Nitric oxide in COVID-19: Too little of a good thing?

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Endogenous nitric oxide (NO) can have beneficial or detrimental effects on vasculature and other systems. For example, NO produced in small amounts by the constitutive synthase, primarily in endothelial cells (eNOS), regulates the basal vasomotor tone and maintains the endothelium in a quiescent state. By contrast, NO produced in excess by the inducible synthase (iNOS) during endothelial infection, damage, and vascular pathology in those who die.

Measuring NO in biological samples is difficult because of the small amounts present and the lability of the gas. NO diffuses from cells into the blood, permeates the erythrocyte membrane, and binds to haemoglobin. The NO levels in the blood can then be estimated from the concentration of the relatively stable reaction products between NO and haemoglobin.

Patients with the novel coronavirus disease (COVID-19) can develop an acute respiratory failure and require hospital admission. Commonly, they present with signs of endothelial and coagulation activation and develop thrombosis. In addition, an autopsy can reveal endothelial infection, damage, and vascular pathology in those who die.

In the March 2022 issue of eBioMedicine, Montiel and colleagues compare the concentration of venous erythrocytic iron-nitrosyl complex (HbNO) between 30 patients with severe COVID-19 and 15 healthy controls matched for cardiovascular risk factors. HbNO was measured as a marker of NO bioavailability with electron paramagnetic spectroscopy. Secondary analyses were performed on 30 patients with less severe COVID-19 and ten with a septic shock of bacterial origin. Patients with severe COVID-19 had less HbNO than controls (116.1 ± 62.1 vs. 163.3 ± 46.7 nmol/L) together with less nitrate/nitrate, end-products of NO metabolism; more lipid peroxides, general markers of oxidative stress; more soluble triggering receptor expressed on myeloid cells-1 and less angiotensin-II, sources of oxidative stress; more soluble von Willebrand factor, soluble intercellular adhesion molecule (sICAM)-1, and endothelin-1, pro-thrombotic and pro-inflammatory molecules of endothelial origin; and more d-dimer, a marker of accelerated clot turnover. In three patients, an autopsy revealed pulmonary endothelial damage. Of note, patients with septic shock had more HbNO than all other groups (451 ± 447 nmol/L). Among patients with COVID-19, HbNO was lower in those with a lower ratio of arterial tension to inspired fraction of oxygen (i.e., with more profound hypoxemia) (coefficient of determination [R2] = 0.13), higher plasma lipid peroxides (R² = 0.07), higher sICAM-1 (R² = 0.21), and higher d-dimer (R²=0.09). The authors conclude that “endothelial oxidative stress with ensuing decreased NO bioavailability appears as a likely pathogenic factor of endothelial dysfunction in ICU COVID-19 patients”.

Montiel and colleagues must be applauded for running such a complex study during the pandemic. The finding of low HbNO and low nitrite/nitrate in the blood is consistent with decreased NO bioavailability. However, some limitations of the work deserve a comment. First, the source of the oxidative stress was not identified. Was it the endothelium? Second, the pathogenic role of a low HbNO was only partly understood. Was it the cause or effect of endothelial dysfunction? Third, the association between HbNO, endothelial dysfunction, and hypoxemia was weak. If all the variables were properly measured, there are three possible explanations for this result: HbNO does not accurately reflect endothelial dysfunction; other biomarkers, such as sICAM-1 and d-dimer, do not accurately reflect endothelial dysfunction; or, more probably, the pathogenesis of COVID-19 is multifactorial. For example, hypoxemia also depends on other variables, most notably pulmonary aeration.

What are the possible clinical implications of the study? First, a low HbNO can explain why patients with COVID-19 do not always present the characteristic physical signs of overt inflammation such as tachycardia, hypotension with hyperlactatemia (i.e., shock), anaemia, hypoalbuminemia, thrombocytopenia, and multi-organ failure. Second, HbNO may be a specific but poorly sensitive marker of endothelial dysfunction. It was abnormally high during septic shock, commonly complicated by endothelial dysfunction. Third, identifying the source of the problem is important as this will affect therapy. Is it an endothelial infection? Or inflammation? Is it a loss of function of eNOS? Or depletion of precursors (i.e., L-arginine) and cofactors (for example, tetrahydrobiopterin)
for the generation of NO? Is it increased NO consumption? This study can be relevant to several aspects of our clinical practice, including the use of oxygen, nutrition, and inhaled NO (iNO). Patients with severe COVID-19 receive highly concentrated oxygen so that inflamed but ventilated alveoli are exposed to hyperoxia, which could worsen the oxidative stress and trigger endothelial dysfunction. Therefore, a more conservative approach may be warranted.\textsuperscript{8} Calorie restriction and dietary supplementation of NO-precursors can induce eNOS and endothelial NO production.\textsuperscript{7,9} Even if iNO at 20–40 ppm and for a few hours does not consistently improve oxygenation in severe COVID-19,\textsuperscript{10} it might exert other beneficial effects when given at higher concentration or for more prolonged periods, even outside of the pulmonary vasculature.\textsuperscript{7}

A valid and readily accessible biomarker will be very useful to assess the impact of different interventions on NO signalling, a key player in the pathogenesis of COVID-19.

Role of the funding source
No funding received.

Contributors
MF and AP conducted the literature search and wrote the original draft together. Both authors read and approved the final version.

Declaration of interests
The authors have no conflict of interests to declare.

References
1. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med. 1993;329:2002–2012.
2. Farah C, Michel LYM, Balligand JL. Nitric oxide signalling in cardiovascular health and disease. Nat Rev Cardiol. 2018;15:292–316.
3. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19. N Engl J Med. 2020;383:1120–128.
4. Montiel V, Lobyshcheva I, Gérard I, et al. Oxidative stress-induced endothelial dysfunction and decreased vascular nitric oxide in COVID-19 patients. eBioMedicine. 2022. https://doi.org/10.1016/j.ebiom.2022.103893.
5. Protti A, Santini A, Pennati F, et al. Lung response to a higher positive end-expiratory pressure in mechanically ventilated patients with COVID-19. Chest. 2021. S0012-3692(21)04100-3. https://doi.org/10.1016/j.chest.2021.10.012.
6. Protti A, Meessen J, Bottazzi B, et al. Circulating pentraxin 3 in severe COVID-19 or other pulmonary sepsis. Eur J Clin Investig. 2021;51:e13530.
7. Lundberg JO, Gladwin MT, Weitzberg E. Strategies to increase nitric oxide signalling in cardiovascular disease. Nat Rev Drug Discov. 2015;14:623–641.
8. Chu DK, Kim LH, Young PJ, et al. Mortality and morbidity in acutely ill adults treated with liberal versus conservative oxygen therapy (IOTA): a systematic review and meta-analysis. Lancet. 2018;391:1693–1705.
9. Nisoli E, Tonello C, Cardile A, et al. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science. 2005;310:314–317.
10. Ferrari M, Santini A, Protti A, et al. Inhaled nitric oxide in mechanically ventilated patients with COVID-19. J Crit Care. 2020;60:159–160.