Tissue damage from neutrophil-induced oxidative stress in COVID-19

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The high neutrophil to lymphocyte ratio observed in critically ill patients with COVID-19 is associated with excessive levels of reactive oxygen species (ROS), which promote a cascade of biological events that drive pathological host responses. ROS induce tissue damage, thrombosis and red blood cell dysfunction, which contribute to COVID-19 disease severity. We suggest that free radical scavengers could be beneficial for the most vulnerable patients.

COVID-19 is caused by the betacoronavirus SARS-CoV-2 and has several unique features compared with other coronavirus infections. In the most vulnerable individuals (for example, older, obese or diabetic individuals), the virus sometimes triggers a cascade of acute biological events that can, unfortunately, lead to patients being ventilated and even dying. A far from negligible number of patients require intensive care, and although most hospital stays are short in duration, this places a huge strain on health systems. It is therefore urgent to gain an in-depth understanding of the critical activators of disease severity in order to reduce mortality and hospitalization rates.

The high neutrophil to lymphocyte ratio reported in critically ill patients with COVID-19 has been found to predict in-hospital mortality[1]. Lung autopsies of deceased patients have revealed neutrophil infiltration in pulmonary capillaries, their extravasation into the alveolar spaces and neutrophilic mucositis[2]. Increased levels of circulating neutrophil extracellular traps (NETs), which are indicative of neutrophil activation, have also been described in patients[3]. Oxidative stress is the result of an imbalance between oxidant production and antioxidant mechanisms that leads to oxidative damage, including lipid peroxidation and DNA oxidation. In addition to the neutrophil infiltration and release of reactive oxygen species (ROS), viral infections are associated with a decrease in antioxidant defences. Exposure to pro-oxidants usually leads to nuclear translocation of the master redox-sensitive transcription factor NRF2, which activates antioxidant defences; however, respiratory viral infections have been associated with inhibition of NRF2-mediated pathways and NF-κB signalling activation, which can promote inflammation and oxidative damage during these infections[4]. Furthermore, there is evidence of a link between decreased expression of the antioxidant enzyme superoxide dismutase 3 (SOD3) in the lungs of elderly patients with COVID-19 and disease severity[5]. Interestingly, children — whose neutrophils are less reactive and adherent, with no alteration of redox balance — are less prone to developing severe forms of COVID-19. The cascade of events triggered by the oxidative stress state in SARS-CoV-2 infection undoubtedly contributes to the severity of host disease and needs to be further explored.

We postulate that, particularly in vulnerable individuals, neutrophilia generates an excess of ROS that exacerbates the host immunopathological response, resulting in more severe disease (FIG. 1). The deleterious action of ROS on the functions of both pulmonary cells and red blood cells (RBCs) can be seen as a major contributor to the hypoxic respiratory failure observed in the most severe cases of COVID-19. Thus, in addition to its damaging effects on alveolar epithelial and endothelial cells with pro-coagulative endotheliitis[6], an excess of ROS can also affect the RBC membrane and haeme group functionality.

Neutrophils usually initiate aggressive responses upon encountering danger signals, which leads to their rapid migration to the targeted tissue, release of NETs, and their production and release of ROS in an oxidative burst. It had been assumed that neutrophil migration from the vascular lumen into extravascular tissues is unidirectional, but recent studies have demonstrated that neutrophils can migrate back into the bloodstream, in a process referred to as neutrophil reverse transendothelial migration (rTEM)[7]. rTEM neutrophils are relatively rigid cells, and this physical characteristic may delay their passage through the tissue's microvasculature and prolong contact with the sinusoids. They may become mechanically entrapped in the microvasculature of major organs, thus contributing to distant organ damage and multiorgan failure. By producing excessive ROS, deregulated neutrophils can spread a local inflammatory response so that it becomes systemic, which explains why they have been involved

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https://doi.org/10.1038/s41577-020-0407-1

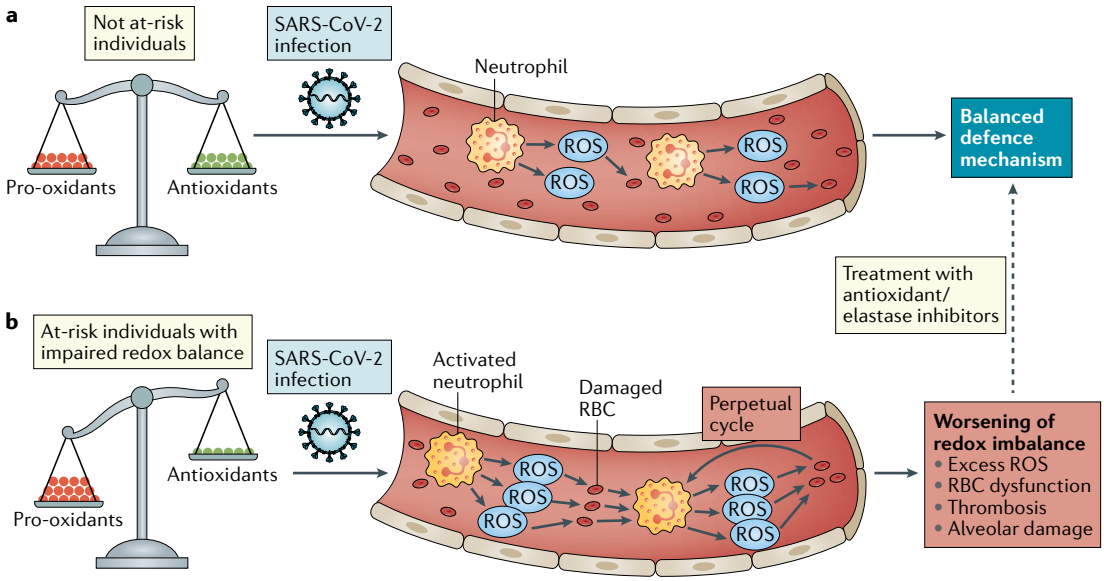

Fig. 1 | **SARS-CoV-2 infection can lead to neutrophilia-induced ROS release. a** | In not at-risk individuals, an excess of reactive oxygen species (ROS) is counterbalanced by an increase in antioxidant defences. **b** | In subjects with impaired redox balance, ROS production is not properly controlled, leading to red blood cell (RBC) membrane peroxidation, which in turn perpetuates neutrophil activation. Excessive oxidative stress might be responsible for the alveolar damage, thrombosis and RBC dysregulation seen in COVID-19. Anti-oxidants and elastase inhibitors may have therapeutic potential.

in whole-body processes such as atherosclerosis and thrombosis[8]. Improper activation of neutrophils is also a potential explanation for the diffuse microvascular thrombosis and capillary leak syndrome observed in critically ill patients with COVID-19 (REF.[9]). In addition, excessive ROS production may affect membrane lipids, integrins and cytoplasmic proteins in various circulating cells. These effects are particularly critical for RBCs, which may become dysfunctional. First, excess ROS can cause oxidation of polyunsaturated fatty acids in the RBC membrane, bringing about a profound modification of the membrane lipids' lateral and transversal distribution and organization at the nanoscale level. This results in biophysical and biomechanical changes in the RBC membrane that affect both the diffusion of oxygen and carbon dioxide and the deformability capability of RBCs in the capillary vessels, thereby favouring thrombocytosis. Reactivation of neutrophils in response to modification of the RBC membrane further fuels this vicious circle. In addition, this modification affects the release of ATP and nitric oxide, both necessary for adequate oxygen transport and vasodilatation between metabolizing tissues and respiratory surfaces. Second, ROS excess may upset the Fe^{2+}/Fe^{3+} balance and disturb iron homeostasis for which iron must be kept in the Fe^{2+} state to bind oxygen. The protonation of superoxide ion associated to Fe^{3+} within the haemoglobin haeme keeps the iron in its higher oxidation state and incapable of binding oxygen, resulting in less efficient oxygen transport despite a high oxygen supply.

In conclusion, the presence of oxidative stress markers (for example, lipid peroxidation, rTEM and a high neutrophil to lymphocyte ratio) in patients with COVID-19 may help to identify high-risk individuals early in the course of the disease and prevent their sudden deterioration. This approach may also pave the way to new therapeutic approaches. We propose that antioxidants such as *N*-acetyl-L-cysteine in combination with elastase inhibitors (for instance, sivelestat)[10] could be used to target rTEM neutrophils in patients with severe COVID-19.

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Acknowledgements
We thank Martin Deotto for his valuable help in drafting the graphic summary and Franck Brouillard and Christophe Bernard for their careful reading of the manuscript.

Competing interests
The authors declare no competing interests.