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Supplementary appendix

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Supplement to: Bonhenry D, Charnley M, Gonçalves J, et al. SARS-CoV-2 infection as a cause of neurodegeneration. *Lancet Neurol* 2024; **23:** 562–63.

SARS-CoV-2 infection as a cause of neurodegeneration

Appendix: Application of the Bradford Hill criteria

The Bradford Hill criteria comprise a widely-used checklist of categories of evidence that should ideally be present in order to infer that a given exposure causes a given harm. The original guidelines date from 1965, and are re-interpreted for modern evidence-based medicine as three categories of evidence each of which really should be present, and each of which themselves divide into sub-categories which individually are more situation-specific (discussed in Howick et al., *Journal of the Royal Society of Medicine* 2009; 102:186-194). Fulfilling the criteria is not a proof, but does indicate the appropriate point of view to hold until contrary evidence should appear. The three categories of criteria are that evidence should be *Direct*, *Mechanistic*, and *Parallel*.

Evidence in the Direct Category

The first of the three categories is *Direct* evidence, for example the direct statistical correlation observed between SARS-CoV-2 infection and subsequent Alzheimers diagnosis, shown in figure 1 of the main text. The statistical power of the direct evidence is ample, for example the quoted study of Zarifkar et al. (*Frontiers in Neurology* 2022; 13:904796) covered >50% of the population of Denmark,[13] and a rigorous stratification and exclusion process for confounders such as age, sex, and co-morbidity was carried out. *Direct* evidence should ideally show appropriate temporal behaviour, and this is a valid point of contention: AD progression rate in general splits into two populations of "fast" and "slow" patients (Thalhauser and Komarova. *Journal of the Royal Society, Interface* 2012; 9: 119-26) but even so it remains difficult to distinguish between AD cases hypothetically initiated by SARS-CoV-2 and those merely accelerated by it from pre-clinical to clinical status. *Direct* evidence is therefore strongly present, but at the same time is the most problematic of the three categories, and must remain so until the full time-course of the effect can be epidemiologically observed.

Evidence in the Mechanistic Category

Reverse causation, where undiagnosed AD increases vulnerability to SARS-CoV-2, is in general difficult to disentangle entirely by relying only on *direct* evidence, this is a strong reason to look also at *Mechanistic* evidence. *Mechanistic* evidence is fulfilled in a general sense although not in detail, by the widely documented tendency to inflammation in patients and by controlled experiments showing prolonged neuroinflammation after mild SARS-CoV-2 infection in macaques (Nieuwland et al. *Journal of Neuroinflammation* 2023; **20**(179)). While no truly comprehensive etiology is yet present, it is shown very strongly that SARS-CoV-2 infection connects to the collection of known neuroinflammatory etiological routes for AD, which are themselves various and often not well documented although the common feature of neuroinflammation is highly salient for Alzheimer's disease and for neurodegenerative disease generally. Review articles discussing mechanistic evidence in detail include (Rahmani et al. *Physiology International* 2022 109**(**2)) and (Strong, *J Neurochem*. 2022 Dec 11).

Shi et al. (in *Int. J. Mol. Sci.* 2023, 24(5)) provide a molecular analysis of overlaps and interactions between SARS-CoV-2 and neurodegenerative disease which is current at the time of submission of this article, and which appears to give a valuable metabolomic overview.

Beside the molecular and pathological evidence of mechanisms connecting SARS-CoV-2 and AD, we place an observation that depression and isolation are themselves identified as risks for AD (Livingston et al. *Lancet* 2020 396:10248) and that SARS-CoV-2 has resulted in a huge increase in demand for treatment of mental health problems in general, including depression.

Parallel Character of Evidence

The third category is that evidence should be *Parallel:* there should be independent research with identical or compatible conclusions, as shown in figure 1 of the main text (for *Direct* evidence) and in the referenced literature above (for *Mechanistic* evidence).

Conclusion

We therefore state formally that SARS-CoV-2 should be considered to present a specific enhancement of risk for Alzheimer's Disease diagnosis, even though the distinction between causation of AD versus acceleration of AD that would eventually have been observed anyway is not possible to resolve with *direct* evidence at this time. The **risk ratio of 3·5** (95% CI: 2·2–5·5) for Alzheimer's Disease diagnosis following a SARS-CoV-2 diagnosis, taken from the Danish study (*Frontiers in Neurology* 2022; 13:904796), should be taken as authoritative until new data should become available, due to the large sample size and rigorous character of the study. Decline of annual excess risk over a decadal timescale cannot yet be guaranteed for SARS-CoV-2 due to the novel mechanistic processes for this infection, however the excess risk of neurodegenerative disease diagnosis typically does decline over **1-15 years** following diagnosis with non-SARS viral infections (Levine et al. *Neuron* 2023 111(7)).

Contributions

DB and JTB wrote the manuscript and prepared figures. NPR, MC, MS, AMB, MM, JG, JM, MH, JCL, PH, RI, and SN spoke at the COVAMINF meeting and discussed the contents of the manuscript. RI discussed and advised in planning the manuscript. The COVAMINF workgroup was funded by the Joint Programme of Neurodegenerative Disease, the Fonds Nationale de la Recherche Luxembourg (grant INTER/JPND22/16969609/COVAMINF) and the Luxembourg Institute for Advanced Studies (audacity project AMINF-2022). No author has any relevant commercial interest, except AMB, an employee of a private medical clinic (listed in affiliation).

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