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Mini review

Hypothesis: Local Hepcidin Controls Abnormal Fibrosis Induced by Sars-Cov-2 via Regulation of Local Iron Homeostasis

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Submitted: 04 August 2021

Accepted: 18 August 2021

Published: 21 August 2021

ISSN: 2573-1033

OPEN ACCESS

Abstract

Abnormal fibrosis is the main cause of severe course and mortality induced by SARS-CoV-2 infection. The abnormality is reflected in the contradictory coexistence of fibrinolysis shutdown and elevated D-dimer levels, which is a marker of hyperfibrinolysis. This paradox may be resolved when coagulation is considered in concert with iron homeostasis. Cytokine storm induced by COVID infection is accompanied by oxidative stress followed by massive erythrocyte destruction and, therefore, it could result in insufficient control of local iron in injured lungs. Given that free ferric iron stabilizes fibrin clots, then interventions aimed to decrease free iron availability in lung tissues might prevent the severe course of ARDS or recover the damaged organ. To date, a cross-talk between local iron homeostasis and coagulation is underestimated. At the same time, research in this direction may provide us both with markers to discriminate the people susceptible to ARDS and to discover a new strategy to treat SARS-CoV-2 related ARDS.

Iron-Mediated Stabilization of Fibrine Clots Is Presumably the Core of ARDS in Covid-19 Patients

Abnormal fibrosis, a critical feature of COVID-induced pathology, is continuously discussed [1-6] since SARS-CoV-2 infection became a global pandemic. The abnormality seems to result from a contradictory coexistence of fibrinolysis shutdown and elevated D-dimer levels in patients with severe COVID-19 infection. The contradiction is not resolved yet. As high D-dimer levels are markers of hyperfibrinolysis thus fibrinolysis is overactivated but physiologically insufficient in COVID-19 patients suffering from ARDS. A link between fibrosis and iron homeostasis might resolve the paradox.

Recently it was supposed [7] that fibrinolysis shutdown in COVID patients [1-3] might result from fibrin clot stabilization by free ferric iron (Fe^{3+}) unraveled in series of research *in vitro* [8-9]. In COVID-19 patients, the fibrin clots presumably contain proteolytically stable links produced by redox reactions catalyzed by ferric iron in addition to regular peptide bonds. If so, the proteolytically stable areas of oxidized fibrin nets slow down the dynamics of fibrinolysis. Thus, fibrinolysis remains persistently activated as there is a need to dissolve the clots with subsequent D-dimer levels elevation. The continuous iron influx in inflammatory conditions stabilizes the clots.

Figure 1 briefly demonstrates how a cross-talk between normal fibrosis and iron homeostasis disturbance may result in abnormal clotting. Destructive events are shown in red while preserving pathways in green. COVID-19 infection induces hyperactivation of the immune system, initiating a cytokine storm followed by oxidative stress and high body temperature presumably induced via IL6. These events increase in vessels permeability. The SARS-CoV-2 virus gets cells via membrane angiotensin-converting enzyme 2 (ACE2) protein, thus suppressing the anti-inflammatory pathway linked to the ACE2. In this way, SARS-CoV-2 virus amplifies inflammation and associated oxidative stress via a disbalance between pro-inflammatory and anti-inflammatory pathways operated by ACE and ACE2, respectively [10]. Proinflammatory stimuli activate NF-κB transcription factor driving tissue factor (TF) expression [11] and subsequent TF release into bloodstream followed by massive clot formation in small vessels and capillaries. TF protein expression was shown 2.1-fold higher in COVID-19 vs. non-COVID-19 ARDS lungs ($p=0.0048$) and 11-fold ($p<0.001$) higher than in control lungs [12]. TF induces temporal fibrin clot formation aimed to prevent bleeding.

Simultaneously, oxidative stress induces massive erythrocytes destruction [4] and the subsequent dramatical influx of catalytically active iron from damaged erythrocytes. Transferrin (Tf) binds free Fe^{3+} in safe state (Tf*Fe) which in physiological condition returns iron to systemic iron turnover. Damaged erythrocytes, senescent and infected cells are engulfed by phagocytes, mainly macrophages. In addition, macrophages

uptake free hemoglobin via CD163, free ferrous iron (Fe^{2+}) via Divalent Metal Transporter (DMT1), and ferric iron (Fe^{3+}) bound to Tf via transferrin receptors (TfRs). In inflammatory conditions, especially when blood circulation is disturbed, local transferrin binding capacity may be overloaded, and free iron ions appear. At this moment, iron becomes a hazard as a catalyst in the Haber-Weiss cycle (yellow square). The cycle both produces harmful hydroxyl radical in Fenton reaction by an interaction of Fe^{2+} with peroxides and recycle free Fe^{3+} iron ions. The free Fe^{3+} ions presumably initiate additional redox links in fibrine clots, which results in a slowdown of fibrinolysis normally mediated by proteases. Therefore, to stop the abnormal clotting and to rebalance fibrosis and fibrinolysis, a removal of excess free iron from inflamed tissues is needed.

In blood samples used for elastography the clots are more stable than in health. Still, they are dissolved with time, while in the infected lungs, due to continuous iron influx followed by iron-induced fibrin clots stabilization, they remain longer. Therefore, iron-mediated red-ox stabilization of fibrin clots is highly likely the core of ARDS in COVID-19 patients. (Figure 1)

What has happened to the local iron homeostasis in COVID19-infected lungs?

A link between acute respiratory distress syndrome (ARDS) and disrupted iron homeostasis is reflected in a growing but still limited number of recent publications. Increased levels of serum ferritin are markers of severity in COVID-19 in patients [13-16]. A correlation between disease severity and serum hepcidin levels in hospitalized COVID patients recently observed by Zhou et al. [16] and Nai et al. [17] confirms an iron involvement in persisting coagulopathy. Nevertheless, to the best of my knowledge, there is still no clinical data regarding a link between local iron homeostasis state and SARS-CoV-2 pathology. In former time, Ghio et al. [18] demonstrated essential modification of iron homeostasis characteristics (total iron, non-hem iron, ferritin, etc.) in lavage of ARDS patients compared to health volunteers. Authors have compared levels of iron, ferritin, haemoglobin, haptoglobin in serum (systemic iron homeostasis) and lavage (local iron homeostasis). The levels of those characteristics in lavage happened to be in about 10 times higher than in serum, demonstrating dramatical local iron homeostasis deregulation in lungs. Similar results were obtained in patients with cystic fibrosis [19]. The authors concluded [18] that successful resolution of ARDS requires a restoration of normal iron homeostasis in the lung. This conclusion should be extended to pathology induced by SARS-CoV-2 infection. (Figure 2)

Basically, tissue resident macrophages recycle iron locally. They engulf the senescent cells, proceed the iron-containing intracellular proteins and release the iron into interstitial fluid to be used for growing and differentiating tissue cells. Infection and subsequent local inflammation initiate local hepcidin expression to prevent the harmful iron activity (see Figure 1). Figure 2 represents an autocrine mechanism of local hepcidin function in SARS-CoV-2 infected lungs. Iron homeostasis is tightly regulated [20] to support a safe concentration of free iron (0-0,3 μM). In inflammatory conditions, when iron becomes catalytically active the task to hold iron in the state unavailable for superoxide radical and peroxides becomes critical. The first line of security is iron binding to transferrin circulating between body fluids (blood, lymph, and interstitial fluids). When iron efflux exceeds the transferrin-binding capacity (in normal conditions Tf saturation is below 40%), an expression of hepcidin, as a master regulator of iron homeostasis, is induced locally in macrophages [21] or epithelial [22] cells by inflammatory cytokines like IL-

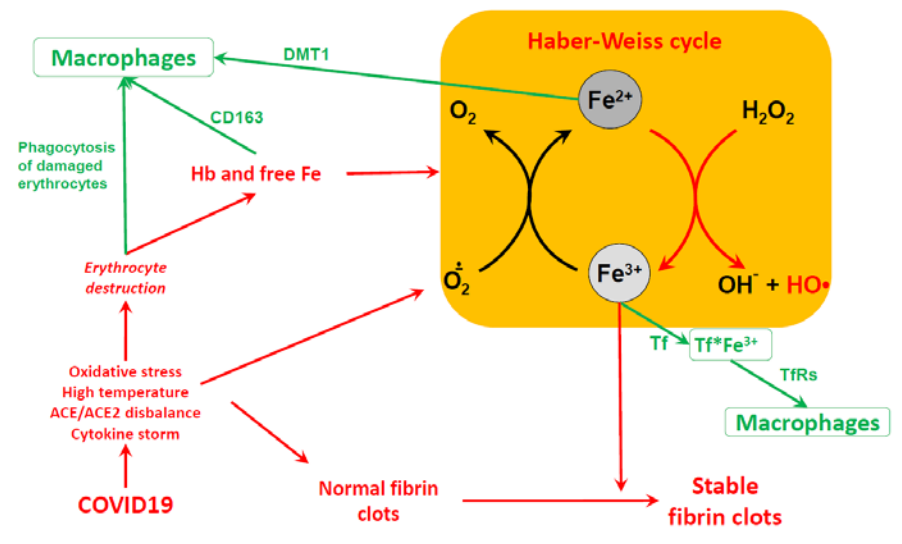

Figure 1 A cross-talk between normal fibrosis and iron homeostasis disturbance may result in abnormal clotting. Inflammatory events result both in the initiation of fibrosis and in excessive free iron release (shown in red). If the iron-binding capacity of transferrin in blood and interstitial fluid is saturated and iron-sequestrating capacity of macrophages (shown in green) is overloaded, free iron ions appear, and the Haber-Weiss cycle (central part) becomes active. The Haber-Weiss cycle recycles Fe^{3+} and produces hydroxyl radicals. The free Fe^{3+} ions are potent to induce abnormal fibrosis. The hydroxyl radicals produced by interaction of Fe^{2+} ions with peroxides (Fenton chemistry) are responsible for the harmful, destructive effect of free iron in oxidative environment.

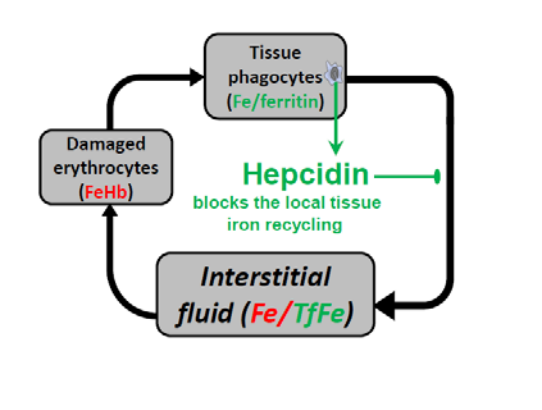

Figure 2 Local iron recycling. The hepcidin expressed locally in COVID-infected lungs presumably prevents free iron accumulation in lung fluids. The hepcidin temporally blocks local iron recycling locking the accumulated iron inside of iron-sequestrating cells.

6. A physiological role of hepcidin is protecting [20,22-25]. Mechanistically, hepcidin down-regulates the only iron exporter ferroportin to lock the iron inside cells, thus, blocking local iron recycling to prevent tissue injury and the subsequent abnormal coagulation. In injured tissues, phagocytes sequestrate iron from extracellular space and accumulate it safely inside of macrophages in the ferritin complex. Therefore, hepcidin does not allow the cells to release iron via ferroportin while ferritin collects it in a safe magnetite-like state inside a stable ferritin complex. Since local iron recycling is blocked by tissue hepcidin then the iron accumulation in the iron-sequestrating cells must be followed by a removal the iron overloaded cells from injured tissue via lymphatics to bone nodes where they could be allowed to release the sequestrated iron into circulation.

When both local transferrin-binding capacity in physiological fluids and iron-sequestrating capacity of macrophages are saturated, the control of iron-mediated fibrin clot stabilization by iron homeostasis is lost, leading to a superposition of elevated D-dimer levels and shut-down of fibrinolysis. If so, the only way to stop clot stabilization is to remove free iron from the clot areas. Figure 3 represents two main ways of iron efflux (green arrows) from damaged tissue: (1) reversible ferric iron binding to the main iron transporting protein transferrin in body fluids with subsequent efflux into the systemic circulation due to link between physiological fluids (interstitial fluid, lymphatics, and blood) to be transferred to iron-sequestrating organs (liver, spleen, bone marrow); (2) iron accumulation in tissue phagocytes (macrophages, dendritic and some other cells) for safe storing in ferritin complex followed by overloaded cells migration to lymph nodes. In inflammation, both ways could be temporarily overloaded, making free iron ions available in extracellular spaces to initiate iron-induced pathology. (Figure 3)

Usually, the disturbance of the local FeH is monitored by serological parameters. Unfortunately, in line with essential difference in local (lavage) and systemic (serum) characteristics of the iron homeostasis [18-19] observed in ARDS, the systemic parameter hardly might be used to monitor the state of iron homeostasis in lungs infected by SARS-CoV-2 without careful investigation of the relationship between local and systemic parameters. To date there is still a limited number of

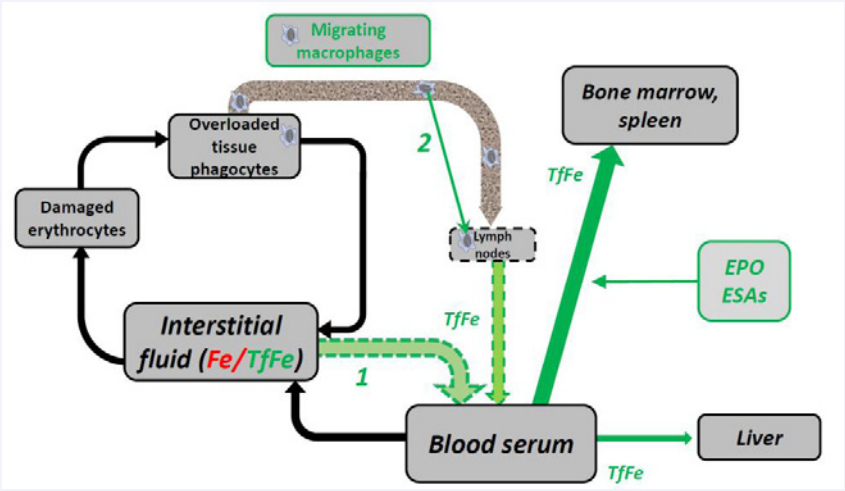

Figure 3 Two ways of iron removal from the damaged organ: (1) transferrin-bound iron efflux via body fluids circulation; (2) supporting of migration of iron-sequestrating macrophages together with the iron incorporated in ferritin to lymph nodes.

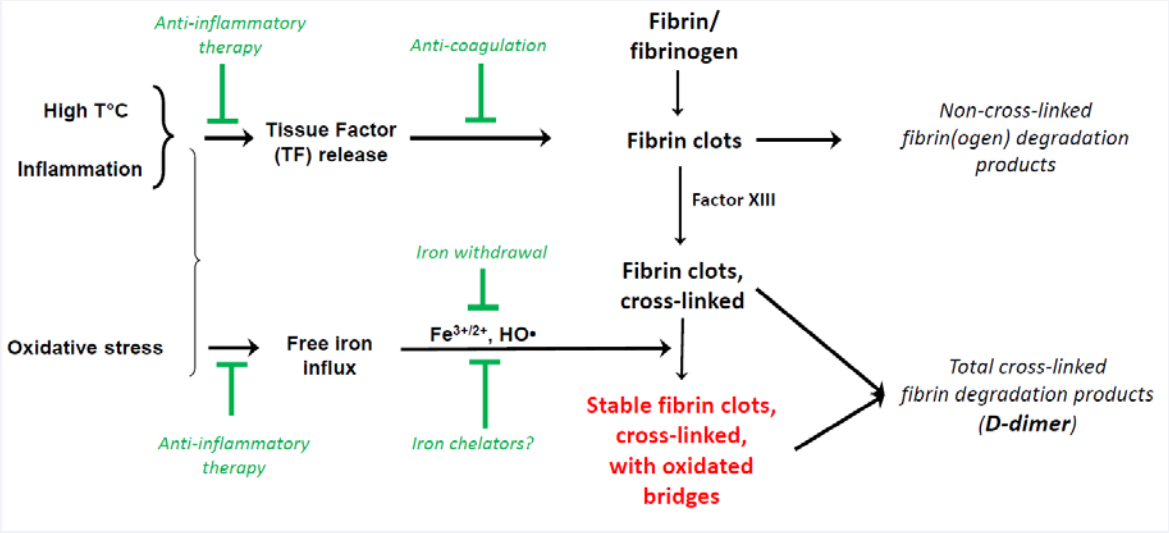

Figure 4 A cross-talk between normal fibrosis and iron homeostasis disturbance may result in abnormal clotting induced by SARS-CoV-2 resulted from the disturbance of local iron homeostasis. Additional redox links in fibrine clots slow down the fibrinolysis. Removing free iron excess from inflamed tissues could stop the abnormal clotting and rebalance fibrosis/fibrinolysis. Possible targets to prevent or treat ARDS are shown in green. These are: (1) anti-inflammatory or antioxidant interventions; (2) appropriate anticoagulant therapy; (3) approaches to decrease iron availability in injured lungs (endogenous hepcidin expression, iron chelation, enhancement of the iron withdrawal from the lung tissue).

publications in which an extension of the disturbance of the local FeH to a systemic level (lymphatics and blood) have been detected. Remarkably, as fibrinolysis shutdown was observed by thromboelastographic method in blood samples of infected patients, therefore, the iron homeostasis disturbance is already extended from local to the systemic level. The extension, in turn, may result in systemic hepcidin increase and subsequent anemia of inflammation manifestation.

Therefore, there is an urgent need to confirm local iron homeostasis disturbance and to look for the ways to recover it.

CONCLUSION AND PERSPECTIVES

A growing number of clinical research [13-17, 22, 26-29] and discussions [7, 23-24, 30-32] argues for crucial role of iron homeostasis disturbance in SARS-CoV-2 pathology. Nevertheless, only a few researches [22, 28] came close to a role of local iron deregulation in lung complications associated with SARS-CoV-2 infection. Obviously, dramatical disturbance of local iron homeostasis formerly observed in ARDS [18] and cystic fibrosis [19] must be checked in the lungs of patients with severe course of SARS-CoV-2 infection. The deep study of interdependence between local iron homeostasis and coagulation in SARS-CoV-2 pathology would reveal the mechanisms responsible for ARDS manifestation, progression, and modulation. Moreover, the research could help to discriminate the people predisposed to severe disease course and to elaborate the way to prevent ARDS. Three main research directions, better in combination, could be suggested to start with to look for reasoned approaches to predict or prevent, or treat ARDS. These are:

1. To compare the iron homeostasis characteristics in lavage and serum of SARS-CoV-2 patients. The comparison could unravel the most informative parameters and provide a possibility to follow the critical changes in lungs via serological markers.
2. To look for a correlation between parameters of both iron homeostasis and coagulation in lavage and serum to find out cause-event relations between the parameters.
3. To follow the crucial parameters in dynamics to define the time course of the iron homeostasis and coagulation cross-talk and criteria when the appropriate intervention may be applied. (Figure 4)

Figure 4 represents schematically how fibrin clots formation interacts with deregulated local iron homeostasis. Each step of the scheme determines the severity of the COVID-induced pathology. Accordingly, intervention to any step may prevent or modulate (shown in green color) the disease course. Both fibrin clots formation and iron homeostasis disturbance result from local inflammation, which in COVID patients usually is associated with cytokine storm and oxidative stress. Several approaches were elaborated to slow down the inflammation and cytokine storm, although they hardly could stop the development of unwanted pathological processes totally. Unfortunately, the step of iron-induced clot stabilization was not modulated by the applied treatments. Exception probably is the Hadadi et al. case report [33], which, hopefully, unraveled that iron withdrawal from injured lungs may help to recover [34]. Unsuccessful attempt to check the ability of epoetin to treat mild course disease [35] indirectly demonstrated that epoetin treatment might help only susceptible patients, presumably, those ones who are suffering from any kind of preceding iron homeostasis disturbance, for instance, already settled anemia of inflammation.

In conclusion, further research of the relationship between local iron homeostasis and fibrosis is promising as it could provide the ways how: (1) to discriminate the persons susceptible

to severe course of SARS-CoV-2 pathology; (2) to look for new strategy for SARS-CoV-2 pathology treatment; (3) to monitor the dynamics of the pathology which could reveal the time when treatment strategy should be changed.

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Cite this article

Sukhomlin T (2021) Hypothesis: Local Hepcidin Controls Abnormal Fibrosis Induced by Sars-Cov-2 via Regulation of Local Iron Homeostasis. Ann Food Process Preserv 5(1): 1028.