

Ferroptosis - a link between iron biology, aging and COVID-19?

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ABSTRACT:

Severe form of COVID-19 is more common in the elderly and although it may include a variety of extrapulmonary manifestations, respiratory dysfunction is primarily present, which includes iron dyshomeostasis. The aging process is characterized by a chronic pro-inflammatory condition of a milder type, and COVID-19 by strong acute inflammation, both resulting in changes in iron metabolism. A new, iron-dependent form of programmed cell death, ferroptosis, is associated with iron dyshomeostasis. The main features of ferroptosis include intracellular iron overload, inhibition of the cellular GSH / GPX4 system, and accumulation of lipid reactive compounds. Clinical manifestations of COVID-19 and changes during aging include GSH depletion, GPX4 inactivation, changes in iron metabolism markers as well as markers of enhanced lipid peroxidation. There is therefore a possibility that SARS-CoV-2 may cause ferroptotic death of lung cells but also cells of other organs.

This review considers the molecular mechanisms of ferroptosis, their relationship to the aging process and the pathogenesis of COVID-19, and the consideration of ferroptosis as a factor linking aging and the pathogenesis of COVID-19.

KEYWORDS: aging, COVID-19, ferroptosis, iron metabolism, inflammation

SAŽETAK:

FERROPTOZA - VEZA IZMEĐU BIOLOGIJE ŽELJEZA, STARENJA I COVID-19?

Teški oblik COVID-19 češći je u starijih osoba i iako može uključivati razne izvanplućne manifestacije, prvenstveno je prisutna respiratorna disfunkcija, što uključuje i dishomeostazu željeza. Proces starenja karakterizira kronično proupalno stanje blažeg tipa, a COVID-19 jaka akutna upala, što rezultira promjenama u metabolizmu željeza. Novi, o željezu ovisan oblik programirane stanične smrti, ferroptoza, povezan je s dishomeostazom željeza. Glavne značajke ferroptoze uključuju intracelularno preopterećenje željezom, inhibiciju staničnog GSH / GPX4 sustava i nakupljanje spojeva reaktivnih lipida. Kliničke manifestacije COVID-19 i promjene tijekom starenja uključuju smanjenje GSH, inaktivaciju GPX4, promjene markera metabolizma željeza kao i markere pojačane peroksidacije lipida. Stoga postoji mogućnost da SARS-CoV-2 može uzrokovati ferroptotičku smrt stanica pluća, ali i stanica drugih organa.

Ovaj pregled razmatra molekularne mehanizme ferroptoze, njihov odnos s procesom starenja i patogeneom COVID-19, te razmatranje ferroptoze kao čimbenika koji povezuje starenje i patogeneom COVID-19.

KLJUČNE RIJEČI: starenje, COVID-19, ferroptoza, metabolizam željeza, upala

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INTRODUCTION

The severe form of COVID-19 that develops in the elderly is often accompanied by an atypical form of acute distress respiratory syndrome (ARDS; in 10–20% of hospitalized patients, ventilation support is required). It is defined by the acute development of bilateral infiltrates, severe hypoxemia, hypoxia and pulmonary edema that cannot be fully explained by common causes. It can also result in acute disorders of heart, kidney, liver function, coagulopathy, and shock. Although severe respiratory disorders in COVID-19 are in part similar to ARDS of other causes, there are evidences to support the view that COVID-19 is a separate entity (1,2). Research is also currently focused on the possible impact of various metabolic changes on phenotype and progression COVID-19.

Numerous discussions also include currently unclear mechanisms that may negatively interfere with the hematopoietic response to respiratory problems (3). Since adaptation to hypoxia implies increased hemoglobin synthesis, it is logical to understand the metabolism of iron, a component of the chemical part of the hemoglobin molecule (4). Disorders of iron metabolism can result in altered iron values not only in the systemic circulation but also in cells of the reticuloendothelial system, especially macrophages. The excess iron that is not stored in ferritin, released e.g. by the ferritinophagy process or by the breakdown of hemoglobin can increase the labile iron pool (LIP) in the cell. Such, free iron will stimulate the process of lipid peroxidation creating lipid-based reactive oxygen species (L-ROS) which can ultimately result in ferroptosis, a new form of regulated cell death (5).

Knowledge of the processes associated with iron is therefore extremely important for understanding the pathogenesis of COVID-19. Due to severe inflammation, patients with COVID-19 may develop hypophremia, or anemia of inflammation, which may complicate the final outcome of the disease (6). At the same time, in such a state

there is an increased concentration of free iron in the cells, which undoubtedly indicates iron dyshomeostasis (7). Among other things, this is supported, for example, by: a) hyperinflammation with massive expression of interleukin-6 (IL-6), and other cytokines e.g. IL-1, IL-6, INF- γ , TNF- α ; IL-6 strongly stimulates the formation of the main regulator systemic and cellular values of iron hepcidin, increase in serum ferritin, decrease of iron and hemoglobin values in the blood and changes of other indicators of iron metabolism (8,9,10), b) data on the similarity of the amino acid sequence of the spike glycoprotein SARS-CoV-2 and hepcidin, which suggests the possibility of simulating the action of hepcidin by the virus (11) and c) the possibility of SARS CoV-2 attack on the β -chain of hemoglobin, via CD147 and CD26 receptors on erythrocytes and their precursors, which may result in hemolysis of erythrocytes and by creating products of heme degradation (12,13).

The inflammation involves a prooxidative state in which endogenous antioxidant defenses are depleted, especially glutathione (GSH), a major cellular antioxidant, and coenzyme Q which

plays a role in preventing lipid peroxidation. Studies have shown that reduced GSH values underlie poor outcomes of COVID-19 patients and that intravenous injection of GSH is effective in alleviating severe respiratory disease symptoms (14,15). A strong inflammatory reaction (“cytokine storm”), as mentioned, favors the accumulation of cellular iron, which can result in further tissue damage and progression of pre-existing inflammation. In this way, the adaptation of hematopoiesis to hypoxia is disturbed. It has also been shown *in vitro* that some viruses *via* specific proteases can themselves modulate the host antioxidant response and generate oxidative stress (16).

The propensity of elderly people to infections, especially respiratory ones, can be associated with changes in the physical properties of tissues (mucus and epithelial barrier function and mucociliary clearance), changes in immune function but also in chronic lower-grade inflammation (17). This is manifested in the incidence and mortality rate of COVID-19, which increases exponentially with age and associated diseases such as hypertension, diabetes, cancer, heart disorders, chronic obstructive pulmonary disease, etc. It has been shown the older age to be one of the most important risk factors for SARS-CoV-2 infection as well as for poor disease outcome (18,19,20). The reasons for this association are still under investigation. It is described, among other things, that the expression of the angiotensin-converting enzyme 2 (*ACE2*) gene, the most well-known receptor for SARS-CoV-2, increases in the lungs with age (21). The transferrin receptor-1, (*TfR1*) also serves as an ideal portal for the entry of various microorganisms into the cells (22). The more severe form of COVID-19 in the elderly with comorbidities is also explained by the dynamic transformation of the innate and acquired immune response (so-called „immunosenescence“) and mild inflammation of the chronic type. Such a pro-inflammatory condition can be caused, for example, by mitochondrial dysfunction, inappropriate autophagy process, endoplasmic reticulum stress and continuous antigenic stimulation (so-called “inflammaging”) (23,24). It results in increased hepcidin expression and disturbance of iron metabolism which can result in cell death by ferroptosis.

The inflammatory process of a certain degree is therefore present in the elderly and in the pathogenesis of COVID-19. It is significantly associated with changes in iron metabolism, which may be reflected in the form of cell death (7,25,26). Namely, various apoptotic and non-apoptotic pathways of host cell death may play an important role in the pathogenesis of the disease after viral infection (27). The pathway that is increasingly associated with the aging process and the pathogenesis of COVID-19 is a non-apoptotic form of cell death - ferroptosis (28). There are therefore more and more papers in the literature describing the complex relationship between inflammation as a characteristic of aging and COVID-19 and the ferroptosis process (29).

Based on the available literature, this paper describes the main and currently known features of the ferroptosis process and selected data on the association of ferroptotic death with the aging

process and with the pathogenesis of COVID-19. One part of the reviewed papers is largely hypothetical, however, the published assumptions are based on known facts from the physiology, biochemistry, and metabolism of iron, so they need to be confirmed or rejected by further research.

For this purpose, PubMed, WHO COVID-19 database, Google Scholar, Embase, Web of Science, Science Direct and BioArX and MedArxiv preprint servers, were reviewed, mostly in the period 2017.-2021. The search strategy was to use different search terms alone and in any combination, such as: "COVID-19", "aging", "SARS-CoV-2", "iron", "hepcidin", "hemoglobin", "ferritin", "hypoxia", "ferroptosis", "inflammation", "cytokine", "oxidative stress", "immunosenescence". Only english articles with available data were included. Primarily review papers are included, but also original research.

WHAT IS FERROPTOSIS?

Ferroptosis (from the Greek "ptosis" and Latin "ferrum" iron) was a recently discovered (2012.) iron-based form of non-apoptotic, programmed cell death. It is characterized by the accumulation of toxic L-ROS and their more stable degradation products e.g. lipid hydroperoxides (L-OOH), malondialdehyde (MDA), 4-hydroxynonenal (4HNE), etc.). It differs from other forms of

cell death by biochemical pathways, major drivers and executors, and morphological features (30). Catalytically active, free ferrous iron (Fe^{2+}), which can trigger the Fenton reaction (Figure 1.) is considered a major participant in the formation of L-ROS during ferroptosis. At the same time, sulfhydryls (-SH) present in peptides (e.g. glutathione, GSH) and proteins (e.g. thioredoxin) provide resistance to the oxidation species (L-ROS) formed (31). Briefly, the triad of the ferroptosis process includes the increased availability of redox active iron, the formation of L-ROS, particularly compounds formed by the oxidation of polyunsaturated fatty acids (PUFA) of membrane phospholipids and decreased GSH-dependent glutathione peroxidase-4 (GPX4) activity (32). Redox active, free iron plays a central role in the non-enzymatic process of L-ROS formation *via* the Fenton reaction. In the enzymatic process, L-ROS are formed by the catalytic action of a group of enzymes containing iron in the active site, lipoxygenases, especially LOX-15 (33,34). In the production of pro-ferroptotic L-OOH, the most common substrates of this enzyme are arachidonoyl- and adrenoyl-phosphatidylethanolamine (35). Enzyme group LOX and indirectly contributes to ferroptosis by affecting immune cells by producing proinflammatory metabolites (leukotrienes, hydroxy eicosatetraenoic acids, oxoecosanoids) (36). A simplified scheme of the ferroptosis process is shown in Figure 1.

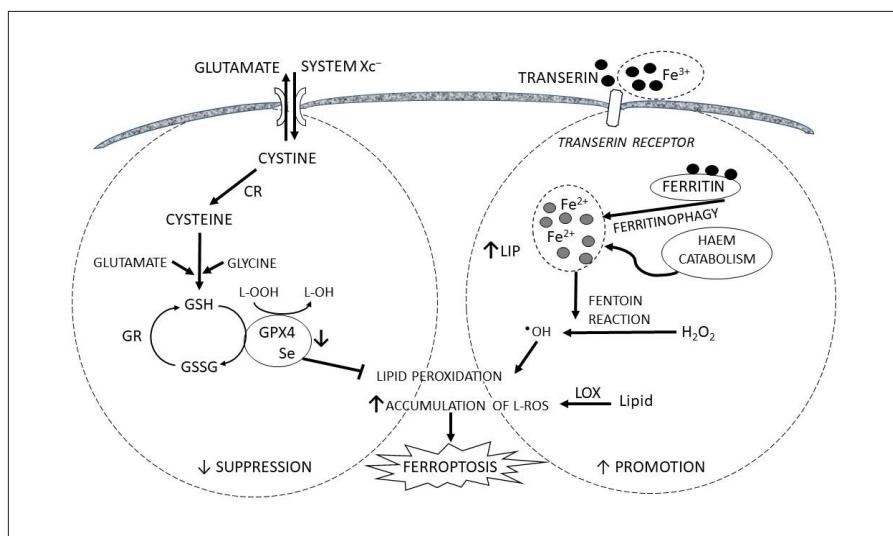


Figure 1. Scheme of the main cellular pathways of ferroptosis.

The main regulatory pathway of the ferroptosis process involves the cysteine-GSH-GPX4-L-ROS axis. Cysteine is transported to the cell via the cystine/glutamate antiporter, the Xc-system where it is reduced to cysteine by cystine reductase. The cells use cysteine in the biosynthetic pathway of GSH, a cofactor of the GPX4 enzyme. Under normal circumstances, GPX4 reduces the L-OOH (equivalent to L-ROS) to their alcoholic, non-toxic form (L-OH). Another possibility of reducing the resulting L-ROS involves ubiquinol from cell membrane compartments, and the resulting ubiquinone is reduced back to the alcoholic form (ubiquinol) by the action of FSP1 (not indicated in the figure). GPX4 and FSP1 therefore have a synergistic effect in terms of inhibiting L-ROS accumulation and thus ferroptosis. The strongest driver of L-ROS formation is the hydroxyl radical (.OH) which is formed by the reaction of ferrous iron with H₂O₂ in the so-called Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$; H₂O₂ is formed by dismutation of the superoxide radical with the catalytic activity of mitochondrial superoxide dismutase). Iron enters the cell by endocytosis of the transferrin/transferrin receptor-1 complex or is released from ferritin via ferritinophagy and/or hemoglobin degradation. FSP1 (ferroptosis-suppressor protein 1); GPX4 (glutathione peroxidase 4); GSH (reduced glutathione); GSSG (oxidized glutathione); GR (glutathione reductase); H₂O₂ (hydrogen peroxide); L-OH (lipid alcohol); L-OOH (lipid hydroperoxides); L-ROS (lipid-based reactive oxygen species); LOX (lipoxygenase); .OH (hydroxyl radical); Se (selenium). For a more detailed mechanism of the ferroptosis process see ref. 37.

Abnormal accumulation of L-ROS and an inefficient GSH / GPX4 system for their elimination result in the destruction of cell membranes and cell organelles as well as irreversible cell death. The ultimate effects of ferroptosis include a change in the physical properties of cell membranes, such as the formation of structured lipid pores. Extensive oxidation further depletes PUFAs resulting in disturbed fluidity and structure of cell membranes and their permeability (38). Different cell types have different susceptibilities to ferroptosis as a result of changes in the metabolism of iron, lipids and some amino acids (cystein, glutamate, glutamin).

In biochemical, genetic, and morphological terms, ferroptosis differs from other forms of cell death, although it is most commonly considered as „regulated necrosis“. Ferroptosis has also been shown to be associated with severe damage to mitochondrial morphology, bioenergetics and metabolism, including mitochondrial GSH (39). This is not surprising because mitochondria are major sites of iron utilization, regulators of oxidative phosphorylation but also major cellular producers of ROS. The morphological features of ferroptosis are therefore the reduction or disappearance of mitochondrial cristae, condensation of mitochondrial membrane density, rupture of the outer mitochondrial membrane and reduction of mitochondrial volume (40). Other cellular organelles, such as the endoplasmic reticulum, Golgi apparatus, and lysosomes, are also involved in ferroptosis (41). It has recently been described that peroxisomes, which synthesize ether phospholipids, more precisely plasmalogens, are significantly contributing to the process of ferroptosis, and are significantly susceptible to the peroxidation process (42).

Signal molecules and signaling pathways directly or indirectly involved in the regulation of ferroptosis include, but are not limited to, activating transcription factor 4, nuclear factor erythroid 2-related factor 2 (Nrf2), tumor suppressor gene p53, Beclin-1 key regulator of autophagy, heat shock proteins, haem oxygenase-1, prostaglandin-endoperoxide synthase 2 gene (*PTGS2*), participants in the mevalonate pathway, etc. (43,44). Most regulators are associated in a specific way with other forms of cell death, so an important goal of future research is to identify those regulators which might distinguish ferroptosis from other types of regulated cell death. Almost all participants in the ferroptotic cascade are target genes of, for example, the Nrf2 transcription factor, indicating its critical role in mediating ferroptotic death (e.g. ferritin light and heavy chain, glutamate-cysteine ligase catalytic and regulatory subunit, ferroportin, GPX4, etc.) (45). It is a promising therapeutic goal for the treatment of some diseases, and therapeutic benefit is also expected from other molecules involved in metabolic/signaling pathways associated with ferroptosis, e.g., inhibition of glutaminolysis, inhibition of ferritinophagia, p53 pathway, and others (46,47).

The potential activators for possible manipulations in the process of ferroptosis are, for example, inhibitors of the X_c^- system (erastin, sorafenib), inhibitors of GSH synthesis and

glutamate-cysteine ligase, a key enzyme in the synthesis of GSH (buthioninesulfoximine), inhibitor GPX4 (RAS-selective lethal 3), accumulation of extracellular glutamate, cystine deficiency etc. In addition to the two main endogenous antioxidant systems (GPX4, FSP1), ferroptosis inhibitors may be an alternative source of cysteine (N-acetylcysteine), L-ROS removers (vitamin E, ferrostatin-1), iron chelators (deferrioxamine mesylate, deferriox) and other compounds (48).

The process of ferroptosis also involves molecules participating in other metabolic pathways that act simultaneously and directly and/or indirectly contribute to ferroptosis by generating L-ROS (33). These are, for example, the cellular mitochondrial tricarboxylic acid cycle and the electron transport chain, lipid metabolism, transsulfuration, mevalonate and pentosaphosphate pathways (49). Ferritinophagy plays a significant role in the control of iron homeostasis and is therefore associated with ferroptosis (47). The process is mediated by nuclear receptor coactivator 4 (NCOA4) which is required for the maintenance of intracellular and systemic iron homeostasis and physiological processes such as erythropoiesis (50). NCOA4 recognizes and binds the heavy subunit of ferritin and then delivers iron-bound ferritin to autophagolysosomes for lysosomal degradation. During this degradation the released iron is available for the Fenton reaction and induction of ferroptosis is enabled (51). Changes in ferritin expression therefore affect ferroptosis by altering the intracellular pool of free, redox active iron. It has recently been discovered that the membrane glycoprotein prominin 2 enhances resistance to ferroptosis by stimulating the export of ferritin from cells (52).

Ferroptosis is an inherently more immunogenic form of death than apoptosis, because the affected cells, in addition to oxidized lipid mediators, also release inflammatory cytokines, damage-associated molecular patterns (DAMPs), alarmin, high mobility group 1 (HMGB1) leading to proinflammatory condition and cell death (29,53). There is a complex relationship between ferroptosis and metabolism, arachidonic acid (AA), the most abundant PUFA in cell membrane phospholipids, and eicosanoid biosynthesis since ferroptosis is associated with increased *PTGS2* expression and prostaglandin release (29,54). It is also possible that cells undergoing ferroptosis also function as AA donors for transcellular biosynthesis of eicosanoids (55).

Despite numerous evidences of the importance of L-ROS accumulation as a consequence of intracellular iron accumulation, the exact mechanism of iron in the ferroptosis signaling pathway itself is still not entirely clear (56). Since iron homeostasis is a complex process and involves the coordination of various proteins e.g. divalent metal transporter 1, ferroportin 1, transferrin, hepcidin, ferritin and other molecules, it is to be expected that altered expression of proteins that regulate iron metabolism may define susceptibility to ferroptosis (30). A more detailed iron metabolism has been reviewed in other publications and is not discussed here (57).

In addition to altered hepcidin values it has recently been discovered that the serum iron transporter, transferrin and its receptor also play a significant role in ferroptosis (58). Furthermore, membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which contains 2 non-covalently bound heme with iron, participates in electron transfer from NADPH to oxygen and thus in the formation of superoxide radical (59). In a situation where GPX4 activity and the required antioxidant systems are inactive, this radical can react with membrane lipids and consequently it can cause cell death. It is not clear, and it is also discussed, whether ferroptosis can “spread” in a paracrine way, therefore, outside the affected cells. The main role in this is given to the relatively stable and easily diffusible end products of lipid peroxidation, 4HNE and MDA (60).

Ferroptosis is of particular interest because of its implication in the pathogenesis of age-related disorders. Unregulated ferroptosis may partly explain cell degeneration and tissue damage of pathogenesis e.g. intracerebral hemorrhage (61), atherosclerosis (62), cardiomyopathy (63), acute kidney disease (64), lung disease (65), and Alzheimer disease (66).

Biochemical, morphological and genetic changes are monitored in scientific research as markers of ferroptosis. Morphological changes of cells such plasma membrane rupture, decreased number of mitochondria, and mitochondrial membrane density, are monitored using an electron microscope. Biochemical changes include monitoring of a) iron accumulation (the changes in labile iron status by fluorescence test, FRET Iron Probe -1), application of inductively coupled plasma-MS, or staining with Perls Prussian Blue), b) lipid peroxidation e.g. peroxidized phospholipids with LiperFluo test, determination of MDA, etc. c) decrease in antioxidants (e.g. GSH concentration), d) increase in expression of specific proteins (e.g. activity of acyl-CoA synthetase long-chain family member 4), e) degradation of specific proteins (e.g. ferritin) and f) release of DAMPs. Of the genetic markers, identification of the regulation of the *PTGS2* gene can, for example, be used (67). Some markers also change in other forms of cell death and are therefore not specific to ferroptosis alone. It is expected that further research with the application of new technologies will reveal and also be able to define more specific markers in order to better understand the process of ferroptosis.

A HYPOTHESIS ON THE ASSOCIATION BETWEEN AGING, COVID-19 AND FERROPTOSIS

Aging is a continuous and inevitable process characterized by a gradual decline in the physiological functions of the organ systems which ultimately results in death. It involves a complex interaction of various unbalanced mechanisms such as altered energy metabolism, anabolism and catabolism, immune response, pro- and antioxidant values, which together also lead to chronic, mild inflammation (“inflammaging”), various disease states and end of life (68,69). The aging process cannot fully encompass

any of the many existing definitions and theories of aging, but according to many of them it is the closest one to the “free radical theory”. It implies the accumulation of oxidative damage as a consequence of the action of ROS over time and can logically be associated with iron dyshomeostasis.

During aging, the rate of metabolism decreases, and, among other things, the synthesis of proteins that require iron for function is reduced. These are, for example, various enzymes in the synthesis of DNA, hemoglobin and Fe-S clusters (26,70). Since there is no sufficiently effective mechanism for the elimination of iron from the body, aging unnecessarily accumulates tissue iron, which is associated with increased serum ferritin (71). In the systemic circulation, however, reduced iron levels are recorded and this change is explained by a chronic pro-inflammatory condition and the consequence of increased hepcidin expression (72). With aging also weakens the process of iron storage in ferritin and LIP increases.

Combined with a progressive decline in endogenous GSH and other antioxidant molecules during aging, the risk of cell death by ferroptosis increases (73). Iron dyshomeostasis that occurs during aging plays an important role in Alzheimer’s disease (iron contributes to aggregation and pathogenicity of β -amyloid) (74), *Mycobacterium tuberculosis* infection (75), Huntington’s disease (76), myocardial infarction (77), pulmonary diseases (78), diabetes (79), etc. It has also been described that T-cell lipid peroxidation during aging induces ferroptosis and thus alters the immune response to infection (80).

Some characteristics of the mitochondria change during aging, resulting in reduced mitochondrial respiration capacity and lack of energy. In infection with SARS-CoV-2, mitochondrial fusion is further increased, mitochondria produce more ROS, there is inappropriate mitophagy, and there is excessive amounts of iron in storage which can cause ferroptosis. This could partly explain the most severe disease outcomes in elderly comorbid patients but also be a guide to research in to COVID-19 therapeutic approaches. The accumulation of iron that damages DNA and blocks genomic repair systems, according to Sphère A. et al. have been termed “ferrosence”. According to preliminary research, this type of cell senescence is also implied as a stimulator of complications in patients with COVID-19 (81,82).

Cellular iron loading and ferroptosis, as a form of cell death, are therefore implied in the pathogenesis of COVID-19. Depending on the pathophysiological context, ferroptosis is characterized by duality in terms of ultimate beneficial effects for the cell (e.g., killing cancer cells) as well as negative, detrimental effects (contributing to neurodegenerative, cardiovascular disorders, type 2 diabetes, etc.). It is therefore justified to consider and define the role of ferroptosis in COVID-19 as well. Some of the clinical manifestations of COVID-19, such as inflammation, hyperferritinemia, hyperkoagulability, immune dysfunction, may indeed be associated with dysregulation of iron homeostasis, suggesting

the possibility of a ferroptosis process. As ferroptosis is an immunogenic process, it stimulates a series of reactions by amplifying the inflammatory response (29,83). Patients with COVID-19 have an increased number of proinflammatory CD4⁺ T cells and CD8⁺ T cells as well as dysregulation of iron homeostasis (84). As previously mentioned ferroptotic cells release DAMPs and activate the advanced glycation end products specific receptor to trigger the inflammatory response of peripheral macrophages and activate NF- κ B in innate immunity.

In the pathogenesis of COVID-19 Banchini F. et al. suggest the possibility of activation of hepcidin synthesis, tissue iron load especially mitochondria, L-ROS production and ferroptotic cell death and in connection with this a certain potential in terms of disease treatment (85). As in a milder form, similar changes occur in the aging process, it is assumed that manipulations in the process of ferroptosis could be a procedure for prolonging a healthy life, and that they have potential in terms of treatment COVID-19. Other researchers come to similar conclusions and recommend the most common iron chelators as an aid in therapy. Lactoferrin, for example, is thought to reduce tissue iron load but at the same time prevent the entry of SARS-CoV-2 into host cells (83).

Because hepcidin synthesis is associated with various protein molecules (e.g., Homeostatic Iron Regulator, IL-6, Toll Like Receptor 4), a disorder in their expression could be reflected in iron homeostasis. The lung macrophages of the elderly are thought to produce IL-6 more pronouncedly, which plays a critical role in creating a pro-inflammatory microenvironment that will contribute to the integrity of the air-blood barrier (alveolar – capillary barrier) (86). IL-6 also increases cellular ferritin and stimulates transferrin receptor-1, internalization of transferrin resulting in iron accumulation. Serum hepcidin concentrations progressively increase with age, are higher in men, and are also increased in COVID-19 patients (87,88). Furthermore, increased values of iron and iron metabolism regulators (transferrin, ferritin) were detected in bronchoalveolar lavage fluid patients with ARDS, disorder present in severely ill COVID-19 patients, and correlated with lung tissue damage (89). The same has been proven in experimental models.

All of the above indicates that important determinants of the ferroptosis process are present in varying degrees in the aging process and in the pathogenesis of COVID-19 and therefore ferroptosis could be an important link in the development of severe COVID-19 in the elderly. In order to better understand the pathogenesis of COVID-19, it is necessary to investigate and define the complex interaction between tissue iron loading, increased expression of hepcidin as well as the other molecules mentioned.

Uncontrolled systemic inflammation in COVID-19 patients is often accompanied by endothelial injuries, activation of the coagulation cascade by disseminated intravascular coagulation, and

consumption of coagulation factors leading to damage to other organs and death. The coagulation mechanism that is assumed appears to be a separate mechanism. Namely, it is hypothesized that in an inflammatory condition accompanied by increased hepcidin expression and iron sequestration, there is the possibility of an iron-activated abnormal coagulation system in small blood vessels and capillaries (90,91). Consequently, proteolytically stable clots form, which are presented as ground-glass opacity, which was previously established in diabetics (92). Consistent with these considerations with respect to iron homeostasis disorder, it would be interesting to consider the ferroptosis process in relation to this form of COVID-19 complication as well.

Sukhomlin T. in the pathogenesis of COVID-19 emphasizes the importance of disorders of local, tissue (lungs but also other organs) iron homeostasis. He suggests accordingly, to begin with on animal models, the measurement of markers of homeostasis disorders locally (hepcidin, total and free iron, ferritin) and not only in serum. In order to assess the risk of complications and for successful treatment, in addition to modulating inflammation and coagulation, he also suggests modulation of iron homeostasis (91,92).

The assumption of the need to modulate iron distribution in patients with COVID-19 is confirmed by a study of the therapeutic effect of erythropoietin. This hormone has an inhibitory effect on hepcidin synthesis and thus stimulates the utilization of iron for erythropoiesis (93). Furthermore, Singh et al. found that SARS-CoV-2 was dependent on binding to S-adenosyl-L-methionine, an intermediate of the homocysteine synthesis transsulfuration pathway. Because participants in this synthetic pathway are indirectly involved in the ferroptosis process, this finding may also link the ferroptosis process to SARS-CoV-2 infection (94). Studies on Vero cells have also demonstrated a direct inhibitory effect of SARS-CoV-2 replication on the expression of some selenoprotein mRNAs, including mRNA-GPX4. This certainly requires further research, especially in light of the established evidence on the association of selenium status in the diet and the outcome of SARS CoV-2 infections (95).

Biochemical markers of iron metabolism disorders, and thus potential cell death by ferroptosis, which are most often determined in the clinical treatment of patients with COVID-19, are generally insufficiently specific only for this disease. Indicators of the antioxidant system are determined more in scientific research. In assessing the presence of the ferroptosis process in the progression of COVID-19, based on published papers, it is currently recommended to determine the concentration of ferritin and hepcidin. In some works, persistent hyperferritinemia in the light of the so-called “long-COVID-19” is defined as a long prognostic marker, which certainly requires further research and confirmation in an appropriate number of patients (96).

Although this work has focused on iron accumulation and ferroptosis, it should be noted that hypophremia is common in elderly patients with COVID-19. The results of the study by Hippchen et al. demonstrate hypophremia (that is decreased

serum iron concentration) and decreased transferrin saturation and an increased hepcidin value, which, however, was weakly correlated with hypophremia (97). Due to the good association of hypophremia with the PaO₂/FiO₂ ratio in patients requiring oxygen therapy, this ratio is considered a reliable prognostic factor in COVID-19 (98). It is assumed that severe hypophremia in severe forms of the disease could be an indicator of a reduced response of the organism to hypoxia, a weakened immune response, which all contributes to the worsening of the disease.

CONCLUSION

Although iron is vital to all living organisms, the chemical property of fluctuating between different oxidation states also makes it a potentially toxic transition metal. Increased accumulation of cellular iron is an important driver of lipid peroxidation and thus ferroptosis and a participant in the occurrence of oxidative stress, a biologically important process that can contribute to increased sensitivity of a person to various strokes. Since ferroptotic cell death may be involved in the pathogenesis of various diseases, understanding of initiation and the underlying regulatory mechanisms of this process have potential therapeutic significance. A significant factor that precedes the ferroptotic process is inflammation of a milder chronic (aging) or stronger, acute (severe form of COVID-19) degree. The molecular interaction between systemic and tissue/cellular regulation of iron, in which hepcidin plays a central role and the inflammatory process, is therefore a new area of research crucial for understanding the aging process and the pathogenesis of COVID-19.

It has been repeatedly confirmed that age contributes to the severity of COVID-19. In the aging process and in the pathogenesis of COVID-19, iron metabolism is disturbed, which is the basis of the process of lipid peroxidation, the trigger of ferroptosis and thus the occurrence of oxidative stress. This certainly leads to depletion of the body's antioxidant systems, especially the cellular GSH/GPX4 system, which prevents the effective removal of the resulting L-ROS, which is also a feature of ferroptosis. There are fundamental risk factors for the process of ferroptosis (iron dyshomeostasis, L-ROS production and sulfhydryl depletion) which, according to the time required for cellular iron accumulation, can develop not only in the lungs but also in other organs (kidney, heart, liver, gut, and nervous system).

A common factor, the cause of iron dyshomeostasis and thus further adverse reactions that end in the process of ferroptosis is, inflammation-induced increased expression of hepcidin. This protein molecule is an indicator of an inflammatory reaction as well as iron dyshomeostasis. While, normal expression of hepcidin has a protective function, its overexpression occurring in COVID-19 can be detrimental and associated with a variety of disease symptoms. The putative common pathway of the aging process and COVID-19 that may result in ferroptosis is shown in Figure 2.

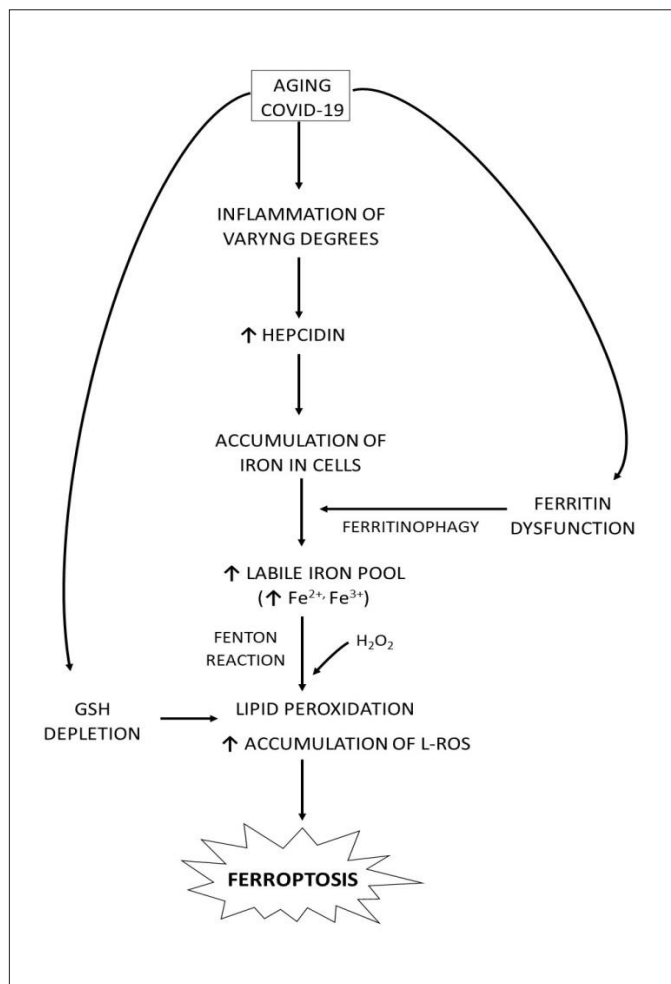


Figure 2. Assumed common pathway of the aging process and COVID-19 resulting in ferroptosis.

Ferroptosis is a recently discovered form of cell death so there are still gaps to be filled. To date, there are no effective therapeutic interventions to prevent accelerated aging and the aging of associated diseases, and the efficacy of some drugs for COVID-19 is still ongoing. In the case of aging and aging associated diseases there is a continuous, and in the case of COVID-19 an urgent need for new treatment strategies. Intracellular iron reduction or a new generation of ferroptosis modulators could be potential candidates for adjuvant therapy in combination with specific antiviral, anti-inflammatory, antioxidant approaches and approaches that more effectively strengthen the immune system of the elderly. The results of some preliminary research suggest that

interventions in the process of ferroptosis could play a significant role. Therefore, *in vitro* and *in vivo* studies on the role of ferroptosis in the aging process, the pathogenesis of COVID-19 (targeted induction or suppression of the process) are intensively continued. We hope that this review will be the basis for some future interesting research.

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