Review of: "[Case Report] Supplementation with Vitamin D, Zinc, and Quercetin to Treat COVID-19: A Case Report"

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Potential competing interests: No potential competing interests to declare.

This case report describes effects of vitamin D, zinc, and quercetin supplementation that were subjectively observed to quickly resolve lingering symptoms in a healthy, 23-year-old female without underlying comorbidities infected by the SARS-CoV-2 virus.

The report is well-written and unbiased, objectively pointing out intrinsic limitations including:

1. single case without controls;
2. assessment was based on subjective numerical scale that can be attributable to the placebo effect;
3. limited data on the synergistic combination in the use of vitamin D, zinc, and quercetin; and
4. the examination was performed remotely with electronic communication that could have been misinterpreted or misrepresented.

Nevertheless, if the subjective report of effectiveness is assumed to be accurate and reliable, then it becomes quite difficult to ignore that there is an unusual, rapid improvement of symptoms at the end of Day 8, or the second day after treatment commenced when the patient began taking quercetin at 1000 mg 3 times a day and the numerical rating for cough was reduced from 7 to 1.

The author presented brief discussions on mechanisms of action including the reduction of inflammation and oxidative stress, as well as the ability to enhance immune function by vitamin D, zinc, and quercetin that could potentially explain the rapid resolution of symptoms. However, an outstanding, but obvious question that required further elucidation was not addressed.

The severity of symptoms recorded in Table 2 clearly showed that it was only after the addition of quercetin that resolution of symptoms became markedly improved. Even though it could have been the result of synergistic reactions that are not clarified, it is also possible that other molecular mechanisms are responsible for the observed effects.

The author explained the choice of dosage based upon previous studies where 5000 IU vitamin D improved cough and ageusia usually in about 5 to 9 days (references 2, 3), and 100 mg of zinc as monotherapy achieved resolution of symptoms in 10-14 days (reference 3). However, the author did not provide a rationale for the selection of the quercetin dose at 1000 mg 3 times daily albeit studies referenced only used 1000 mg twice daily (reference 21).

At this point, it would appear that without further illumination of potential relevant molecular mechanism(s) responsible for

the dramatic difference experienced by the patient between the first and third day after treatment commencement, this report is at best an interesting account of an inconsequential case report involving the subjective, successful use of vitamin D, zinc, and quercetin in a COVID19 patient.

However, if the author can offer an extensive discussion on the fundamental molecular processes used by all viruses, including SARS-CoV-2, for replication and explain the possible associations between the three supplements with these processes, then this report will become significantly more meaningful, offering deeper insight into how natural molecules such as vitamin D, zinc, and quercetin may affect viral replication cycles.

Phase Separation Regulates Viral Life Cycles

It is now widely accepted that all viruses hijack host resources in order to manipulate phase separation to enhance viral genome assembly, replication, and dissemination [Li et al. 2022], [Zhang et al. 2023].

Roden et al. in 2022 reported for the first time that the SARS-CoV-2 dsRNA promoted nucleocapsid protein condensate formation, and the addition of dsRNA altered the material properties of the resulting membraneless organelles. Furthermore, increasing dsRNA could reduce the lower critical solution temperature (LCST) of N-protein to that of physiological body temperatures. In other words, dsRNA facilitates N-protein phase separation to form viral factories in mammalian cells by lowering LCST independent of total RNA length/sequence [Roden et al. 2022].

As such, it is reasonable to assume that any significant reversal of symptoms should be attributable to the suppression of viral replication and associated phase separation processes.

Since quercetin has been shown experimentally to bind to dsRNA with high affinity [Rusak et al. 2009], [Marinić et al. 2006], [Septembre-Malaterre et al. 2021], it is entirely plausible that the administration of quercetin at adequate concentrations, such as 1000 mg x 3/day, could theoretically halt viral phase separation effectively by binding to dsRNA.

This would also explain why dramatic resolution of symptoms happened at the end of the second day of treatment commencement, after the patient has taken 3000 mg of quercetin.

However, several outstanding issues remain and should be addressed for a thorough discussion in this context:

Vitamin D

Vitamin D is known to react synergistically with dsRNA to upregulate host defense gene cathelicidin [Hansdottir et al. 2008]. How quercetin may affect this specific immune response is unclear. It is not improbable that suppressing viral replication by interfering with phase separation processes is more potent than targeting immune responses after viral replication has already started (Day 8).

Zinc

There is a significant inverse correlation between serum zinc levels and the severity and mortality of patients infected by SARS-CoV-2 where serum zinc levels were found to be markedly lower in the severe group compared to controls, mild,

and moderate groups [Pvsn et al. 2023]. Thus the addition of zinc supplementation may benefit the patient because zinc has critical roles in gene transcription, DNA repair, and antioxidant defenses [Ho 2004].

Furthermore, zinc is also indispensable in the formation of DNA-binding proteins known as zinc fingers. Zinc fingers are protein residues that form a motif that is folded around a central zinc ion. Each motif forms an independent minidomain so that adjacent zinc fingers are combined to form DNA-binding domains where the modules are seen as ‘gripping’ the DNA, hence the term zinc fingers [Klug and Schwabe 1995]. However, the use of zinc can be pro-viral due to the molecular functions of zinc fingers.

The Role of Zinc Fingers in SARS-CoV-2 Replication

Metals such as zinc and copper play vital roles in the survival and pathogenesis of viruses. Metal ions bind to viral proteins to facilitate strand transfer during reverse transcription of the vRNA, nucleic acid annealing and integration, transcription, and vRNA maturation [Chaturvedi and Shrivastava 2005], [Monette and Mouland 2020].

For example, nonstructural proteins (nsps) of the SARS-CoV-2 virus participate in viral genome replication and transcription. As such, the survival and dissemination of the virus are critically dependent on the optimal functioning of nsps. Deletion of nsp2 in SARS-CoV can result in suppression of growth and RNA synthesis. Interestingly, the crystal structure of the N-terminal of the SARS-CoV-2 nsp2 at a high resolution of 1.96 Å revealed a molecular structure consisting of three zinc fingers with the ability to bind with nucleic acids [Ma et al. 2021].

Moreover, it is now understood that zinc may assist the nucleocapsid protein to undergo phase separation during viral replication. Experimental studies reported prion-like domains in metal-coordinated nucleocapsids initiate phase separation events that lead to nucleation and assembly of viruses, contributing to their replication and persistence as cell-free infectious aerosol droplets [Monette and Mouland 2020]. Zinc has also been observed to promote SARS-CoV-2 N protein phase separation with RNA in vitro [Chen et al. 2020].

Since viruses such as SARS-CoV-2 must use host resources for all processes during its replication, the formation of viable, functional nsps with zinc fingers and promotion of N protein phase separation will utilize valuable host zinc stores. That may be the reason why serum zinc levels are inversely correlated with disease severity. However, if this is true, then supplementation with zinc becomes a double-edged sword that can also fuel viral replication under favorable conditions.

At present, the role of zinc is highly controversial in the context of viral replication, and hence a more detailed discussion is fully warranted to avoid confusion and misunderstanding. Additionally, the precise timing in the administration of zinc during the viral infection cycle may become critical in the determination of the effectiveness of zinc monotherapies.

Quercetin

Even though quercetin may be extremely effective in suppressing viral phase separation by binding to dsRNA to inhibit the formation of viral replication ‘factories’ [Rusak et al. 2009], [Marinić et al. 2006], [Septembre-Malaterre et al. 2021], the use of quercetin should include caution against the genotoxic effects in the suppression of non-homologous end joining and homologous recombination pathways in hematopoietic stem and progenitor cells at continuously high concentrations

[Biechonski et al. 2017]. 3000 mg/day is a relatively high concentration. Therefore, the continued use of quercetin at this level requires further examination for potential genotoxic effects.

Additional Comment

The discussion and the inclusion of the fact that the patient was vaccinated subtly implies that previous vaccination may have failed to confer adequate protection. This is a plausible inference as increased understanding shows that IgG4 induced by repeated vaccinations may generate immune tolerance [Uversky et al. 2023], [Irrgang et al. 2022]. However, without blood work from the patient that can substantiate this assumption, it may be advisable to clarify that it is unknown how previous vaccinations may have contributed to the development of symptoms in the patient.

References:

Biechonski, S.; Gourevich, D.; Rall, M.; Aqaqe, N.; Yassin, M.; Zipin-Roitman, A.; Trakhtenbrot, L.; Olender, L.; Raz, Y.; Jaffa, A. J.; *et al.* Quercetin Alters the DNA Damage Response in Human Hematopoietic Stem and Progenitor Cells via TopoII- and PI3K-Dependent Mechanisms Synergizing in Leukemogenic Rearrangements. *Int. J. Cancer* **2017**, *140*, 864–876. https://doi.org/10.1002/ijc.30497

Chaturvedi, U. C.; Shrivastava, R. Interaction of Viral Proteins with Metal Ions: Role in Maintaining the Structure and Functions of Viruses. *FEMS Immunol. Med. Microbiol.* **2005**, *43*, 105–114. , https://doi.org/10.1016/j.femsim.2004.11.004

Chen, H.; Cui, Y.; Han, X.; Hu, W.; Sun, M.; Zhang, Y.; Wang, P.-H.; Song, G.; Chen, W.; Lou, J. Liquid-Liquid Phase Separation by SARS-CoV-2 Nucleocapsid Protein and RNA. *Cell Res.* **2020**, *30*, 1143–1145. https://doi.org/10.1038/s41422-020-00408-2

Hansdottir, S.; Monick, M. M.; Hinde, S. L.; Lovan, N.; Look, D. C.; Hunninghake, G. W. Respiratory Epithelial Cells Convert Inactive Vitamin D to Its Active Form: Potential Effects on Host Defense. *J. Immunol.* **2008**, *181*, 7090–7099. https://doi.org/10.4049/jimmunol.181.10.7090

Ho, E. Zinc Deficiency, DNA Damage and Cancer Risk. *J. Nutr. Biochem.* **2004**, *15*, 572–578. https://doi.org/10.1016/j.jnutbio.2004.07.005

Irrgang, P.; Gerling, J.; Kocher, K.; Lapuente, D.; Steininger, P.; Habenicht, K.; Wytopil, M.; Beileke, S.; Schäfer, S.; Zhong, J.; *et al.* Class Switch towards Non-Inflammatory, Spike-Specific IgG4 Antibodies after Repeated SARS-CoV-2 mRNA Vaccination. *Sci Immunol* **2022**, eade2798. DOI: 10.1126/sciimmunol.ade2798

Klug, A.; Schwabe, J. W. Protein Motifs 5. Zinc Fingers. *FASEB J.* **1995**, *9*, 597–604. https://doi.org/10.1096/fasebj.9.8.7768350

Li, H.; Ernst, C.; Kolonko-Adamska, M.; Greb-Markiewicz, B.; Man, J.; Parissi, V.; Ng, B. W.-L. Phase Separation in Viral Infections. *Trends Microbiol.* **2022**, *30*, 1217–1231. https://doi.org/10.1016/j.tim.2022.06.005

Ma, J.; Chen, Y.; Wu, W.; Chen, Z. Structure and Function of N-Terminal Zinc Finger Domain of SARS-CoV-2 NSP2. *Virol. Sin.* **2021**, *36*, 1104–1112. https://doi.org/10.1007/s12250-021-00431-6

Marinić, M.; Piantanida, I.; Rusak, G.; Zinić, M. Interactions of Quercetin and Its Lanthane Complex with Double Stranded DNA/RNA and Single Stranded RNA: Spectrophotometric Sensing of Poly G. *J. Inorg. Biochem.* **2006**, *100*, 288–298. https://doi.org/10.1016/j.jinorgbio.2005.11.013

Monette, A.; Mouland, A.J. Zinc and Copper Ions Differentially Regulate Prion-Like Phase Separation Dynamics of Pan-Virus Nucleocapsid Biomolecular Condensates. *Viruses* **2020**, *12*, 1179. https://doi.org/10.3390/v12101179

Pvsn, K. K.; Tomo, S.; Purohit, P.; Sankanagoudar, S.; Charan, J.; Purohit, A.; Nag, V.; Bhatia, P.; Singh, K.; Dutt, N.;*et al.* Comparative Analysis of Serum Zinc, Copper and Magnesium Level and Their Relations in Association with Severity and Mortality in SARS-CoV-2 Patients. *Biol. Trace Elem. Res.* **2023**, *201*, 23–30. https://doi.org/10.1007/s12011-022-03124-7

Roden, C. A.; Dai, Y.; Giannetti, C. A.; Seim, I.; Lee, M.; Sealfon, R.; McLaughlin, G. A.; Boerneke, M. A.; Iserman, C.; Wey, S. A.; *et al.* Double-Stranded RNA Drives SARS-CoV-2 Nucleocapsid Protein to Undergo Phase Separation at Specific Temperatures. *Nucleic Acids Res.* **2022**, *50*, 8168–8192. https://doi.org/10.1093/nar/gkac596

Rusak, G.; Piantanida, I.; Bretschneider, S.; Ludwig-Müller, J. Complex Formation of Quercetin with Lanthanum Enhances Binding to Plant Viral Satellite Double Stranded RNA. *J. Inorg. Biochem.* **2009**, *103*, 1597–1601. https://doi.org/10.1016/j.jinorgbio.2009.08.008

Septembre-Malaterre, A.; Bedoui, Y.; Giry, C.; Gasque, P.; Guiraud, P.; Sélambarom, J. Quercetin Can Reduce Viral RNA Level of O'nyong-Nyong Virus and Resulting Innate Immune Cytokine Responses in Cultured Human Synovial Fibroblasts. *Sci. Rep.* **2021**, *11*, 6369. https:doi.org/10.1038/s41598-021-85840-z

Uversky, V. N.; Redwan, E. M.; Makis, W.; Rubio-Casillas, A. IgG4 Antibodies Induced by Repeated Vaccination May Generate Immune Tolerance to the SARS-CoV-2 Spike Protein. *Vaccines (Basel)* **2023**, *11*. https://doi.org/10.3390/vaccines11050991

Zhang, X.; Zheng, R.; Li, Z.; Ma, J. Liquid-Liquid Phase Separation in Viral Function.*J. Mol. Biol.* **2023**, 167955. https://doi.org/10.1016/j.jmb.2023.167955