bjh commentary

# COVID-19-induced endotheliitis: emerging evidence and possible therapeutic strategies

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## Introduction

The coronavirus disease 2019 (COVID-19) pandemic, a viral illness caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2),[1] has produced at the time of this writing nearly 33 million cases of infection, with over a million deaths in 235 countries,[2] causing an unprecedented burden on healthcare systems and a severe global socioeconomic crisis. As the pandemic spreads, knowledge on the disease course, as well as potential risk factors and predictors of severity is increasing daily, and initial data from randomised controlled studies have allowed care providers to refine therapeutic strategies. Nonetheless, mortality is markedly elevated among those presenting with severe disease, long-term sequelae among survivors are unknown, and vaccine-based therapies currently remain at early stages of development.

Most reported cases are asymptomatic or present with mild symptoms; however, 7–26% of hospitalised patients experience severe disease, often requiring admission to intensive care units (ICUs), with progressive multiple organ dysfunction and high mortality.[3–5] Such differences in clinical outcomes have led physicians to initiate diverse pharmacological therapies at various stages of the disease, generating challenges as to the most appropriate therapeutic choice for COVID-19. In this context, the use of dexamethasone has significantly reduced mortality rates in critically ill patients requiring supplemental oxygen or mechanical ventilation,[6] and remdesivir has demonstrated clinical benefit in hospitalised patients, but with unknown survival benefit to date[7]; additional effective treatment options are therefore urgently needed.

In an initial attempt to provide a uniform and widely reproducible methodology to guide systematic treatment strategies, a three-stage classification of COVID-19 has been proposed.[8] The Stage I or 'early infection' occurs at the initial establishment of disease with high viral replication, and commonly presents with a range of complaints that can include mild and often non-specific influenza-like signs and symptoms. Stage II is the 'pulmonary phase', with preferential viral-mediated injury of the lung parenchyma and this is characterised by shortness of breath, hypoxia and pulmonary infiltrates with some degree of lung inflammation. Stage III is characterised by an exaggerated host immune-inflammatory response to the virus, leading to acute respiratory distress syndrome (ARDS) and multi-organ failure (MOF).

## Endothelial cells are a preferential target of COVID-19 resulting in widespread endotheliitis

Emerging evidence suggests that endothelial damage and subsequent morphological and functional changes in the endothelium play important roles in COVID-19-induced hyperinflammation. The virus, which binds to the

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angiotensin-converting enzyme 2 (ACE2) receptor,[9] displays a profound tropism for human lung and small intestine epithelium, as well as the vascular endothelium.[10] In an important case series from Varga *et al.*,[11] postmortem histology from three patients affected by late-stage COVID-19, revealed viral inclusions in endothelial apoptotic cells and microvascular lymphocytic endotheliitis, with infiltration of inflammatory cells around the vessels and endothelial cells (ECs), as well as evidence of endothelial apoptotic cell death in the lung, kidney, small bowel and heart. Additionally, autopsy findings of 27 patients in another series confirmed the detection of the SARS-CoV-2 in multiple organs, including the respiratory tract, pharynx, heart, liver, brain, and kidneys.[12] Immunofluorescence of kidney specimens from six of the 27 patients showed the presence of SARS-CoV-2 protein in all renal compartments, and in three of the patients preferentially in the endothelium of the glomerulus. Similar microscopic findings were also noted in lung specimens from seven patients with COVID-19, which displayed small vessel endotheliitis, microvascular thrombosis and angiogenesis, along with the presence of SARS-CoV-2 in pulmonary ECs, an observation strongly supporting the vascular tropism of the virus.[13] Lastly, Stahl *et al.*[14] have identified in the plasma and serum of 19 critically ill patients with COVID-19 evidence of disruption of the endothelial glycocalyx, reflected by increased levels of the Tie-2 receptor and syndecan-1 (SDC-1), a heparan sulphate (HS) proteoglycan. This particular observation is of interest as the endothelial glycocalyx covers the luminal surface of ECs, and its integrity is vital for the maintenance of vascular homeostasis.

Such findings suggest that virus-mediated apoptosis may promote endothelial barrier disruption with interstitial oedema and increased recruitment of circulating activated immune cells, thus causing widespread endothelial dysfunction, as well as activation of platelets and the coagulation cascade leading to venous and arterial thrombosis.[15] Altered pro-inflammatory and pro-thrombotic status is confirmed by the presence of elevated inflammation-related indices (e.g. C-reactive protein and serum ferritin), humoral biomarkers [interleukin (IL)-2, IL-6, IL-7, granulocyte-colony stimulating factor (G-CSF), tumour necrosis factor-alpha (TNF-α)], and indicators of an increased pro-coagulant-fibrinolytic state [e.g. von Willebrand factor (VWF), D-dimer, fibrinogen]. Further, factor VIII (FVIII), a potent and key factor in the coagulation process, is greatly increased in ICU patients with COVID-19.[16]

In this setting, it has been proposed that the systemic hyperinflammation observed in severe COVID-19 is comparable to a cytokine release syndrome (CRS), or cytokine storm. IL-6 has a central role in the generation of the cytokine storm, and is commonly elevated in the serum of severely ill patients with COVID-19.[17] High levels of IL-6 activate ECs, thus resulting in vascular leakage, further cytokine secretion and activation of the complement and coagulation cascades.[18] Interestingly, a population of IL-6 producing monocytes was found to be expanded in the peripheral blood of ICU patients[19] and an aberrant macrophage response exhibiting increased levels of pro-inflammatory cytokines has been detected in bronchoalveolar fluid, especially in severely ill patients.[20] Although the exact driver of monocyte activation remains unclear, such cells are attracted to the endothelium, where the release of highly noxious molecules, such as reactive oxygen species (ROS), contributes to endothelial dysfunction and promotes hyperinflammation.[21] Further, activated monocytes enhance tissue factor expression and form aggregates with platelets through P-selectin interaction and hence augmenting the pro-coagulant response.[22] Indeed, significantly increased levels of VWF and FVIII and thrombomodulin,[16] aberrant coagulation, thrombosis and microangiopathy are very common in critically ill patients with COVID-19, resulting in a disseminated intravascular coagulation (DIC)-like syndrome characterised by massive fibrin formation and organ dysfunction.[23] Likewise, adaptive immunity actively participates in the establishment of the inflammatory response; specifically, activated and proliferating $CD8^+$ T cells are prevalent in mild COVID-19, whereas critically ill patients display higher levels of hyperactive IL-6-producing $CD4^+$ T cells, which may contribute to disease severity, even after viral clearance.[17] Interestingly, T cells show phenotypical signs of an exhausted, functionally unresponsive state, thus allowing viral escape from immune surveillance.[22] Once initiated, the endotheliitis and resultant cytokine storm become self-sustaining, leading to widespread organ damage. Some patients may also display features of haemophagocytic lymphohistiocytosis, such as cytopenias, hyperferritinaemia and rapid onset of MOF.

Overall, once hyperinflammation and CRS develop, rates of mortality significantly increase.[24–26] As direct viral activation of the vascular endothelium has an important role in initiating and maintaining the hyperinflammatory response, attempting to blunt such a response with endothelial-protective agents is a very rational strategy. Controlled clinical trials focussing on the use of anti-cytokine antibodies, including tocilizumab (IL-6 inhibitor), have failed to show significant activity in this stage of the disease.[27] However, increasing evidence suggests that the altered homeostasis of the endothelium may be a key initiating event in the pathogenesis of the disease, therefore representing a potentially more promising target.[28]

## Endothelial cell-related disorders in haematology: post-bone marrow transplantation syndromes and sickle cell disease and the overlap with the pathobiology of COVID-19

Clinically and histopathologically, COVID-19-associated endotheliitis resembles a spectrum of post-bone marrow and stem cell transplantation (BMT) syndromes characterised by disruption of endothelial homeostasis and consequently

dysregulation of coagulation, vascular tone, endothelial permeability and vascular inflammation.[29] These disorders include hepatic veno-occlusive disease (VOD)/sinusoidal obstruction syndrome (SOS), idiopathic pneumonia syndrome (IPS), transplant-associated thrombotic microangiopathy and graft-*versus*-host disease (G*v*HD).

Hepatic VOD/SOS develops as a result of endothelial damage to hepatic sinusoids and subsequent hepatocyte necrosis[30]. Damage to the ECs leads to a hypercoagulable state, production of inflammatory mediators, and the upregulation as well as release of heparanase.[31] Heparanase degrades the heparan sulphate scaffold of the subendothelial basement membrane, consequently allowing the extravasation of blood-borne cells, including activated T lymphocytes, neutrophils and macrophages.[32] This cascade of events leads to postsinusoidal hypertension, hyperinflammation and ultimately MOF. Severe VOD/SOS associated with MOF without effective therapy is fatal in >80% of cases.[33] Interestingly, the histopathological examination of lung lesions in VOD/SOS shows early alveolar epithelial and lung endothelial injury, resulting in accumulation of protein- and fibrin-rich inflammatory oedematous fluid in the alveolar space and progression to interstitial fibrosis,[34,35] as is also seen in fatal COVID-19 cases.

Similarly, IPS, a widespread alveolar injury in the absence of identifiable infectious or non-infectious causes, is characterised by histological evidence of EC injury with fibrin accumulation, luminal thrombosis and fibrotic processes. Adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and/or vascular cell adhesion molecule 1 (VCAM-1), are commonly upregulated, thus reflecting profound endothelial activation.[36] It has been suggested that TNF-α directly causes endothelial injury, and increased levels of angiopoietin-2 (Ang-2), have been recently reported in cases of acute exacerbations of IPS,[37] similar to that seen in severely ill patients with COVID-19.[38]

Likewise, multifactorial endothelial damage has been also implicated in the development of transplant-associated thrombotic microangiopathy, where micro-vessel intimal swelling and necrosis lead to the formation of luminal microthrombi and subsequent microangiopathic haemolytic anaemia. Plasma levels of markers of EC injury and inflammation, such as thrombomodulin, plasminogen activator inhibitor-1 (PAI-1), ICAM-1, VCAM-1, IL-1, TNF-α, interferon gamma and IL-8 are commonly elevated.[39,40] Endothelial dysfunction predominantly affects the kidneys and the brain, but may become widespread and progress to MOF, which in turn is associated with high mortality.

Lastly, acute G*v*HD (a*v*HD) develops as a consequence of the activation of the immune system. Antigen-presenting cells become activated by endothelial and tissue damage derived from direct toxicity of the conditioning regimen, thus initiating an alloreactive T-cell response directed against recipient tissues.[41] As a result, SDC-1 is commonly elevated in the serum of patients with G*v*HD and correlates with disease severity.[42] In addition to cell-mediated cytotoxic damage, the cytokine storm generated in response to T-cell activation and proliferation causes targeted organ damage involving mainly the skin, liver and gut.[43] It has recently been suggested that endothelial vulnerability and pro-thrombotic shift precedes clinically evident a*v*HD and that angiogenesis driven by early endothelial activation is an initiating event.[44] Indeed, increased plasma levels of VWF,[45] Ang-2[46] and TNF receptor 1[47] have been detected in patients prior to development of a*v*HD, correlating with response to therapy.

Similarly, markers of endothelial dysfunction and inflammatory activation have been detected also in the serum of patients with sickle cell disease (SCD), especially during vaso-occlusive episodes. SCD is characterised by a chronic course of relapsing-remitting episodes of ischaemia and then reperfusion. The polymerisation of defective haemoglobin S upon deoxygenation initiates many pathological processes, such as complement activation, generation of ROS and pro-thrombotic molecules, secretion of numerous pro-inflammatory cytokines and chemokines and ultimately leucocyte recruitment.[48] Oxidative stress and endothelial dysregulation plays a key role in vaso-occlusion; ECs activated by substances released by the haemolytic process and by red blood cell adhesion initiate production and release of soluble mediators such as IL-1β, IL-8, IL-6, IL-1α and PAI-1,[49,50] and increase the expression of adhesion molecules such as VCAM-1, ICAM-1, E-selectin and P-selectin,[51,52] reflecting a pro-inflammatory and pro-thrombotic shift. Vaso-occlusive phenomena commonly affect the lung vasculature, provoking acute chest syndrome (ACS), a spectrum of diseases ranging from mild pneumonia to ARDS and MOF, which is the leading cause of morbidity and mortality in SCD.[53] Lung specimens from ACS cases showed micro-thrombotic occlusion, endothelial VWF deposition and arterial vessel re-modelling with initial fibrotic processes,[54] fascinatingly all comparable to the histopathological findings in COVID-19.[55] Importantly, heme-mediated endothelial damage to alveolar cells is regulated by the p38 mitogen-activated protein kinase (MAPK) pathway, which plays a crucial role in the biosynthesis of pro-inflammatory cytokines and collagen production.[56] This key pathway is also upregulated in COVID-19 as a result of decreased ACE2 tissue functionality consequent to viral binding, and may consequently promote endotheliitis, hypercoagulation and end-stage fibrosis.[57,58]

In summary, post-BMT syndromes, vaso-occlusive organ dysfunction in SCD and COVID-19-associated endotheliitis share common pathological mechanisms including: i) dysregulation of the homeostasis of the endothelial milieu toward a pro-inflammatory and pro-thrombotic phenotype with thrombotic microangiopathy; ii) hyperproduction of inflammatory cytokines such as IL-6, IL-8 and TNF-α;[59–62] and iii) small vessel endotheliitis and endothelial barrier dysfunction, leading to oedema of the microvascular bed, protein and fibrin accumulation and subsequent fibrotic shift.[34,36] All these conditions if untreated irremediably lead to MOF and display similar microscopic and macroscopic features in target

organs upon pathological examination. At the molecular level, the p38 MAPK pathway may also be critical in promoting vasoconstrictive and inflammatory phenomena; its activation is described in SCD, COVID-19 and also as a result of conditioning regimen-induced endothelial damage in BMT.[63] Together, these findings support the notion that the pleiotropic character of the endothelium as a key regulator of the internal homeostasis, vascular tone, blood coagulation and the inflammatory process and therefore of so called 'immune-thrombosis' events, make it an intriguing therapeutic target for post-BMT disorders, SCD, and COVID-19.

## Agents targeting EC-related disorders

### *Heparins*

Classically, heparins have been the most widely used drugs for the treatment and prevention of endothelial cell disorders. Several animal studies and clinical trials have suggested that, in addition to its well-known anticoagulant effects, heparin also possesses anti-inflammatory properties, mainly mediated by inhibition of IL-6 release and its activity,[64] a phenomena also demonstrated in patients with COVID-19 treated with low-molecular-weight heparin.[65] Further, heparin is structurally related to HS,[66] a negatively charged glycosaminoglycan as described earlier, which serves as binding sites for growth factors, cytokines, selectins, extracellular-matrix molecules, and a large number of human viruses,[67] including the SARS-CoV-2 virus.[68,69] Indeed, Clausen *et al.*[70] have recently demonstrated that the SARS-CoV-2 spike protein must bind both the ACE2 receptor and HS to enter human cells. The structural analogies between heparin and HS may result in competitive inhibition, where heparin and related compounds compete with the cell surface HS for viral binding to target cells,[68,69] thus potentially blocking or at least attenuating viral entry. The beneficial effects of heparin-based therapies are also linked to their inhibition of circulating heparanase enzymatic activity.[32] Heparanase, an endo-β-glucuronidase, physiologically cleaves HS chains located in extracellular matrices and on cell surfaces.[32] It is often overexpressed during viral infections and act as a regulator of virus release after replication has occurred, promoting its dissemination.[71–74] Additionally, it may be upregulated by pro-inflammatory molecules such as IL-1 and TNF-α. Once activated, heparanase stimulates the expression and release of pro-inflammatory cytokines, including TNF-α, IL-1 and IL-6.[75] The enzyme has been implicated in cancer progression, inflammation,[76] VOD/SOS development[77] and other vascular pathologies.[71]

Currently, numerous clinical trials are underway to investigate the therapeutic potential of intravenous and subcutaneous heparin, as well as the appropriate dose regimen in COVID-19. Further, nebulised heparin delivered directly to the airways may be effective in preventing infection and mitigating lung disease (clinicaltrials.gov; NCT04545541, NCT04511923). Notwithstanding their anticoagulant, anti-inflammatory and anti-viral properties, the use of heparins is associated with a substantially increased risk of systemic bleeding, and other challenging 'off-target' effects, making its use potentially part of the standard of care, but not without qualification, as well as highlighting the need for combination approaches.

### *Defibrotide*

The use of defibrotide (DF), which has both comparable but distinct properties from heparins and negligible haemorrhagic risk[78] may therefore be warranted, especially given the established propensity for the development of DIC later in the COVID-19 clinical course. DF is a naturally derived, complex mixture of poly-deoxyribonuleotides extracted originally from bovine lung and now exclusively from porcine gut mucosa.[79,80] Since its original isolation >30 years ago, DF has demonstrated locally acting pro-fibrinolytic,[81-84] antithrombotic,[85,86] anti-ischaemic and anti-inflammatory activities, which exert protective effects on small vessel endothelia. It is currently approved for the treatment of paediatric and adult hepatic VOD/SOS with MOF.[87–89] In this setting, DF has demonstrated efficacy and safety in critically ill patients with MOF, as well as a significant reduction in PAI-1 and other markers of endothelial stress in patients with VOD/SOS and MOF successfully treated with DF.[89-91] Furthermore, in a pivotal Phase III trial, DF prophylaxis reduced the incidence and severity of VOD/SOS in high-risk children undergoing BMT.[92] In a more recent study, Palomo *et al.*[93] demonstrated that DF directly interacts with the cell membrane and becomes internalised by ECs, thus providing physical evidence of its endothelial-protective properties. In particular, DF appears to decrease levels of pro-inflammatory proteins, such as TNF-α,[94] IL-6, vascular endothelial growth factor (VEGF)[95] and to downregulate major histocompatibility complex (MHC) Class I and Class II molecules,[96,97] therefore attenuating both the inflammatory and immune responses. Furthermore, it appears to decrease interaction between leucocytes and ECs by downregulating P-selectin,[98] ICAM-1[95] and VCAM-1.[99] Lastly, DF displays potent adenosine agonism.[100] Such activity may be clinically relevant, not least based on substantial improvement observed in an animal model of acute lung injury upon treatment with adenosine receptor agonists.[101]

Based on such properties, the use of DF can be reasonably extended to other post-BMT syndromes and other microangiopathies involving CRS complicating a variety of disease states and treatment modalities, such as chimeric antigen receptor (CAR) T-cell therapy.[102] Indeed, paediatric and adult patients receiving DF as VOD/SOS prophylaxis also exhibited a reduced incidence of a*G*v*H*D,[92,103] a finding that is strongly supported by a preclinical model of a*G*v*H*D.[99] Additionally, a retrospective survey from paediatric patients treated with DF for transplant-associated thrombotic microangiopathy showed resolution of clinical disease in

77% of patients.[104] Currently, Phase II studies investigating the use of DF for prevention of transplant-associated thrombotic microangiopathy and VOD, and in the same context the treatment of ACS are ongoing (clinicaltrials.gov; NCT03384693, NCT03805581, NCT02675959). Notably, DF suppresses the expression of heparanase transcripts, cell surface expression and enzymatic activity,[95] suggesting that DF may have anti-viral properties, although this remains to be confirmed.[71–73] Heparanase is putatively upregulated by the cytokine storm of advanced COVID-19 and may contribute to further inflammation, oedema of the microvascular bed and coagulopathy.[28,75,105,106] DF is a potent inhibitor of heparanase in terms of both cell surface and gene expression, and therefore is especially attractive. Furthermore, the therapeutic use of DF in a murine model of IPS significantly improved survival compared to untreated controls by reducing, among other biomarkers, the levels of Ang-2,[107] which is known to correlate with ARDS and is markedly elevated in critically ill patients with COVID-19.[38]

In addition, ICU-admitted patients with COVID-19 may display increased platelet activation and subsequent formation of platelet-monocyte aggregates upon interaction with P-selectin, thus stimulating monocyte-induced inflammation and thrombosis.[22] By reducing P-selectin and other adhesion molecules expression, DF may inhibit monocyte-derived inflammatory and pro-coagulant signals. Lastly and most importantly, DF has also been shown to decrease the activity of p38 MAPK and its pathway,[93] the importance of which is increasingly recognised in the pathogenesis of the COVID-19 hyperinflammation syndrome and this may be a key therapeutic target in this process.[58,108]

In summary, the multitargeted endothelial-based therapeutic properties of DF and its relative safety, as well as its regulatory approval, make it an ideal potential therapeutic candidate for the treatment of COVID-19 vascular complications.[28] In contrast to heparin, DF also exhibits broader anti-cytokine, anti-inflammatory and endothelial-stabilising properties. Importantly, by acting on the heparanase-HS axis,[74,102]

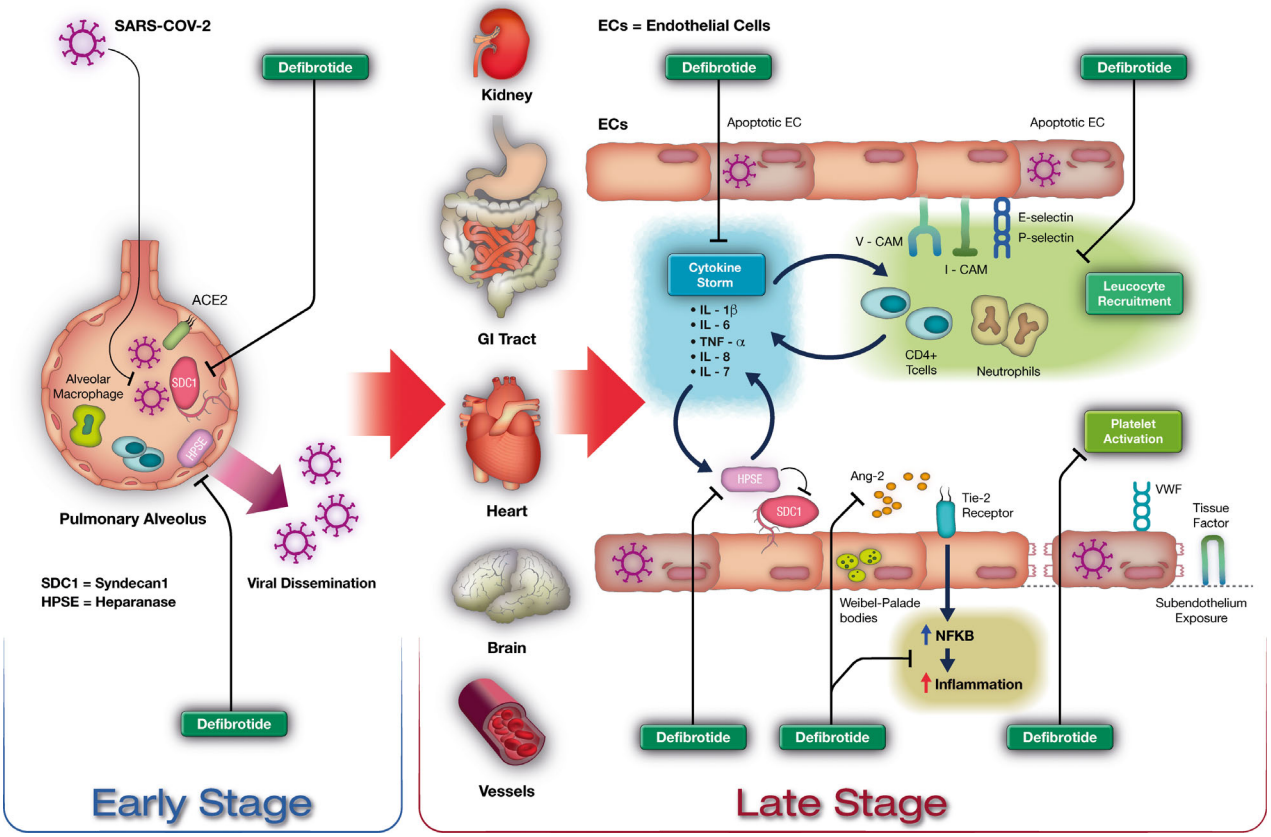


**Fig 1.** Potential mechanisms of action of defibrotide in the treatment of COVID-19. Left, defibrotide limits viral attachment by interfering with Syndecan-1, the primary cell surface heparan sulfate on ECs, and reduces viral dissemination, by inhibiting HPSE-mediated viral release. Right, effects of defibrotide on endothelial-mediated pathological processes. Viral infection of ECs promotes apoptosis with breakdown of endothelial barrier and exposure of the subendothelium, with subsequent platelet activation and thrombotic phenomena. Defibrotide inhibits platelet activation and leukocyte recruitment and blocks the generation of the cytokine storm; specifically, HPSE-mediated activation of immune cells is suppressed, thus limiting the development of cytokine release syndrome. Sars-Cov-2, severe acute respiratory syndrome coronavirus-2; ACE2, angiotensin-converting enzyme 2; Ang-2, angiopoietin-2; GI, gastrointestinal; IL, interleukin; NFKB, nuclear factor kappa-light chain-enhancer of activated B cells; TNF-α, tumor necrosis factor-alpha; VWF, von Willebrand Factor. [Colour figure can be viewed at wileyonlinelibrary.com]

DF may limit viral infectivity given its capacity to **i)** compete with HS and thereby possibly inhibit virus–cell adhesion and entry, **ii)** inhibit heparanase enzymatic activity and thereby attenuate virus detachment/release and spread[74] and **iii)** inhibit heparanase-mediated activation of immune cells and thereby upregulation of pro-inflammatory cytokines and the associated self-sustaining systemic inflammatory host response (Fig 1). Actively accruing, international Phase II clinical trials are now underway and should shed critical light on DF's therapeutic potential in patients with COVID-19 (examples include clinicaltrials.gov; NCT04348383, NCT04335201). Strikingly, two critically ill paediatric patients treated with DF for a SARS-CoV-2-associated multisystem inflammatory syndrome experienced complete resolution and no attributable toxicity, with correlative studies supporting the mechanistic effects described above, as well as favourable effects seen on complement activation.[109] Similarly, preliminary results from the current studies as part of the international DEFACOVID (Defibrotide as Prevention and Treatment of Respiratory Distress and Cytokine Release Syndrome of COVID-19) study group support both safety and promising potential efficacy to date.

### *Other heparanase inhibitors*

Given the heparanase-inhibiting activity of heparin, effort has been directed towards modifications of its structure to endow candidate molecules with potentiated anti-heparanase activity while limiting anticoagulant effects. Specifically, N-acetylated and glycol-split heparins are promising agents presenting such characteristics. Indeed, administration of N-acetylheparin (NAH) in murine models of sepsis ameliorated lung and intestinal injury and subsequent oedema by reducing tissue neutrophilic infiltration and suppressing IL-6, IL-1β and TNF-α production.[110,111] Furthermore, roneparstat, the most developed glycol-split NAH, restored pathological renal cellular damage caused by ischaemia-reperfusion by reducing release of pro-inflammatory cytokines and reverted established fibrotic processes, thus restoring normal tissue histology in preclinical models.[112] This aspect is especially relevant, considering the extensive formation of fibrosis and irreversible end-organ damage in post-BMT syndromes, SCD and advanced COVID-19.

Additionally, much interest has been directed towards the novel heparanase-inhibiting agent pixatimod, a modified oligosaccharide glycoside with heparan sulphate-mimetic properties. Pixatimod is a potent inhibitor of Type 1 T-helper cells (Th1)/Th17 effector functions,[113] IL-6 expression,[114] M2 macrophage activation,[115] angiogenesis and tumour progression *in vivo*. Furthermore, it exhibits mild anticoagulant activity and despite transient infusion reactions is otherwise generally well tolerated. Guimond *et al.*[116] have recently demonstrated that pixatimod interacts with the SARS-CoV-2 spike protein binding site, and this is coherent with its heparan sulphate-mimetic activity. Moreover, pixatimod was found to markedly inhibit SARS-CoV-2 infectivity,[116] supporting its clinical application as a novel therapeutic intervention for prophylaxis and treatment of COVID-19. Taken together, heparanase emerges as a host-encoded virulence factor that once activated enhances viral spread and triggers downstream inflammatory cascades. These preliminary data indicate that heparanase inhibitors currently under development are possible candidates for multisystem inflammatory conditions, such as COVID-19, sepsis, thrombotic microangiopathies and cancer, but as of now studies remain preclinical with clinical application pending.

## Conclusions

In conclusion, increasing evidence suggests that the SARS-CoV-2 directly targets ECs, promoting the release of pro-inflammatory and pro-thrombotic molecules. Endothelial dysfunction appears to be a crucial initiating step in the pathogenesis of the disease and its ensuing morbidity and mortality. Endotheliitis with the hyperproduction of cytokines leading to CRS, hypercoagulability and thrombotic microangiopathy are hallmarks shared by COVID-19, VOD/SOS and other endothelial injury syndromes, underpinned by inflammation and including the vaso-occlusive crises of SCD, so providing a common pathobiology across these respective syndromes. Most importantly, endothelial-protective agents, such as DF, represent a promising and rational therapeutic strategy in COVID-19, with DF currently under investigation in a variety of settings and combinations. As a unifying concept, heparanase inhibition, with the modulation of related pathways and other effects on endothelial stress responses may thus be crucial in mediating anti-viral and anti-inflammatory activity. In particular, as this relates to endotheliitis, it may directly abrogate CRS and its sequelae, which in turn may lead to improved patient outcome.

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## Author Contributions

Eleonora Calabretta, Jose M. Moraleda, Israel Vlodavsky, Ruben Jara, Carmelo Carlo-Stella and Paul Richardson drafted the manuscript; all authors participated in the critical revision and approval of the final report.

## Conflict of interest

Jose M. Moraleda declares Advisory Board fees from Jazz Pharmaceuticals; Antonio Pagliuca has received Advisory Board and Speaker fees from Jazz Pharmaceuticals; Rebecca M. Baron is on a Merck Advisory Board and a Consultant

for Genentech. Robert Soiffer serves on the Board of Directors for Kiadis and Be The Match/National Marrow Donor Program; provided consulting for Gilead, Rheos Therapeutics, Cugene, Precision Bioscience, Mana Therapeutics, VOR Biopharma, and Novartis; and Data Safety Monitoring Board for Juno/Celgene; Paul Richardson is an Advisory Committee Member for Jazz Pharmaceuticals; Carmelo Carlo-Stella is a Consultant/Advisory Board Member for Genente Science srl, ADC Therapeutics, Novartis, Roche, Karyopharm, Sanofi, Boehringer Igelheim and Servier. The remaining authors declare nothing to disclose.

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