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

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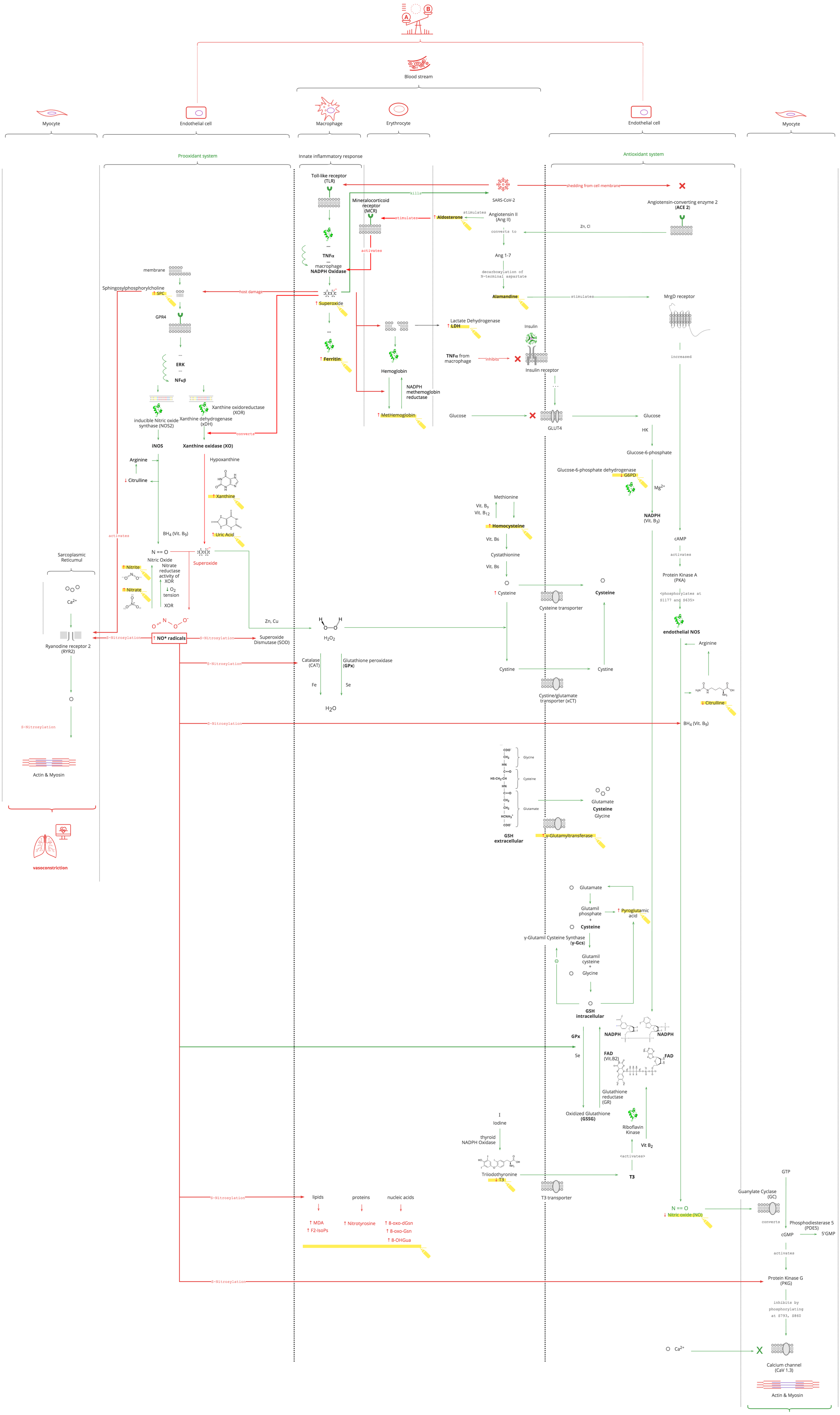
Graphical Abstract



Abstract

At the time of this article, there is no data-proven treatment for COVID-19. There are several empiric proposals, yet these must respect "Primum non nocere." Thinking pathophysiologically, along with data, will ultimately lead to safe and effective treatment.

The goal of this article is to review the relevant biomarkers as diagnostic armamentarium that can assess the body's compensatory response to SARS-CoV-2 infection, monitor their clinical course and help navigate safe treatment.

OBSERVATION: Clinical manifestations of COVID-19 vary significantly, from asymptomatic to lethal outcomes. Moreover, mortality rates differ varying on age, underlying health conditions (e.g. obesity, diabetes, hypertension) and ethnicity.

PROBLEM: Since, in modern world more than half of all noninfectious diseases are diseases of accumulation i.e. obesity-related, it is difficult to determine whether a person is vulnerable to SARS-CoV-2 infection based on history alone.

QUESTION: How does SARS-CoV-2 interact and interfere with normal biologic processes? What are the biomarkers which measure these and the body's compensatory response?

METHODS: Domain experts provided starting points (SARS-CoV-2, ARDS, age, ...) to trigger chain reaction-like facts extraction and reasoning by machine operating on publications available in Pubmed and PMC (https://sci.ai/). Extracted facts and presynthesized steps were validated by experts to form (1) an evidence-supporting dataset, (2) normal, (3) pathological pathways, and (4) a list of relevant biomarkers.

RESULTS: 1. This paper presents a detailed description of the two-component innate immune response: the pro-inflammatory (prooxidant - PO) and the anti-inflammatory (antioxidant - AO) systems. The PO system attacks pathogens and unfortunately also damages host's cells causing oxidative stress. The AO system alleviates oxidative stress and balances a normal immune response. The balance can be monitored by the biomarkers highlighted in the graphical abstract.

2. As with any other pathogen, SARS-CoV-2 triggers an inflammatory PO response. Importantly, it also has a distinctive feature: it suppresses the AO response. This occurs specifically through the down regulation of the ACE2 pathway. This pathway is a protective mechanism of the renin-angiotensin-aldosterone system (RAAS).

3. Patients with genetic or acquired glucose-6-phosphate dehydrogenase (G6PD) deficiency already have PO/AO dysbalance and this is aggravated by COVID-19.

CONCLUSION: Nonspecific PO immune response to any pathogen is a normal biological process. The Achilles' heel of COVID-19 patients is the AO response which is blocked specifically by SARS-CoV-2. G6PD deficient patients have background latent inflammation that depletes their AO system and renders them vulnerable to SARS-CoV-2 infection.

Keywords: COVID-19, ARDS, ACE2, nitric oxide, ROS, G6PD, glutathione, pyroglutamic acidosis, alamandine, MrgD receptor, aldosterone

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

PO/AO Balance

Human cells constitute just up to 40-45 % of the body's total cell count. The rest is microbiota. The host maintains borders between sterile sites and microbiota by using the biocidal properties of inflammation, an innate immune response which has developed through evolution. The goal of inflammation is to destroy all non-self proteins: viruses, bacterias, fungi, protozoas, helminthics, lice, scabies, cancer cells, unrecognized own cell with advanced glycation end-products (AGEs) constantly, on a daily basis. It utilizes Reactive Oxygen Species (ROS). All organisms have DNA (some viruses have RNA that is similar to DNA) and ROS, especially an hydroxyl radical: this mediates DNA cleavage by initial abstraction of hydrogen atom from C'5 and C'4 of DNA backbone. Therefore, ROS is a general biocidal product for everything that contains DNA or RNA.
This property of ROS is used in chemotherapy against cancer cells. However, for normal cells it acts as mutagen simultaneously. Radiation causes abnormally high ROS production which overwhelms the antioxidant (AO) system causing an independent disease known as the Acute Radiation Syndrome (ARS).

The AO system balances the Prooxidant (PO) system to protect the host during the ROS attack. Thus, PO and AO work in balance as one system. Homeostasis is maintained by PO/AO balance. Although, these systems work in balance, they use different proteins.

Where the PO/AO systems are not in balance pathology ultimately develops.

PO/AO Dysbalance

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 infection.

Biomarkers can be used to assess status of the underlying health condition, monitor the PO/AO dysbalance and its degree of deviation from normal.

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 enzyme complex of macrophages leading to decreased production of ROS.

AO response predominance

In health, the molecules of AO system should be in reduced form to neutralize the ROS. In disease, the AO molecules are in their oxidized form. Transformation of molecules from their oxidized to reduced forms requires body's normal AO system performance and exogenous antioxidants on daily basis since the human organism cannot accumulate them.

PO response predominance

The human organism's PO system addresses insults (infection and non-infection diseases) with inflammation. This is mediated by ROS. The more insult, the more ROS.

SARS-CoV-2, as any infection, triggers a nonspecific PO response from macrophages through toll-like receptor (TLR) activation. This results in tumor necrosis factor alpha (TNF alpha) activation of NADPH oxidase in macrophages. NADPH oxidase mediates ROS production. Macrophages produce ferritin to protect themselves from ROS. The resulting ROS targets the virus and destroys it.
In addition, ROS also oxidizes hemoglobin to methemoglobin and it induces latent chronic hemolysis. This is why the erythrocytes leak lactate dehydrogenase (LDH).
Furthermore, ROS damages the host's endothelial cell membranes and, under ischemic or inflammatory conditions, xanthine dehydrogenase (XDH) of xanthine oxidoreductase (XOR) complex is converted to xanthine oxidase (XO) via the oxidation of sulfhydryl residues or proteolysis of XDH. In the presence of oxygen, XO catalyses the oxidation of hypoxanthine to xanthine and then to uric acid (UA), with consequent production of the superoxide anion (O2) and hydrogen peroxide (H2O2). The consequence of this is the production of NO* radicals that through reaction of S-nitrosylation inactivate proteins of AO system and activate Ca2+ channels in myocytes leading to vasoconstriction.

In summary, patients with underlying health conditions and their accompanying inflammation, the consequence of which is increased ROS that manifests clinically as increased blood pressure.

SARS-CoV-2 potentiates this already pathological PO pathway. The body responds with compensatory mechanisms, e.g. tachycardia and tachypnea responding to vasoconstriction in pulmonary vessels. This is commonly referred to clinically as the systemic inflammatory response syndrome (SIRS). Patients with underlying health conditions are predisposed to exaggerated SIRS. Accordingly, it is important to optimally control chronic diseases.

Biomarkers such as ferritin, LDH and uric acid are all prognostic and predictive of response to therapy. They can be used to monitor the body's compensatory homeostatic response to inflammation and therapeutic intervention, respectively. The goal of therapy is to maintain these parameters as normal as possible and certainly not to give any treatment that worsens them first of all. Primum non nocere.

Treatment that potentiate oxidative stress.

When a patient requires respiratory support, oxygen therapy is necessary. However, since an inflamed person has the potential to convert excessive oxygen to ROS, we should give only enough oxygen to maintain the blood oxygen levels at their lowest normal boundary. SpO2 = 96% is an

acceptable value. In addition, low blood oxygen level significantly inhibits aldosterone secretion and stimulates nitrite reductase activity of XOR to convert NO* radicals back to nitric oxide (NO). It is worth noting that aggressiveness of invasive mechanical ventilation is equal to a surgical procedure with all potential general postoperative complications.

A multitude of antimicrobial drugs as diverse as the anti-malarial chloroquine and anti-parasitic ivermectin induce ROS production in vitro and in vivo, leading to their therapeutic potential against coronaviruses. Furthermore, common antibiotics such as amoxicillin and azithromycin and several non-antimicrobial drugs, such as NSAIDs, also increase ROS production.

However, as discussed previously, ROS is harmful to the human organism. Therefore, it is critically important to study the clinical efficacy, or lack thereof, of these drugs.

In summary, we should minimize all interventions that contribute to oxidative stress because they can make predictive biomarkers mentioned before worse. Primum non nocere.

We must strive to keep PO/AO system balanced.

AO response deficiency

The AO system at least is responsible for:

1. Oxidative stress alleviation,
2. ROS neutralization.

1. Oxidative stress alleviation

The angiotensin-converting enzyme 2 (ACE2)/endothelial nitric oxide synthase (eNOS)/nitric oxide (NO) pathway is one of the protective components of blood vessels. The NO mediates vasodilation. Effective pulmonary gas exchange requires alignment between alveoli and relaxed pulmonary capillaries.

The novelty of SARS-CoV-2 is that it binds to ACE2, interacts with it to enter the cell, and destroys it. This carries particular relevance. Its consequence is decreased NO production. Moreover, decreased ACE2 activity leads to inability to convert angiotensin II to angiotensin 1-7. This pathway causes angiotensin II accumulation that cause vasoconstriction by itself as a short term support of blood pressure and stimulates aldosterone secretion. Physiologically, aldosterone causes sodium and water absorption and potassium and hydrogen excretion as a long term support of blood pressure. Interestingly, in healthy patients without underlying health conditions there is a phenomenon of “aldosterone escape” before edema formation even in patients with primary hyperaldosteronism. It is explained by the fact that patients with primary hyperaldosteronism have higher plasma levels of atrial natriuretic peptide (ANP) which counteract the action of aldosterone.

On the contrary, underlying health conditions such as obesity and metabolic syndrome are accompanied with renin and K+-independent hyperaldosteronism and reduced ANP. That is why, this subpopulation have increased aldosterone that maintains edema and hypertension caused by inflammation.

In summary, patients with underlying health conditions clinically have hypertension and predisposition to edema development.

Moreover, aldosterone induces NADPH oxidase of macrophages through mineralocorticoid receptor (MCR) activation. NADPH oxidase is responsible for ROS production. Therefore, the SARS-CoV-2 triggers PO system unspecifically as any other virus and specifically blocks AO system potentiating the ROS production by PO system. And there are evidences that ROS play a key role in the initiation phase of ARDS.
Clinically, it was confirmed that increased aldosterone level was independently associated with increased overall mortality in patients with ARDS.
Therefore, the SARS-CoV-2 blockage of the ACE2/eNOS/NO pathway leads to the edema formation and the inability of blood vessels to dilate. This vasoconstriction and edema causes increased pulmonary arterial pressure and make gas exchange worse. According to the Euler-Liljestrand mechanism, hypoxia aggravates the condition. Because of its unique pathogenesis, it occurs early during COVID-19, even before the immune system is triggered.

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.

2. ROS neutralization

Glutathione in its reduced form (GSH) and glutathione peroxidase (GPx) are the most essential antioxidants. Both are intracellular and extracellular. They neutralize ROS and convert them to non-toxic products, namely H20.
GSH is made up of 3 amino acids: glycine, cysteine and glutamate. The sulfhydryl (-SH) moiety of cysteine neutralizes ROS. During this reaction, GSH is oxidized by GPx and converted to oxidized glutathione (GSSG). GSSG is unable to neutralize ROS. It is useless. Yet, by reduction reaction catalyzed by glutathione reductase (GR) it can be converted to GSH that is able to neutralize ROS again. In addition, there are two other pathways to regenerate GSH: the gamma-glutamyl cycle and restoration from extracellular GSH by gamma-glutamyl transferase (GGT).

In brief, NO and GSH are antioxidants. Both require (A) NADPH and (B) FAD co-factors.

(A) GSH and NO production require NADPH

NADPH is a derivative of niacinamide or vitamin B3 produced via pentose phosphate pathway (PPP). It is catalyzed by glucose-6-phosphate dehydrogenase (G6PD), the rate limiting enzyme.

Glucose-6-phosphate dehydrogenase deficiency

G6PD deficiency can be inborn by genetic mutation or acquired in patients with underlying health conditions. As discussed previously, the human organism's PO system addresses insults (infection and non-infection diseases) with inflammation. Acquired G6PD deficiency develops when macrophage-derived TNF alpha inhibits insulin receptor signaling. The consequence of this is insulin resistance. G6PD takes part in the PPP of glucose metabolism. This requires normal insulin receptor signaling for glucose entrance into cells. Without glucose, G6PD gene expression

is not triggered.
In brief, inflammation-induced TNF alpha causes decreased G6PD expression.

In summary, comparing the normal to abnormal processes:

The normal process

Insulin allows glucose to enter cells.
Glucose undergoes intracellular metabolism.
Intracellular glucose metabolism stimulates G6PD expression.

The abnormal process: G6PD deficiency

Underlying health conditions cause inflammation.
Inflammation causes insulin resistance.
Insulin resistance decreases intracellular glucose.
Decreased intracellular glucose decreases G6PD expression.

Therefore, patients with underlying health conditions develop G6PD deficiency.

In addition, there are several systems which require NADPH as a co-factor and compete with each other for it:
macrophage NADPH oxidase which is intended for defense against viruses in the PO system;
NADPH methemoglobin reductase which is intended for hemoglobin recovery;
thyroid NADPH oxidase which is intended for T3 production;
NADPH glutathione reductase which is intended for GSH reduction;
NADPH nitric oxide synthase which is intended for NO production in the AO system.

Thus, the inability of G6PD to supply enough NADPH for the PO/AO overload in inflammation, along with these other demands, aggravates acquired G6PD deficiency.

(B) GSH and NO production require FAD

NADPH is a co-factor for NADPH oxidase that catalyzes T3 production in the thyroid gland. G6PD deficiency therefore causes T3 deficiency. This clinically manifests as relative transient hypothyroidism also known as low T3 syndrome or euthyroid sick syndrome.
Moreover, T3 activates riboflavin kinase to produce FAD, a vitamin B2 derivative. FAD is a co-factor for the GR in GSSH reduction to GSH. It is also a co-factor for eNOS in NO production.

Therefore, T3 deficiency is an indirect biomarker of G6PD deficiency.

Since both NO and GSH require NADPH and FAD to function, G6PD deficiency ultimately decreases (a) GSH and (b) NO levels.

(a) G6PD deficiency ultimately decreases GSH.

As a consequence of intracellular GSH depletion, GGT activity is up-regulated in order to restore it from extracellular GSH.
When there is abundant GSH, it suppresses its own production by blocking -glutamyl cysteine synthase (-Gcs) in the gamma-glutamyl cycle. Otherwise, GSH depletion results in increased -Gcs and accumulation of pyroglutamic acid, an intermediate metabolite of the gamma-glutamyl cycle.

It is worth noting that GSH depletion can also occur when paracetamol is used as an antipyretic to avoid NSAIDs. This occurs by direct binding of the highly reactive metabolite of paracetamol, N-acetyl-p-benzoquinoneimine (NAPQI) to GSH.

Thus, GSH depletion in critically ill patients with G6PD deficiency, or paracetamol use, clinically manifests as unexplained anion gap metabolic acidosis. This should be considered as pyroglutamic acidosis until proven otherwise. Acidemia by itself is not so important, but is a sign of serious metabolic stress.

In brief, in critical patients, GSH depletion causes GGT up-regulation and pyroglutamic acidosis.

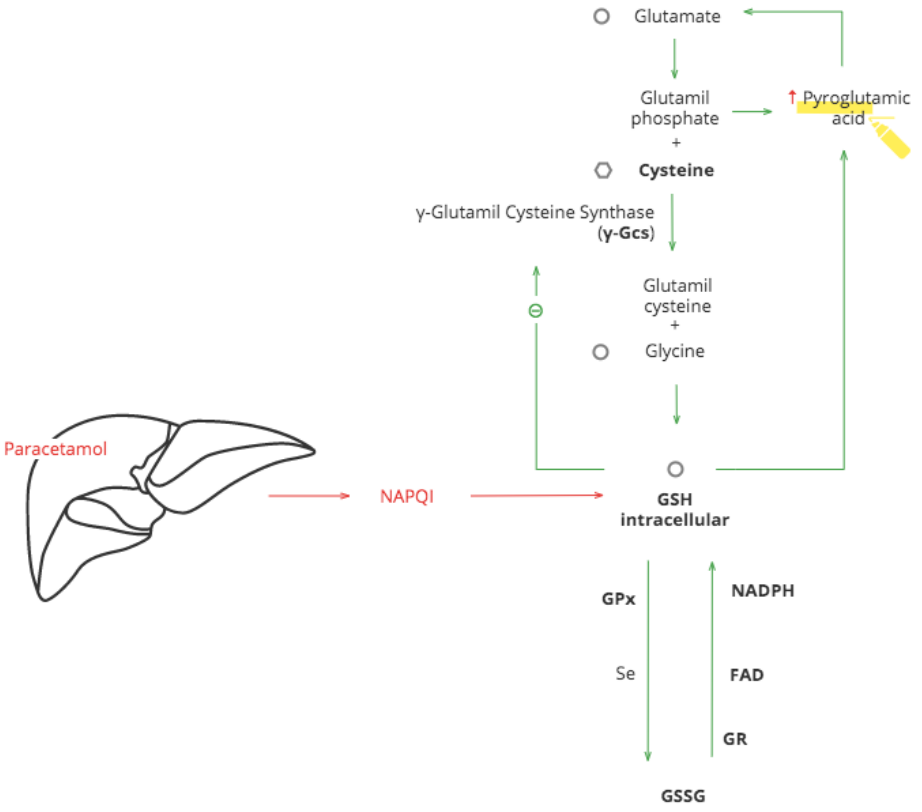

Fig.1 GSH depletion caused by excessive use of paracetamol

G6PD deficiency causes hemolysis through severe GSH deficiency.

Severe G6PD deficiency in patients with underlying health conditions, especially when critically ill, or patients with the genetic G6PD mutation and accompanying ROS overproduction, can cause hemolysis. This occurs in particular after treatment that potentiates oxidative stress. If a patient's hemoglobin decreases after 2-3 days on certain medications, e.g. chloroquine or excessive oxygen therapy, and the LDH level has increased, G6PD deficiency should be strongly suspected and the causative agent should be stopped.

Therefore, it is important to establish a patient's G6PD status before any treatment.
In summary, comparing the normal to abnormal processes:

The normal process

G6PD produces NADPH.
NADPH is required for production of T3.
T3 is required for production of FAD.
FAD is required for regeneration of GSH.
GSH neutralizes ROS.
Neutralized ROS does not cause hemolysis.

The abnormal process: G6PD deficiency

G6PD deficiency produces NADPH deficiency.
NADPH deficiency leads to T3 deficiency.
T3 deficiency produces FAD deficiency.
FAD deficiency produces GSH deficiency.
GSH deficiency does not sufficiently neutralize ROS.
Increased ROS causes hemolysis.

(b) G6PD deficiency ultimately decreases NO.

When GSH is depleted, the body responds to replete it through gamma-glutamyl cycle. This occurs by the signaling of the production of cysteine from methionine through homocysteine. This process requires a derivative of folic acid (Vit.B9) 5'methyltetrahydrofolic acid (5-MTHF).
Folic acid is also necessary for NOS enzymes. In particular, induced NOS (iNOS) uses the co-factor tetrahydrobiopterin (BH4), a vit.B9 derivative. This occurs in the PO system to synthesize NO. In inflammation, a superoxide radical (O2*) molecule is produced by xanthine oxidase (XO). It reacts with NO to form a NO* radical. This occurs in endothelial cells. The goal of NO* radical production is to kill pathogens. The NO produced by endothelial NOS (eNOS) is also used for vasodilation. This is a component of the AO system and also uses BH4. As was previously mentioned, vasodilation is a protective mechanism of vessels that facilitates gas exchange and balances ROS-induced vasoconstriction.

In the acute phase of COVID-19, SARS-CoV-2 suppresses the ACE2/eNOS/NO pathway. In addition, when inlammation-induced PO/AO overload occurs, homocysteine and iNOS require Vit.B9 to function and this depletes it. In the convalescent phase, eNOS competes for Vit.B9

to produce NO which is responsible for recovery. Therefore, recovery is compromised by Vit.B9 deficiency. It must be supplemented.

Biomarkers such as G6PD activity, T3, pyroglutamic acid, GGT and homocysteine levels are all prognostic and predictive of response to therapy. They can be used to monitor the body's ability for compensatory homeostatic response to inflammation and therapeutic interventions, respectively. The goal of therapy is to maintain these parameters as normal as possible and certainly not to give any treatment that worsens them first of all. Primum non nocere.

The G6PD level carries a particular relevance in critically ill patients. Its deficiency adversely affects the patient's clinical course. Therefore, we should minimize stressful interventions in these patients. If the G6PD levels are adequate, the AO system is able to neutralize ROS leading to recovery.

Conclusion

1. SARS-CoV-2 triggers the innate immune response. This is normal. It also suppresses the AO ACE2/eNOS/NO pathway. This is abnormal and is unique to SARS-CoV-2 and leads to poor recovery, especially in patients with underlying health conditions.

2. Patients with genetic and/or acquired G6PD deficiency are vulnerable to SARS-CoV-2 infection.

3. We strongly recommend biomarker monitoring prior to any interventions, especially in critically ill patients in order to assess the body's status of inflammation:

- ferritin as a biomarker of macrophage involvement in the innate immune response;

- lactate dehydrogenase (LDH) level as a biomarker of hemolysis by ROS;

- uric acid as a biomarker of endothelial cell involvement in inflammation.

We also recommend biomarker monitoring to assess the status of their AO system:

- G6PD activity directly, or a T3 level as an indirect biomarker of G6PD activity, which is particularly important for critically ill patients.

- pyroglutamic acid and GGT levels as biomarkers of GSH depletion and as indicators of impending oxidative catastrophe in severely ill COVID-19 patients.

- homocysteine levels negatively correlate with serum Vit.B9 and can be used as a biomarker of folic acid deficiency and predictive of potential NO depletion.

A basic understanding of these first principles of COVID-19 pathophysiology is essential to manage it appropriately.

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