

COVID-19 and haemoglobin oxygen affinity: some clarity?

We thank Hurutyunyan *et al.*¹ for their interest in our short communication² and for comprehensively reviewing the importance of haemoglobin (Hb) oxygen affinity. They raise a number of interesting points, some of which require a response.

Firstly, in their review of the literature, they discuss the respiratory and oxygenation problems associated with COVID-19 infection. They suggest, but without any direct proof, that increasing Hb oxygen affinity by allosteric modulators may have therapeutic potential in COVID-19. While this may indeed provide symptomatic relief in terms of increasing SaO₂ and oxygen loading at the lungs, they seem to have overlooked that a non-specific increase in oxygen affinity could limit oxygen unloading and exacerbate hypoxia at the tissues. It is far from certain, and remains to be tested, whether such interventions would therefore be beneficial.

Furthermore, Hurutyunyan *et al.* are of the opinion that patients with COVID-19 pneumonia have unexplained hypoxaemia and therefore the only logical conclusion is that there must be a change in Hb affinity in these patients. While we obviously agree that Hb O₂ affinity is important and must be investigated, it is not the only explanation. Hurutyunyan *et al.* exclude a significant role for reduced gas exchange, despite the fact (as pointed out in our original communication) that the primary pathology involves SARS-CoV-2 entering cells via the ACE2 receptor and, in pulmonary endothelial cells, this results in activation of the renin-angiotensin system. After downregulating ACE2, the production of angiotensin-II is increased and its counter-regulating molecule, Ang-(1-7), is reduced. This results in much-increased vasoconstriction, inflammation and oedema.³ The observation that inhaled nitric oxide reduces the hypoxaemia in patients with COVID-19 suggests that the hypoxaemia is, at least in part, due to inappropriate vasoconstriction.⁴

Hurutyunyan *et al.* raise four specific points in relation to our short study and our conclusions. Taking these each in turn:

Heterotropic allosteric effectors

Hurutyunyan *et al.* suggest that our study fails to account for the possibility that allosteric regulators of Hb O₂ affinity may be somehow differentially altered in COVID-19. We completely agree. Investigating this was never the intention of our study. Our study was inspired by the *in silico* modelling study of Lui and Li,⁵ who suggested virally-derived ORF and surface proteins could directly interact with both the porphyrin and haem moieties of Hb. Our study therefore aimed simply to ask if there is any evidence for such a direct interaction and if this

manifests as a change in the oxygen affinity of Hb. It would be a highly speculative and contorted argument to ascertain, with no evidence, that these virally-derived proteins only interact with Hb in the presence of other allosteric regulators.

We fully appreciate the importance of well-characterised allosteric regulators in both modifying Hb oxygen loading and unloading. However, it is unclear which of these Hurutyunyan and colleagues think may be differentially regulated by COVID-19 in a way that distinguishes this pathology from other respiratory conditions. They mention CO₂ (and, by inference, pH), but it is unclear how they hypothesise this might be differentially altered by COVID-19 in a manner that distinguishes it from other respiratory or hyperventilatory pathologies.

Hill slopes, curve fits and equations

In order to understand the rationale for the choice of curve-fitting equations, and the meaning of the variables estimated, it is important to appreciate what the *Hemox Analyzer* (as used in our studies)² actually measures. Although used extensively in haematology labs around the world, this type of analyser actually does not measure oxygen content (as might be measured by the more laborious but more direct Van Slyke technique). The *Hemox Analyzer* uses a spectrophotometric method to estimate absorbance in the red spectrum – fully oxygenated blood obviously being bright red and deoxygenated blood much darker (but still red). The important point to note here is that fully deoxygenated blood does not return a value of zero (as would be reported by the Van Slyke method) as it remains red, albeit darker. Initially, we attempted to fit our data with the equations described by O’Riordan *et al.*,⁶ as these equations derive real physiologically attributable variables. Unfortunately, these equations deviate very slightly from the measured values, particularly at extremely low PO₂ values. Our numerical data actually showed a much higher degree of correlation when fitted with a sigmoidal function. This is because, as explained above, zero SaO₂ does not equate to zero absorbance using this spectrophotometric method, and hence our curve fits were unconstrained to the ‘Bottom’ value. Having accepted this, it then questions whether absorbance provides a reliable estimate of O₂ affinity. Pulse-oximetry, based on identical spectrophotometric principles as used in our analysis, is the method most commonly used to routinely estimate SaO₂ clinically and it reports accurately the P50 value that remains a standard clinical indicator of Hb oxygen affinity. It is unclear how a direct change in Hb O₂ affinity due to viral protein-Hb interactions would be missed by such a spectrophotometric or curve-fitting analysis.

With regard to the Hill Slopes – we largely agree. However, the spectrophotometric nature of this assay, and the fact that the curve fits to a ‘Bottom’ value that is negative and well below zero % O₂ saturation, mean that the absolute values of the derived variables are not numerically comparable to those measured when O₂-content is derived directly. This results in co-operativity values that are numerically low and should not be compared in absolute terms to those measured using other methods. The slope of these curves, however, is still a measure of co-operativity, which clearly remains unchanged in Hb from COVID-19 patients.

Hemox Analyzer

CO₂ is, of course, close to zero in these analyses; although, as stated in our communication, pH and temperature were controlled to 7.4 and 37°C, respectively. However, even when equilibrated with room air (saturated) or pure N₂ (desaturated), CO₂ is substantially lower than *in vivo*. Hurutyunyan *et al.* are therefore correct to ascertain that these conditions do not replicate those found *in vivo* – either at the lungs or at the tissues. However, this is an accusation that can be levelled at most clinical measures of oxygen affinity. Also, what is true at the lungs will be different at the tissues, so replicating *in vivo* conditions is difficult if not impossible. Non-labile modulators such as 2,3-DPG, however, would be assumed to still be present in our samples and, hence, any COVID-19-specific effects mediated by these modulators should still be apparent.

As described above, it was never our intention to replicate *in vivo* conditions (which remain poorly defined and largely unknown in COVID-19). We simply set out to ask if there is measurable evidence for a change in the basic properties in Hb derived from patients testing positive to COVID-19. Our studies suggest there is not. This does not preclude a COVID-induced difference in labile allosteric modulators *in vivo*, but this would be highly speculative and without any experimental substantiation.

Conclusions

Finally, in their concluding statement, Hurutyunyan *et al.* assert that “a decrease in affinity of haemoglobin to oxygen in patients with severe COVID-19 may be a contributing factor to the pathophysiology of hypoxia in this patient group”.

This may or may not be true, but they have no experimental evidence to back up this statement.

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