Review 3: "Serum Sphingosine-1-Phosphate as Novel Prognostic and Predictive Biomarker for COVID-19 Severity and Morbidity and Its Implications in Clinical Management"

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RR:C19 Evidence Scale rating by reviewer:

Not informative. *The flaws in the data and methods in this study are sufficiently serious that they do not substantially justify the claims made. It is not possible to say whether the results and conclusions would match that of the hypothetical ideal study. The study should not be considered as evidence by decision-makers.*

Review:

The manuscript with the title: "Serum sphingosine-1-phosphate as novel prognostic and predictive biomarker for COVID-19 severity and morbidity and its implications in clinical management," by Giovanni Marfia and colleagues is a comprehensive analysis of 111 patients with COVID-19 and 47 healthy subjects. The authors report that the S1P and ApoM concentrations in serum of COVID-19 patients are significantly diminished compared to healthy volunteers. While S1P surprisingly did not significantly correlate with ApoM, which binds S1P and which is mainly found in HDL as the predominant carrier molecule for S1P in plasma, it correlated well with RBC, HGB and HCT values. This correlation can be expected because RBC is one of the major sources for S1P in plasma. Albumin, the second main carrier molecule for S1P in plasma, and HDL-C correlated well with S1P and with ApoM, suggesting a potential role of carrier molecule concentration and S1P concentration in plasma. The authors also subdivided the COVID-19 patient cohort in those requiring and not requiring intensive care and found significantly reduced levels of S1P, ApoM, albumin and HDL-C in serum of patients that were transferred to the intensive care unit. ApoM and S1P levels additionally correlated with the pneumonia severity index (PSI) and the neutrophils to lymphocytes ratio (NLR). S1P, but not ApoM additionally correlated with the time after admission. Finally, the authors demonstrate different risks for ICU admission and mortality in COVID-19 patients depending on the determined S1P concentration.

While the data presented in this manuscript appear to be sound, there are several obstacles in the presented manuscript that hamper the conclusions. First of all, the authors state that they measured S1P and other biomarkers only once for each patient, but they do not mention any particular time point after admission, and they do not mention the severity state of COVID-19 patients when serum was collected. In fact, Fig. 4B suggests that blood sampling was done at very different time points from 1 to 75 days after admission. Even if days were hours, these different time points for blood withdrawal could severely skew the results, especially when S1P-levels significantly correlate with the time from admission as demonstrated here. It could be that some patients already required intensive care at the time of blood withdrawal. In that case the S1P-level may still serve as a severity factor, but not as a prognostic biomarker. The uncertainty of the time point of blood withdrawal and the severity score of patients included in this study renders the interpretation of the presented data really difficult. For the

same reason, the data comparing COVID-19 patients requiring (ICU) and not requiring (noICU) intensive care are probably biased by the fact that at least some blood samples were included from patients that required immediate transfer to an ICU or were already admitted to an ICU (Fig. 3). Again, a separation of the patients according to a severity score at the time of blood withdrawal would be much more informative. Fig. 5 showing the prognostic value of S1P in COVID-19 patients is hardly interpretable. The risk of ICU admission (Fig. 5A) seems to be lower with high S1P-levels in serum, while mortality seems to be increased (Fig. 5B). In both cases, the y-axis is labelled with "cumulative survival," and the text is not really helpful in interpreting these opposite results. Maybe it is just the labelling of the y-axis, but this needs to be clarified. It should be noted that the authors do not provide receiver operating characteristic curves to demonstrate the specificity and sensitivity of S1P as a prognostic or severity marker, the statement made in the title is therefore somewhat over-interpreted. It should also be noted that the error bars of healthy and COVID-19 groups as well as noICU and ICU groups largely overlap, indicating that a clear separation of patient groups will be difficult. Also, a comparison of healthy subjects with COVID-19 patients that do not require intensive care is missing. It would be interesting if S1P-levels in serum of COVID-19 patients with a milder disease progression are also lower compared to healthy volunteers. If not, lower S1P-levels may just be a severity marker for patients requiring intensive care similar to studies with sepsis patients.

With regard to the clinical data, the authors do not mention any treatment or applied drugs. E.g., volume resuscitation performed during intensive care management of the patients may result in dilution effects of all parameters provided in this study, which may be completely missed in this study. No data are presented whether or not the Covid-19 patients received serum albumin or other colloids or crystalloids during hospitalization. It is obvious that these issues should be taken into account for strict normalization of data in order to avoid misinterpretation of any therapy-driven effects. Furthermore, there are no defined endpoints of this clinical study, the data are purely observational and correlative, the authors do not provide any mechanistic details or further evidence for the presented observations and correlations. Stating that "[...] restoring abnormal S1P levels to a normal range [...] may have the potential to be a therapeutic target for reducing the risk of disease progression and death [...] in patients with COVID-19" implies a functional role of lowered (but not zero) S1P-levels in COVID-19 patients, which is not proven at all and also not covered by the presented data.

In conclusion, the presented manuscript supports the notion that patients requiring intensive care can be identified by lower S1P-levels. The presented data need more analyses to substantiate the statement that S1P could be a prognostic and predictive biomarker for COVID-19 severity and morbidity.