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

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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 effective and safe treatment. The goal of this article is to review the relevant biomarkers that can assess COVID-19 patients and monitor their clinical course.

OBSERVATION: Clinical manifestations of COVID-19 vary significantly, from asymptomatic to lethal outcomes. Moreover, mortality rates differ varying on age, comorbidities and ethnicity.

PROBLEM: It is difficult to determine whether a person is vulnerable to SARS-CoV-2 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, diabetes, 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) evidence-supporting dataset and (2) normal and (3) pathological pathways, (4) list of relevant biomarkers.

RESULTS:

1. A detailed description of the two-component innate immune response, proinflammatory (prooxidant - PO) and antiinflammatory (antioxidant - AO) systems is presented. The PO system attacks pathogens and unintentially damages host’s cells. The AO system alleviates oxidative stress, as a condition of host damage, and balances normal immune response. The balance can be monitored by the biomarkers highlighted in the Figure.

2. As for any other pathogen, SARS-CoV-2 triggers an inflammatory PO response. But it also has a distinctive feature to suppress the AO response. This occurs specifically through the down regulation of the ACE2 pathway as a protective mechanism of the renin-angiotensin system (RAS).

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.

Research data is available at https://doi.org/10.6084/m9.figshare.12121389
In addition to the pathway in the graphical abstract, here we elaborate further on the normal processes affected by SARS-CoV-2 and how initial defensive mechanism of innate immune response contributes unintentionally to complications in patients with comorbidities.

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, bacteria, 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 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. Importantly, subclinical abnormal background conditions, e.g. prediabetes, can be accelerated by the stress of infection.

Biomarkers can be used to assess the status of the background condition and its degree of deviation from normal.

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 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 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. This results in the production of NO⁺ radicals. In turn, these activate Ca²⁺ channels through S-nitrosylation in myocytes leading to vasoconstriction.

In summary, patients with comorbid conditions – both non-infectious and infectious – with their accompanying inflammation, results in increased ROS that manifests clinically as increased blood pressure.

SARS-CoV-2 potentiates this 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 comorbidities are predisposed to exaggerated SIRS. Accordingly, it is important to optimally control chronic diseases.

Biomarkers such as ferritin, LDH and uric acid can be used to monitor the status of inflammation.

Treatment options that aggravate oxidative stress.

Interestingly, drugs such as chloroquine (anti-protozoa) or ivermectin (anti-helminthic) that induce ROS production against their primarily targets will work perfectly against viruses on cell cultures. In fact, organism made up of "cell lines" with their own DNA sensitive to ROS also. NSAIDs induce ROS production too. Mechanical ventilation and intense oxygen therapy are intended to support respiratory-distressed patients but its aggressiveness is equal to surgical procedure and excessive oxygen is used as added “fuel” to the respiratory burst in macrophages.

That is why we should minimize the therapeutic interventions that contribute to the oxidative stress. To keep balance act in balance.

AO response deficiency

AO system is responsible for ROS neutralization and oxidative stress alleviation.

Oxidative stress alleviation - ACE2/eNOS/NO pathway

ACE2/nitric oxide (NO) pathway is one of the protective components of vessels. SARS-CoV-2 down-regulates ACE2 specifically and NO insufficiency predisposes vessels to inability to balance the increased tone caused by ROS overproduction compromising vessels’ physiology. So, PO predominance and AO deficiency (PO/AO overload) with ROS overproduction in vessels is a start condition at early stages of COVID-19. Thereby, damage of vessels make the increased pulmonary arterial pressure symptom as the main pathophysiological component in ARDS lungs of COVID-19 patients.
ROS neutralization - GSH

Glutathione (GSH) with glutathione peroxidase (Gpx) as the most important and most abundant antioxidants that neutralize ROS and convert them to non-toxic products, such as H2O. GSH is made up of 3 amino acids: glycine, cysteine and glutamate. The sulfhydryl (-SH) moiety of cysteine is responsible for ability to detoxify ROS. GSH is being oxidized by GPx and converted to GSSG – oxidized glutathione during the reaction of ROS neutralization. GSH can be regenerated from reduction of GSSG with the help of glutathione reductase (GR) or restored by gamma-glutamyl cycle or restored from extracellular GSH by gamma-glutamyl transferase (GGT).

GSH and NO production require NADPH

NADPH is produced via pentose phosphate pathway (PPP) and glucose-6-phosphate dehydrogenase (G6PD) as the rate limiting enzyme.

Glucose-6-phosphate dehydrogenase deficiency

Decreased G6PD activity can be inborn or acquired in patients with diabetes, obesity, old age and hypertension. Patients with comorbidities develop an acquired G6PD deficiency because macrophages' TNF alpha inhibits insulin receptor signaling leading to insulin resistance. Since G6PD takes part in glucose metabolism, it requires insulin to enter glucose into cell to metabolize it. No glucose - no substrate for G6PD down-regulating it’s activity.

Normal process
(Insulin) take into (glucose) activates (intracellular glucose metabolism) stimulates (G6PD)
G6PD deficiency
(Comorbidity) maintains (inflammation) induces (insulin resistance) DECREASES (intracellular glucose metabolism) stimulates (G6PD)

Therefore, patients with comorbidities have G6PD deficiency.

Additionally, patients develop relative NADPH deficiency that mimics G6PD deficiency because PO system - macrophage’s NADPH oxidase (intended for antiviral defense) and AO system - NADPH methemoglobin reductase (intended for hemoglobin recovery), NADPH glutathione reductase (intended for GSH reduction), thyroid NADPH oxidase (intended for T3 production), NADPH nitric oxide synthase (NOS) – all of them compete for NADPH as a cofactor. G6PD’s inability to supply NADPH sufficiently for overloaded PO/AO system is one of the reasons of the acquired relatively decreased G6PD activity.

GSH and NO production require FAD

Since NADPH is a cofactor for T3 synthesis in thyroid gland and G6PD deficient patients have deficit in NADPH thus such patients have relative transient hypothyroidism. Moreover, T3 activates riboflavin kinase to produce FAD (vitamin B2 derivative), a cofactor for the GR in GSSH reduction to GSH and for NO production. Someway, T3 deficiency is an indirect biomarker of G6PD deficiency.

In severe G6PD deficiency ROS overload can lead to hemolysis of erythrocytes, especially after administration of certain drugs, e.g. chloroquine, NSAIDs that induce ROS production additionally.
If patient drops hemoglobin after 2-3 days of NSAIDS or antimalarial drugs treatment and LDH level is increased, G6PD deficiency should be strongly suspected and causative medication should be stopped.

Normal process

(G6PD) produces (NADPH) produces (T3) produces (FAD) produces (GSH) neutralizes (ROS)
induces (hemolysis)

G6PD deficiency

(G6PD deficiency) produces (NADPH deficiency) produces (T3 deficiency) produces (FAD deficiency) produces (GSH deficiency) DOESN’T neutralize (ROS) induces (HEMOLYSIS) is present

Therefore, G6PD deficiency ultimately decreases GSH and NO levels.

GSH deficiency

As a consequence of intracellular GSH depletion, GGT activity is up-regulated in order to restore it from extracellular GSH. Moreover, GSH provides negative feedback on enzyme –glutamyl cysteine synthase (–Gcs) in gamma-glutamyl cycle and when GSH depletion continues, the activity of –Gcs is increased and stimulates accumulation of pyroglutamic acid, an intermediate metabolite of gamma-glutamyl cycle. At the same time, avoidance of other NSAIDs in COVID-19 management can lead to overuse of acetaminophen and highly reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) of which depletes GSH directly. Thus, GSH depletion caused by PO/AO overload or APAP overuse leads to unexplained anion gap metabolic acidosis in critical patients, which should be considered as pyroglutamic acidosis until proven otherwise. Acidemia by itself is not so important, but as a sign of serious metabolic stress. N-acetyl-L-cysteine (NAC) administration should be considered in the light of acetaminophen overuse.

GSH synthesis in gamma-glutamyl cycle involves cysteine production where homocysteine uses folic acid (vitamin B9) derivatives to intensify this process. But, at the same time, NOS competes for folic acid to use its derivative as tetrahydrobiopterin (BH4) for NO production. As NO synthesis is increased in PO system by induced NOS (iNOS) to produce NO* radicals when superoxide (O2*) is present and in AO system by endothelial NOS (eNOS) to relax vessels, iNOS and eNOS compete for folic acid already in the case of PO/AO overload.

Conclusion

1. SARS-CoV-2 triggers normal innate immune response (PO system) unintentionally and abnormally suppresses ACE2/eNOS/NO pathway of AO system intentionally;
2. Patients with genetic and/or acquired G6PD deficiency are vulnerable to SARS-CoV-2;
3. We strongly recommend monitoring of biomarkers prior to any treatment in order to find out compensatory capacity of PO system:
   - ferritin as a biomarker of macrophages involvement in innate immune defense;
   - 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.
   and biomarkers of AO system:
   - G6PD activity directly or T3 level as an indirect biomarker of G6PD activity, particularly important for critical patients;
   - homocysteine level as a biomarker of folic acid deficiency and potential NO depletion.
- pyroglutamic acid and GGT levels as a GSH depletion biomarkers and as an indicators of impending oxidative catastrophe in severe COVID-19 patients.

Clinicians need to have a basic understanding of the pathophysiology in order to manage it appropriately.

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