study by Huang et al. also found that diabetes was associated with higher IgG levels. [13] This could also mean that subjects with a more severe disease due to one or more comorbidities had a better antibody titer at 14 months. Similar findings were observed by studies by

Gudbjartsson DF et. al, Chirathaworn C et al, Huang M et al, Terpos E et al, and Zhao J et. al. [14-17] However, larger studies are needed to draw stronger conclusions regarding these associations.

Role of Loss of smell and taste on antibody titers

It was observed that the subjects who experienced loss of smell and taste during infection, with respect to disease severity, developed a better antibody titer at 14 months. ($p=0.043$ and $p=0.031$ respectively). Although similar p values of significance were observed for lower antibody titers developed at 14 months in healthcare workers as compared to non-healthcare workers ($p=0.023$), a generalized comment is not justified. A significant p value in such a situation could be due to $n=6$ subjects in the healthcare workers group and $n=26$ for non-healthcare workers resulting in bias.

Serologic status at 14 months post-infection

At 14 months post-infection in May 2021, the percentage of anti-SARS-CoV-2 S-RBD IgG positive subjects were analyzed and 97.14% (34 out of 35) patients were positive for anti-SARS-CoV-2 RBD IgG at 14 months. This was also observed for the preceding 12 and 13 months (34/35 positive for anti-S-RBD antibodies) but the median neutralization titer differed at each time point. [Figure 2] In terms of disease severity, it was observed that in both the groups, the antibody titers developed at different Time periods (TPs) were discrete and statistically significant (p value <0.001 for the mild group and the Moderately- Severe group). The results are described in Table 3 [Table 3] Since the data repeats over a period of time, multiple comparison test; i.e The Friedman's Two-way ANOVA test was applied. [Figure 3] In order to highlight the significance of the TPs with respect to each other, pairwise multiple comparison tests; i.e Two/More related samples test was applied.

Role of disease severity on antibody titers

Median neutralization titer (MNT) was calculated for both disease severity groups. It was observed that the subjects of the moderately-severe group developed a higher median antibody titer (14.78 AU/ml) at 14 months when compared to the mild group (5.55 AU/ml) but the dispersion or variation was higher for the Moderately-Severe group, indicating a larger degree of variability in development of antibodies. The box plot shows the Median line at the 25th percentile in both the cases indicating that the data is positively skewed i.e., some values are towards the higher end. The subjects in the mild group show less variability in terms of developing antibody titers and have a smaller median while subjects of the Moderately-Severe group exhibit larger variation and also has a higher median. [Table 4]

DISCUSSION

The SARS-CoV-2 is an enveloped virus with four structural proteins: spike (S) protein, membrane (M) protein, envelope (E) protein and nucleocapsid (N) protein. Among these four structural proteins, the S and N proteins are the main immunogens. The S protein is a major protective antigen that elicits highly potent neutralizing antibodies (nAbs) and plays an essential role in viral attachment, fusion, entry, and transmission. The S protein comprises of an N-terminal S1 subunit responsible for virus-receptor binding and a C-terminal S2 subunit responsible for virus-cell membrane fusion. The S1 sub-unit is further divided into an N-terminal domain (NTD) and a receptor-binding domain (RBD). The RBD within S1 interacts directly with host receptors, human angiotensin converting enzyme 2 (hACE2). [18] The immunity against any infectious disease is comprised of two arms: innate immunity and adaptive or acquired immunity. Each of these arms contain humoral (B cells) and the cell mediated (T cell) immune elements.

Antibodies are synthesized and secreted by plasma cells that are derived from the B cells of the immune system and can be used as a correlate of immunity. Antibody tests also

known as serological tests, detect the presence of antibodies against a particular disease-causing agent in the blood, to evaluate the immune response against it. Antibodies can come in different varieties known as isotypes or classes which differ in their biological properties and ability to deal with different antigens and are called Immunoglobulins. Immunoglobulin (IgM) eliminates pathogens in the early stages of B Cell mediated immunity and is a marker of active infection, while immunoglobulin G (IgG) provides long lasting antibody-mediated immunity and is the only antibody capable of crossing the placenta to give passive immunity to the fetus.

In a recent study by Turner et al it was observed that the SARS-CoV-2 infection induces a robust antigen-specific, long-lived humoral immune response in humans. The patients who experienced mild infections (n=77), serum anti-SARS-CoV-2 spike (S) antibodies declined rapidly in the first 4 months after infection and then more gradually over the following 7 months, remaining detectable at least 11 months after infection. The S-binding bone marrow plasma cells (BMPCs) are quiescent, indicating that they are part of a long-lived compartment. [19]

The neutralizing antibodies (nAbs) are capable of preventing an infectious agent from infecting a cell by neutralizing or inhibiting its biological effect. The most critical target for SARS-CoV-2 nAbs is the RBD within the S1 subunit of S protein. Such nAbs can interrupt the interaction of RBD and its receptor ACE2. Thus, SARS-CoV-2 S-RBD IgG antibody level in human serum or plasma correlates with protective immune responses in individuals who have recovered from SARS-CoV-2 infection and could also reflect herd immunity at a population level aiding in planning of clinical management of patients with past or ongoing COVID-19 infection. Anti-spike nAbs produced by COVID-19 patients can block viral infection of human cells in vitro and counter viral replication in vivo. [20-22]

Neutralizing antibody titers (and total Spike antibody titers) have a positive correlation with COVID-19 disease severity in large cohort studies. [7,11,23]. This was also observed in our study findings. The subjects in the moderately-severe group developed a higher Median Neutralisation Titre (14.78 AU/ml , $p=0.003$) at 14 months when compared to the Mild group (5.55 AU/ml , $p<0.001$). [Table 4] This result was statistically significant. However, the relationship between the neutralizing antibodies, T follicular helper cells (Tfh cells), and COVID-19 disease severity appears to be complex. A higher neutralizing antibody titer is associated with severe disease and potentially “extrafollicular B cell responses” [23] whereas the SARS-CoV-2-specific Tfh cells are associated differently. Moreover, antibodies could act like a useful surrogate marker of CD4⁺ T cell responses in many infections, since antibody assays are much easier to perform and more sensitive in small blood volumes when compared to antigen-specific T cell assays. [24]

A recent study by Abu-Raddad LJ et al in Qatar, assessed the cumulative risk as well as incidence rate of SARS-CoV-2 reinfection in a nationwide cohort of 43,044 antibody-positive individuals. This study with a follow period of up to 35 weeks, demonstrated and confirmed through viral genome sequencing that SARS-CoV-2 reinfection occurs, but “only rarely” with a cumulative risk of ~2 per 1000 persons and reinfection incidence rate of <1 per 10,000 person weeks as compared to the complement cohort of 149,923 antibody-negative persons with a much higher cumulative risk of re-infection (~31 per 1000 persons after 46 weeks of follow-up) and estimated incidence rate of infection (~14 per 10,000 person-weeks.). The estimated efficacy of natural infection against reinfection was 95%. Moreover, this study showed no evidence of waning protective immunity against reinfection in this cohort for over 7 months. [25]

An important point that needs to be highlighted in our study is zero cases of re-infection despite the fact that the Umbria region has been experiencing multiple waves with mutant strains. [11]

Adoption of anti-S-RBD immunoassay

The S1 subunit has low evolutionary protein homologies within the coronavirus family suggesting less cross-reactivities among the endemic coronaviruses, but the N protein-based antibody assays exhibit a higher false-negative rate compared with the S1 subunit, making the anti-S-RBD assays more specific. Although the Nucleocapsid and Spike IgG titers are highly correlated [24], spike is the target of SARS-CoV-2 neutralizing antibodies, and the RBD of Spike is the target of >90% of neutralizing antibodies in COVID-19 cases [23,26-28], with some neutralizing antibodies instead targeting the N-terminal domain (NTD). [29] Therefore, after following up the cohort for 10 months for anti NCP antibodies, a more specific assay was adopted that detected the presence of antibodies against the S-RBD. It was observed in our study the subjects , n=7 who were seronegative for anti-NCP antibodies at T5 , were found to be seropositive at T6, T7 and T8 for anti-S-RBD emphasizing on the fact that N protein-based antibody assays exhibit a higher false-negative rate when compared with the anti-S-RBD assays. [Figure 4]

Antibody seropositivity in the cohort

In this study, at T0, 2 months after the initial infection , 24 out of 30 (80%) subjects were positive for SARS-CoV-2 NCP IgG antibodies, followed by a slight dip with 20 out of 30 (66.7%) subjects with antibody seropositivity at T1, 3 months after infection. This antibody seropositivity trend remained stable in 23 out of 30 (76.7%) at T2, T3, T4 and a second dip was observed at T5. However, after the adoption of anti-S-RBD immunoassay, 31 out of 32 (96.8%) subjects showed antibody persistency at all the three time points; T6, T7 and T8, 12, 13 and 14 months post infection respectively.

Our results are in line with previous studies showing a similar longevity and pattern of anti-SARS-CoV-2 Ab responses, with Ab levels reaching a peak at 23 days following symptom onset and being maintained for at least 4 months, [11,14,30-36] yet contradictory to others, in which a low prevalence and rapid decay (within 3 months) of

anti-SARS-CoV-2 Abs in COVID-19 patients with either mild or severe disease were observed.[37,38]

Average Antibody titers [Anti-NCP IgG and Anti-S-RBD IgG] developed at 14 months

The estimated marginal mean or average of antibody titers (in AU/ml) developed over different TPs such as T0-T5 and T6-T8 with respect to two different immunological assays against NCP and Spike protein respectively. It can be observed that the average of *Anti-NCP IgG* antibody titers tends to show a decreasing trend across T0-T5, whereas *Anti-S-RBD IgG* shows a better average of antibody titers, in terms of magnitude (seropositivity) across T6-T8.

In Conclusion, our study findings are consistent with recent studies reporting robust antibody persistency suggesting that induced SARS-CoV-2 immunity through natural infection, might be very efficacious against re-infection (>90%) and could persist for more than six months. Our study followed-up patients up to 14 months demonstrating presence of anti-S-RBD IgG in 96.8% of recovered COVID-19 subjects.

In such a scenario, prioritizing vaccination for “naive” individuals (with no previous history of COVID-19 infection) and recovered but antibody-negative individuals would be helpful by saving time, effort and resources that could be used for the vulnerable populations.

This study also provides valuable information for future implementation and vaccine distribution policies. Further studies need to be conducted to determine antibody responses in patients infected by mutant strains when compared to the original wild type.

References:

1. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020 Feb 22;395(10224):565-574. doi: 10.1016/S0140-6736(20)30251-8. Epub 2020 Jan 30. PMID: 32007145; PMCID: PMC7159086.
2. Wu LP, Wang NC, Chang YH et al. Duration of antibody responses after severe acute respiratory syndrome. *Emerg Infect Dis*. 2007 Oct;13(10):1562-4. doi: 10.3201/eid1310.070576. PMID: 18258008; PMCID: PMC2851497.
3. Hall VJ, Foulkes S, Charlett A, et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). *Lancet*. 2021 Apr 17;397(10283):1459-1469. doi: 10.1016/S0140-6736(21)00675-9. Epub 2021 Apr 9. PMID: 33844963; PMCID: PMC8040523.
4. Matusali G, Colavita F, Lapa D, Meschi S, Bordi L, Piselli P, Gagliardini R, Corpolongo A, Nicastri E, Antinori A, Ippolito G, Capobianchi MR, Castilletti C, Inmi Covid-Laboratory Team. SARS-CoV-2 Serum Neutralization Assay: A Traditional Tool for a Brand-New Virus. *Viruses*. 2021 Apr 10;13(4):655
5. Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jadi, R.S., et al. (2020). Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 181, 1489–1501.e15.
6. Juno, J.A., Tan, H.-X., Lee, W.S., Reynaldi, A., Kelly, H.G., Wragg, K., Esterbauer, R., Kent, H.E., Batten, C.J., Mordant, F.L., et al. (2020). Humoral and circulating follicular helper T cell responses in recovered patients with COVID19. *Nat. Med.* 26, 1428–1434.

7. Robbiani, D.F., Gaebler, C., Muecksch, F., Lorenzi, J.C.C., Wang, Z., Cho, A., Agudelo, M., Barnes, C.O., Gazumyan, A., Finkin, S., et al. (2020). Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* 584, 437–442
8. MAGLUMI 2019-nCoV IgM/IgG - Letter of Authorization (fda.gov)
9. World Health Organization. International guidelines for certification and classification (coding) of COVID-19 as cause of death. Available from: https://www.who.int/classifications/icd/Guidelines_Cause_of_Death_COVID-19-20200420-EN.pdf?ua=1. Document Number: WHO/HQ/DDI/DNA/CAT. Accessed on June 1, 2020.
10. Xydakis MS, Dehgani-Mobaraki P, Holbrook EH, et al. Smell and taste dysfunction in patients with COVID-19. *Lancet Infect Dis.* 2020 Sep;20(9):1015-1016. doi: 10.1016/S1473-3099(20)30293-0. Epub 2020 Apr 15. PMID: 32304629; PMCID: PMC7159875.
11. Dehgani-Mobaraki P, Kamber Zaidi A, Porreca A et. al. Antibody persistency and trend post-SARS-CoV-2 infection at eight months. *Ann Ig.* 2021 May. doi: 10.7416/ai.2021.2455. Online ahead of print.
12. https://www.snibe.com/zh_en/en_newsView.aspx?id=647
13. Huang M, Lu QB, Zhao H, et al. Temporal antibody responses to SARS-CoV-2 in patients of coronavirus disease 2019. *Cell Discovery.* 2020;6:64. doi: <https://dx.doi.org/10.1038/s41421-020-00209-2>. PMID: 32983570.
14. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. *N Engl J Med.* 2020 Sep 1. doi: <https://dx.doi.org/10.1056/NEJMoa2026116>. PMID: 32871063.
15. Chirathaworn C, Sripramote M, Chalongviriyalert P, et al. SARS-CoV-2 RNA shedding in recovered COVID-19 cases and the presence of antibodies against SARS-CoV-2 in recovered COVID-19 cases and close contacts, Thailand, April-June 2020.

- PLoS ONE. 2020;15(10):e0236905. doi: <https://dx.doi.org/10.1371/journal.pone.0236905>. PMID: 33119712.
16. Terpos E, Politou M, Sargentanis TN, et al. Anti– SARS-CoV-2 Antibody Responses in Convalescent Plasma Donors Are Increased in Hospitalized Patients;Subanalyses of a Phase 2 Clinical Study. *Microorganisms*. 2020 2020;8(12):1885-. doi: <https://dx.doi.org/10.1080/22221751.2020.1762515>.
  17. Zhao J, Yuan Q, Wang H, et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. *Clin Infect Dis*. 2020 11 19;71(16):2027-34. doi: <https://dx.doi.org/10.1093/cid/ciaa344>. PMID: 32221519
  18. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell*. 2020. <https://doi.org/10.1016/j.cell.2020.03.045>.
  19. Turner, J.S., Kim, W., Kalaidina, E. et al. SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans. *Nature* (2021). <https://doi.org/10.1038/s41586-021-03647-4>
  20. Zohar, T. et al. Compromised humoral functional evolution tracks with SARS-CoV-2 mortality. *Cell* 183, 1508–1519 (2020). e12.
  21. Wu, F. et al. Evaluating the association of clinical characteristics with neutralizing antibody levels in patients who have recovered from mild COVID-19 in Shanghai. China *JAMA Intern. Med*. 180, 1356–1362 (2020).
  22. Wang, X. et al. Neutralizing antibody responses to severe acute respiratory syndrome coronavirus 2 in coronavirus disease 2019 inpatients and convalescent patients. *Clin. Infect. Dis*. <https://doi.org/10.1093/cid/ciaa721> (2020).
  23. Piccoli L, Park YJ, Tortorici MA, et al. Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell*. 2020 Nov 12;183(4):1024-1042.e21. doi: 10.1016/j.cell.2020.09.037. Epub 2020 Sep 16. PMID: 32991844; PMCID: PMC7494283

24. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell*. 2021 Feb 18;184(4):861-880. doi: 10.1016/j.cell.2021.01.007. Epub 2021 Jan 12. PMID: 33497610; PMCID: PMC7803150.
25. Abu-Raddad LJ, Chemaitelly H, Coyle P, et al. SARS-CoV-2 antibody-positivity protects against reinfection for at least seven months with 95% efficacy. *EClinicalMedicine*. 2021 May;35:100861. doi: 10.1016/j.eclinm.2021.100861. Epub 2021 Apr 28. PMID: 33937733; PMCID: PMC8079668.
26. Brouwer PJM, Caniels TG, van der Straten K, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. *Science*. 2020 Aug 7;369(6504):643-650. doi: 10.1126/science.abc5902. Epub 2020 Jun 15. PMID: 32540902; PMCID: PMC7299281.
27. Rogers TF, Zhao F, Huang D, et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. *Science*. 2020 Aug 21;369(6506):956-963. doi: 10.1126/science.abc7520. Epub 2020 Jun 15. PMID: 32540903; PMCID: PMC7299280.
28. Suthar MS, Zimmerman MG, Kauffman RC, et al. Rapid Generation of Neutralizing Antibody Responses in COVID-19 Patients. *Cell Rep Med*. 2020 Jun 23;1(3):100040. doi: 10.1016/j.xcrm.2020.100040. Epub 2020 Jun 8. PMID: 32835303; PMCID: PMC7276302.
29. Liu L, Wang P, Nair M.S, et al. Potent neutralizing antibodies directed to multiple epitopes on SARS-CoV-2 spike. *bioRxiv*. 2020; <https://doi.org/10.1101/2020.06.17.153486>
30. Bolke, E., Matuschek, C., and Fischer, J.C. (2020). Loss of anti-SARS-CoV-2 antibodies in mild covid-19. *N. Engl. J. Med*. 383, 1694–1695.
31. Iyer, A.S., Jones, F.K., Nodoushani, A., Kelly, M., Becker, M., Slater, D., Mills, R., Teng, E., Kamruzzaman, M., Garcia-Beltran, W.F., et al. (2020). Persistence and

- decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Sci. Immunol.* 5, eabe0367.
32. Isho, B., Abe, K.T., Zuo, M., Jamal, A.J., Rathod, B., Wang, J.H., Li, Z., Chao, G., Rojas, O.L., Bang, Y.M., et al. (2020). Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci. Immunol.* 5, eabe55115.
  33. Wajnberg, A., Amanat, F., Firpo, A., Altman, D.R., Bailey, M.J., Mansour, M., McMahon, M., Meade, P., Mendu, D.R., Muellers, K., et al. (2020). Robust neutralizing antibodies to SARSCoV-2 infection persist for months. *Science* 370, 1227–1230.
  34. Hartley, G.E., Edwards, E.S.J., Aui, P.M., Varese, N., Stojanovic, S., McMahon, J., Peleg, A.Y., Boo, I., Drummer, H.E., Hogarth, P.M., et al. (2020). Rapid generation of durable B cell memory to SARS-CoV-2 spike and nucleocapsid proteins in COVID-19 and convalescence. *Sci. Immunol.* 5, eabf8891.
  35. Roltgen, K., Powell, A.E., Wirz, O.F., Stevens, B.A., Hogan, C.A., Najeeb, J., Hunter, M., Wang, H., Sahoo, M.K., Huang, C., et al. (2020). Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. *Sci. Immunol.* 5, eabe0240.
  36. Dan, J.M., Mateus, J., Kato, Y., Hastie, K.M., Yu, E.D., Faliti, C.E., Grifoni, A., Ramirez, S.I., Haupt, S., Frazier, A., et al. (2021). Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 371, eabf4063.
  37. Ibarrondo, F.J., Fulcher, J.A., Goodman-Meza, D., Elliott, J., Hofmann, C., Hausner, M.A., Ferbas, K.G., Tobin, N.H., Aldrovandi, G.M., and Yang, O.O. (2020). Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. *N. Engl. J. Med.* 383, 1085–1087.
  38. Kutsuna, S., Asai, Y., and Matsunaga, A. (2020). Loss of anti-SARS-CoV-2 antibodies in mild covid-19. *N. Engl. J. Med.* 383, 1695–1696

STUDY DESIGN

N=30

N=42

N=32

STUDY RESULTS

PARAMETER			Mild (n=19)	Moderately- Severe (n=13)	P- value
CLINICAL SYMPTOM					
	Shortness of breath	Yes	6(31.6%)	10(76.9%)	0.029
		No	13(68.4%)	3(23.1%)	
	Fatigue	Yes	9(47.4%)	12(92.3%)	0.011
		No	10(52.6%)	1(7.7%)	
	Headache	Yes	5(26.3%)	8(61.5%)	0.046
		No	14(73.7%)	5(38.5%)	
CO-MORBIDITIES					
	Cardiovascular disease	Yes	0(0.0%)	5(38.5%)	0.006
		No	19(100.0%)	8(61.5%)	

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FIGURE 1

Table 1: Baseline clinical, demographic features and disease characteristics of subjects having COVID-19, in March, 2020:

PARAMETER		Mild	Moderate-Severe	p-value
		n=19	n=13	
Age [q1-q3] (in years)		31(29-59)	56(35-69)	0.147
Sex	Male	6(54.5%)	5(45.5%)	0.687
	Female	13(61.9%)	8(38.1%)	
Blood Group	A+	8(42.1%)	5(38.5%)	0.773
	AB+	0(0.0%)	2(15.4%)	
	B+	1(5.3%)	0(0.0%)	
	O+	5(26.3%)	3(23.1%)	
	A-	1(5.3%)	1(7.7%)	
	AB-	1(5.3%)	0(0.0%)	
	O-	3(15.8%)	2(15.4%)	
COMORBIDITIES				
Asthma/Seasonal Allergies	Yes	6(31.6%)	2(15.4%)	0.420
	No	13(68.4%)	11(84.6%)	
Diabetes	Yes	2(10.5%)	4(30.8%)	0.194
	No	17(89.5%)	9(69.2%)	
Hypertension	Yes	5(26.3%)	6(46.2%)	0.246
	No	14(73.7%)	7(53.8%)	
Cardiovascular	Yes	0(0.0%)	5(38.5%)	0.006
	No	19(100.0%)	8(61.5%)	
SYMPTOMS				
Fever	Yes	15(78.9%)	13(100.0%)	0.128
	No	4(21.1%)	0(0.0%)	
Rhinorrhea	Yes	3(15.8%)	5(38.5%)	0.219
	No	16(84.2%)	8(61.5%)	
Dry Cough	Yes	9(47.4%)	4(30.8%)	0.471
	No	10(52.6%)	9(69.2%)	
Sore Throat	Yes	5(26.3%)	4(30.8%)	1.000
	No	14(73.7%)	9(69.2%)	
Shortness of breath	Yes	6(31.6%)	10(76.9%)	0.029
	No	13(68.4%)	3(23.1%)	
Fatigue	Yes	9(47.4%)	12(92.3%)	0.011
	No	10(52.6%)	1(7.7%)	
Headache	Yes	5(26.3%)	8(61.5%)	0.046
	No	14(73.7%)	5(38.5%)	

Skin Eruption	Yes	1(5.3%)	3(23.1%)	0.279
	No	18(94.7%)	10(76.9%)	
Muscle Ache	Yes	14(73.7%)	11(84.6%)	0.671
	No	5(26.3%)	2(15.4%)	
Diarrhea	Yes	4(21.1%)	7(53.8%)	0.072
	No	15(78.9%)	6(46.2%)	
Conjunctivitis	Yes	3(15.8%)	2(15.4%)	1.000
	No	16(84.2%)	11(84.6%)	
Loss of smell	Yes	16(84.2%)	11(84.6%)	1.000
	No	3(15.8%)	2(15.4%)	
Loss of taste	Yes	15(78.9%)	11(84.6%)	1.000
	No	4(21.1%)	2(84.6%)	
Chest Pain	Yes	5(26.3%)	5(38.5%)	0.467
	No	14(73.7%)	8(61.5%)	

Table 2: Baseline clinical, demographic features and disease characteristics of subjects having COVID-19, after March, 2020 at 14 months along with Severity:

Age-Group	Less than 45	More than 45
	n=15	n=17
Severity	9.67(5.18-20.12)	9.71(4.31-35.39)
Time at 14 months		
p- value	0.296	0.054
Comorbidities	With comorbidities	Without comorbidities
	n=18	n=14
Severity	7.28(5.17-22.01)	14.08(5.77-32.14)
Time at 14 months		
p- value	0.033	0.257
Sex	Male	Female
	n=11	n=21
Severity	6.22(3.27-27.97)	13.38(5.53-24.06)
Time at 14 months		
p- value	0.100	0.111
Loss of smell	Yes	No
	n=27	n=5
Severity	9.702(5.18-23.97)	7.49(3.17-45.95)
Time at 14 months		
p-value	0.043	0.564
Loss of taste	Yes	No
	n=26	n=6
Severity	9.68(5.17-24.01)	13.81(4.24-44.38)
Time at 14 months		
p-value	0.031	0.643
Medico	Yes	No
	n=6	n=26
Severity	14.91(8.64-24.74)	7.22(4.96-24.97)
Time at 14 months		
p-value	0.827	0.023

Table 3: IgG titres for mild (n=19) and moderately-severe (n=13) groups evaluated at each time point, T0-T8 where the first blood sample was collected two months after infection in the month of May 2020 (T0) and then, one month (T1), three months (T2), five months (T3), six months (T4), eight months(T5), ten months(T6), eleven months(T7) and twelve months(T8) after T0.

Parameter (IgG)	Mild	p-value	Pairwise Comparison
T0	1.84(0.47-3.53)	<0.001	T1≠T7 T4≠T6 T4≠T8 T4≠T7 T5≠T7
T1	1.81(0.45-2.90)		
T2	2.18(0.77-5.02)		
T3	2.25(0.66-3.99)		
T4	1.04(0.32-1.86)		
T5	2.26(0.56-5.37)		
T6	5.81(4.85-19.12)		
T7	5.79(5.05-21.64)		
T8	5.55(5.14-24.15)		

Parameter (IgG)	Moderate-Severe	p-value	Pairwise Comparison
T0	10.86(1.53-31.94)	<0.001	T5≠T6 T5≠T7 T5≠T8
T1	10.01(1.50-35.44)		
T2	10.60(3.40-16.25)		
T3	6.82(2.24-12.54)		
T4	6.68(3.62-12.47)		
T5	4.38(0.91-7.45)		
T6	25.09(9.87-42.85)		
T7	20.89(11.76-43.09)		
T8	13.38(8.58-33.39)		

Table 4: Table shows Inferential statistics for which data was analyzed for the Outcome variable i.e., Group has either mild or moderately-severe symptoms individually to the exposure variable which is Continuous. The main characteristics are expressed as Median (q2) and First and Third quartiles i.e., (q1-q3) for continuous variable. The continuous variable was non-normal and so the p-values result from one sample Kolmogorov–Smirnov test.

IgG. T8 SPIKE 12m after T0 (14m after infection)	Mild	Not- Mild (Moderate- Severe)
	n=19	n=13
	5.55(4.44-20.12)	14.78(9.68-34.66)
p-value	<0.001	0.003

Bar Graph of for Mild (Figure 2A) and Moderately-severe group (Figure 2B) when followed up from T0-T8, (14 months) using Friedman Test

Figure 2A MILD GROUP

Figure 2B MODERATELY SEVERE GROUP

Figure 2

KEY FOR TIME POINTS T0-T8

- T0: First serological sample, 2 months post infection in MARCH 2020 (MAY 2020)
- T1: Second serological sample, 3 months post infection (JUNE 2020)
- T2: Third serological sample, 5 months post infection (AUGUST 2020)
- T3: Fourth serological sample, 7 months post infection (OCTOBER 2020)
- T4: Fifth serological sample, 8 months post infection (NOVEMBER 2020)
- T5: Sixth serological sample, 10 months post infection (JANUARY 2021)
- T6: Seventh serological sample, 12 months post infection (MARCH 2021)
- T7: Eighth serological sample, 13 months post infection (APRIL 2021)
- T8: Eighth serological sample, 14 months post infection (MAY 2021)

Figure 2

Comparison between Antibody titers for n=7 subjects at different time points (TPs) (Anti-NCP IgG titres Vs Anti-Spike-RBD IgG titres)

Figure 3