Revision to the Manuscript No: 77094

Clinical Significance of Anti-nucleocapsid IgG sero-positivity in SARS-CoV-2 Infection in Hospitalized Patients in North Dakota.

Response to Reviewer's comments:

Reviewer # 1: Comment:

This paper presents a case study concerning several epidemiologic-clinical parameters when they are taken against SARS-CoV-2 in patients hospitalized with severe COVID-19 illness. The authors provide statistical analysis of a certain group of patients tested for COVID-19 who were admitted to a hospital in North Dakota. The authors conclude that the admission rate to the ICU and the use of BiPAP, HFNC and VENT support were not significantly distinct across IgG status. However, an important parameter related to monitoring the patients' condition - the length of stay - when assessed by IgG status was found to be significant. In overall, the paper is well-written and flows logically. Technically, the authors apply regression models to better fit their data, hence improving the quality of the results and their analysis. Although the discussion and data analysis have been performed adequately, I believe that a correlation study involving the examined variables would bring interesting insights and a richer discussion of the results.

Author response:

We would like to thank you for your comments and suggestions and we appreciate you for your time to review our manuscript for its scientific content and clinical value.

We have included a short analysis using the Phi coefficient (analogous to a Pearson correlation coefficient for binary variables) for the binary outcome variables. This has been described in the "Statistical analyses; Correlation of Outcomes on page-7.

Reviewer # 2: Comments:

#1: Introduction – This part is well written and comprehensive. There is some evidenced-based data to indicate that IgG-S levels upon admission are not associated with disease outcomes and it should be addressed in the second paragraph of the introduction. The authors should use the following work, which include a good example for such evidence: https://doi.org/10.1371/journal.pone.0268050

Author response:

We sincerely thank the reviewer for the suggestion. The reference has been added to our manuscript bibliography, as well as addressed in the second paragraph of "Introduction".

#2: Methods:

Reviewer's comment:

Study population – How were patients selected? What was the randomization process? Were they consecutive patients? Were only patients with severe COVID-19 included?

Author response:

All patients admitted to the hospital within the study period with severe or critical Covid-19 infection and who were screened for IgG-N within 48 hours of admission were included in the study. Patients with mild and moderate symptoms were excluded because most of these patients were not hospitalized. Retrospective data was collected from those patients who had an IgG-N test done at the time of admission or within 48 hours of admission.

Reviewer's comment:

When was the N-IgG taken? Upon admission?

Author response:

Patients were included if they had IgG-N antibody test done at the time or within 48 hours of admission. We have updated this in the " Methods".

Reviewer's comment:

A lot of variables are missing from your analysis. First, the duration from symptoms onset to hospital arrival should be included as it is obvious that a patient with illness duration of 10 days might have higher N-IgG levels

upon admission than others with shorter duration. Second, it is known that many co-morbidities effect COVID-19 disease outcomes. By not including them (HTN, DM, immunodeficiency state, etc.) there can be a strong bias in your results. In my opinion, as the groups are quite small, a regression model doesn't fit, and you should concentrate on describing your groups better.

Author response:

We would like to thank the reviewer for a very valid suggestion. We agree with the reviewer that the duration of symptoms before presentation to the hospital may affect levels of SARS-CoV2-antibodies. The study does not measure the level of the antibody titer but rather only qualitatively measures the presence or absence of IgG-N antibodies at the time of hospitalization. We think however that the duration of symptoms is important and have updated our data to include this. We now include duration of symptoms in days (DOS) in the analyses along with the other confounding variables (age, sex, BMI).

We agree with the reviewer that comorbidities such as Hypertension, Diabetes, Chronic lung diseases may confound the results. However, given that there was no strong evidence about the IgG-response to SARS-CoV-2 is affected by the above-mentioned comorbidities, we chose to analyze our data independent of the comorbidities. We acknowledged this limitation of our study in the manuscript and we hope to consider it as a potential future research project.

Reviewer's comment:

I don't think you should exclude a patient only because of his extended hospital stay. Such actions lead to biased results. Instead, you should use median for age and not mean (median should generally be used for small groups).

Author response:

In the relevant analyses, we ran models both with and without the patient with extreme value. In the final model, the results were not significant regardless of inclusion / exclusion.

#4: Results:

Reviewer's comment:

Results should first include a general description of the study cohort (size, age etc.)

Author response:

"Results" now includes the description of the study cohort.

Reviewer's comment:

I am not familiar with presenting the t-value and f-value

Author response:

t and f values are test statistics, with the corresponding p-values that tell whether those test statistics were significant.

Reviewer's comment:

Figures 2-6 does not add significant information to justify their inclusion. They show the same information as appear in table1 and because of the small sample size all include 0 in their standard deviation.

Author response:

We agree with the reviewer's suggestions. However, Figure 2-6 have been included for graphical demonstration of individual group for the convenience of the readers.

Reviewer/s comment:

You should refrain from describing the analysis with the extreme LOS which clearly was the only reason for the significant correlation

Author response:

As suggested by the reviewer, under # 3, we have included the analyses both with and without the patient with extreme value for further

consideration. As noted above, we ran relevant models with and without the extreme LOS value and in the final analysis, the results were non-significant regardless.

Reviewer comment:

Table 1 – all means should include a standard deviation. All medians should include an IQR. All percentiles should include the absolute number of patients. All abbreviations should be explained in a caption.

Author response:

Table 1 now includes means with standard deviation and medians with IQR for age, BMI, duration of symptoms, and length of stay.

Reviewer comments:

I find it hard to believe that over 50% of patients needed invasive or non-invasive ventilation. Did you include each patient only once for the maximal oxygen support method which he needed? What about other oxygen support methods – via oxygen mask or nasal cannula?

Author response:

We included the patients only with severe to critical Covid-19 diseases into our study. This means all patients in our study group were hypoxic and required supplemental oxygen or advanced respiratory support during

their course of hospitalization. The patients were included in each method of respiratory support that they used, so patients may be in more than one category (e.g., used both BiPAP and Ventilation).

In exploratory analysis, we created a combined categorical variable for ventilation with four categories (Nasal canula / Mask, High flow nasal canula, BiPAP and mechanical ventilation). We ran a Fisher's exact test (analogous to Chi-square test for small sample size) to determine if the use of different ventilatory support methods were different across IgG-N status. Here we counted the ventilatory support system only once based on the maximal amount of oxygen support / ventilatory support needed. The test was not significant (p=0.6837), which means that there was no evidence that severity of respiratory failure was different across IgG-N status, and therefore we did not include it in the article.

5: Discussion:

Reviewer's comment:

As noted above, the authors should use the example given in the discussion as it is highly relevant to paragraph 2.

A short paragraph with conclusion should be included at the end.

Author response:

Discussion has been addended with a paragraph with conclusion.