PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Temporal trends and differences of SARS-CoV-2-specific antibody responses in symptomatic and asymptomatic subjects. A longitudinal study from Umbria in Italy
AUTHORS	Abraha, Iosief; Eusebi, Paolo; Germani, Antonella; Pasquarelli, Erica; Pascolini, Sofia; Antonietti, Rossana; Argenti, Sandro; Fioravanti, Alessandra; Martini, Elisa; Aristei, Luana; Mancinelli, Paola; Ottaviani, Maria Letizia; Roselli, Martina; Barzacca, Milena; Belardinelli, Erika; Micheli, Marta

VERSION 1 – REVIEW

REVIEWER	Braun, Ralf Danube Private University
REVIEW RETURNED	22-Sep-2021

GENERAL COMMENTS	The authors could confirm that the serological response upon SARS-CoV-2 infection correlates with COVID-19 disease severity, and that the spike-specific antibodies are more stable that the antibodies against the nucleocapsid protein. All the points of the manuscript are confirmative to previous data. I think this is fine, however, the manuscript should be streamlined and focused, for the benefit of the reader. Major points: 1.) Title: The title is too long. I recommend using a more concise one, e.g., " Temporal trends and differences of SARS-CoV-2-specific antibody responses in symptomatic and asymptomatic subjects. A cross-sectional study from Umbria in Italy." 2.) Please shorten the introduction and the discussion to a maximum of 1.5 pages each. 3.) The aims of the study should be clearly defined, and answered by the following part of the paper. 4.) Figure legends and table descriptions need to be more informative. The reader should be able to understand figures and tables without reading the complete text. 5.) How do you know that the observed increase in antibody levels in number of participants is "probably due to reinfection" (see abstract)? If not supported by data, this is a mere speculation, and not a result. "None of the participants reported clinical reinfection with SARS-Cov-2 virus" (see abstract). What do you mean with this statement? None of the participants were reinfected in the hospital OR were not re-hospitalized upon reinfection OR could not show any diagnostic test for reinfection? But how does this fit to your above statement? 6.) Isn't it trivial that symptomatic and hospitalized participants have been pharmacologically treated whereas asymptomatic were not? What is the research question behind these observations?

	Minor points: 1.) Abstract: Please build complete sentences instead of using key words. 2.) Abstract-Conclusion: Please tone down your statement "probably provide protection", as it still an open date which classes of antibodies and which antibody levels provide protection from reinfection. 3.) Whole manuscript: - Please use upper and lower cases in a correct fashion. - Please use "SARS-CoV-2" and "COVID-19" throughout the manuscript. Do not switch between SARS-CoV-2 and Sars-CoV-2 or Sars-Cov2, or COVID-19 or Covid19 or Covid-19. 4.) Figure 2: Duration of symptoms in days? This is unclear in the figure. 5.) Figure 3: Number and units of antibody prevelance remain mysterious in this figure. 6.) How are "symptomatic" and "oligo-/asymptomatic" participants definded (see Table 1).The number of symptoms? The duration of symptoms? If you have a non-hospitalized participant with some symptoms of different duration, how did you decide to put in the oligoasymptomatic or in the symptomatic category?

REVIEWER	Harris, Ross Public Health England, Immunisation, Hepatitis, Blood Safety and Countermeasures Response
REVIEW RETURNED	16-Dec-2021

GENERAL COMMENTS	This paper presents a largely descriptive study of antibody levels following SARS-COV-2 infection. The long follow up is of interest, and the persistently higher antibody levels in those with symptomatic and severe infection vs. asymptomatic. I would have preferred more formal statistical analysis, which is largely in terms of non-parametric comparisons between groups. The logged values of antibody titres tend to have an approximately normal distribution, and can therefore be thought of in terms of both mean differences and trends over time and analysed using more powerful parametric models. Formal comparisons within a regression framework can provide quantification of the difference in trends for the three groups. More sophisticated models can also allow for individual variation in AB responses, which are substantial – see e.g., https://www.journalofinfection.com/article/S0163-4453(21)00132-8/pdf In particular some further thought could be given to whether those starting at a higher point decline faster, or at the same rate, for instance. This has a particular bearing on the idea that some increases in AB levels over time correspond to further exposure to SARS-COV-2 and boosting of antibodies: a formal model, allowing for individual variability in responses, is required in order to detect a signal beyond what is potentially random noise. The discussion on p19 (lines 15-36) is therefore somewhat speculative without statistical quantification of any “boosting”. It is also somewhat anecdotal: no reinfections were reported, but is the sample size and background case rate sufficient that zero observed infections is meaningful?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Ralf Braun, Danube Private University
Comments to the Author:
The authors could confirm that the serological response upon SARS-CoV-2 infection correlates with COVID-19 disease severity, and that the spike-specific antibodies are more stable that the antibodies against the nucleocapsid protein. All the points of the manuscript are confirmative to previous data. I think this is fine, however, the manuscript should be streamlined and focused, for the benefit of the reader.

First of all we would like to thank Dr. Braun for spending his time to review our manuscript. We appreciate very much Dr. Braun's comments and we did our best to revise the manuscript in accordance with the suggestions provided.

Major points:
1.) Title: The title is too long. I recommend using a more concise one, e.g., " Temporal trends and differences of SARS-CoV-2-specific antibody responses in symptomatic and asymptomatic subjects. A cross-sectional study from Umbria in Italy."
We thank Dr. Braun for this interesting suggestion. We changed the title accordingly.

2.) Please shorten the introduction and the discussion to a maximum of 1.5 pages each.
We thank Dr. Braun for this interesting suggestion. We reduced the introduction to 1.5 pages. We shortened also the Discussion though it was not materially possible reduce it to 1,5 pages.

3.) The aims of the study should be clearly defined, and answered by the following part of the paper.

We agree with Dr. Braun that the aims are not clearly presented. The amended version of the aims is as follows:

"The objectives of our study were (a) to describe differences in clinical and treatment characteristics between clinical categories (oligo/asymptomatic, symptomatic and hospital admitted) of subjects who had Sars-Cov-2; (b) to assess the correlation between serological titers and the clinical categories; (c) to evaluate the trend of anti-Sars-Cov-2 titers among the clinical categories over a follow-up of 12 month. In addition, we performed a clinical and history evaluation of the participants for a possible viral infection at every time follow-up."

Accordingly, we restructured the Results section by modifying titles, subtitles and the text as necessary; text in the Discussion was also modified. Additionally, we provided the definition of the clinical categories the Methods section as follows:

"For our analysis participants were categorized as follows: (a) oligo/asymptomatic, (b) symptomatic, and (c) hospital admitted. Oligosymptomatic patients were those with symptoms enduring for less than three days or with only one symptom (anosmia/ageusia or asthenia) that may last more than three days. Conversely, symptomatic patients were those with more than one symptom lasting at least three days and without any hospital admission."

4.) Figure legends and table descriptions need to be more informative. The reader should be able to understand figures and tables without reading the complete text.
We agree and we revised text and notes of Tables and Figures as required.

5.) How do you know that the observed increase in antibody levels in number of participants is "probably due to reinfection" (see abstract)? If not supported by data, this is a mere speculation, and not a result. "None of the participants reported clinical reinfection with SARS-Cov-2 virus" (see abstract). What do you mean with this statement? None of the participants were reinfected in the hospital OR were not re-hospitalized upon reinfection OR could not show any diagnostic test for reinfection? But how does this fit to your above statement?
We thank Dr. Braun for this comment that gives us the chance to clarify and revise our manuscript. We admit that it was necessary to underline that we intended "clinical reinfection" and/or "re-

hospitalized upon infection". Hence we removed the above mentioned sentence from the Abstract and amended the main text as follows:
Methods: "*During follow-up, at the time of specimen collection participants were evaluated for potential COVID-19 related clinical reinfection or re-hospitalized upon reinfection*."
Results: "*During serologic follow-up participants underwent a history examination and clinical visit. When participants were not available for clinical visit their health status and history examination of recent or past reinfection was ascertained through telephone call. None of the participants in any of the group had any sign or symptom that could be attributed to a possible clinical Sars-Cov-2 reinfection or was hospitalized upon reinfection. Since the study did not consider the application of PCR or antigenic test we cannot exclude that some participants might have developed asymptomatic Sars-Cov-2 reinfection.*"

6.) Isn't it trivial that symptomatic and hospitalized participants have been pharmacologically treated whereas asymptomatic were not? What is the research question behind these observations?
We admit that that this part might be less significant that the issue regarding the correlation and trend of the antibodies. However, it was part of our aims describing the characteristics of the three groups of participants according to the severity of the symptoms.

Minor points:
1.) Abstract: Please build complete sentences instead of using key words.
We thank dr. Braun for this suggestion. We tried our best to build complete sentences
2.) Abstract-Conclusion: Please tone down your statement "probably provide protection", as it still an open date which classes of antibodies and which antibody levels provide protection from reinfection.
We removed the sentence and limited our reflection in the discussion section.
3.) Whole manuscript:
- Please use upper and lower cases in a correct fashion.
- Please use "SARS-CoV-2" and "COVID-19" throughout the manuscript. Do not switch between SARS-CoV-2 and Sars-CoV-2 or Sars-Cov2, or COVID-19 or Covid19 or Covid-19.
We thank very much dr. Braun for this suggestion. We revised the whole manuscript and amended as necessary
4.) Figure 2: Duration of symptoms in days? This is unclear in the figure.
We revised the description of the Figure as necessary
5.) Figure 3: Number and units of antibody prevelance remain mysterious in this figure.
The Figure 3 has been replaced in agreement with the statistical amendment.
6.) How are "symptomatic" and "oligo-/asymptomatic" participants definded (see Table 1).The number of symptoms? The duration of symptoms? If you have a non-hospitalized participant with some symptoms of different duration, how did you decide to put in the oligoasymptomatic or in the symptomatic category?
We thank very much dr. Braun for pointing out this important omission. We have now provided in the revised version the definition of the different categories

Reviewer: 2
Dr. Ross Harris, Public Health England
Comments to the Author:
This paper presents a largely descriptive study of antibody levels following SARS-COV-2 infection. The long follow up is of interest, and the persistently higher antibody levels in those with symptomatic and severe infection vs. asymptomatic.

I would have preferred more formal statistical analysis, which is largely in terms of non-parametric comparisons between groups. The logged values of antibody titres tend to have an approximately normal distribution, and can therefore be thought of in terms of both mean differences and trends over time and analysed using more powerful parametric models. Formal comparisons within a regression framework can provide quantification of the difference in trends for the three groups. More sophisticated models can also allow for individual variation in AB responses, which are substantial – see e.g., https://www.journalofinfection.com/article/S0163-4453(21)00132-8/pdf

In particular some further thought could be given to whether those starting at a higher point decline faster, or at the same rate, for instance. This has a particular bearing on the idea that some increases in AB levels over time correspond to further exposure to SARS-COV-2 and boosting of antibodies: a formal model, allowing for individual variability in responses, is required in order to detect a signal beyond what is potentially random noise. The discussion on p19 (lines 15-36) is therefore somewhat speculative without statistical quantification of any “boosting”. It is also somewhat anecdotal: no reinfections were reported, but is the sample size and background case rate sufficient that zero observed infections is meaningful?

We thank Dr. Harris for spending his time to review our work. We accepted his suggestion in performing more statistical analysis and we involved as a co-author Paolo Eusebi who has an excellent curriculum in statistics.
We performed mixed-effects regression models for repeated measures that allow us to characterize the individual responses. We modeled the logarithm of anti-Sars-Cov-2 titers at each follow-up as the dependent variable (continuous) or the persistence of anti-Sars-Cov-2 response (binary). Furthermore, we wanted to explore whether those starting at a higher point decline faster, or at the same rate. We fitted a model of changes of anti-Sars-Cov-2 titers at follow-ups 2 and 3 including the baseline as covariate. The baseline term was not significant, so we can say that the rate of decline of anti-Sars-Cov-2 titers is not influenced by the baseline levels. In the text we included the following sentences:
"*The value of the antibody anti-S titer at first visit did not influence the rate of change at follow-up*."
"*The value of the antibody anti-N titer at first visit did not influence the rate of change at follow-up*."

Regarding clinical follow-up we clarified what we intend as reinfection:
Methods: “*During follow-up, at the time of specimen collection participants were evaluated for potential COVID-19 related clinical reinfection or re-hospitalized upon reinfection*.”
Results: “*During serologic follow-up participants underwent a history examination and clinical visit. When participants were not available for clinical visit their health status and history examination of recent or past reinfection was ascertained through telephone call. None of the participants in any of the group had any sign or symptom that could be attributed to a possible clinical Sars-Cov-2 reinfection or was hospitalized upon reinfection. Since the study did not consider the application of PCR or antigenic test we cannot exclude that some participants might have developed asymptomatic Sars-Cov-2 reinfection.*”

We revised also the corresponding part in the discussion. We tried to reduce the tone of the speculation by removing some sentences and revising the paragraph.

Reviewer: 1
Competing interests of Reviewer: None

Reviewer: 2
Competing interests of Reviewer: Nothing to declare

VERSION 2 – REVIEW

REVIEWER	Braun, Ralf Danube Private University
REVIEW RETURNED	07-Mar-2022

GENERAL COMMENTS	The authors addressed all my comments adequately.