Review 2: "COVID-19 and its clinical severity are associated with alterations of plasma sphingolipids and enzyme activities of sphingomyelinase and ceramidase"

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RR:C19 Evidence Scale rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:

Activation of the sphingolipid metabolizing pathways is described in the manuscript as a hallmark of the body's response to SARS-CoV-2 infection, which is discussed and recently described in the literature using similar or independent methods. Herein, the authors describe an up-regulation of two rate-limiting enzymes of ceramide generation and down-regulation of one ceramide degrading enzyme during the course of the disease. For these aims, they determined the activity upon release into circulating. Most interestingly, in a large cohort of patients with different grades of severity, there is a parallel running change with respect to enzymes activity and concentration of respective substrates and products, respectively. This observation is of great interest due to the fact that the monitoring of ceramide generation (activity) or bio-effective concentration in the circulation might serve for a better understanding of mechanisms of organ dysfunction (already observed in these patients with a severe manifestation of the disease); as well as for the elucidation of these changes for improved stratification of the patients with an anti-viral therapy (administration of antibodies or virustatics) as a decision-permitting biomarker.

Unfortunately, the clinical information on the patients and the methodology of the sampling process is very limited, which makes interpretation of results difficult. There are many possible sources of bias depending on sampling time point in respect to the timeline of the disease process, therapeutic interventions, and methods of severity grading. The authors should clarify the following points to further improve the quality of the manuscript:

- At what time point of the disease or hospital stay were patients sampled, e.g. day of admission, day of consent, or other?

- Over which time period were patients sampled, as clinical characteristics and management were highly variable over time?
- What kind of immunomodulating or COVID-specific therapy did patients receive? As you mentioned e.g. chloroquine might modulate the measured effects and therapeutic interventions differ depending on disease severity and available knowledge at the time of treatment.
- How was clinical severity assessed? Was it at the time of blood draw or the worst over the whole hospital stay? Were patients transferred from other hospitals to your ICU included? Why were patients without the need for oxygen admitted in such large numbers? Were any patients on ECMO during blood draw and is there any information on the effects of ECMO itself on the measured outcomes?
- How were patient numbers per group planned? Was there a pre-defined protocol with fixed groups of 50 patients and if so, was there some kind of power calculation? Or were the analyzed patients selected from a larger cohort and if so, how was the sample drawn?

Description and operative implementation of analytical methods are well done, pre-analytical preparation of the samples, the performance of activity measurements, and profiling of sphingolipids are fine. From our point of view, this study as yet is the largest one with a comprehensive panel determining sphingolipid degrading enzymes paralleled with the pattern of related compounds from circulating plasma. With respect to interpretation of results, there is the only reference to the classification of patients by the authors, comparison or analyses with established markers of disease severity and/or mortality are missing.

For the discussion section, a short debate on the source of the enzymes released into circulation might improve the understanding of the interpretation of the results. From their data, authors speculate that patients might benefit from inhibition of sphingomyelinase activity, a notion that is supported by independent studies showing beneficial effects of antidepressant medication such as fluoxetine acting as a functional inhibitor of activation in the development of COVID-19. This is a very important issue, but in line with that point, we also encourage the authors to present a comparison of activity levels of sphingomyelinase with an antecedent treatment of patients with these compounds.