Taken by Storm:
The Legacy of Covid-19 and the Future of Heparin

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Abstract: Despite exposure mitigation and vaccination, the SARS-CoV-2 infection continues to be an enormous threat to the vulnerable and an ongoing challenge to the medical community. Investigations into how the virus infects, how it spreads, and how the immune system responds have identified therapeutic approaches that can halt the cascade of events that lead to the loss of life. In this

regard, heparin has emerged as a particularly effective therapeutic, capable of addressing several key pathological events that occur during the course of the disease. Notably, heparin can limit the severity of an immunological defense mechanism known as immunothrombosis. In immunothrombosis, platelet-driven clot formation serves to isolate, immobilize, and contain the invading pathogen, impeding its spread from the alveolar microcirculation into the general circulation. Immunothrombosis is the platelets' attempt to avert a disaster and save a life. Aside from clot formation, the platelet is programmed to respond to the viral threat in a variety of ways. The platelet can engulf and internalize the virus with lethal intent. The platelet can release antiviral molecules that disable and destroy. And the platelet can act to recruit other immune cells and compel them to participate in a coordinated defense against the virus, shaping their functions and directing their actions. Given these behaviors, the platelet should be regarded as an immune cell and recognized as the immune cell relied upon to orchestrate the final defense against SARS-CoV-2 invasion. However, this defense is not without collateral damage, even death, and should be therapeutically addressed. Let's see how it all plays out in this narrative review.

Introduction

> *During infection, pathogens and their products influence the platelet response and can even be toxic. However, platelets are able to sense and engage bacteria and viruses to assist in their removal and destruction. Platelets greatly contribute to host defense by multiple mechanisms, including forming immune complexes and aggregates, shedding their granular content, and internalizing pathogens and subsequently being marked for removal.* **∼Page and Pretorius, 2020**

> *Severe SARS-CoV-2 infection* ***mostly presents with coagulation abnormalities****, pulmonary microvascular thrombosis, and severe inflammatory response.* **∼Fard et al., 2021, emphasis added)**

> *The cause for which patients with severe COVID-19 die is not related to the damage caused by the replication of the SARS-CoV-2 virus but rather it seems to be linked to the so-called cytokine storm.* **∼Lete, 2021**

Although the virus known as SARS-CoV-2 is new to the human experience, the immune response to the virus is not. The invading virus awaits an immune

system capable of mounting a formidable defense. And most often, the virus is vanquished with little notice and with little fanfare. However, for many, the war effort is a little messy, yet a successful outcome follows, and life goes on. On the other hand, should the infection be sufficiently advanced and viral replication accelerates unabated, a life is threatened, and it is all-out war. Enter the platelet.

Surprisingly, in COVID-19, the platelet plays a major role in host defense, as the review will outline. But before all-out war breaks out and the platelet takes matters into its own hands, and does so under the most difficult of circumstances, SARS-CoV-2 must first establish a foothold (Koupenova, 2020). To infect and position itself to replicate, it must succeed in locating and establishing physical contact with a surface bound cellular receptor known as ACE2 (Janardhan et al., 2020; Savla et al., 2021). Fortunately, for the virus, the ACE2 receptor is found residing on several easily accessible cell types of the human body (Janardhan et al., 2020). Receptor engagement is regarded as a requirement for successful viral entry and subsequent replication (Tree et al., 2021)—in the life cycle of a virus that supposedly isn't even alive. But it's not as if we are defenseless. We have our ways and we have our means of preventing a virus from reaching ACE2 and other relevant structures, at least that's the plan.

Our initial defense against respiratory viruses is a layer of mucus that covers the ACE2-expressive cells that line the upper airway (Bridges et al., 2021; Gupta et al., 2021). These cells, including the vulnerable cells that surround the eye (Gupta et al., 2021), are the initial targets of the SARS-CoV-2 virus (Bridges et al., 2021), simply because they are the cells most easy to infect. Fortunately, within the layer of mucus we have endogenous antivirals, prepositioned to bind viruses that challenge this initial layer of defense. Lactoferrin serves as an example (Kell et al., 2020; Weinberg, 2001; Zimecki et al., 2021). But there are vulnerabilities, allowing the virus to succeed.

"In the nose, ciliated cells are the primary target cells for SARS-CoV-2 viral infection, replication and release. Infected cells shed their cilia, which disables mucociliary clearance." (Bridges et al., 2021). Furthermore, and perhaps for reasons not entirely clear, the vulnerable may have a *"suppressed or incompletely activated innate immune response to SARSCoV-2 infection in the upper airways."* (Bridges et al., 2021). Yet, even at this point in the disease process, it may all be asymptomatic (Bridges et al., 2021), meaning you never know you are infected,

and you never know you are passing it on. Indeed, *"Asymptomatic individuals can still have a productive viral infection and infect others."* (Bridges et al., 2021) This is but one reason why the virus spreads so easily throughout a population. It is quite unstoppable.

Unfortunately, SARS-CoV-2 is not content to infect the upper airway (or an eye socket or two). Its next objective is to reach the air-exchange units of the lung, the alveoli, also rich in ACE2 (Janardhan et al., 2020). *"The extension of SARS-CoV-2 infection into* ***the gas exchange portions of the lung is the primary cause of severe morbidity and mortality*** *in patients with COVID-19."* (Bridges et al., 2021, emphasis added). It is in the alveoli where the invasion must be stopped.

Perhaps the alveolar epithelial cell, particularly type II (Yavuz et al., 2020), is the ultimate destination for SARS-CoV-2, a place where the ambitious virus can do some serious replication and some serious sheading, and from which to establish a legacy. Indeed, it is in the alveoli where *"More than 80% of total ACE2 expression is found."* (Yavuz et al., 2020) And, although the virus is intently focused on replicating and spreading, it also seems to have a consuming interest in killing. Needless to say, it does a pretty good job of this—with over 5.5 million deaths under its belt thus far in the pandemic, with many more deaths to come. Can it be stopped? Let's hope the alveolar macrophage has something to say about this, for *"It is in the alveoli where the infection can turn deadly."* (Bridges et al., 2021) To underscore the gravity of the situation,

> *Symptoms of COVID-19 are highly variable with common hyperactivity of immune responses known as a "cytokine storm". In fact, this* ***massive release of inflammatory cytokines <u>into in the pulmonary alveolar structure</u> is a main cause of mortality*** *during COVID-19 infection.* (Zimecki et al., 2021, emphasis added)

At this point in the SARS-CoV-2 infection, all eyes are turned to the alveoli, the location where a life and death struggle will be played out. Fortunately, the alveoli have their defenses; and, if all goes as planned, they will prevail and minimize the threat. The alveolus, the individual gas-exchange unit of the alveoli, is home to a resident defender called the macrophage. This cell, the alveolar macrophage, is tasked with defending against a variety of insults, viral invasion

included (Chan et al., 2021). It defends by ingulfing, processing, then characterizing the threat, leading to cytokine expression and antigen presentation (Budinger et al., 2021). The alveolar macrophage will do its best to prevent the spread of SARS-CoV-2.

So, it would be wise to come up with something to aid in the struggle against SARS-CoV-2 at the level of the alveoli. Sorry, nothing comes to mind . . . Oh, except for heparin.

> *Previous studies demonstrated that heparin and its analogues are able to bind to the SARS-CoV-2 spike protein and block SARS-CoV-2 infection using therapeutic relevant concentrations.* (Bertanha et al., 2021)

> *For COVID-19, the heparin anti-viral effect is expected to be related to the fact that* ***heparin blocks viral interaction with host cell receptors*** *thereby inhibiting viral interaction with the cell and, consequently, the viral infection.* (Bertanha et al., 2021, emphasis added)

> *Nebulized heparin decreased proinflammatory cytokines in lung tissue and the expression of NF-κB effectors in alveolar macrophages.* (Chimenti et al., 2017)

I apologize, I'm getting a little ahead of myself. You're not quite ready for our discussion of heparin. I'll try to contain my enthusiasm.

Returning now to the epic battle being waged within alveoli, we see the macrophage gallantly fighting the viral onslaught.

> *After gaining access to the distal lung, SARS-CoV-2 productively infects the small number of ACE2-expressing alveolar epithelial cells, releasing SARS-CoV-2 virions into the alveolar space. These viral particles are ingested by or infect alveolar macrophages. Alveolar macrophages harboring the virus respond by increasing the transcription of T cell chemokines and perhaps activating the NLRP3 inflammasome. These events may induce the activation of coronavirus cross-reactive memory T cells.* (Budinger at al., 2021)

But there is a problem, one that involves the concept of macrophage polarization. Quite remarkably (and quite dreadfully, may I add), SARS-CoV-2 can take over this cell, too, and use it to further replicate and spread (Lv et al., 2021).

> *Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) invades the alveoli, where abundant alveolar macrophages (AMs) reside. How AMs respond to SARS-CoV-2 invasion remains elusive. Here, we show that* ***classically activated M1 AMs facilitate viral spread; however, alternatively activated M2 AMs limit the spread. M1 AMs utilize cellular softness to efficiently take up SARS-CoV-2. Subsequently, the invaded viruses take over the endo-lysosomal system to escape****. M1 AMs have a lower endosomal pH, favoring membrane fusion and allowing the entry of viral RNA from the endosomes into the cytoplasm, where the virus achieves replication and is packaged to be released. In contrast, M2 AMs have a higher endosomal pH but a lower lysosomal pH, thus delivering the virus to lysosomes for degradation.* (Lv et al., 2021, emphasis added)

Highly relevant to its propagation, *SARS-CoV-2* favors the M1 macrophage over the M2. Indeed, *"Viral infection may induce M1 polarization of macrophages, which is generally* ***considered to be of paramount importance in viral clearance****, due to the release of pro-inflammatory cytokines."* (Lv et al., 2021, emphasis added) But apparently, a cell sworn to defend may unwittingly serve the enemy. *"M1 AMs are hijacked by SARS-CoV-2 allowing for viral infection and spread; however, M2 AMs possess the ability to degrade the virus and limit its spread."* (Lv et al., 2021)

Unfortunately, given the dominate M1 macrophage phenotype now in play, within the alveoli, an overwhelming infection is likely to take hold. I hope there are beds available in the ICU, as things are about to get ugly. All-out war is about to break out. The future may just depend upon the deliberate, decisive actions taken by the platelet.

> *Platelets contain many proinflammatory and anti-inflammatory cytokines and chemokines and, upon activation, can release them to the extracellular space.* ***The culmination of these events makes platelets a main immunomodulatory host.*** (Fard et al., 2021, emphasis added)

The platelet in host defense

> *Platelets are also active participants in the host response to viruses, and have been shown to be protective in viral infections.*
>
> *Activation of platelets leads to their consumption into aggregates with other platelets, leukocytes, and the endothelium. Platelets with bound antibody are targets of phagocytes, and platelets with a bacterial or viral load are sequestrated and also cleared from the circulation.*
>
> *Platelets are among the first cells to accumulate at sites of infection and inflammation, and can be considered as first responders to invading pathogens. Here,* ***platelets have a key role in sensing and effecting the first wave of responses to microbial and viral threat.*** **∼Page and Pretorius, 2020, emphasis added**

The platelet is quite the immune cell. Ordinarily, it monitors and maintains the integrity of the alveolar capillary bed (Fard et al., 2021), a thankless task, and to be honest, a task quite boring. But when circumstances change, things get a little more interesting. Under viral threat, the platelet **1)** can engulf a virus (Barrett et al., 2021b; Maouia et al., 2020; Seyoum et al., 2018); **2)** can secrete antivirals (Allaoui et al., 2021; Seyoum et al., 2018); **3)** can release an impressive array of chemokines and cytokines, molecules created to attract and influence the behavior of other immune cells (Bonaventura et al., 2021; Rolla et al., 2021); **4)** can physically attach to neutrophils, presumably to enhance clot formation (Bonaventura et al., 2021); and **5)** can create and display what are called "antigens" from the body parts of pathogens, to pass on detailed information about the enemy to other immune cells so they can formulate an appropriate response (Brambilla et al., 2021). And when the dust settles and victory is attained, the platelet will begin the task of repairing the damage inflected upon the vascular endothelial lining, damage which occurred during the battle with the virus (Koupenova, 2020). So, as you can see, there is a lot on the plate of a platelet, both during and after viral invasion. And with respect to the battle against SARS-CoV-2, we find the platelet about to play a crucial role.

As the disease progresses to involve the alveolus, we see the alveolar macrophage struggling to contain the viral onslaught. The platelet senses the likelihood of an overwhelming invasion and knows exactly what to do. As previously mentioned, the platelet sends out warning signals to the other

immune cells in the region, compelling them to arrive at the scene and prepare for battle—all done with a great sense of urgency. And while all are welcomed, it is the neutrophil that will be called upon to take centerstage. It has a very important job to do. "*Recruited to the battlefield by platelet signaling, the neutrophil acts to further immobilize the viral threat."* (Bonaventura et al., 2021) In addition, the neutrophil can defend *"by participating in the elaboration of cell signaling networks involving cytokines, chemokines, survival and growth factors that cause downstream pro-inflammatory effects."* (Thierry and Roch, 2020) Now back to the platelet.

At this point in the battle, the platelet is one very busy cell, extremely busy, acting to *"reduce the circulating viral load by engulfing viruses and presenting them to neutrophils"* (Brambilla et al., 2021). And aside from signaling, recruiting, presenting, and eating, the platelet is also responding to direct physical contact with the SARS-CoV-2 spike protein.

> *Here, we . . . demonstrate, for the first time, that platelets express ACE2 and TMPRSS2.* ***SARS-CoV-2 and its Spike protein directly bind platelet ACE2 and enhance platelet activation in vitro.*** *The Spike protein also potentiates thrombus formation in vivo. Moreover, we were able to demonstrate that SARS-CoV-2 and its* ***Spike protein directly stimulate platelets*** *resulting in coagulation factor release, inflammatory cytokine secretion, and leukocyte–platelet aggregates (LPAs) formation."* (Zhang et al., 2020, emphasis added)

Once the platelet finds itself in physical contact with the spike protein projections of SARS-CoV-2, things have gone beyond serious. The initial wave of viruses has arrived at the gates. A few have already trickled in. The platelet is keenly aware that it is now at the front lines and fully in charge of the war effort and must rise to the occasion to stop the spread of an advancing enemy, and at all costs. Instinctively, the platelet knows it must deploy the nuclear option. It must turn to immunothrombosis in the battle against SARS-CoV-2. At this point in time, it has no other choice. And there is no time to lose.

Immunothrombosis

> *Of importance,* ***activated platelets are critically involved in neutrophil extracellular trap (NET) formation****, which is* ***an essential element*** ***of immunothrombosis****.* **∼Bonaventura et al., 2021, emphasis added**

> *Netosis at the site of infection* ***is necessary*** *for protection against viral challenge.* **∼Koupenova, 2020, emphasis added**

When you want to freeze the enemy in place, indeed capture it and detain it on the field of battle, a NET is what you use. The platelet somehow knows this and is aware that the neutrophil is built for NET formation and deployment, a cellular event knows as NETosis. And having signaled for neutrophils to arrive at the point of invasion, the platelet will direct the neutrophil to explosively-release a highly developed portion of its inner self, to form a sticky structure that will ensnare the pathogen and anything else that stands in the way (Page and Pretorius, 2020). *Splat!* is one of the noises that can be repeatedly heard. (Screams, too.) Notably, the release of a NET from the belly of a neutrophil can be triggered very quickly, as needed, in a matter of a few minutes (Thierry and Roch, 2020). And with respect to the SARS-CoV-2 infection currently underway, and becoming increasingly serious, the NET can trap and immobilize SARS-CoV-2 to prevent further interaction with host cells. Apparently, the greater the threat, the greater number of NETs are deployed and strewn all over the battlefield, both within the microvasculature and within the alveoli (Dixon et al., 2020). At this point in the time, a NET is essential because immunothrombosis is essential.

Neutrophils also do a number of other things besides the casting of nets. For example, the neutrophil can activate coagulation cascades, and otherwise help in clot formation (Bonaventura et al., 2021). But this point in the battle against SARS-CoV-2, the NET is the weapon of choice—a platelet can only eat so much, a neutrophil can only eat so much. Interestingly, a NET can not only directly entrap a virus, but it can also electrostatically attract a free-floating virus, which can then become ensnared and immobilized, steadfastly bound to the NET (Thierry and Roch, 2020). A NET can also keep antimicrobials in prolonged contact with the immobilized pathogen (Gozzo et al., 2020), likely to enhance their effectiveness. Given the circumstances, at this point in the infection, a NET is quite

indispensable. A NET can defend. A NET can protect. However, a ventilator should be standing by.

Although necessary at this point in the battle, NETs can cause substantial harm to the individual who is mounting this defense—interfering with gas exchange and creating pulmonary compromise which can lead to respiratory failure (Bonaventura et al., 2021; Kvietys et al., 2021). But don't blame the NET! Blame the virus. Okay, you can blame the NET if you wish (at least that freedom hasn't yet been taken away).

It is important to consider, NETs are not supposed to kill the very individual it is trying to protect. NETs are supposed to generate *"an intravascular scaffold that facilitates the recognition, containment and destruction of pathogens, thereby protecting host integrity without inducing major collateral damage to the host."* (Engelmann and Mossberg, 2013) And with NET formation, the pathogen is *"restricted to the intravascular compartment"* to minimize damage to the host (Engelmann and Massberg, 2013). But tell that to the dead and dying. Although intentional and beneficial, this whole business can get way out of hand and threaten a life.

At this point, in what has developed into a true life and death struggle against SARS-CoV-2, I wonder if heparin could come in handy. Things are now getting very serious. A ventilator is no longer on standby. The "worst-case scenario" is currently being played out (Osuchowski et al., 2021). Someone is struggling just to survive. And in the absence of effective therapeutics, I don't even want to know what will happen next. Actually, I already know. At this point in the course of the infection we are rapidly approaching "the endgame," the final stage of the infection (Libby and Lüscher, 2020). Everything is on the line.

Taken by storm

> *SARS-CoV-2 causes lung inflammation which progresses to cytokine storm in the most severe cases.* **∼Wool and Miller, 2021**

> *Cytokines, protein pro-inflammatory mediators, serve as key danger signals that shift endothelial functions from the homeostatic into the defensive mode. The endgame of COVID-19 usually involves a cytokine storm . . .."* **∼Libby and Lüscher, 2020**

> *Mortality in COVID-19 patients has been linked to the presence of the so-called "cytokine storm" induced by the virus. Excessive production of proinflammatory cytokines leads to ARDS aggravation and widespread tissue damage resulting in multi-organ failure and death.* **∼Ragab et al., 2020**

> *Symptoms of COVID-19 are highly variable with common hyperactivity of immune responses known as a "cytokine storm". In fact,* ***this massive release of inflammatory cytokines into in the pulmonary alveolar structure is a main cause of mortality during COVID-19 infection."*** **∼Zimecki et al., 2021, emphasis added**

The above quotations pretty much tell the story of this, the final stage of the SARS-CoV-2 infection. If you have had COVID and never reached "the endgame," consider yourself fortunate. Perhaps you benefited from the outstanding work performed by the alveolar macrophage. Or perhaps it is the platelet that saved your . . . well, you know. Regardless, and most unfortunately, the final stage of COVID-19 is the experience of far too many individuals. Yet for those who are about to be lost, there are still therapeutic opportunities to peruse.

At this stage of the infection a lot is going on. Cells are dying at a rapid rate, expelling components that once resided within cell nuclei, structural proteins called histones (Gozzo et al., 2020; Shaw et al., 2021a). Ectopic histones are cytotoxic (Van Haren et al., 2020), and raise alarms that further drive inflammation (Buijsers et al., 2020). As a result, systemic inflammation intensifies (Shaw et al., 2021b) and a *"massive release of inflammatory cytokines into in the pulmonary alveolar structure"* is ongoing (Zimecki et al., 2021). Additionally, the production of coagulation factors increases, leading to disseminated intravascular coagulation (Bonaventura et al., 2021)—well beyond the bounds of the defensive measure we call immunothrombosis. And while all this occurring and intensifying, the pulmonary vascular becomes leaky (Shi et al., 2020), and an individual is about to drown and suffocate in a respiratory distress sequelae called ARDS (Osuchowski et al., 2021).

> *In patients affected by SARS-CoV-2, the proinflammatory response and, in particular,* ***the cytokine storm represent a centerpiece of COVID-19 pathogenesis****, causing great destructive consequences for the host. When the immune system is not more able to counteract the virus and to conclude the inflammatory response, the aberrant production of the cytokines led to macrophage hyperactivity, with consequences for the whole body, including*

fever, anemia, and organs malfunction. ***At some point, the cytokine storm becomes unstoppable,*** *leading to irreversible end-organ dysfunction and even death.* (Castelli et al., 2020, emphasis added)

So, this is how it all ends . . . unless we intervene in a timely manner. No time to lose.

Ideal agent

The high incidence of thromboembolic events in COVID-19 patients suggests that coagulopathy plays an important role in the SARS-CoV-2 pathogenesis. ***This already makes heparin a unique, potentially curative agent that can be used immediately to help resolve the ongoing crisis associated with SARS-CoV-2 infection and COVID-19 disease. We demonstrate here in vitro that heparin does indeed inhibit SARS-CoV-2 infection.*** **∼Conzelmann et al., 2020, emphasis added**

Heparin prevents the entry of SARS-CoV-2 into cells*, binding to the recombinant surface receptor-binding domain (SARS-CoV-2 S1 RBD) and inducing a significant structural change.* **∼Pisani et al., 2020, emphasis added**

Several immunomodulatory properties have been associated with unfractionated heparin (UH), which can inactivate inflammatory cytokines, inhibit neutrophil chemotaxis and leukocyte migration, neutralize complement factor C5a and sequester acute phase proteins . . .. **∼Guglielmetti et al., 2020**

Heparin is more than a trusted anticoagulant. It is a versatile therapeutic, ready to be used **to the fullest** in the battle against SARS-CoV-2, and for a number of reasons.

To begin with: Heparin can prevent the entry of the SARS-CoV-2 into the cell. "*Mechanistically, we observed that heparin binds and destabilizes the RBD protein and furthermore, we show heparin directly inhibits the binding of RBD to the human ACE2 protein receptor."* (Tree et al., 2021) Additionally, heparin inactivates/immobilizes a critical part of the SARS-CoV-2 spile protein, particularly the receptor binding domain (RBD), *"hindering presentation of the RBD to ACE2"* and impeding entry into the host cell (Paiardi et al., 2022). Indeed, following direct contact, an *"extremely strong (and nearly irreversible) binding"* between

heparin and the SARS-CoV-2 spike protein can be established (Hippensteel et al., 2020). Oh, I've got more.

Conveniently, heparin delivered by inhalation can limit the buildup of fibrin, a clotting protein that pathologically accumulates within the alveoli in COVID-19 and interferes with oxygen exchange (Dixon et al., 2020; Van Harem et al., 2020; Whyte et al., 2020). This attribute allows heparin to prevent a deterioration in oxygen exchange, lessening the need for higher levels of oxygen support, and perhaps averting the need for mechanical ventilation (see Van Haren et al., 2020). But don't wait too long. Timely intervention is advised (Shaw et a., 2021a). Indeed, inhalational heparin could be administered at the onset of COVID-19 symptoms and thus *"may attenuate disease progression."* (Van Haren et al., 2020).

So, as long as we are preventing fibrin buildup and stabilizing oxygen exchange, we might as well dissolve NETs, too. Likely, they have served their purpose or have been expelled in excess, so it is time to intervene before ARDS becomes insurmountable. *"Indeed, ARDS can be considered as a NETopathy; higher levels of NETs were described in the plasma and bronchoalveolar fluid of patients with transfusion-associated and pneumonia-related ARDS . . .."* (Bonaventura et al., 2021) And fortunate are we; heparin is capable of digesting NETs (Bonaventura et al., 2021).

"What else can heparin do for us in our battle against SARS-CoV-2?" you ask. I like the question. It tells me you are paying attention.

> *Heparin demonstrated a **strong affinity for extracellular histones** and prevents their interaction with platelets, a potential mechanism contributing to the regulation of inflammation.* (Gozzo et al., 2020, emphasis added)

It should be noted, the presence of large quantities of circulating histones triggers the systemic release of IL-6 (Shaw et al., 2021b), with IL-6 being a principal driver of the dreaded cytokine storm (Litov et al., 2020; Libby and Lüscher, 2020). Fortunately, heparin can block IL-6 release (Shi et al., 2021), disable the IL-6 molecule, and limit the ability of IL-6 to generate a cytokine storm (Litov et al., 2020). *"Timely control of the cytokine storm **in its early stage** is the key to improving the treatment success rate and reducing the mortality rate in*

patients with COVID-19." (Litov et al., 2020, emphasis added). Indeed, ***"Reducing the levels of IL-6 and decreasing its activity may prevent or even reverse the cytokine storm syndrome****, thereby improving the condition of patients with COVID-19."* (Shi et al., 2020, emphasis added)

And it looks like heparin, delivered by inhalation, can have a direct, anti-inflammatory effect on the alveolar macrophage. *"Nebulized heparin decreased proinflammatory cytokines in lung tissue and the expression of NF-κB effectors in alveolar macrophages."* (Chimenti et al., 2017) This, in the context of excessive pro-inflammatory responses, is a win-win.

> *These findings suggest that heparin exerts its anti-inflammatory effects by suppressing the production of cytokines and chemokines swiftly in macrophages. The production and release of cytokines occurs more rapidly with macrophages as compared to alveolar cells and fibroblasts, so, small changes in the production of proinflammatory cytokines by these cells could also induce a huge inhibition of inflammation in the lung. So, the quick de-activation of macrophages would be useful to control the classical pro-inflammatory cascade activation.* (Camprubí–Rimblas et al., 2017)

Is there more that heparin can do? Of course there is. Have you got all day?

> *Heparin also has other actions of potential benefit including* ***inhibition of inflammatory cytokines, prevention of bronchospasm, increased nitric oxide release and limiting adhesion of microbes*** *to respiratory epithelium.* Dixon et al., 2020, emphasis added)

And let me add this:

> *It has also been shown that heparanase [sic] can promote viral infection and spread. LMWH [low molecular weight heparin] can inhibit the enzymatic activity of heparinase, inhibiting viral spread.* (Vitiello and Ferrara, 2021)

As you can see, there are several outstanding reasons to the use of heparin in the battle against SARS-CoV-2. And the future is already here. The use of heparin for COVID-19 is a reality—recommended by the WHO (Shi et al., 2021) and apparently in widespread use (Guglielmetti et al., 2020; Di Micco et al., 2021;

Perna et al., 2020). However, the **optimal utilization** of heparin is still in the future. It is as if we do not know what we have.

> *Heparin and its derivatives are an under-exploited antiviral drug class, despite possessing broad-spectrum activity against a multitude of distinct viruses, including SARS-associated Coronaviridae.* (Lazzaroni et al., 2021)

The use of heparin in the hospital setting is typically prophylactic, employed to prevent the clotting disorders that typically befall the critically ill patient, such as deep vein thrombosis. And this is good! However, *"Although prophylactic doses [of heparin] may be adequate in most patients, it would be important to administer therapeutic dosage based on the individual risk of coagulopathy and thrombosis."* (Gozzo et al., 2020) Therapeutic heparin is what you reach for when life is in the balance. We can ask Negri and associates about this.

In a case series report, Negri et al outline the experiences of 27 critically ill patients with COVID-19 who received heparin in therapeutic doses. Of the 27, *"92% of the patients were discharged home within a median time of 11 days."* The story goes something like this:

> *All patients received initially enoxaparin 0.5 mg/kg SC every 24 h. Patients with a creatinine clearance under 30 mL/min received subcutaneous unfractionated heparin at a dose of 5,000 units every 8 h. If an abrupt decrease in oxygenation or an increase in D Dimer levels was observed, enoxaparin dose was raised to 0.5 mg/kg SC every 12 h and, in the event of thrombotic phenomena or worsening hypoxia, the dose was further increased to 1 mg/kg SC every 12 h. Patients with a BMI (body mass index) of 35 or higher were also considered for the higher dose regimen. Patients in shock or intubated were treated from the beginning with intravenous heparin, targeting an APTT ratio around 1.5 to 2.0 times the normal range. If a patient presented any acute thrombotic event, heparin dosing was increased to obtain an APTT approximately 2.0 to 2.5 times the normal range.* (Negri et al., 2020)

That's how it's done! You individualize heparin. You give it in therapeutically relevant amounts. And you use laboratory parameters to reduce the risk of over-anticoagulation and bleeding. And the results?

> *We observed no deaths due to any cause or haemorrhagic complications due to anticoagulation during the study period. Moreover, after three months, all but one patient were discharged home without supplementary oxygen.* (Negri et al., 2020)

And apparently, heparin can be helpful in the treatment of cytokine storm.

> One retrospective study, demonstrated *"for the first time,"* a ***"significant beneficial effect of LMWH in controlling cytokine storm and delaying disease progression."*** (Shi et al., 2020, emphasis added)

On the other hand, there are reports that tell a different story; and authors who caution the clinician not to become overly enthusiastic about the use of heparin in the battle against SARS-CoV-2. Examples of such are Swan et al., 2021 and Ten Cate, 2021. However, overall, the reports that favor the use of heparin seem to outnumber those that dissuade. And in the reports that strike a cautionary note, methodology issues are likely present, such as differences in study populations, differences in disease severity, inconsistent methods of heparinization, varied dosages used, and late-entry intervention—methodology issues similar or identical to those acknowledged in Ten Cate, 2021.

The reports of success with LMWH may be impressive indeed and should not be minimized. However, the use of unfractionated heparin (UHF), the "natural heparin" (Litov et al., 2020), may be a better approach, as *"low-molecular weight formulations may be less likely to have direct antiviral activity through competitive spike protein binding."* (Hippensteel et al., 2020) In this regard,

> *It has been demonstrated that unfractionated heparin has a 150-fold higher antiviral effect against SARS-CoV-2 than low molecular-weight heparin (LMWH). Recent data show that* ***long chain heparin inhibits spike–cell interactions,*** *whereas different LMWHs were less effective.* (Paiardi et al., 2022)

> *Another interesting non-anticoagulant function is its antiviral role. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein interacts directly and strongly with heparan sulfate, meaning that exogenous heparin could competitively inhibit viral entry into cells.* ***This may***

not be applicable to LMWHs, which do not show this capability. (Swan et al., 2021)

Likely, the clinician will want to "*competitively inhibit viral entry into cells*," and will be pleased to learn that UHF can do this. And why not do this, if circumstances allow? *"Unfractionated heparin (UFH) could be used in patients with renal impairment or at high bleeding risk who need rapid reversal; however, it requires expertise in the management."* (Mattioli et al., 2020)

To be brief, in the opinion of the author, in COVID-19, a greater degree of success can be achieved with UFH, IV or SQ, than with the use of LMWH. Bleeding risk can be minimized with careful monitoring and dosing. Furthermore, UFH has a relatively short half-life and is pharmacologically reversable (Weeks et al., 2021), allowing for rapid correction in the event of bleeding from over-heparinization. But should the risk of systemic heparinization be deemed inappropriate, or heparin needs to be scaled back or held do to bleeding concerns, nebulized heparin is available, ready to serve, safely and effectively serve.

Nebulised UFH targets pulmonary fibrin deposition and inflammation, and local administration to the lungs allows higher dosages and increases local efficacy, reduces the risk of systemic bleeding and ***is more effective than intravenous administration.*** *Importantly, previous studies have shown that following nebulisation, UFH does not enter the systemic circulation significantly which means it can be used in addition to systemic therapeutic or prophylactic anti-coagulation* ***without concerns of furthering systemic anti-coagulation.*** *The use of nebulised UFH in other respiratory settings was not associated with local side effects in the lung including bleeding.* (Van Haren et al., 2020, emphasis added)

Furthermore,

Nebulised heparin at the dose proposed in this study has not been found to increase the risk of major non-pulmonary bleeding or of blood transfusion. In clinical trials where more than 150 intensive care patients were treated with nebulised heparin for ARDS and related conditions, there were no cases of pulmonary haemorrhage with patient deterioration. Bloodstaining of the airway secretions of invasively ventilated intensive care

unit patients is common. Approximately 1 out of 20 patients treated with nebulised heparin experience greater-than-usual bloodstaining of secretions and, although this bloodstaining can be unsightly, rarely is it clinically deleterious. The risk of medically important haemoptysis is estimated to be less than 1 out of 100. (Dixon et al., 2020)

Regarding dose and frequency:

Nebulisation allows for the targeting of lung tissue directly and therefore impact upon the local hyperinflammatory response and alveolar coagulation resulting from SARS-CoV-2 viral load in the lung. During the UK trial, UFH (Wockhardt) will be administered at 25,000 IU (130 mg) every 6 h to patients. (Tree et al., 2021)

The pulmonary route of administration of heparin is tried in some types of respiratory diseases and non-COVID-19 related ARDS conditions before. There are no reports of major (or even minor) [complications] even with a maximum 120,000 IU unfractionated heparin per day. (Hursitoglu et al., 2021)

The CHARLI study helps somewhat in these aspects—it is important to see that nebulised heparin at dosages of 25000 UI every 6 h, as used in most studies to date, is a safe strategy, with concomitant use of systemic low molecular weight or unfractionated heparin. Despite the increase in the activated partial thromboplastin time (aPTT), suggesting some systemic effect of nebulised heparin, the number of transfusions and major bleeding events was not affected. Withholding of treatment was only necessary in small proportion of patients in response to blood-tinged sputum or an excessive prolongation of aPTT. (Ball et al., 2021)

It is important to recognize that nebulized heparin can be given along with heparin administered in other forms (Ball et al., 2021; Van Haren et al., 2020).

Importantly, previous studies have shown that following nebulisation, UFH does not enter the systemic circulation significantly which means it ***can be used in addition to systemic therapeutic or prophylactic anti-coagulation without concerns of furthering systemic anti-coagulation.*** *The use of nebulised UFH in other respiratory settings was not associated with*

local side effects in the lung including bleeding. (Van Haren et al., 2020, emphasis added)

Let's end our discussion on nebulized heparin with the following:

COVID-19 is associated with the development of ARDS displaying the typical features of diffuse alveolar damage with extensive pulmonary coagulation activation resulting in fibrin deposition in the microvasculature and formation of hyaline membranes in the air sacs. The anticoagulant actions of nebulised heparin limit fibrin deposition. Serendipitously, unfractionated heparin also inactivates the SARS-CoV-2 virus and prevents its entry into mammalian cells. Nebulisation of heparin may therefore limit fibrin-mediated lung injury and inhibit pulmonary infection by SARS-CoV-2.

A recent (not yet published; under journal review) pre-pandemic double-blind multi-centre randomised study of 256 mechanically ventilated patients with or at risk of developing ARDS led by our group found, clinically important pre-specified secondary outcomes were significantly improved with nebulised heparin. There was no evidence of harm. (Dixon et al., 2020)

Finally, and briefly, there is yet another way to exploit the use of heparin in the battle against SARS-CoV-2, referred to as extracorporeal heparinized bead hemofiltration, using the Seraph® 100 filter (Seffer et al., 2021). This procedure can be accomplished at the bedside or in an outpatient facility. It involves circulating the patient's blood through the "heparinized" filter, thereby heparin-binding the virus and removing it from the circulation. *"Viremia has been shown to be present in 41% [32] of patients in general and in up to 50% of critically ill patients with SARS-CoV-2."* (Seffer et al., 2021) Importantly, *" . . . recent in vitro data have also shown that SARS-CoV-2 can be removed by the Seraph® 100."* (Seffer et al., 2021)

Conclusion

To date, no doubts should be raised about the use of anticoagulant therapy in patients with COVID-19, having guaranteed a good safety profile in prophylactic and therapeutic doses. **~Di Micco et al., 2021**

Heparin prophylaxis should be virtually prescribed to all COVID-19 hospitalized patients, in the light of hyper-coagulation provided by the inflammation and the virus, but also of the frequent concomitant cardiovascular risk factors. **~Lazzaroni et al., 2021**

Ideally, heparins should be administered to COVID-19 patients as early as possible. ~Drouet et al., 2020

Need I say more? Probably. I should caution against unnecessary delay.

Unfortunately, current trials are mostly examining these agents in isolation and there may be a significant delay before evidence-based practice can be implemented. (Bhattacharyya et al., 2020)

Perhaps now is the time for evidence-based medicine to take a back seat, step aside to allow our clinicians to throw everything we have at SARS-CoV-2, to learn new things and to freely implement. Later, when many lives are spared and the pandemic is in the rear-view mirror, we can take the time to go over things more thoroughly. But for now, you, me, and those whom we care for are at risk, not only from SARS-CoV-2 but also from inadequate therapeutic intervention. And, for those whose very existence is under threat, we must implement what we believe to be the best options available, and without delay. The legacy of COVID-19 will certainly include all the lives taken by storm, but it will also include the many lives spared by the strategic use of heparin in the battle against this disease.

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