Highlights of COVID-19 pathogenesis. Insights into Oxidative Damage.

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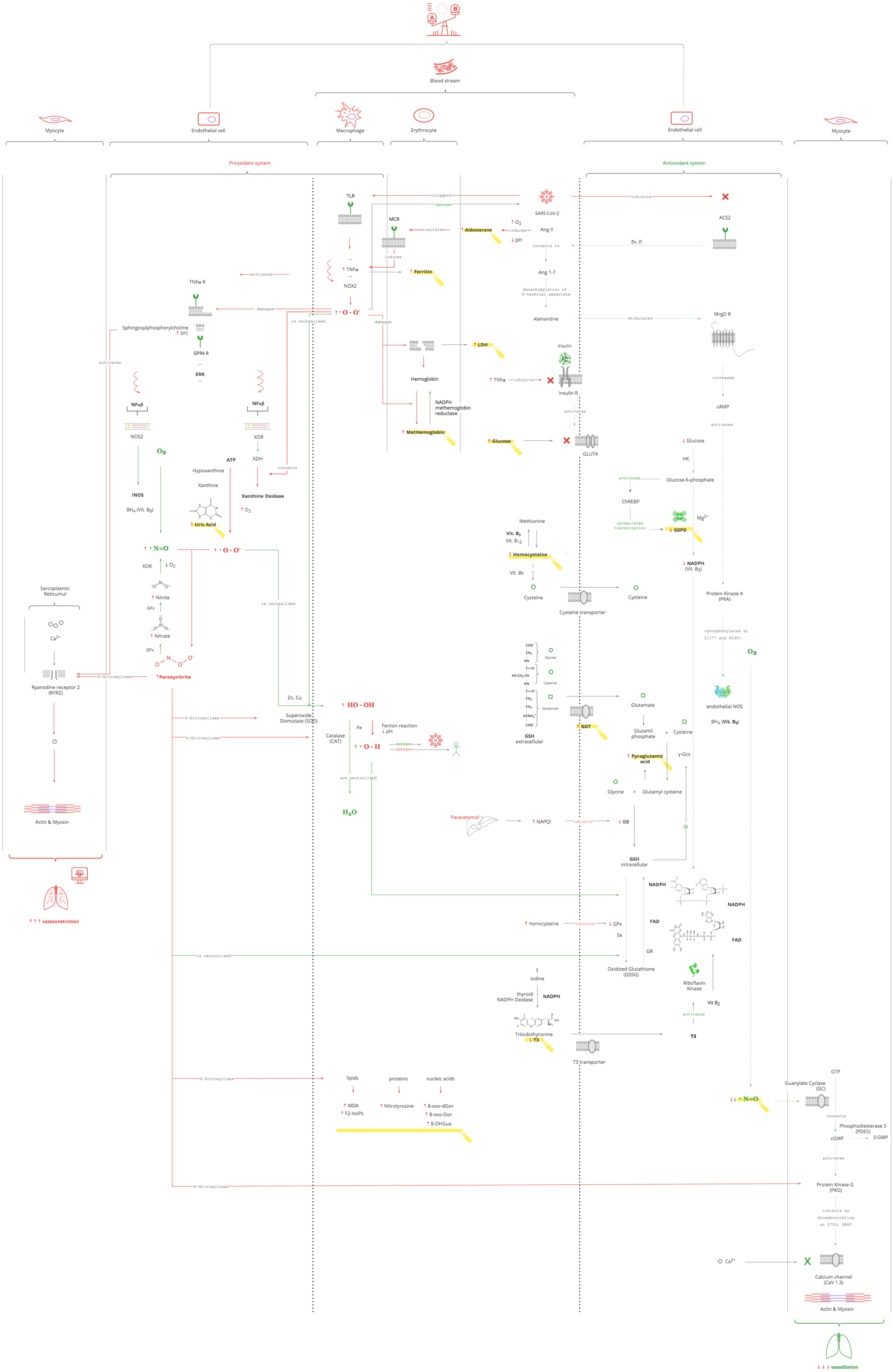
Graphical Abstract



Abstract

OBSERVATION: Clinical manifestations of COVID-19 vary significantly, from asymptomatic to lethal outcomes where mortality rates differ varying on underlying health conditions and ethnicity. Moreover, there is no data-proven treatment for COVID-19. Symptomatic approach does not work appropriately. There are several empiric proposals, yet these must respect "Primum non nocere."
PROBLEM: Based on history alone, it is difficult to determine the vulnerability to SARS-CoV-2 infection at the individual level and to personalize treatment.
The goal of this article is to review biomarkers relevant to COVID-19 pathogenesis that can assess the vulnerability to SARS-CoV-2 infection and help monitor the clinical course to navigate safe treatment in order to follow "Primum non nocere."
We investigated the biochemical rationale behind these observations using machine reasoning by the sci.AI system (https://sci.ai/). Facts were extracted and linked from publications available in nlm.nih.gov and Europe PMC to form the dataset which was validated by medical experts.
RESULTS: This paper presents a detailed description of the impact of SARS-CoV-2 on two-component innate immune response: the pro-inflammatory (prooxidant - PO) and the balancing anti-inflammatory (antioxidant - AO) systems.
1. As with any other pathogen, SARS-CoV-2 triggers a PO response which attacks pathogens with free radicals of the reactive oxygen species (ROS).
2. Importantly, it also has a distinctive feature: it suppresses the AO response. This occurs specifically through the downregulation of nitric oxide (NO) production.
3. The PO/AO system dysbalance caused by SARS-CoV-2 infection results in an excessive inflammatory response which damages the host's cells. This condition is aggravated in patients with underlying health conditions which is accompanied by glucose-6-phosphate dehydrogenase deficiency (G6PDd), where AO mechanisms, NO, and glutathione (GSH), are compromised.
The PO/AO status can be monitored by the biomarkers highlighted in the Graphical Abstract.
CONCLUSION: The Achilles' heel of COVID-19 patients is the AO response which is blocked specifically by SARS-CoV-2. It makes the normal nonspecific PO immune response excessive causing host damage. Inability to counteract oxidative damage due to inadequate AO system functioning renders G6PDd patients vulnerable to SARS-CoV-2 infection.

Keywords: COVID-19 ARDS ROS glucose-6-phosphate dehydrogenase nitric oxide glutathione

Human cells constitute just up to 40-45% of the body's total cell count. The rest is microbiota. The host maintains borders between them and defends itself against exogenous infection through its immune response which have two cooperative phases. The first phase occurs early, is innate, nonspecific, and has two components, PO and AO. The second phase occurs with a delay, is adaptive and specific. It is mediated through antibody expression.

PO/AO Balance

A pro-inflammatory PO system mediates inflammation. The goal of inflammation is to destroy all recognized as non-self agents such as viruses, bacterias, fungi, etc. and unrecognized as own cell such as cancer cells constantly, on a daily basis. It attacks with free radicals of the ROS because all organisms have vulnerable biomacromolecules such as proteins and nucleic acids. Therefore, ROS is a general biocidal product for all organisms including human.
An anti-inflammatory AO system balances the PO response to protect the host during the ROS attack.

Thus, PO and AO work in balance as a whole innate immune response to keep homeostasis intact, otherwise pathology ultimately develops.

PO/AO Dysbalance

PO response deficiency

This condition is caused by a primary inherited immunodeficiency, e.g. chronic granulomatous disease. This is characterized by a deficiency in the NADPH oxidase (NOX2) enzyme complex of macrophages leading to decreased production of ROS and vulnerability to infections as a consequence.

AO response predominance

In health, the molecules of AO system mostly in reduced form that is able to neutralize endogenous and exogenous toxic molecules. In disease, the AO molecules, mostly, are in their oxidized form because of increased demand. To work properly, transformation of molecules from their oxidized to reduced forms requires normal enzyme functioning and their co-factors, and exogenous antioxidants intake on daily basis since the human organism cannot accumulate them. Moreover, oxidized exogenous antioxidants use endogenous AO system for reduction process. Otherwise, they act as PO molecules. Thus, under stress of life, more is better than less.

PO response predominance and AO response deficiency

When there is increased production and/or decreased neutralization, a heightened level of ROS occurs, and excessive levels cause collateral damage to normal cells and is referred to as "oxidative stress," "cytokine storm" and "systemic inflammatory response syndrome" (SIRS). Acute respiratory distress syndrome (ARDS) is one of the clinical manifestations of SIRS and SARS-CoV-2 infection.
We investigated the biochemical rationale behind these observations to make up COVID-19 pathogenesis. Pathways were derived with the help of machine reasoning by sci.AI system (https://sci.ai/).

Domain experts provided starting points (SARS-CoV-2, ARDS, SIRS,..) to trigger chain reaction-like facts extraction and reasoning by machine operating. It is achieved by discovering and grouping complementary facts from publications available in Pubmed and Europe PMC. Extracted facts and presynthesized steps were validated by experts to form (1) an evidence-supporting dataset (https://doi.org/10.6084/m9.figshare.12121389), (2) normal (see Appendix), (3) pathological pathways, and (4) a list of relevant biomarkers.
Detailed sci.AI methodology is at: https://doi.org/10.6084/m9.figshare.12198021

In addition to the pathway in the Graphical Abstract, here we elaborate further on the normal processes affected by SARS-CoV-2 and how compromised innate immune response contributes, accidentally, to complications in patients with underlying health conditions.

SARS-CoV-2 interacts with PO system

SARS-CoV-2, as does any pathogen, triggers macrophages, the first-line cell of the PO system, through toll-like receptor (TLR) activation. This results in tumor necrosis factor alpha (TNFα)-induced activation of NOX2. Activated NOX2 produces ROS, particularly superoxide anion (O2*-) from oxygen (O2). Then a hydroxyl radical (OH*) is produced through the Fenton reaction. The OH* is able to cleave surrounding biomacromolecules of virus and, in excessive levels, a host. Ferritin production is induced by TNFα and can be used to monitor the degree of macrophage' involvement in the PO response.
Thus, SARS-CoV-2 interacts with the normal PO system and normal ROS level is a first-line antimicrobial defense.

PO response predominance

Individuals probably contract COVID-19 at similar rates. However, once infected, some persons do worse than others. There are a lot of potential reasons for this. For example, the inoculum of infection is an important variable and can make PO response excessive but will not be further discussed here. Here we suggest that the excessive PO response makes difference between COVID-19 and other respiratory infections regardless of viral load. The specific impact of SARS-CoV-2 on the renin-angiotensin-aldosterone system (RAAS) compromises one of the component of AO system, NO, and, in turn, increases aldosterone (Ald) level making PO response excessive.

SARS-CoV-2 interferes with AO system through RAAS

The AO system balances the PO response. There are many mechanisms but here we will focus on two of them: suppression of NOX2-induced ROS production by NO and ROS neutralization by GSH. SARS-CoV-2 inhibits NO production and induces excessive ROS production through Ald increase.

SARS-CoV-2 inhibits NO production SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor in order to enter a cell and in turn destroys this receptor. The ACE2 receptor is involved in the protective ACE2/eNOS/NO pathway of the RAAS. It leads to the

suppression of the most prevalent isoform of nitric oxide synthase (NOS): endothelial NOS (eNOS) and consequently decreases NO levels. NO is essential for suppression of NOX2-induced ROS production, vasodilation, prevention of platelet aggregation and inhibition of the replication of SARS-CoV. For more details, see Appendix below.

Additionally, suppression of ACE2 activity also leads to an inability to convert angiotensin II (Ang II) to angiotensin 1-7 (Ang 1-7). Ang II is a potent vasoconstrictor and also stimulates Ald. This results in a transient increase in Ald that induces TNFα through mineralocorticoid receptor (MCR) on macrophages. NOX2 hyperactivation by TNFα, which is induced by the virus and Ald, and its disinhibition by virus-induced NO inhibition, perpetuate ROS production, making it excessive.

Thus, SARS-CoV-2 interferes with the RAAS system compromising the AO system and increase of Ald production rendering the PO response excessive.

Host damage

Blood cell damage. An antimicrobial response stresses the host simultaneously, especially platelets, lymphocytes and erythrocytes. Damage of erythrocyte membranes results in latent hemolysis leaking lactate dehydrogenase (LDH) and hemoglobin (Hb) is oxidized to methemoglobin (MetHb).

Endotheliocyte damage. Released macrophage' TNFα induces NFkb to start transcription of xanthine oxidoreductase (XOR) complex and inducible NOS (iNOS). Macrophage' ROS damages the host's endothelial cell membranes and converts xanthine dehydrogenase (XDH) of XOR complex to xanthine oxidase (XO) via proteolysis. XO catalyses the oxidation of hypoxanthine from ATP to xanthine and then to uric acid (UA). Moreover, XO has higher reactivity for molecular O2 in comparison to XDH and additionally produces the O2*-. Under inflammatory conditions, iNOS becomes the primary source of NO. But XO-derived O2*- effectively reduce NO bioavailability by its diffusion-limited reaction to form NO* radicals (RNS) such as peroxynitrite (O=NOO). UA can react with O=NOO losing its antioxidant properties. Moreover, inactivation of AO proteins occurs through reaction of S-nitrosylation. For more details, see Appendix below.

Myocyte damage. RNS through reaction of S-nitrosylation activate Ryanodine receptors (RyR) to open Ca2+ channels in myocytes resulting in vasoconstriction. Because of its unique pathogenesis, it occurs early during COVID-19. Accordingly, the vasoconstriction is the main component of COVID-19-associated ARDS. This can explain the phenomenon we observe regarding the fail of mechanical ventilation in such patients.

COVID-19-induced immune dysregulation becomes clinically significant in patients with compromised AO response.

AO response deficiency

As we mentioned above, the COVID-19-induced excessive PO response needs to be balanced by AO mechanisms: NO and GSH. These two mechanisms are dependent on the co-factor, NADPH. It is produced uniquely by G6PD, a rate-limiting enzyme in the pentose phosphate pathway (PPP) of glucose metabolism. While congenital G6PDd is well known, its acquired deficiency is less appreciated. It accompanies diabetes mellitus and hypertension grouped together as the metabolic syndrome. In addition, advancing age also lowers it. We will focus on the role of underlying health conditions and we will relate this to the PO/AO systems which we discussed above.

The role of underlying health conditions

It is worth to note that condition of patients with subclinical underlying health conditions, e.g. prediabetes, can be accelerated and aggravated by the stress of any infection. But due to specific impact of COVID-19 it becomes life-threatening. We demonstrate the biochemical rationale of these findings and why these cohorts do worse with COVID-19.

In contrast to COVID-19 that causes acute TNFα increase, most underlying health conditions are accompanied by chronic TNFα-induced insulin resistance. And G6PDd develops as a consequence of it. This is because G6PD activity requires normal insulin receptor signaling for glucose entrance into cells. Under normal conditions, glucose is phosphorylated to glucose-6-phosphate which activates carbohydrate response element-binding protein (ChREBP). ChREBP regulates the expression of rate-limiting enzymes in glucose metabolism, particularly G6PD. Thus, with decreased intracellular glucose G6PD gene expression is less triggered decreasing NADPH production.

Thus, the innate immune response to underlying health conditions is mediated through chronic TNFα stimulation which causes G6PDd. In addition to chronic NO underproduction which was abolished by SARS-CoV-2, compromised GSH mechanism in G6PDd cohort aggravates COVID-19 course.

The GSH system. GSH is an essential endogenous antioxidant. As depicted in Fig. 5, it is composed of 3 amino acids: glycine, cysteine, and glutamate. The sulfhydryl (-SH) moiety of cysteine is responsible for the neutralization of toxic substances, both endogenous such as ROS and exogenous such as xenobiotics. During this reaction, GSH is oxidized to its inactive form, GSSG. The recycling requires NADPH and FAD.
GSH depletion can be caused by G6PDd, which leads to an inability to recycle it. It can also be caused by excessive levels of toxic substances which overload the capacity for its neutralization. Furthermore, the GSH system can be compromised by exogenous substances e.g. the paracetamol metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which inactivates glutathione synthetase (GS) of GSH production, and by endogenous substances e.g. homocysteine, which inactivates glutathione peroxidase (GPx) of GSH functioning. The body responds with γ-glutamyl transferase (GGT) up-regulation to replete intracellular amino acids from extracellular GSH; and also by de novo production of cysteine from methionine. These amino acids then enter the γ-glutamyl cycle. When there is abundant GSH, it suppresses its own production by blocking γ-glutamyl cysteine synthase (γ-Gcs). Otherwise, GSH depletion results in increased γ-Gcs leading to accumulation of

pyroglutamic acid.

In addition to NO and GSH, there are several other systems that require NADPH and compete for it: macrophage NADPH oxidase (NOX2) for antimicrobial defense; NADPH methemoglobin reductase for Hb recovery; and thyroid NADPH oxidase for triiodothyronine (T3) production. The inability of G6PD to supply enough NADPH for the excessive immune response, along with these other demands, aggravates the condition.
Thus, G6PD is essential for both components of innate immune response and, particularly, for the AO system to balance the PO system.

NO and GSH are also dependent on FAD. FAD production is catalyzed by T3 which requires NADPH for its synthesis by thyroid NADPH oxidase.
Thus, G6PDd ultimately decreases T3, NO, and GSH, thereby compromising the body's compensatory mechanisms.

Conclusion

Here we propose our understanding of the first principles of COVID-19 pathophysiology to recognize the degree of vulnerability before and manage it appropriately after.

1. SARS-CoV-2 triggers normal PO system of innate immune response and abnormally interferes with the RAAS inhibiting the AO component, NO, and increasing aldosterone level uniquely. It makes PO response excessive;
2. Patients with underlying health conditions can develop G6PDd making them vulnerable to SARS-CoV-2 infection. This is because its deficiency can adversely affects these patient's clinical course. Therefore, it is important to recognize a body's compensatory status before start of treatment.
3. Biomarkers can be used to assess the status of ongoing condition and dynamic of its deviation from normal during treatment, especially, in critical patients. All proposed biomarkers are prognostic and predictive of response to therapy. They can be used to monitor the body's response to COVID-19-induced inflammation and to therapeutic intervention, respectively.

In order to assess the body's inflammation status:

- ferritin as a biomarker of macrophages involvement in innate immune defense
- lymphocytopenia, thrombocytopenia and erythrocytopenia are biomarkers of oxidative stress
- lactate dehydrogenase (LDH) level as a biomarker of erythrocyte damage by ROS and hemolysis
- uric acid as a biomarker of involvement of endothelial cells into inflammation
- aldosterone is a specific biomarker of COVID-19

In order to assess the status of it's AO system:

- G6PD activity directly

- T3 level as an indirect biomarker of G6PD activity
- pyroglutamic acid and GGT levels as biomarkers of GSH depletion which indicate impending oxidative catastrophe in severe COVID-19 patients
- uric acid as a biomarker of involvement of endothelial cells into inflammation
- homocysteine level negatively correlates with serum Vit.B9 and can be used as a biomarker of folic acid deficiency and potential NO depletion

Thinking pathophysiologically, along with data, will ultimately lead to safe and effective treatment.

Declarations:
Availability of data and materials
Research data is available at https://doi.org/10.6084/m9.figshare.12121389
All canonical pathways were validated in Reactome and Kegg databases.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
All authors contributed to the work equally. All authors read and approved the final manuscript.

Appendix

To show the difference between normal and pathological processes, here we provide some details from physiological pathways.

One of the main function of endothelial cells (EC) is to produce NO and its adequate level should be maintained regardless of pathological factors.
The main source of NO is the nitric oxide synthase (NOS) family of proteins which requires the presence of O2. The involvement of a particular isoform depends on location and biological processes. Normally, endothelial NOS (eNOS) is present in EC and is the main source of NO. Abnormally, inflammatory cytokine NFkb transcription factor induces inducible NOS (iNOS) expression. Therefore, under oxygenated conditions NO production is NOS-dependent. And under hypoxic conditions, there is another mechanism, XOR-dependent.

EC contains XOR that is responsible for the final step in purine degradation resulting in UA production which, at physiological concentrations, is a powerful ROS scavenger that protects the erythrocyte membrane from lipid peroxidation. XOR is a complex protein with two distinct enzymatic forms. It acts as an oxidase for purine analogs and as a reductase for certain drugs and nitrites similarly to the bacterial nitrite reductase. Which form, oxidative or reductive, depends on several conditions, the most important being the level of O2 in serum.

Oxygenated condition.
Under the normal, non-inflammatory, condition XOR acts as an oxidase in the form of XDH. It

catalyzes the oxidation of hypoxanthine to UA without any toxic byproducts. And eNOS is a primary source of NO.
In inflammation, XOR acts as an oxidase in the form of XO with O2*- production. And iNOS is a primary source of NO.

Hypoxic condition. In certain cells with reduced O2 consumption such as adipocytes or during transient hypoxia in non-inflammatory condition, eNOS-dependent NO production is diminished due to the absence of O2. Reductase activity of XOR catalyzes the reduction reaction of nitrites to NO. Food is a source of nitrites.
In inflammation, iNOS-mediated NO production is diminished due to the absence of O2. In the presence of elevated nitrite levels derived from RNS neutralization by the GSH system or from food, XOR becomes the main source of NO production.

Since XOR is a "paradox molecule" with opposing roles, the goal is to guide the therapy toward stimulation of reductase activity and inhibition of XO-induced oxidase activity, especially under inflammatory conditions.