Review 2: "SARS-CoV-2 Infection Impacts Carbon Metabolism and Depends on Glutamine for Replication in Syrian Hamster Astrocytes"

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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:

The preprint by Gomes de Oliveira and colleagues is an interesting and detailed report on the effects of SARS-CoV-2 infection on astrocytes. Using a variety of techniques, including primary cultured astrocytes, in vivo infection on Syrian Hamsters (that are permissive for SARS-CoV-2 infection), infected brain slices, metabolomics on cultured astrocytes, and single nuclei transcriptomics of brain patients autopsy samples, the authors show that SARS-CoV-2 viral infection alters glutamate/glutamine pathways hijacking the astroglial metabolism for viral replication/assembly. The findings are of enormous importance keeping in mind the main role of astrocytes in sustaining neuronal and oligodendroglial metabolism by the lactate shuttle (i.e. by providing lactate and pyruvate for the neuronal/oligodendroglial TCA cycle) in the normal brain. In this scenario, the findings of the preprint may justify an astroglial malfunctioning the neurological and cognitive alterations produced not only in acute COVID-19 but also in the long COVID-19 pathology. Even the observed acceleration in neurodegenerative diseases and symptoms observed after SARS-CoV-2 infection in older and/or susceptible patients may find an explanation when considering these experimental findings.

I have some specific comments about the findings:

- As seen in Supplementary Figure 1, not all astrocytes become infected by SARS-CoV-2 in vitro—at least at the MOI tested by the authors. In fact, the infected astrocytes look very elongated and not stellated or amoeboid as expected in most astrocytes in vitro. I wonder if there is some preference of SARS-CoV-2 to infect a population of astrocytes in vitro (and in vivo). Improving in vitro images of infected astrocytes and including brain sections from infected animals may adequately answer this question. These images may also open an interesting avenue to studying the astroglial

heterogeneity and susceptibility to SARS-CoV-2 by different astroglial populations and different brain regions. As an example, perivascular astrocytes with their end-feet contacting endothelial cells may be in the first row if SARS-CoV-2 reaches the CNS by the hematogenous route secondarily to respiratory tract infection. Brain regional susceptibility is also another interesting question that the brain slices processed for immunohistochemistry may answer.

- As shown by the authors, SARS-CoV-2 infection seems to induce astroglial proinflammatory gain-of-function, with alterations in the TCA cycle and glycolysis, increased mitochondrial fragmentation, and induction of ER stress pathways. However, mitochondrial respiration only shows a trend to be affected by infection—probably due to a large error and a small number of repeats in the experiments shown by the authors. Improving this experiment is essential because the large error may not be an artifact and could potentially represent a degree of heterogeneity in the astroglial susceptibility to infection or survival to SARS-CoV-2.
- Metabolomics findings showed alterations in the molecules of the TCA cycle. I am focusing especially on lactate and pyruvate since they are key molecules to be transferred to neurons and oligodendrocytes and the reduced availability of these intermediates may seriously affect neuronal survival and perhaps oligodendrocytes. These data have been complemented by the authors with elegant loss-of-function studies blocking different steps of glycolysis and glutamine/glutamate pathways. These studies may support further investigation in potential blockers of SARS-CoV-2 replication of great interest in the clinics.
- Regarding in vivo infection and SARS-CoV-2 presence in the hamsters: CNS has been followed by authors during 3-5-7-14 days post-infection and viral load in the CNS seems to diminish with time. I wonder why this is happening. A discussion about these findings could be interesting. Is the SARS-CoV-2 reaching the CNS by the trans-epithelial-olfactory pathway and then moving away from the CNS? Is it that SARS-CoV-2 can not amplify in the CNS? Lessons from other viruses that reach the CNS and stay “hidden” in astrocytes (i.e. HIV) could be interesting to compare with the findings presented in this preprint.

In summary, the preprint shows a collection of very interesting findings that will open new lines of research in the proposed neurotropism of SARS-CoV-2. The study is reliable, uses in vitro and in vivo approaches (in animals and human samples), and the conclusions are supported by the data. There are only minor limitations that lie in the small number of infected astrocytes in the in vitro experiments and the lack of images

of infected Syrian Hamster brains (or human patients samples) that may allow further interpretations of the data in light of the recognized heterogeneity of astroglial cell population. Improving the discussion (or performing co-culture studies) of the consequences of the altered lactate/pyruvate metabolism for neurons and oligodendrocytes could be interesting in this context.