

THE IMPLICATIONS OF OXIDATIVE STRESS IN LONG COVID PATHOGENESIS

IMPLIKACIJE OKSIDATIVNOG STRESA U PATOGENEZI PRODUŽENOG COVID-A

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Abstract

As far as clinical presentation is concerned, following an episode of acute sickness, the SARS-CoV-2 infection may lead to the development of a number of complications known as post-acute sequelae of SARS-CoV-2 infection (PASC). The definition of PASC, as well as its estimated prevalence evolved over the course of time and acquired knowledge. Although COVID-19 was initially characterized as an acute respiratory illness, convalescents frequently report diverse clinical manifestations related to several organ systems, referred to as long COVID. However, the fundamental molecular mechanisms that are responsible for the incapacitating symptoms, occurring in patients with long COVID, remain largely unexplained at this time. From a molecular medicine point of view, one of the proposed postulates favors the impaired redox balance, which may serve as a central hub responsible for mechanisms disturbing the cellular homeostasis, innate immune response and metabolism. This review will try to tackle the current knowledge about the underlying mechanisms comprising the proposed interplay of the disturbed redox balance and inflammation, that may potentially contribute to the occurrence of tissue or organ damage that is linked with COVID-19, as well as the eventual manifestation of symptoms observed in individuals with long COVID. One might assume that in certain individuals, there are mechanisms that may dominate over others. Genetic variability may offer some answers - especially in the case of polymorphisms occurring in genes that encode for antioxidant proteins and enzymes.

Keywords:

long COVID,
oxidative stress,
inflammation,
polymorphisms,
antioxidant defense

Sažetak

Što se kliničke prezentacije tiče, nakon epizode akutne bolesti, infekcija SARS-CoV-2 može dovesti do razvoja brojnih komplikacija poznatih kao post-akutne sekvle infekcije SARS-CoV-2 (engl. *post-acute sequelae of SARS-CoV-2 infection* - PASC). Definicija PASC, kao i njihova procenjena prevalencija, menjala se tokom vremena sa sticanjem novih znanja o ovom stanju. Iako je COVID-19 prvobitno okarakterisan kao akutna respiratorna bolest, osobe koje su je preležale navode različite kliničke manifestacije, koje se odnose na nekoliko sistema organa, poznate pod nazivom produženi COVID. Međutim, osnovni molekularni mehanizmi koji su odgovorni za simptome koji se javljaju kod pacijenata sa produženim COVID-om su u ovom trenutku uglavnom nerazjašnjeni. Sa stanovišta molekularne medicine, jedan od predloženih postulata ističe značaj narušene redoks ravnoteže, koja može biti zajednički imenilac odgovoran za poremećaje ćelijske homeostaze, urođenog imunskog odgovora i metabolizma. Ovaj pregledni rad ima za cilj da pruži uvid u trenutno znanje o osnovnim mehanizmima koji ukazuju na međusobu povezanost narušene redoks ravnoteže i inflamacije, što potencijalno može doprineti nastanku oštećenja tkiva ili organa, povezanih sa COVID-19, kao i eventualnim kliničkim manifestacijama uočenim kod osoba sa produženim COVID-om. Moguće je pretpostaviti da kod određenih osoba postoje mehanizmi koji mogu biti dominantni u odnosu na druge. U ovom smislu, genetska varijabilnost može ponuditi objašnjenje za ove pojave, posebno u slučaju polimorfizama koji se javljaju u genima koji kodiraju antioksidativne proteine i enzime.

Ključne reči:

produženi COVID, oksidativni stres, zapaljenje, polimorfizmi, antioksidativna odbrana

Introduction

The definition of post-acute sequelae of SARS-CoV-2 infection (PASC) evolved over the course of time and acquired knowledge. According to the Centers for Disease Control and Prevention (<http://www.CDC.gov>), Long COVID or post-COVID syndrome represents “a condition marked by the prolongation of COVID-19 symptoms - or the emergence of new ones - 4 or more weeks after infection with SARS-CoV-2 “. On the other hand, according to the National Institute of Health (<http://www.NIH.gov>), this syndrome “accounts for those patients that are persistently symptomatic for more than 30 days from the onset of infection”. Alternatively, the World Health Organization (<http://www.WHO.int>) describes PASC as post COVID-19 condition “occurring in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis”. Recently, the RECOVER Initiative, an NIH-funded project (<https://recovercovid.org/>), has been introduced, defining the syndrome as “ongoing, relapsing, or new symptoms, or other health effects occurring after the acute phase of SARS-CoV-2 infection (i.e., present four or more weeks after the acute infection)”.

As the definition itself, the estimated prevalence of PASC varies as well, ranging from 3.3%, followed by 13.9%, to 39% (in the UK, USA and Denmark, respectively) (1). The administrated vaccines were highly effective in preventing the development of severe forms of COVID-19, thereby exhibiting the capacity to prevent long COVID (2). As far as clinical presentation is concerned, following an episode of acute sickness, the SARS-CoV-2 infection may lead to the development of a number of complications known as sequelae. However, the convalescents

frequently report diverse complications related to neurologic, cardiac/cardiovascular, gastrointestinal, psychiatric, pulmonary body systems, etc. Still, the fundamental molecular mechanisms that are responsible for these incapacitating symptoms remain largely unexplained at this time. The initial hypothesis postulates that PASC results from a long-term organ damage due to the acute-phase infection, where specific mechanistic pathways aggravate the initial illness itself, therefore harming many organs, which will eventually lead to the development of later symptoms (1). From molecular medicine point of view, one of the proposed postulates favors the impaired redox homeostasis, that may serve as a central hub responsible for mechanisms disturbing the cellular homeostasis, innate immune response and metabolism (3). Existing hypotheses propose that the persistent burden of long COVID in persons with post-acute sequelae of COVID-19 may be attributed to the intertwined and continuous presence of oxidative stress and inflammation, hence influencing its development and persistence.

This review will try to tackle the current knowledge about the underlying mechanisms comprising the proposed interplay of the disturbed redox balance and inflammation, that may potentially contribute to the occurrence of tissue or organ damage, that is linked with COVID-19, as well as the eventual manifestation of symptoms observed in individuals with long COVID. A comprehensive literature analysis was conducted by scanning the PubMed database for papers published in the English language from 2021 to 2023. Furthermore, a supplementary manual search was conducted by examining the reference lists of the discovered articles to augment the first selection with additional pertinent publications. Studies that were not linked, articles written in languages other than English, articles without available full text, and conference papers

were considered unsuitable and hence eliminated from further analysis. In order to account for the diversity of research methods and the complexity of the subject matter, the scope of the literature search was not restricted to a particular study design. The article is solely focused on the human population.

Redox imbalance

According to its first definition postulated by Helmut Sies, oxidative stress is a condition that occurs when there is an imbalance between the levels of prooxidants and antioxidants in the body, with prooxidants dominating the equation (4). Prooxidants may be represented by free radicals, which by definition represent atoms, molecules or ions with an unpaired electron, which makes them very reactive. Due to the tendency to take over an electron and thus complete their orbital, free radicals enter the chain reactions that can result in oxidative damage towards important macromolecules, as a part of a process labeled as “oxidative distress” (5). The term “reactive oxygen species” (ROS) is used to denote a set of products of partial reduction of molecular oxygen: superoxide anion ($O_2^{\cdot -}$), then hydrogen peroxide (H_2O_2), which is not a classic radical, and the most dangerous of all reactive oxygen species - hydroxyl radicals (OH^{\cdot}). The oxygen molecule, otherwise

necessary for our aerobic life, is a biradical, which means that it has two unpaired electrons, that are individually orbiting, and it is also very reactive. In addition to ROS, reactive nitrogen species (RNS), especially nitric oxide and peroxyxynitrite, are of great biological and medical importance (6).

Of note, free radicals are created in a controlled manner during the normal metabolic activity of the cells. On the other hand, an imbalance between mechanisms that promote oxidative stress and lead to high production of ROS as opposed to defense mechanisms, can lead to molecular and cellular damage with the involvement of inflammation pathways, as well (7). In this context, it is noteworthy that oxidative stress is observed in a multitude of disorders. Recently, the role of oxidative stress has been confirmed in numerous infectious diseases, such as viral infections, and also in the onset and progression of the COVID-19 (8). Namely, high production of ROS is often found in respiratory infections, which lead to increased production of pro-inflammatory cytokines, pronounced inflammatory response, induction of cell apoptosis and other pathophysiological processes, which lead to intense oxidative stress (9). The virus of SARS-CoV-2 has also been shown to stimulate the generation of ROS (10). This postulation agrees with the presumption that specific mechanistic pathways aggravate the initial COVID illness

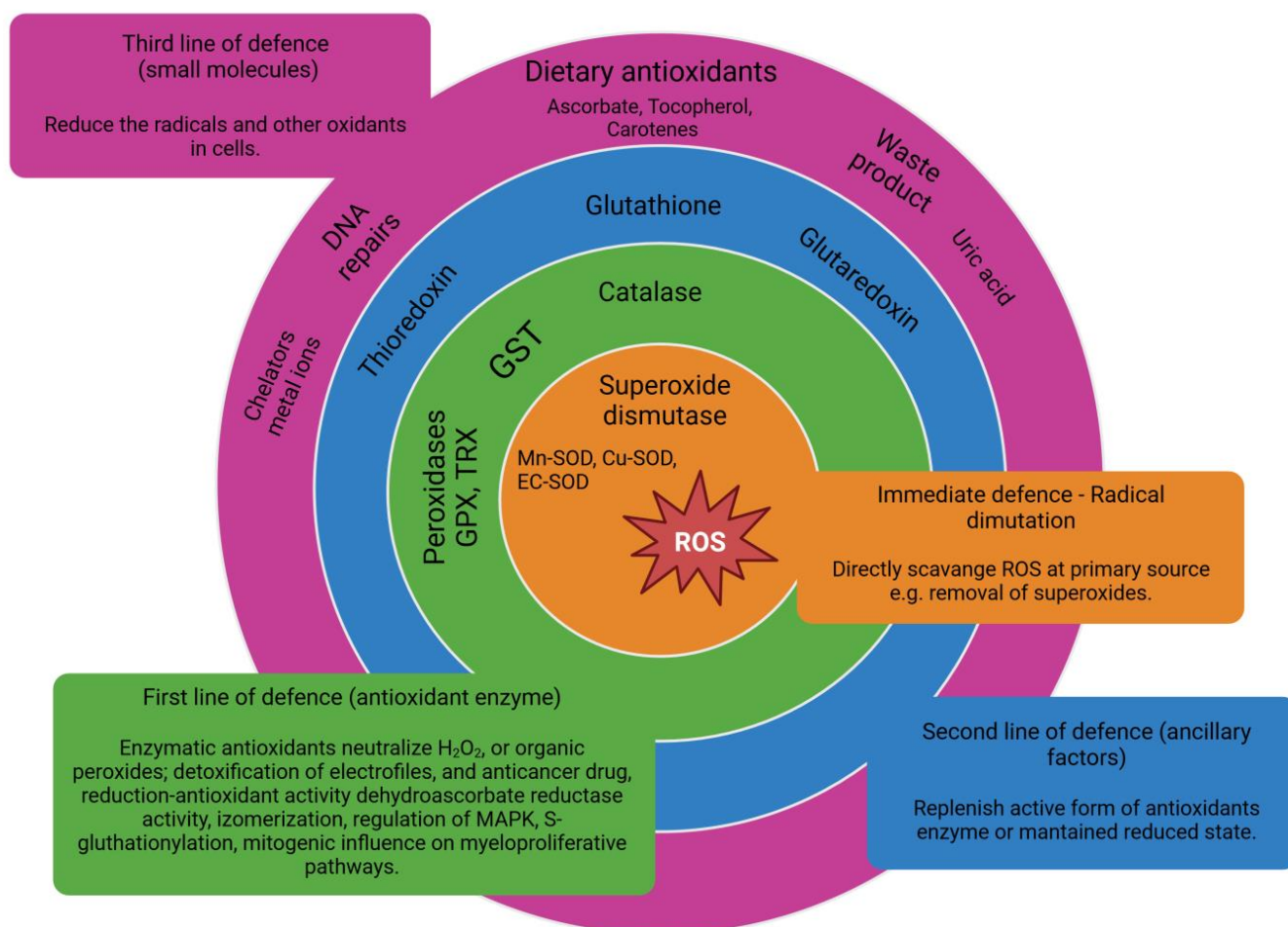


Figure 1. Lines of antioxidant defense. The enzymes constitute the immediate antioxidant defense (SOD, superoxide dismutase) as well as the first line of antioxidant defense: GPX, glutathione peroxidase and GST, glutathione S-transferase and catalase (13).

itself, therefore harming many organs, which will eventually lead to the development of symptoms associated with long COVID (1).

In the pathophysiology of the SARS-CoV-2 infection, ROS represent both cause and consequence of its pathophysiology, constituting the vicious circle of oxidative stress and inflammation interplay (11, 12) where each process mutually reinforces each other. Oxidative stress is observed when the production reactive oxygen species (ROS) prevails the concentration/ activity of antioxidant system (**figure 1**). To second these premises, it is observed that in more severe COVID-19 forms, inflammatory parameters, as well as multiorgan impairment biomarkers correlate with markers of oxidative stress (3). Presumably, individuals who might be more at risk for developing post-COVID conditions are those who have suffered from more severe forms of COVID-19 illness, especially those who were hospitalized or with existing co-morbidities, where the underlying mechanism significantly contributes to oxidative stress (3).

Oxidation of biologically important macromolecules such as lipids, proteins, and nucleic acids forms part of the pathophysiological basis for the development, as well as progression of numerous pathological conditions, including COVID-19 (9, 10). Namely, it is known that free radicals react with polyunsaturated fatty acids of lipids, with aminoacyl chains of proteins or with residues of bases and sugars of nucleotides or even nucleic acids themselves. Indicators of oxidative damage can be determined by routine or more advanced laboratory methods and include measuring the concentration of biomarkers of oxidative protein damage (the level of thiol groups, advanced oxidation protein products (AOPP)), oxidative lipid damage (content of malondialdehyde, isoprostane) and oxidative DNA damage: 8-hydroxy 2'-deoxyguanosine (8-OHdG), but also the overall pro-oxidant-antioxidant balance (14, 15).

Genetic variability in antioxidant defense associated with the development of specific clinical phenotypes of long COVID

The substantial heterogeneity of long COVID makes deciphering this new condition rather challenging. It has been suggested that development of specific clinical phenotypes of long COVID can be partially explained by specific host genetic variations, especially in genes linked to oxidative stress and inflammation, as well. Namely, individuals carrying genetic variants associated with altered redox balance are more prone to the development of clinical phenotypes associated with long COVID. These genetic variations can result in great inter-individual differences underpinning the mechanisms maintaining the redox homeostasis. So far, genome-wide association study meta-analyses that investigated the contribution of common genetic variation to COVID-19 have confirmed the association of 20 genomic loci with disease severity and

susceptibility, with the strongest effect on severity at the 3p21.31 locus (16). However, much remains unknown about the genetic basis of susceptibility to long COVID.

The evaluation of genetic polymorphisms that are recognized to impact the cellular antioxidant capacity, as well as detoxification mechanisms, in individuals with the history of SARS-CoV-2 infection and consequently long COVID, has the potential to make a significant and meaningful impact in the realm of academic inquiry. Nuclear factor-erythroid-2-related factor 2 (Nrf2) represents a key redox-sensitive basic leucine zipper transcription factor, generally providing the regulation of intracellular antioxidant activity, therefore maintaining the cellular redox homeostasis along with cellular detoxification processes (17). Much of this effectiveness is being achieved through Nrf2 ability to upregulate antioxidant response element (ARE)-mediated gene expression, including genes encoding for quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1), NAD(P)H (nicotinamide adenine dinucleotide phosphate), superoxide dismutase (SOD), and certain glutathione S-transferase (GST) (18-21). The most significant functional polymorphism is *Nrf2* rs6721961 (-617C/A), which is located in the promoter region. It was discovered that this single nucleotide polymorphism (SNP) decreased the transcription activity of Nrf2, connected to weakened binding of Nrf2 to the aforementioned ARE, resulting in decreased Nrf2-dependent gene transcription (22).

As a short-lived protein whose stability is regulated by Kelch-like ECH associating protein 1 (Keap1), Nrf2 has dependent ubiquitination and subsequent proteasome degradation. Under balanced physiological condition, Nrf2 occupies majority of time in the cytoplasm, complexed with the regulatory Keap1 subunit. As a result of oxidative stress, however, Keap1-Nrf2 complex disassembles and Nrf2 protein moves to the nucleus, where it engages with the gene promoters located inside the antioxidant response element (ARE) sequences, thereby initiating the activation of antioxidant defense genes (23, 24). This Nrf2 translocation is enabled by one prominent feature of Keap1. Namely, Keap1 is thought to be an electrophile and ROS biosensor (17). The biosensor feature is grounded on the fact that this particular protein is rich in cysteine, constituting so called cysteine sensors numerated as C151, C273, and C288. In the state of oxidative stress, electrophiles modify the structure of Keap1, particularly the sensors that seem to be responsible for introducing Nrf2 into the nucleus (25). The expression of Keap1 might be polymorphic, as well. The functional polymorphism of *Keap1* rs1048290 results in C, cytosine → G, guanine substitution which was reported to be related to the level of Keap1 protein expression, presumably affecting the regulation of Nrf2. Of note, there is evidence supporting another way of regulation of Nrf2 activity by the multifunctional autophagy adapter, p62 (sequestosome 1), which plays a role in non-canonical Nrf2 regulation (26) by interacting with the Keap1-Nrf2 pathway (27). Namely, the accumulation of p62 and inhibition of Keap1 mediated Nrf2 activation constitutes signaling Keap1/Nrf2/p62 pathway that has been involved

in the mitigation of cellular senescence, which is expedited by an overabundance of oxidative stress within cells as a result of COVID-19 infection (28). Consequently, the aforementioned vicious circle of oxidative stress and inflammation increases the risk of severe complications in SARS-CoV-2 infection, imposing a risk for long COVID syndrome (17). However, the sole role of this Keap1/Nrf2/p62 pathway in long COVID is yet to be elucidated, especially in the light of Keap1 and Nrf2 polymorphic expression (figure 2).

Among NRF2 targeted genes, there are those encoding for certain glutathione S-transferase (GSTs), known for their catalytic antioxidant capability and detoxification activity, in addition to their non-catalytic roles, as negative regulators of protein kinases involved in cell lifecycle, by the means of protein-protein interactions (29). Members of the glutathione S-transferase superfamily comprise the most important cytosolic GST families such as *mi* (GSTM3), *pi* (GSTP1), *omega* (GSTO1 and GSTO2). Previous studies have shown that host GST genetic profile affects susceptibility, as well as severity of clinical

manifestations in COVID-19 patients (30,31). Regarding its functional significance, it was shown that *GSTM3* polymorphism rs1332018 (C/A) influences gene expression, whereas polymorphisms of *GSTP1*, *GSTO1* and *GSTO2* are characterized as SNPs. In case of *GSTP1* rs1695 polymorphism, substitution of A313G causes change of isoleucine with valine at position 105 (Ile105Val), whereas the presence of T instead of C at position 341 in case of *GSTP1* rs1138272 polymorphism results in coding protein with valine (Val) as a replacement of alanine (Ala), both modifying substrate specificity. Haplotype *GSTP1ABCD* represents the combination of these two polymorphisms. In both *GSTO* genes, the most commonly studied are two functional polymorphisms: *GSTO1* Ala140Asp (rs4925) and *GSTO2* Asn142Asp (rs156697). Indeed, the results on the association between polymorphisms of glutathione transferases P1, M3, O1, O2 and COVID-19 susceptibility and/or severity have shed some light on the involvement of genetic variants on susceptibility and/or probability of developing severe COVID-19, whereas *GSTO* polymorphisms influenced inflammation and coagulation parameters

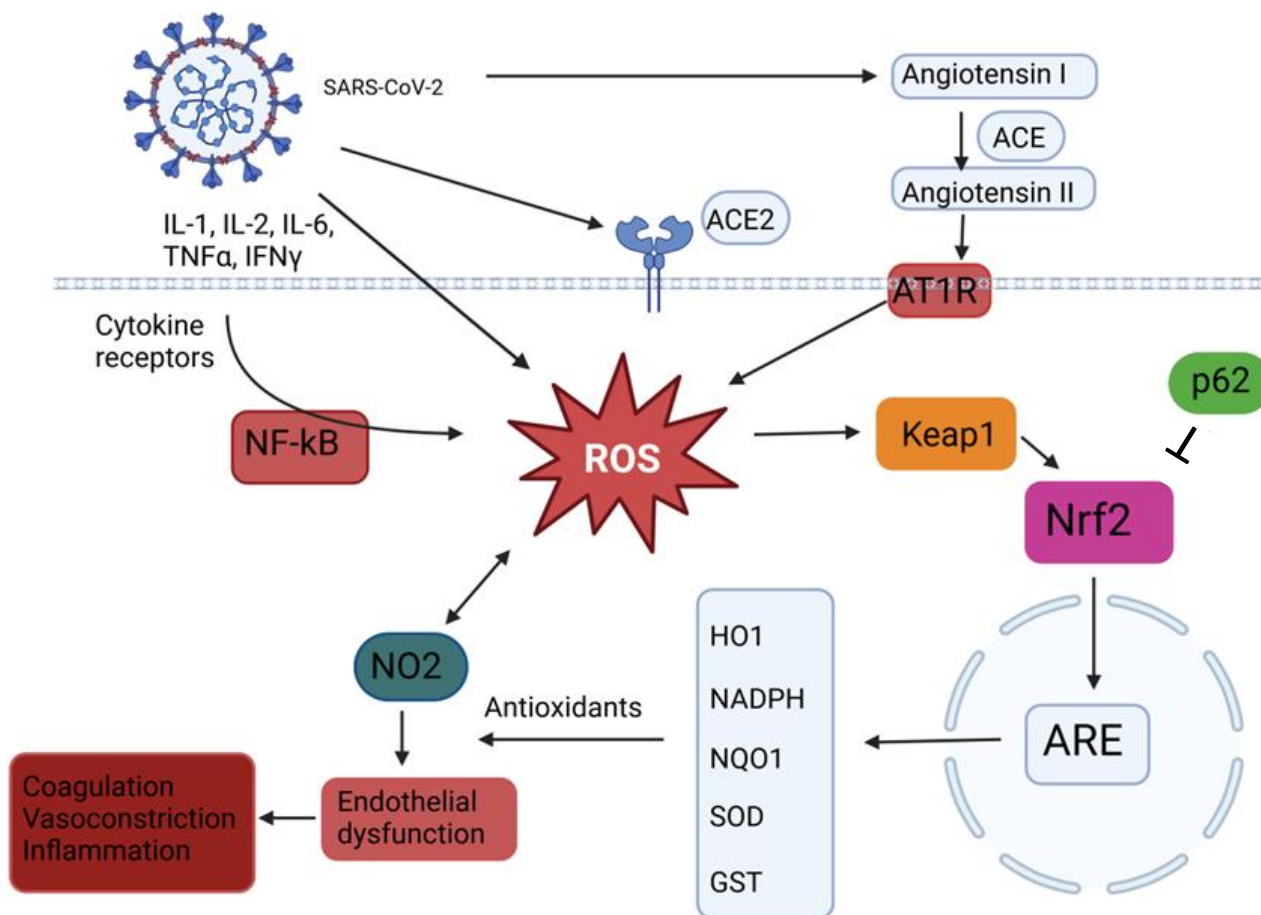


Figure 2. The hypnotized role of Keap1/Nrf2/p62 pathway in long COVID; Created in Biorender www.biorender.com; SARS-CoV-2 - Severe Acute Respiratory Syndrome coronavirus 2, IL-1 - Interleukin 1, IL-2 - Interleukin 2, IL-6 - Interleukin 6, TNFα - Tumour necrosis factor α, IFNγ - Interferon γ, ACE - Angiotensin Converting Enzyme, AT1R - Angiotensin II receptor type 1, ACE2 - Angiotensin converting enzyme 2, receptor, NF-κB - Nuclear factor kappa-light-chain-enhancer of activated B cells, ROS - Reactive oxygen species, Keap1 - Kelch-like ECH-associated protein 1, Nrf2 - nuclear factor erythroid 2-related factor 2, ARE - Antioxidant response element, HO1 - Heme oxygenase, NADPH - Reduced nicotinamide adenine dinucleotide phosphate, NQO1 - NAD(P)H Quinone Dehydrogenase 1, SOD - Superoxide dismutase, GST - Glutathione S transferase.

(32).

Beside GSTs, Nrf2 modulates the expression and therefore affects the overall activity of glutathione peroxidases (GPX) and glutathione reductases. Along with GSTs, GPX constitute the first-line of antioxidant defense along. The GPX family comprises several isozymes that reduce hydrogen peroxide and other organic peroxides to oxygen and water. Special attention has been given to GPX3, an extracellular enzyme involved in catalyzing the neutralization of hydro- and soluble lipid hydroperoxides, using the reduced glutathione (33). *GPX3* T-65C (rs8177412) polymorphism is a part of identified segment inside the *GPX3* promoter haplotype, accountable for the suppression of gene transcription, leading to a reduction in plasma GPX3 activity levels (34). The gene polymorphism *GPX1* (rs1050450) is distinguished by the occurrence of nucleotide substitution, specifically the replacement of cytosine (C) with thymine (T). This substitution leads to the replacement of the amino acid proline (Pro) with leucine (Leu). Consequently, this alteration is linked to a reduction in GPX1 activity (35). However, the so called immediate-line antioxidant defense is represented by superoxide dismutase (SOD) isoenzyme that catalyzes the simultaneous reduction and oxidation of superoxide into oxygen and hydrogen peroxide. The gene polymorphism *SOD2* (rs4880) is characterized by a nucleotide substitution (T, thymine → C, cytosine), resulting in an amino acid alteration from valine (Val) to alanine (Ala). Research has demonstrated that the existence of a genetic variant, specifically the Val-*SOD2* allele, leads to a decrease in the effectiveness of *SOD2* transportation within mitochondria, resulting in a reduction of approximately 30 - 40% (36).

Clinical manifestations of PASC pathologies potentially associated with redox imbalance

Simultaneous determination of genetic susceptibility biomarkers might identify an individual redox profile and enable timely prevention and adequate treatment of long COVID sequelae. For instance, a very recent study found a noteworthy association between the *GPX3* rs8177412 variant genotype and the odds of acquiring severe forms of COVID-19 (OR = 2.42), taking into account the inflammatory markers as well (37). Precisely, further investigation of COVID-19 convalescents after recovery from moderate to severe forms of disease will substantially contribute to clarifying the exact roles of antioxidant gene variants in the development of long COVID clinical manifestations, as well as define the potential biological mechanisms underlying pathogenesis of the sequelae.

The significant dysregulation of redox homeostasis in cardiac complications, seen as a part of a long COVID syndrome, might be associated by previously described polymorphisms in genes encoding for catalytic and regulatory antioxidant proteins. Namely, the results of a recent study of Asanin et al. have shown that *SOD2* rs4880, *GPX1* rs1050450, *GPX3* rs8177412, and *Nrf2* rs6721961

polymorphisms influence the individual sensitivity on the occurrence of cardiac symptoms within the context of long COVID puzzle, that persists even with instances of mild to moderate COVID-19 cases (38). Namely, the carriers of variant *GPX* alleles were found to have a substantial association with altered left atrial and right ventricular echocardiographic parameters, specifically LAVI (Left Atrial Volume Index), REAC (Right Ventricle Fractional Area Change) and RV-EF (Right Ventricular Ejection Fraction). Moreover, the variant *SOD2* allele carriers may exhibit modest left ventricular systolic dysfunction, as shown by elevated levels of left ventricular echocardiographic measures such as EDD (End-Diastolic Diameter), LVMI (Left Ventricular Mass Index) and GLS (Global Longitudinal Strain), as well as troponin T concentrations (38).

Hematologic pathologies and their mechanisms represent one of the fields that require additional attention. There is still a lack of evidence supporting the mechanisms behind sustained systemic inflammation associated with hypercoagulability on one side, and endotheliopathy on the other, even after the 6-month period past the acute phase of the COVID-19. Of note, there are evidence supporting the fact that SARS-CoV-2 virus itself does not have intrinsic procoagulant effects (39). As far as hypercoagulability is concerned, what is known is that over the course of COVID-19 associated coagulopathy (CAC), hypercoagulability along hypofibrinolysis prevail, contributing to the poorly controlled proinflammatory milieu (40,41). Consequently, increased levels of pro-inflammatory markers related to the coagulation status (e.g. CRP, IL-6, and D-dimer) and lymphopenia have been associated with long COVID (42). This outcome is not unexpected as patients infected with SARS-CoV-2 that are carriers of certain variant alleles of genes encoding for antioxidant enzymes have elevated levels of inflammation and coagulation markers. Indeed, it has been observed that variations in the *SOD2* rs4880 and *GPX1* rs1050450 genes have an impact on the laboratory biochemical profile of individuals affected with COVID-19. Specifically, the *SOD2**Val allele exhibited a statistically significant correlation with elevated concentrations of both fibrinogen and ferritin. Conversely, the *GPX1**Leu allele shown an association with greater levels of fibrinogen, with a particularly pronounced effect on D-dimer concentrations (32). Regarding the previously mentioned endotheliopathy, such chronic low, however, poorly controlled proinflammatory status has been associated to endothelial alterations, with a cytotoxic immune attack towards endothelium itself (43). Subsequent microclots, which are resistant to fibrinolysis, may be the cause of oxidative stress along with secretion of proinflammatory cytokines, establishing an aforementioned vicious cycle that may be implicated in the plethora of clinical manifestations observed in long COVID (44). Disturbance of not only systemic, but organ-related redox balance may follow, supporting further mechanism underlying phenotypic aspects in long COVID intensity and persistency (45). This might potentially be utilized as the focal point for therapy selection in patients during the

acute phase. In a particular study conducted on a mouse model, it was observed that antiviral pill *Paxlovid* (Food And Drug Administration) exhibited a notable inhibitory effect on the secretion of extracellular matrix proteins by chondrocytes. Furthermore, *Paxlovid* was found to induce endoplasmic reticulum stress, oxidative stress, and subsequent ferroptosis, thereby expediting the process of senescence and degeneration in chondrocytes (46). Overall, inflammatory mediators, reactive oxygen species, matrix metalloproteases, glycocalyx fragments, and maybe viral proteins are believed to play a role in the development of hypercoagulability and endotheliopathy in individuals affected by COVID-19. These factors may also contribute to the occurrence of long COVID symptoms in affected individuals (47).

Earlier mentioned events, such as ROS triggered inflammation, consequently damaged endothelium, resulting in microthrombi formation, may affect the central nervous system as well, due to the ongoing neuroinflammation, and promote the formation of immuno-endothelial-neurological vicious circle (48). Although disturbed redox homeostasis and neuroinflammation are suggested as prevailing in the development of long COVID neurological sequelae, the comprehension is still very unclear (49). Undoubtedly, there exists a certain degree of genetic predisposition to this phenomenon. Some research was implicated in investigating the impact of antioxidant genetic profiles on the odds of developing neurological sequelae in individuals with long COVID. Patients who have viral infections, such as new coronavirus disease 2019 (COVID-19) or influenza, frequently present with myalgia, as one of their symptoms. In such cases, myalgia is a reflection of systemic inflammation and the cytokine response (50). In relation to the myalgia associated with long COVID, individuals who possessed at least one *GSTP1AB* Val allele exhibited a significantly reduced susceptibility to this particular neurological symptom, with a risk reduction of almost two-fold. Moreover, the presence of the *GSTO1A* Asp allele was associated with a roughly two-fold reduction in the odds of experiencing long COVID myalgia (51). The very same study also revealed that there is a positive association between genetic variability in glutathione peroxidases and the odds of developing myalgia related to long COVID. Namely, the *GPX1* Leu and *GPX3* CC alleles exhibited significant odds, surpassing a threefold increase in those who possessed the combined *GPX1LeuLeu/GPX3CC* genotype. When all aforementioned genotypes were assessed in combination, it was shown that the individuals carrying combined *GPX1LeuLeu/GPX3CC /GSTP1AB* Ile/Ile/*GSTO1AlaAla/* genotype had 10-fold increased odds of having this neurological manifestation compared to the carriers of *GPX1ProPro/GPX3TT /GSTP1ABValVal/GSTO1A* Asp/Asp/ genotype (51).

“Brain fog” represents a recently hypothesized syndrome which includes attention abnormalities, processing speed impairments, challenges in linguistic fluency, memory difficulties, and disorders in executive function. There exist multiple potential explanations about the

pathogenesis of the aforementioned condition. One possible reason is to the presence of an active reservoir of viruses within the neurological system, coupled with neuronal damage (52-54). However, the role of the redox imbalance related to active-virus reservoir is still unclear. When it comes to the predisposition caused by antioxidant related genetics, the findings of the study conducted by Ercegovic et al. revealed that individuals with the *GSTM1*-null genotype and *GPX1* Leu alleles exhibited a more than two-fold increase in the odds of experiencing “brain fog” symptoms in long COVID. The observed effect was further enhanced when these genotypes were present in combination, resulting in nearly a 13-fold increase in odds when individuals were carrying *GSTM1*-null/*GPX1LeuLeu* genotype in contrast to carriers of *GSTM1*-active/*GPX1ProPro* genotype. The presence of *Nrf2* polymorphism is associated with a 50% increase in the likelihood of experiencing long COVID related cognitive impairment, commonly referred to as “brain fog.” (51).

Additional attention should be paid towards sexual dimorphism related to the interplay of oxidative stress and inflammation in long COVID pathogenesis. Sex-specific variations in immune system functionality may play a crucial role in the development of disparities in the context of long COVID-19 syndrome. Women exhibit quicker and more vigorous innate and adaptive immune responses, which can shield them from initial infection and reduce its severity. Nevertheless, this disparity can make females more susceptible to enduring autoimmune-related disorders (55). The underlying mechanism is yet to be deciphered. Mitochondria are known source of free radicals and have the ability to facilitate inflammatory response. The decreased immunological response in men can potentially be attributed to female-biased mitochondrial damage, which leads to a reduced level of functioning in male mitochondria (56,57).

Laboratory findings in long COVID

Due to the multi-faceted nature of long COVID, at this point there are no specific laboratory findings that can be used for its diagnosis and severity assessment. Therefore, the diagnostic laboratory evaluation should be individualized. Besides the most prominent hematologic and biochemical biomarkers, the laboratory assessment may comprise biomarkers of systemic inflammation, immune profiling, biomarkers reflecting SARS-CoV-2 persistence or those assessing humoral and cellular response against SARS-CoV-2 in long COVID, biomarkers reflecting reactivation of latent viruses or autoimmunity, as well as endothelial or vascular biomarkers, biomarkers of coagulation and fibrinolysis, hormonal and metabolic biomarkers, various proteomics biomarkers, metabolites or even cerebrospinal fluid biomarkers (44). So far, there are no suggested redox biomarkers of oxidative macromolecule damage that may individually or in combination with others aid in diagnosis and severity assessment of long COVID.

Concluding remarks on hypotheses for PASC development and sustainability: future perspectives

There are several prevailing hypotheses underlying long COVID syndrome. A number of them underpin the role of either immune system uncontrollable damage after the initial virus infection or long-lasting inflammation due to continued presence of viral particles, as well as body response similar to an autoimmune disease (2). The role of the redox imbalance with regard to the level of oxidative damage is yet to be deciphered. One might assume that in certain individual, there are mechanisms that may dominate over others. Genetic variability may offer some answers, especially in the case of polymorphisms occurring in genes that code for antioxidant proteins and enzymes (44). Simultaneous determination of genetic susceptibility biomarkers might identify an individual redox profile and enable timely prevention and adequate treatment of long COVID sequelae. Potential biomarkers could be employed for PASC sufferers until the underlying individual or mutual mechanisms within long COVID are clearly delineated.

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