Sera neutralizing activities against SARS-CoV-2 and multiple variants six month after hospitalization for COVID-19

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Summary:

Anti-SARS-CoV-2 antibodies decline overtime. In patients previously hospitalized for COVID-19, we found a sustained humoral response for at least 6 months. The antibodies cross-react and should confer a similar protection against emerging variants, with the notable exception of B.1.351 variant.

Abstract

Background: Humoral response to SARS-CoV-2 occurs within the first weeks after COVID-19. Those antibodies exert a neutralizing activity against SARS-CoV-2, whose evolution overtime after COVID-19 as well as efficiency against novel variants are however poorly characterized.

Methods: In this prospective study, sera of 107 patients hospitalized with COVID-19 were collected at 3- and 6-months post-infection. We performed quantitative neutralization experiments on top of high-throughput serological assays evaluating anti-Spike (S) and anti-Nucleocapsid (NP) IgG.

Findings: Levels of sero-neutralization and IgG rates against the ancestral strain decreased significantly over time. After 6 months, 2.8% of the patients had a negative serological status for both anti-S and anti-NP IgG. However, all sera had a persistent and effective neutralizing effect against SARS-CoV-2. IgG levels correlated with sero-neutralization and this correlation was stronger for anti-S than for anti-NP antibodies. The level of sero-neutralization quantified at 6 months correlated with markers of initial severity, notably admission in intensive care units and the need for mechanical invasive ventilation. In addition, sera collected at 6 months were tested against multiple SARS-CoV-2 variants and showed efficient neutralizing effects against D614G, B.1.1.7 and P.1 variants but a significantly weaker activity against B.1.351 variant.

Interpretation: Decrease of IgG rates and serological assays becoming negative did not imply loss of neutralizing capacity. Our results indicate a sustained humoral response against the ancestral strain and the D614G, B.1.1.7 and P.1 variants for at least 6 months in patients previously hospitalized for COVID-19. A weaker protection was however observed for the B.1.351 variant.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been shown to induce a humoral immune response with seroconversion occurring in most patients between 7 and 21 days after diagnosis[1,2]. This early humoral response is mostly composed of IgA, IgM and IgG directed against the viral surface glycoprotein Spike (S), the nucleocapsid protein (NP) or the spike Receptor Binding Domain (RBD)[2]. The detection of such antibodies may reflect a neutralizing activity believed to be a key point in viral clearance[3,4], as well as conferring a relative protection to the disease in the convalescent phase. Similarly to other coronaviruses, anti-SARS-CoV-2 antibodies decline overtime[5], which raised questions about the extent of the protection conferred and the potential risk of reinfection. Furthermore, the recent emergence of multiple SARS-CoV-2 variants raised additional questions on cross-reactivity of the acquired antibodies after COVID-19[6].

Some publications have reported an association between the level of antibodies and the clinical severity, a higher level being observed in patients presenting the most critical form of the disease[4,7–10]. However, if there is consistent evidence that outpatients usually develop weaker immune response, there is fewer data relating to patients in intensive care units, a valuable population whose described antibodies response could set the upper limit of the humoral immunity against SARS-CoV-2.

While vaccination is ongoing worldwide, the insufficient supply of doses makes prioritization strategies still needed. Being able to shape levels of immunity required to protect against severe reinfection would considerably assist public health strategies in this regard, in addition to being critical information to estimate if vaccines stand the test of time and emerging variants.

Our study explores the longitudinal evolution of antibody levels and of sera neutralizing activities in a French monocentric cohort of patients hospitalized for COVID-19 during the first wave of SARS-CoV-2 pandemic and followed-up for 6 months after hospital discharge. In addition to the ancestral viral strain, sera-neutralization activities against the emerging SARS-CoV-2 variants (B.1.1.7, B.1.351 and P.1) were evaluated.

Material and Methods

Cohort description

We conducted a single-center prospective observational study on adult patients with laboratory positive SARS-CoV-2 real-time reverse-transcriptase polymerase chain reaction (RT-PCR) admitted to Hôpital Européen Georges Pompidou (APHP, Paris, France) for at least 48h. All patients were initially enrolled from March 17th to April 29th 2020 and were then proposed for a clinical and serological follow-up at month 3 (M3) and M6 post-infection. The study is part of The French Covid cohort (NCT04262921) sponsored by Inserm and was authorized by the French Ethics Committee CPP Ile-de-France VI (ID RCB:2020-A00256-33). This study was conducted with the understanding and the consent of each participant or its surrogate.

Data collection

Demographic, clinical presentation, and comorbidity data during the index COVID-19 hospitalization were extracted from the electronic medical records collected in a standardized data collection form in the Clinical Data Warehouse (CDW) of our hospital. The dedicated medical records were stored on an i2b2 platform in a CDW together with all other hospital health records.

Serological assays

Abbott SARS-CoV-2 IgG assays (Des Plaines, IL, USA) targeting SARS-CoV-2 nucleoprotein were done on Architect™ i2000SR analyzer (Abbott), according to manufacturer's instructions. Index value threshold for positivity was 1.4 as recommended. Beckman Coulter Access SARS-CoV-2 IgG assays (Brea, CA, USA) targeting the RBD of SARS-CoV-2 spike surface protein, were done on UniCel DxI 800 Access Immunoassay System (Beckman Coulter), according to manufacturer's instructions. Index value threshold for positivity was 1 as recommended. Qualitative results as well as index values were used for analysis for both assays.

Virus strains

The ancestral non-D614G SARS-CoV-2 strain (BetaCoV/France/IDF0372/2020) was isolated in France from an imported case from Wuhan by the National Reference Center for Respiratory Viruses (NRC) hosted by Institut Pasteur. The reference D614G strain (hCoV-19/France/GE1973/2020) was supplied by the NRC. This viral strain was supplied through the European Virus Archive goes Global (EVAg) platform. The B.1.1.7 strain originated from an individual in Tours (France) who returned from the United Kingdom[11]. The B.1.351 strain (CNR 202100078) originated from an individual in Créteil (France)[11]. The P.1. strain (TY7-501), first identified in Brazil, was obtained from Global Health security action group Laboratory Network (GISAID sample ID: hCoV-19/Japan/TY7-501/2021; GISAID ID: EPI_ISL_833366). Individuals provided informed consent for the use of their biological materials. The variant strains were isolated from nasal swabs on Vero E6 cells and amplified by one or two passages. Of note, the sequence of TY7-501 contains a G181V mutation on its S protein compared with the original clinical sample sequence. Titration of viral stocks was performed on Vero E6 cells, with a limiting dilution technique allowing a calculation of the 50% tissue culture infectious dose, or on S-Fuse

cells [11]. Viruses were sequenced directly on nasal swabs and after one or two passages on Vero cells.

S-Fuse neutralization assay

Neutralization was performed using the S-Fuse reporter system, as previously described[11]. Briefly, U2OS-ACE2 GFP1–10 or GFP 11 cells, which become GFP^+ upon infection with SARS-CoV-2, were mixed (1:1 ratio) and plated at 8×10^3 cells per well in a μClear 96-well plate (Greiner Bio-One). SARS-CoV-2 strains were incubated with sera at the indicated dilutions for 15 min at room temperature and added to S-Fuse cells. All sera were heat inactivated 30 min at 56 °C before use. After 18 h incubation at 37°C 5%CO2, cells were fixed with 2% paraformaldehyde, washed and stained with Hoechst (1:1,000 dilution; Invitrogen). Images were acquired on an Opera Phenix high-content confocal microscope (PerkinElmer). The GFP area, the number of syncytia and nuclei were quantified using the Harmony software (PerkinElmer). The percentage of neutralization was calculated using the number of syncytia with the following formula: $100 \times (1 - (\text{value with serum} - \text{value in 'noninfected'})/(\text{value in 'no serum'} - \text{value in 'noninfected'}))$. Neutralizing activity of each sera was expressed as the ED_{50}, calculated using the percentage of neutralization at each different concentration. Cells were tested negative for mycoplasma. Neutralization determined with the S-Fuse reporter system correlates to pseudovirus neutralization assay[12].

Statistical analyses

Statistics were performed using NCSS 2012 software (G Hintze, Kaysville, UT, USA). All numerical data were checked for normality and non-normal distributions were transformed (using ExpNorScore function on NCSS, which returns the expected value of the normal order statistic corresponding to X).

Continuous variables are reported as means (SDs). Discrete variables are described as counts and percentages. Groups were compared using Two sample T-Test or Wilcoxon Rank test when necessary for continuous variables and Fisher's exact Test or $\chi 2$ for discrete variables.

We also performed a multiple regression analysis to assess variables correlated to the sero-neutralization at M6. For analyses, P values <0.05 were considered significant.

Role of the funding source:

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. The corresponding author (J.S.H) had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

Results

Between March 17th and April 29th, 2020, 354 patients were hospitalized at Hôpital Européen Georges Pompidou (Paris, France) with a confirmed SARS-CoV-2–positive pneumonia. By November 16th, 2020, 85 deaths (24.0%) had occurred during or after hospitalization. From June 17th to November 16th 2020, we were able to manage a complete follow-up for 107 of these patients with two time-point visits at 3 and 6 months after hospital discharge (see Supplementary Figure 1). The 3-month and 6-month visits were performed with a median interval of 98 days (IQR: 91-101) and 203 days (IQR: 191-216), respectively. Among those patients with a complete follow-up, 32.7% (35/107) had required medical care in Intensive Care Unit during the acute COVID-19 phase. Fifteen (15/35, 42.9%) of them required invasive mechanical ventilation (MV). The oxygenation maximal flow in patient in non-ICU unit, in patients not requiring MV in ICU unit and in patients before MV in ICU was 2.0 ± 1.6, 10.7± 5.2 and 13.0 ± 3.6 L/min respectively (p<0.0001). A majority of patients were male (73/107, 68.2%) with a mean age of 58.7 ± 14.0 years-old. A past history of cardiovascular risk factor (chronic cardiac disease, diabetes, obesity, hypertension, chronic kidney disease) was found in 51.4% (55/107) of them and 10.2% (11/107) had immunosuppressive diseases (cirrhosis, asplenia, sickle cell anemia, solid organ or stem cell transplantation, HIV infection, primary immune deficiency, chronic hematological disease, malignant neoplasm, autoimmune disorder). Moreover, 5.6% (6/107) of them were previously treated with an immunosuppressive therapy. All patients' characteristics are listed in Table 1.

Longitudinal serological assays at 3- and 6-months post-infection

We quantitively assessed the presence of IgG recognizing the nucleocapsid protein (NP) or spike (S) domain in serum samples from these 107 patients. At M3 after COVID-19 infection, anti-S and anti-NP antibodies were detected in all patients. Both anti-S and anti-NP IgG levels significantly decreased between M3 and M6 (respectively 17.1±13.6 to 7.8±9.2, $p<0.0001$ and 6.6±2.3 to 3.8±2.4, $p<0.0001$, Figure 1). Six months after COVID-19 infection, anti-S serology was considered negative (i.e., antibodies below the commercial threshold) in 9 samples (9/107, 8.4%) and anti-NP in 17 samples (17/107, 15.9%). However, only 3 patients had a negative serological status with both assays (3/107, 2.8%) at M6.

Neutralizing activities against ancestral SARS-CoV-2 at 3- and 6-months post-infection

We then aimed to assess if a persistent serum neutralizing activity was detected up to 6 months following COVID-19 infection, independent of anti-S or anti-NP levels. To do so, we used S-Fuse cells (specifically designed to become GFP+ when productively infected by SARS-CoV-2) to evaluate our patients' sera propensity to prevent such infection. At minimum dilution (1/30), sero-neutralization was observed in all samples at M3 and M6, even when anti-S and anti-NP IgG were considered as negative regarding the commercial kit threshold.

We next quantified sero-neutralization by performing serial dilutions in order to define the ID50 neutralization (maximum dilution to maintain a 50% neutralization capacity). ID50 neutralization significantly decreased between M3 and M6 (Figure 2), with residual values nevertheless indicating a high neutralizing activity at M6.

Impact of the initial clinical severity on residual humoral immunity

As all measures were highly variable between patients, we then sought factors associated with higher levels of neutralizing activities. In a multiple regression analysis, we found that an initial management in ICU and the need for an invasive mechanical ventilation were the only two factors significantly associated with a higher rate of ID50 neutralization at M6 (Table 2). When considering ID50 neutralization according to ICU hospitalization and the need for mechanical ventilation, we found that

patients in ICU had significantly higher neutralizing activities as compared to non-ICU patients with the highest levels observed in ICU patients who had invasive mechanical ventilation (Figure 3A).

A similar trend was observed at M3, however not reaching significance (Figure 3A).

We then observed that anti-S modestly correlate with sera ID50 neutralization (anti-S IgG : R = 0.54, 95%CI[0.39-0.67], p value <0.0001, with a weaker association for anti-NP IgG (anti-NP IgG : R = 0.33, 95%CI[0.25-0.49], p value 0.0007).

We then analyzed anti-S and anti-NP IgG levels at M3 and M6 according to the initial management in ICU and the need for an invasive mechanical ventilation. We found higher levels of anti-NP IgG in mechanically ventilated patients in ICU vs. non mechanically ventilated patients admitted to ICU vs. patient in non-ICU medical departments at M3 (7.6±1.8 vs. 7.2±2.2 vs. 6.2±2.3, p=0.03 by Kruskall-Wallis test, Figure 3B) with a significantly higher rate in ICU patients vs. no ICU patients (7.4±2.0 vs. 6.2±2.3 p value = 0.01). We confirmed this result at M6 (5.6±1.8. vs. 4.4±2.0 vs. 3.3±2.4, p=0.0005 by Kruskall-Wallis test, Figure 3B) with a significantly higher rate in ICU patients vs. no ICU patients (4.9±2.0 vs. 3.3±2.4 p value = 0.001). In contrast, this pattern was not observed with the anti-S antibodies as there were no significant differences between the ICU vs. the no ICU groups at M3 and at M6 (Figure 3C).

Neutralizing activities against SARS-CoV-2 emerging variants

We then used sera collected at 6 months following COVID-19 infection to assess their neutralizing activities against multiple variants, including D614G, B.1.1.7, B1.351 and P.1 variants. At minimum dilution (1/30), sero-neutralization was observed in all samples against the D614G and B.1.1.7 variants. We then quantified sero-neutralization by performing serial dilutions in order to define the ID50 neutralization for the 4 strains. D614G, B.1.1.7 and P.1 strains were similarly sensitive to the sera with a high neutralizing activity observed for these three variants. In contrast, the neutralization titers against B.1.351 were significantly lower with a 3-fold decrease in ID50 between D614G and B.1.351 strains (p value<0.0001) (Figure 4A). ID50s for the B.1.351 variant were also significantly lower as compared to the B.1.1.7 and the P.1 variants (Figure 4B).

Discussion

In the present study, we described the longitudinal evolution of IgG levels and sero-neutralization at 3- and 6-months post-infection in a relatively large prospective cohort of 107 hospitalized patients – with a third of severe cases tending to be scarce in literature – and thus provides important data about the evolution of humoral immunity after a hospitalization for COVID-19 infection. We found that at least one serology assay was still positive at 6 months in 97.2% of the studied patients. Although antibodies levels decreased significantly over time, with rates dropping under the positivity threshold in a few cases, all patients' sera conserved an effective neutralizing activity at 6 months post-infection against the ancestral strain. Sero-neutralization remained higher at 3 and 6 months in patients who had required intensive care. We also used our sera collection to estimate the levels of humoral protection against the emerging SARS-CoV-2 variants. In these additional *in vitro* experiments, we found that sera-neutralizing activities was also effective against the B.1.1.7 and P.1 variants (also known as the UK and Brazilian variants), but was potentially weaker for the B.1.351 strain (also known as South-African variant).

Higher ID50 against the ancestral strain were observed in patients with more severe presentations, even at distance of infection. This correlation between sero-neutralization and clinical severity has been previously described[7,10,13,14], and our results now indicate that this trend might persist over time. Interestingly, we found that anti-NP IgG titers were higher according to the stage of severity, which was not observed for anti-S IgG. Early after symptoms onset, anti-NP response had already been reported as a possible marker of severity, associated to delayed viral clearance and disease severity[8]. Whether this exacerbated humoral response in severe patients is a

protective adaptation to a more intense viral load or if it plays a putative role in pathogenicity remains subject to debate[15,16].

At 6 months post-infection, we found that anti-S IgG titers correlated with sera ID50 neutralization, but not anti-NP IgG. This was generally in line with other works that had underlined, at different times post infection, a relatively strong correlation between neutralizing antibodies and anti-S or anti-RBD antibodies, and a usually poorer correlation to anti-NP antibodies[9,14,17,18].

The evolution of sera-neutralization overtime and in response to emerging variants is one important element to consider when questioning the extent of effective protection conferred by a priori infection and thus helps evaluating the strength of shield immunity during this pandemic. In line with first encouraging results[11, 13,17,19–21], we confirmed in this study the persistence of neutralization up to 6 months post-infection against the ancestral strain, but also the existence of a broader and similarly effective neutralizing activities against novel variants including B1.1.7 and P1 variants. These results are in favor of antibodies cross-reactivity and potential protection against reinfection with these variants. As compared to other strains, we observed a weaker protection against the B.1.351 variant, however with a substantial neutralizing activity observed in most of the patients. These data suggest that these antibodies acquired during a prior COVID-19 infection might not confer a complete protection against this emerging variant firstly described in South African patients[22]. We took benefit of the available sera collection and the development of a novel assay to estimate these activities but cannot extrapolate on a higher risk of reinfection in these convalescent patients. Several publications based on pseudovirus or virus neutralizing assays

outlined that variants could partially evade humoral immunity – in exposed patients as well as vaccinees[23–25].

Our cohort of patients with the most critical forms of COVID-19 represents a valuable population to explore maximal antibodies response and set the upper limit of the humoral immunity against the SARS-CoV-2. Our results suggest that our patients should be protected at least 6 months against future re-infection. So far, there are few cases of reinfection published in the literature[26]. Reinfections rates have been estimated as low in large recent observational studies despite waning neutralizing antibodies[27,28]. However, it is impossible to extrapolate on future infections with novel variants. Interestingly, a recent model predicted a relationship between neutralizing levels and immune protection against the ancestral strain and novel variants, as well as a protection against severe disease[29]. Further studies are nonetheless required, especially regarding the B.1.351 variant, in order to determine if partial humoral escape can clinically lead to severe events. Cellular immunity also appears as a major shield against SARS-CoV-2, with the development of durable T memory cells[20], whose reactivity could be only slightly impacted by variants[30].

Overall, patients who survived the most critical forms of COVID-19 consequently developed an intense and prolonged humoral immunity. These levels tend to correlate with the severity of the initial presentation, with patients in ICU who had invasive mechanical ventilation having the highest neutralizing activities as compared to ICU and non-ICU patients. The developed antibodies are cross-reacting and should confer a similar protection against emerging variants, with the notable exception of the B.1.351 variant. This pattern may be considered in global deployment of vaccination worldwide. Future studies will establish whether acquired antibodies also protect against re-infections with the emerging SARS-CoV-2 variants. Our data indicate the need for a specific attention to the B.1.351 variant.

NOTES

Contributions:

JSH, MB, ML, DV and ET designed the study. JSH, ML and AF had full access to all of the data, and take the responsibility of data integrity and accuracy of the data analysis. JSH drafted the paper with the help of MB, ML, AF, DP and BM. AF and JSH performed all the analyses. MB, ML, BV, NR, AB, HPé, DL, and DV collected the data. DP, BM, TB and OS performed the neutralization experiments. All authors critically revised the manuscript for important intellectual content and gave final approval for the version to be published.

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References :

1. **Vabret N, Britton GJ, Gruber C, et al. Immunology of COVID-19: Current State of the Science. Immunity 2020; 52:910–941.**

2. **Bastos ML, Tavaziva G, Abidi SK, et al. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ 2020; 370. Available at: https://www.bmj.com/content/370/bmj.m2516. Accessed 22 November 2020.**

3. **Tang MS, Case JB, Franks CE, et al. Association between SARS-CoV-2 Neutralizing Antibodies and Commercial Serological Assays. Clinical Chemistry 2020; 66:1538–1547.**

4. **Wang Y, Zhang L, Sang L, et al. Kinetics of viral load and antibody response in relation to COVID-19 severity. J Clin Invest 2020; 130:5235–5244.**

5. **Post N, Eddy D, Huntley C, et al. Antibody response to SARS-CoV-2 infection in humans: A systematic review. PLoS One 2020; 15:e0244126.**

6. **Prévost J, Finzi A. The great escape? SARS-CoV-2 variants evading neutralizing responses. Cell Host & Microbe 2021; 29:322–324.**

7. **Long Q-X, Tang X-J, Shi Q-L, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nature Medicine 2020; 26:1200–1204.**

8. **Zhao J, Yuan Q, Wang H, et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clinical Infectious Diseases 2020; 71:2027–2034.**

9. **Terpos E, Politou M, Sergentanis 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; 8.**

10. **Garcia-Beltran WF, Lam EC, Astudillo MG, et al. COVID-19-neutralizing antibodies predict disease severity and survival. Cell 2021; 184:476-488.e11.**

11. **Planas D, Bruel T, Grzelak L, et al. Sensitivity of infectious SARS-CoV-2 B.1.1.7 and B.1.351 variants to neutralizing antibodies. Nat Med. 2021 Mar 26. doi: 10.1038/s41591-021-01318-5.**

12. **Sterlin D, Mathian A, Miyara M, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci Transl Med 2021; 13.**

13. **Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat Microbiol 2020; 5:1598–1607.**

14. **Legros V, Denolly S, Vogrig M, et al. A longitudinal study of SARS-CoV-2-infected patients reveals a high correlation between neutralizing antibodies and COVID-19 severity. Cellular & Molecular Immunology 2021; 18:318–327.**

15. **Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. Nature Reviews Immunology 2020; 20:339–341.**

16. **Cao X. COVID-19: immunopathology and its implications for therapy. Nature Reviews Immunology 2020; 20:269–270.**

17. **Figueiredo-Campos P, Blankenhaus B, Mota C, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset. European Journal of Immunology 2020; 50:2025–2040.**

18. **Wu F, Liu M, Wang A, 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 2020; 180:1356–1362.**

19. **Grzelak L, Velay A, Madec Y, et al. Sex differences in the decline of neutralizing antibodies to SARS-CoV-2. medRxiv 2020; :2020.11.12.20230466.**

20. **Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021; 371.**

21. **Iyer AS, Jones FK, Nodoushani A, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. Science Immunology 2020; 5. Available at: https://immunology.sciencemag.org/content/5/52/eabe0367. Accessed 12 March 2021.**

22. **CDC. Cases, Data, and Surveillance. 2020. Available at: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html. Accessed 26 March 2021.**

23. **Zhou D, Dejnirattisai W, Supasa P, et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell 2021;**

24. **Hu J, Peng P, Wang K, et al. Emerging SARS-CoV-2 variants reduce neutralization sensitivity to convalescent sera and monoclonal antibodies. Cell Mol Immunol 2021;**

25. **Garcia-Beltran WF, Lam EC, St. Denis K, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. Cell 2021; Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7953441/. Accessed 21 March 2021.**

26. **Stokel-Walker C. What we know about covid-19 reinfection so far. BMJ 2021; 372:n99.**

27. **Perez G, Banon T, Gazit S, et al. A 1 to 1000 SARS-CoV-2 reinfection proportion in members of a large healthcare provider in Israel: a preliminary report. medRxiv 2021; :2021.03.06.21253051.**

28. **Hansen CH, Michlmayr D, Gubbels SM, Mølbak K, Ethelberg S. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. The Lancet 2021; 0. Available at: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)00575-4/abstract. Accessed 21 March 2021.**

29. **Khoury DS, Cromer D, Reynaldi A, et al. What level of neutralising antibody protects from COVID-19? medRxiv 2021; :2021.03.09.21252641.**

30. **Tarke A, Sidney J, Methot N, et al. Negligible impact of SARS-CoV-2 variants on CD4 + and CD8 + T cell reactivity in COVID-19 exposed donors and vaccinees. bioRxiv 2021;**

Table n°1: Demographic, comorbidities and treatment during the acute phase

	Overall (n=107)	ICU (n=35)	No ICU (n=72)	P value
Age, mean (SD), y	58.7 ± 14.0	59.0 ± 11.9	58.5 ± 15.0	0.86
Women, No (%)	34 (31.8)	10 (28.6)	24 (33.3)	0.61
BMI, mean (SD), kg/m²	27.2 ± 4.5	27.6 ± 4.0	27.0 ± 4.7	0.46
Past medical History				
Previous Heart Disease, No (%)	14 (13.1)	4 (11.4)	10 (13.9)	1.00
Hypertension, No (%)	36 (33.6)	14 (40.0)	22 (30.6)	0.33
Diabetes, No (%)	17 (15.9)	8 (22.9)	9 (12.5)	0.17
Chronic Renal Disease, No (%)	8 (7.5)	2 (5.7)	6 (8.3)	1.00
Chronic Pulmonary Disease, No (%)	10 (9.3)	2 (5.7)	8 (11.1)	0.49
Asthma, No (%)	12 (11.2)	2 (5.7)	10 (13.9)	0.33
Hepatic Disease, No (%)	2 (1.9)	1 (2.9)	1 (1.4)	0.55
Active Cancer, No (%)	4 (3.7)	1 (2.9)	3 (4.2)	1.00
Hematologic Disease, No (%)	7 (6.5)	2 (5.7)	5 (6.9)	1.00
HIV, No (%)	2 (1.9)	0 (0.0)	2 (2.8)	1.00

Rheumatic Disease, No (%)	5 (4.7)	2 (5.7)	3 (4.2)	0.72
Sickle Cell Disease, No (%)	2 (1.9)	0 (0.0)	2 (2.8)	0.32
Immunosuppressive treat, No (%)	6 (5.6)	3 (8.6)	3 (4.2)	0.39
Covid-19 Treatment				
Oxygenation maximal flow, mean (SD), L/min	4.9 ± 5.0	10.8 ± 4.3	2.0 ± 1.6	**<0.0001**
Invasive mechanical Ventilation, No (%)	15 (14.2)	15 (42.9)	0 (0.0)	**<0.0001**
Antiviral Therapy*, No (%)	2 (1.9)	2 (5.7)	0 (0.0)	0.10
Anti-IL6 antibody, No (%)	11 (10.3)	10 (28.6)	1 (1.4)	**<0.0001**
Anti-IL1 antibody, No (%)	1 (0.9)	1 (2.9)	0 (0.0)	0.32
Hydroxychloroquine, No (%)	11 (10.3)	6 (17.1)	5 (6.9)	0.10
Corticosteroïds, No (%)	3 (2.8)	3 (8.6)	0 (0.0)	**0.03**

*antiviral therapy : lopinavir for 1 patient and remdesivir for 1 patient

Table 2. Multiple regression analysis of variables associated with sero-neutralization at 6 months

Variables	Adjusted* regression coef (± SE)	p value
ICU hospitalization	0.372 ± 0.099	**0.0003**
Body mass index	0.036 ± 0.021	0.10
Pre-existing heart disease	0.1064 ± 0.1466	0.47
Hypertension	0.1397 ± 0.1087	0.20
Diabetes	0.129 ± 0.1339	0.34
Chronic pulmonary disease	-0.0259 ± 0.1718	0.88
Immune disorders	0.00294 ± 0.1374	0.98
Invasive mechanical ventilation	0.4155 ± 0.1352	**0.0028**
Anti-IL6 antibody	0.2215 ± 0.1591	0.17
Corticosteroïds	0.2538 ± 0.2938	0.39
Serology		

3 months anti-NP	0.3368 ± 0.0973	**0.0008**
3 months anti-Spike	0.4662 ± 0.0923	**<0.00001**
6 months anti-NP	0.35857 ± 0.0960	**0.0003**
6 months anti-Spike	0.6434 ± 0.0899	**<0.0001**

* Regression beta are adjusted on age and sex

Figures legends :

Figure 1: temporal evolution of anti-SARS-CoV-2 antibodies. Level of anti-NP and anti-S IgG, in the cohort, 3 months and 6 months after the hospitalisation for RT-PCR confirmed SARS-CoV-2 infection. The IgG level was determined quantitatively by chemiluminescent microparticle immunoassay. Difference between time-points were analyzed with t-test, ******** $p<0.0001$. The red line represents the median for each time-points.

Figure 2: Temporal evolution of neutralizing activity **a.** sera collected 3 (blue) and 6 (red) months post hospitalization were tested with serial dilutions for their ability to neutralize SARS-CoV-2. Shown is the mean activity at each serum dilution with the 95% confidence interval. **b.** The inhibitory dose of 50% (ID50) of neutralization is depicted 3 months and 6 months after the hospitalization. Statistical analysis: Wilcoxon tests were performed. ****$p < 0.0001$. The red line represents the median for each time-points

Figure 3: Evaluation of the humoral response according to the initial clinical presentation. **A**. Level of ID50 neutralization, **b**. anti-NP, and **c.** anti-S IgG, at 3 and 6 months post infection, in 3 different subgroups of the cohort: patients requiring mechanical ventilation (+/+), patients requiring ICU admission without mechanical ventilation (+/-), patients hospitalized in conventional care (-/-). The IgG level was determined quantitatively by chemiluminescent microparticle immunoassay. Inhibitory dose of 50% (ID50) was determined by serial dilution. Differences between the 3 subgroups in each time-point were analyzed with one-way anova then

by multiple comparison between groups, ✱✱✱ p<0.001, ✱ p<0.05 for comparison between groups. The black line represents the median for each time-points.

Figure 4: Neutralizing activity against D614G, B.1.1.7, B.1.351 and P.1. **a.** Sera collected 6 months post hospitalization were serially diluted and tested for their ability to neutralize the four indicated SARS-CoV-2 strains (D614G, B.1.1.7, B.1.351 and P.1). Shown is the mean activity at each serum dilution with the 95% confidence interval. **b.** Inhibitory dose of 50% (ID50) of neutralization for the 4 viral isolates. Each dot represents an individual. Statistical analysis: one-way anova then by multiple comparison between groups were performed. ****p < 0.0001. The red line represents the median for each time-points

Figure 1

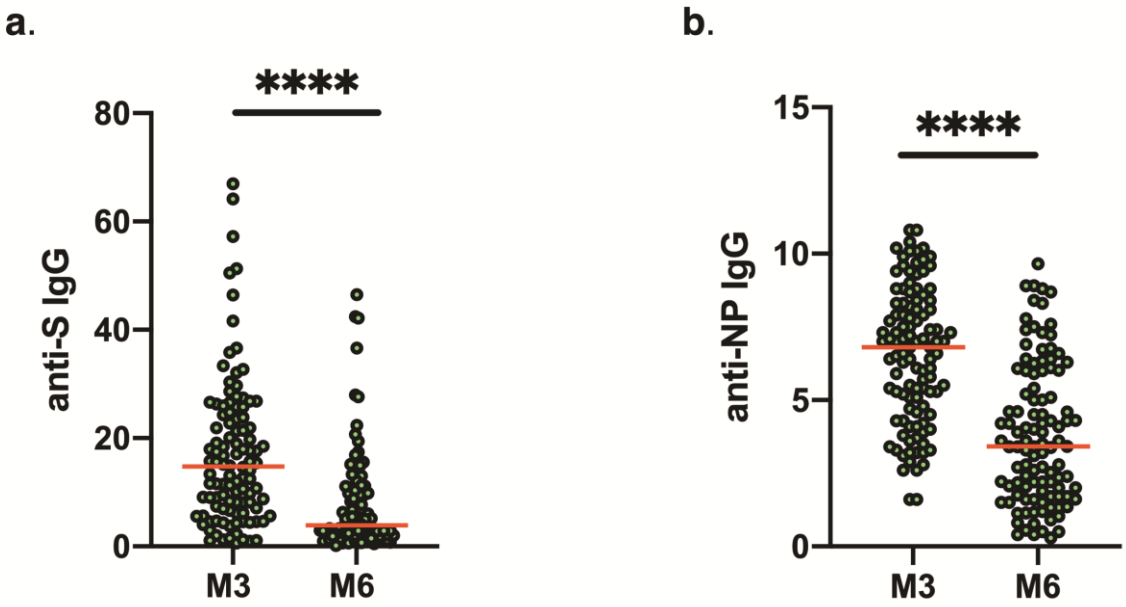

Figure 2

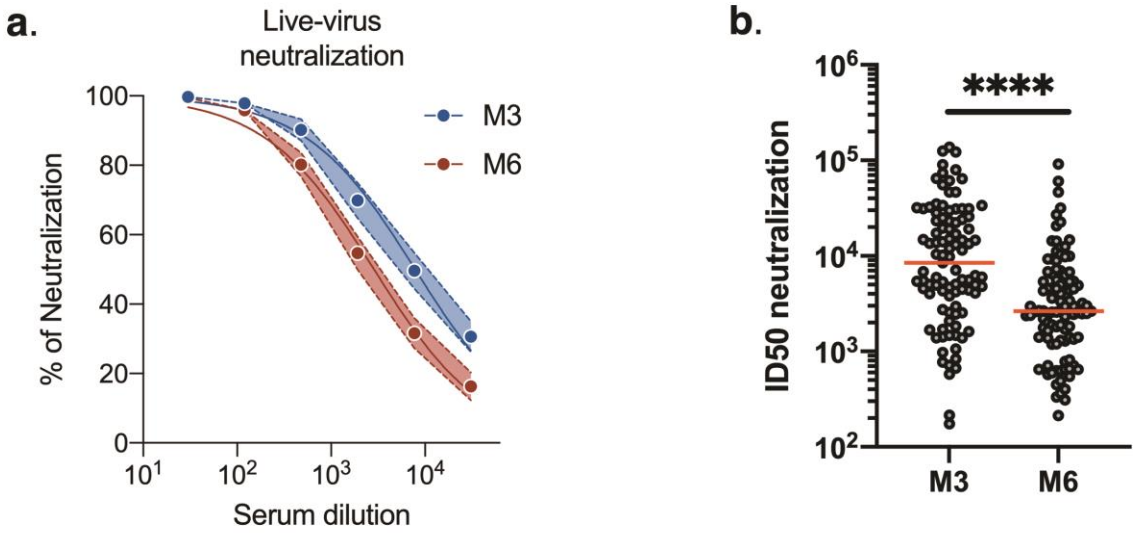

Figure 3

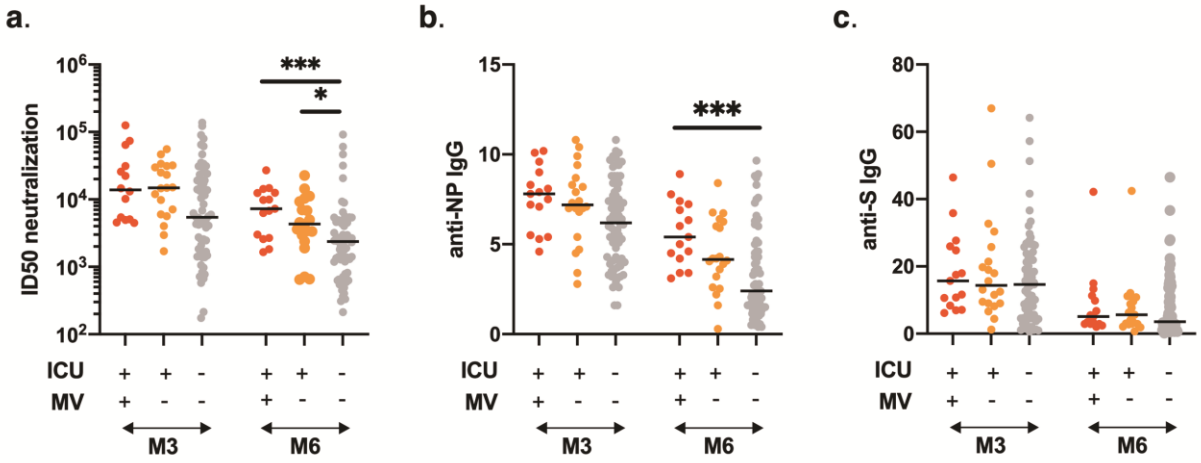

Figure 4