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Iron: A double-edged sword

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A mechanistically oriented study in the current issue of Transfusion, by Suffredini et al., uses an animal model to investigate whether intravenous iron therapy or transfusion with "fresh" RBCs is better for treating anemia in critically ill patients.[1] This report is particularly timely given that concerns about the risks of blood transfusion have inspired patient blood management efforts to reduce transfusions by using alternative therapies, such as intravenous iron.[2] Furthermore, there are concerns whether the acute delivery of iron, either in soluble form or packaged in RBCs, predisposes patients to new infections, converts "benign" bacterial colonization into virulent infection, or enhances the virulence of existing infections.[3] These authors carefully address this issue by exploiting a well-described canine model of bacterial pneumonia and septic shock that they characterized previously.[4] This large animal model is valuable for studying underlying mechanisms and therapeutic interventions that could improve the care of human patients with septic shock.

The focus on iron is relevant because of its importance for the biology of humans and their disease-causing pathogens. Given the ease and rapidity with which iron interconverts between ferrous and ferric forms, it plays key roles in redox reactions and, therefore, has important functions in many diverse proteins. For example, in addition to its role in oxygen delivery by hemoglobin and myoglobin, it functions in the reaction centers of multiple enzymes found throughout the body, including in the immune system. However, iron's redox biology can also produce damage; for example, "free" iron produces reactive oxygen species in the Fenton reaction, which cause oxidative stress and harm host tissues by oxidative damage of proteins, lipids, and nucleic acids. Thus, because iron behaves as a two-edged sword, tight regulation of its availability and biological accessibility are critically important. To this end, multiple chaperones and iron-binding molecules prevent adverse redox effects by minimizing the availability of "free" iron. For example, transferrin, the primary iron-binding and iron-transport protein in the circulation, has an incredibly high affinity constant for iron. In addition, under normal conditions, an excess of non-liganded transferrin circulates (i.e., transferrin saturation is normally <30%); in this way, when "free" iron appears in the circulation, it rapidly binds to transferrin, thereby preventing its participation

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in redox reactions. Only when transferrin is almost fully saturated does non-transferrin bound iron appear, which can induce multiple pathological consequences.[3] Analogously, ferritin, the primary iron-binding and iron-storage protein inside cells, helps maintain low levels of "free" cytosolic iron. Thus, although the human body contains 3-4 grams of iron (mostly in hemoglobin), only vanishingly small concentrations of free, non-protein-bound iron are available under normal circumstances.

From a research standpoint, we are currently in a "golden age" of iron biology.[5] Over the last decade or so, many molecules relevant for iron metabolism were identified and their mechanisms continue to be elucidated. These include, but are not limited to, haptoglobin and its receptor CD163, hemopexin and its receptor CD91, hepcidin, ferroportin, erythroferrone, human hemochromatosis protein (HFE), hemojuvelin, Steap family proteins, ZIP14, Natural resistance-associated macrophage protein-1, divalent metal transporter-1, and Lipocalin-2.[5] These discoveries suggested novel therapeutic interventions, pinpointed the genetic underpinnings of multiple human disorders, and provided new tools for further investigation of humans and animal models. Similarly, there has been an explosion of new knowledge and new tools relevant to understanding iron metabolism in microorganisms.[6,7]

Of course, iron has a prominent place in transfusion medicine based on its role in hemoglobin function. In addition, there are active on-going discussions about iron deficiency in volunteer blood donors, whether it is medically important, and whether steps should be taken to avoid it.[8] There is also active discussion regarding whether non-transfusion bound iron appears following stored RBC transfusions, and whether it causes clinically relevant adverse outcomes.[9] This issue led to the focus of the current paper;[1] that is, should one treat anemia in critically ill patients with RBC transfusions, which could potentially induce circulating non-transferrin bound iron? Or would it be better to treat these patients' presumed iron deficiency with intravenous iron infusions, which definitely would increase non-transferrin bound iron levels? Although there were prior concerns about the use of intravenous iron because of its association with severe anaphylactic reactions, these concerns have been mitigated by the development of new formulations, which can be safely used in multiple settings.[10,11] Therefore, intravenous iron is now used more frequently and for a wider array of indications, thereby supporting the timeliness of the current paper.[1]

To put the potential risks of non-transferrin bound iron into a broader context and explain the concerns regarding infectious diseases, a short discussion about nutritional immunity is warranted, with a specific focus on iron. Nutritional immunity is a process by which a host organism sequesters trace nutrients, such as iron, in an effort to limit infection. The resulting decline in freely available iron starves the invading pathogens. Thus, vertebrates can sequester iron within cells and in circulating iron-binding proteins, making it an important defense mechanism against both intracellular and extracellular pathogens.[7,12-14] In addition to its important role in cooperating with innate immunity, nutritional immunity can also modulate adaptive immune responses; indeed, both iron deficiency and iron overload affect cellular immunity.[15.16]

In relationship to mammalian hosts, various microorganisms behave as commensal "parasites" (i.e., the microbiome) or as pathogens. Some microbes are only commensals

(e.g., lactobacilli) or pathogens (e.g., rabies virus), whereas others, which are typically considered to be commensal, can also be pathogenic under the right conditions (e.g., *Staphylococcus epidermidis* in the setting of iron overload[17]). Thus, there is a delicate balance and interplay between the host and invading pathogens. Because iron is existentially important for virtually all living organisms, with both benefits and drawbacks, there is fierce competition for iron between the host and the commensal/pathogen. To this end, multiple mechanisms allow the host to "withhold" iron from the pathogen, both intracellularly and extracellularly. For example, inflammatory responses can produce interleukin-6, which induces hepcidin production. Hepcidin then down-regulates ferroportin expression, thereby decreasing gastrointestinal iron absorption and increasing iron retention by macrophages, resulting in iron being withheld from extracellular pathogens (e.g., in *S. aureus* sepsis). Unsaturated transferrin, which binds free iron in the circulation and subsequently delivers it to cells for storage in ferritin, also limits iron's availability to extracellular pathogens.

Taken together, given the complexity of mammalian and prokaryotic iron metabolism and biology, it is difficult to make generalized statements, and more nuanced conclusions are frequently required. One example is the distinction between extracellular pathogens (e.g., *S. aureus*) and those adapted to intracellular life (e.g., *Salmonella typhimurium*).[18,19] To this end, iron-withholding mechanisms that protect the host from *S. aureus* infection, by increasing macrophage iron retention and inducing a state of hypoferremia, can thereby enhance the virulence of *S. typhimurium*, which preferentially infects macrophages.[19] Therefore, the protective nature of nutritional immunity for one pathogen can put the host at extreme risk for another pathogen. For example, in the context of the current paper,[1] elevated levels of circulating non-transferrin bound iron are, indeed, extremely dangerous for *S. aureus* sepsis, but would be less so for a sub-clinical *S. typhimurium* infection.

In contrast, multiple mechanisms allow pathogens to adapt and obtain the iron necessary for their growth and metabolism, even in "hostile" environments. For example, bacteria can "go fishing" by secreting siderophores, which are small molecules that chelate iron with higher affinity than transferrin, to extract and capture iron from the host.[6,7,14] In particular, abundant literature describes iron acquisition mechanisms by *S. aureus* and iron withholding strategies by the host during infection by this pathogen.[6]

A related issue involves the concept of ferrophilic and non-ferrophilic microbes; that is, although iron is very important, the ability of some organisms to survive and thrive depends more critically on adequate iron availability, as compared to others. In addition, some pathogens adapt better to iron withholding environments than others. The most extreme example of this phenomenon is a non-ferrophilic pathogen, *Borrelia burgdorferi*, the etiologic agent of Lyme disease, which is one of the very few organisms, along with *Lactobacillus*, that does not require iron at all;[20] therefore, "extra" iron availability would not enhance this pathogen's virulence *per se*.

In addition, different outcomes result from different methods of iron delivery, different pathways to iron overload, and different cell type specificities of iron overload. That is, in many studies, different types of iron overload predisposed patients to different types of infection. Thus, patients with chronic iron overload due to transfusion-induced

hemosiderosis experience specific infections, such as *Salmonella*-induced osteomyelitis in sickle cell disease patients and related infections in thalassemia patients.[21-23] Analogously, *Yersinia* infections are classically seen in hereditary hemochromatosis.[24] In contrast, acute iron "toxicity," such as after iron supplementation in humans[1,25,26] or after stored RBC transfusions in animal models,[3,27] enhances different types of infection depending on whether excess iron is delivered intracellularly to macrophages, or circulates extracellularly as non-transferrin bound iron. Finally, concepts regarding nutritional immunity and infection are not limited to bacterial pathogens, but are also relevant to mycobacteria, fungi, viruses, and parasites.[7,13,24] Thus, given the current philosophical focus on "precision medicine" and the complexity of iron biology for both host and pathogen, it is difficult to endorse generalized concepts to the effect that "iron is bad for infection;" the more modest conclusion is "it depends."

Given the issues described above, although the paper by Suffredini et al. is highly focused with clean and interpretable data,[1] some questions remain. For example, although the title describes a model of pneumonia, which it certainly is, it might be better described as a model of septic shock, for which it was initially developed.[4] In addition, they grafted an acute hemorrhage-resuscitation model onto their original model. Although the 25% blood loss was described as "mild anemia," this new model actually adds a significant acute hemorrhage onto the already fairly dramatic event of septic shock. Indeed, this disease model is so dramatic that ~50% of the dogs receiving the control therapeutic intervention died (i.e., "fresh" RBC transfusion), even when they used doses of bacteria that produced no mortality in their earlier publications,[4] which did not involve acute hemorrhage. Thus, the current model may not represent the "typical" Intensive Care Unit patient (if there is such an entity),[2] but would be more analogous to a critically-ill trauma patient, or one with a significant gastrointestinal or post-partum bleed, or following surgical hemorrhage. In this case, it would be surprising if physicians only chose between intravenous iron versus RBC transfusion to address an acute blood loss requiring immediate intervention, because iron supplementation requires ~7 days to produce a reticulocytosis. In contrast, in a different, more typical, Intensive Care Unit patient, who might have a more slowly developing anemia, its likely source would be iatrogenic phlebotomy for laboratory testing combined with the anemia of chronic disease; the latter is an iron-withholding state due to elevated hepcidin levels and would be less likely to respond to iron therapy. However, despite these caveats, their model is valuable and reproducible, and provides useful insights.

Although not explicitly stated in the current paper,[1] the *S. aureus* strain used, a human clinical isolate,[28] was presumably sensitive to oxacillin (a β-lactamase resistant penicillin); they previously documented its sensitivity to ceftriaxone,[4] a cephalosporin with similar therapeutic activity. Thus, it is interesting that the animals' illness progressed despite their receiving oxacillin, beginning at 4 hours after study initiation and continuing throughout the 4-day observation period. Nonetheless, this phenomenon is similar to their previous reports[4] and to what is seen in human sepsis, where septic shock can proceed despite antibiotic treatment and negative blood culture results. In addition, although not definitively documented, the mortality seen in their model is presumably due to septic shock, as seen previously.[4,28] However, it would have been instructive to perform bacterial counts in various organs; these additional data could have provided some insight regarding whether

the intravenous iron led to worse outcomes by enhancing proliferation of this pathogen or by enhancing its virulence (e.g., increasing toxin production[6]).

Finally, the degree of hemoconcentration seen in their infected animals was significant and surprising, at least to us. Indeed, it was so significant that there were no differences between animals that had, or had not, received transfusions. In addition, these hemoglobin levels were higher than those at baseline, even though the animals lost 25% of their RBC volume. Interestingly, they observed this phenomenon previously using a similar model that did not involve hemorrhage.[4] Although the cause of this rather impressive hemoconcentration is not completely clear, it may be due to the high splenic reserve in dogs, which is released in response to various stressors;[29] thus, these results differ from what one would expect in humans.

In summary, this new paper[1] is an important contribution to our understanding of the roles of RBC transfusion and intravenous iron supplementation/repletion in the setting of septic shock caused by *S. aureus*, a Gram-positive, ferrophilic, extracellular pathogen. However, given the complexities described above, we believe that statements such as “treatment of patients with anemia with IV iron should be undertaken with caution in the setting of established infection,” although true, may be too generalized based on the data presented. Instead, we believe that more nuanced conclusions are appropriate, particularly now that “precision” or “personalized” medicine approaches are in vogue. Indeed, bacterial infections (along with RBC transfusions) were one of the earliest instances of “personalized medicine,” in which the invading pathogen is “genetically” characterized to the genus and species level, and then tested for its sensitivity or resistance to small molecule therapeutics (e.g., antibiotics), thereby enabling physicians to provide the right drug to the right patient at the right time.

Therefore, although precise clinical recommendations regarding intravenous iron therapy in the critical care setting are not currently clear, the results of the recently completed IRONMAN trial[2] may point the way forward. In this small, double-blind, randomized clinical trial in Intensive Care Unit patients without severe sepsis, intravenous iron did not reduce transfusion requirements, as the primary outcome. Nonetheless, at discharge, there was a statistically significant, albeit modest, increase in hemoglobin levels (107 vs. 100 g/L in iron vs. placebo, respectively), as a secondary outcome. Although the rate of nosocomial infection was fairly high in both groups (28.6% and 22.9% in iron vs. placebo, respectively), the difference in this secondary outcome was not statistically significant; however, the study was not powered to address this issue. Therefore, although providing clinical advice is difficult at this time, one must balance the potential risk that this intervention may worsen certain infections (Suffradinin et al.[1]) against the potential benefit that it may only modestly increase hemoglobin levels,[2] because these patients may not have an erythropoietic response to iron. Thus, the field would benefit from the completion of a sufficiently powered, randomized clinical trial, similar in design to the IRONMAN trial, with infection as the primary outcome (with infecting pathogens identified specifically), and which would take a more individualized approach towards identifying anemic Intensive Care Unit patients who might benefit from iron therapy (e.g., those with both low iron and low hepcidin levels).

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