REVIEW

SARS-CoV-2, platelets, and endothelium: coexistence in space and time, or a pernicious ménage à trois?

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Abstract

As we enter year 3 of SARS-CoV-2 pandemic, long-term consequences of COVID-19 have become a major public health issue worldwide; however, the molecular and cellular underpinnings of 'long COVID' remain very poorly understood. A paradigm has recently emerged that thrombo-inflammatory consequences of SARS-CoV-2's impact on endothelial cells and platelets likely play a significant role in the development of chronic symptomatology associated with COVID-19. In this brief overview, we discuss the recent findings pertaining to the detection of SARS-CoV-2 virions in vascular cell subtypes, the contribution of the coagulation system to the development of 'long COVID', and the potential role of stem/progenitor cells in the viral and thrombotic dissemination in this disorder.

Introduction

Thrombosis is one of the most perilous and frequently occurring clinical manifestations of the novel infectious disease COVID-19 caused by a single-stranded RNA (ssRNA) coronavirus SARS-CoV-2. Thrombi have been detected in the lung and other anatomical locations/vascular beds. Thrombotic disorders are not typically observed during the initial stages of COVID-19, which is confined to the epithelial lining of the upper respiratory tract; thrombi are more likely to occur if there is systemic progression. The pathogen infects lung tissue and replicates in alveolar cells; the subsequent disruption of the basal membrane facilitates SARS-CoV-2's access to pericytes and endothelial cells in the nearby capillaries, leading to the development of thrombotic micro-vasculitis (1). Upon ensuing physical damage to the vascular barrier, the pathogen reaches systemic circulation (2). Viremia and viral sepsis – the hallmarks of severe COVID-19 – carry a high risk of thrombotic vasculitis, thrombosis, and thromboembolism (3) that can manifest long-term in the latent form, as well as a persisting (so-called 'long COVID') and/or progressive process; they are also prevalent in COVID-19's terminal stage.

At the present time, the prevailing views of the pathophysiological underpinnings of SARS-CoV-2-induced hemostatic derangements emphasize thrombogenic phenomena that are secondary to the infection itself, for example, generalized immune and/or inflammatory mechanisms such as cytokine storm, complement activation, and various types of immune responses (4, 5, 6, 7, 8). It remains an open question whether ssRNA viruses can directly disrupt cellular effectors of hemostasis to an extent that would yield a pro-thrombotic shift. Even though the amount of information on this effect is very modest and its interpretation is

controversial, it is reasonable to explore a hypothesis that some of COVID-19's thrombotic sequelae – those that develop relatively early, as well as later on upon the onset of an unfavorable, prolonged course of the disease – can be caused at least in part by virus-induced cellular disruption. Specifically, SARS-CoV-2 virions themselves and its substructures, for example, intact ssRNA, viral proteins, and/or fragments thereof may serve as triggering agents/contributors to the damage of the cellular effectors of hemostasis. As outlined below, the damage caused to endothelial and/or platelet precursors in particular may set the stage for viral dissemination throughout the body, that is, the development of COVID-19 septicemia, as well as the conversion of the disease into a chronic disorder (long COVID).

Detection of SARS-CoV-2 virions in cellular effectors of hemostasis

Platelets and endothelial cells are hardly at the forefront of active ssRNA viral invasion and/or virion production – if detected at all, the rate of viral replication in these cell types is minimal and virions are not commonly detected in the supernatants of infected cultured cells (4, 9, 10). At the same time, a number of morphological, molecular, and physiological signs of cellular damage have been described that are tied to either the virions themselves, or its specific components; this damage may lead to significant clinicopathological consequences.

Platelets

There is abundant evidence that platelets are hyperactive in patients with COVID-19, with some studies implicating SARS-CoV-2 as a direct contributor. Manne *et al.* (11) demonstrated that platelet hyperactivation is partly due to the changes in nucleic acid content in their cytosol. Zaid *et al.* (12) detected SARS-CoV-2 RNA in hyperactive platelets that produced – and released into plasma – high quantities of inflammatory cytokines, serotonin, and platelet factor 4 (PF4) that can act as a chemokine (13). Thus, infected activated platelets may contribute to cytokine storm in COVID-19. In a detailed study, Zhang *et al.* (14) concluded that platelet hyperactivation in severe COVID-19 is positively associated with viremia (RNA-emia). *In vitro*, SARS-CoV-2 virions as well as isolated S-protein were shown to directly activate healthy human platelets as evidenced by platelet aggregation and spreading, PAC-1 binding, SELP expression, α-granule secretion, dense granule

release, and clot retraction; MAPK signaling pathway has been implicated. This leads to the elevation of coagulation factors F5/(F)V and F13/(F)XIII, several inflammatory cytokines (PF4, TNF, IL1B, and CXCL8), P-selectin, and platelet–leukocyte aggregates in the circulation (14).

Vascular endothelium

Several studies reported clinical and morphological signs of infectious endotheliitis in COVID-19. Various organs and tissues seem to be affected; electron microscopy, molecular, and immunohistochemical (IHC) approaches revealed the presence of SARS-CoV-2 virions and/or their fragments in the endothelium (1, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23). The expression of mRNA encoding IL6 and IFN-α proteins is increased in infected human microvascular endothelial cells (10). The levels of some of the markers of endothelial dysfunction (VCAM1, ANGPT2, and ICAM1) are positively associated with viral load in the circulation (RNA-emia) (24).

Transgenic mice expressing human angiotensin-converting enzyme 2 (hACE2) and non-human primates are susceptible to infection with SARS-CoV-2; in both of these experimental models, colocalization of the virus (readouts being RNA and protein) and the endothelial marker CD31 have been described, along with the activation of KRAS and MAPK signaling pathways (22). SARS-CoV-2 can also infect mature, pre-activated murine endothelial cells that become ACE2-positive, which was demonstrated using IHC and electron microscopy approaches (22). Thus, SARS-CoV-2 virus is able to infect and directly damage mature vascular endothelial cells *in vitro* and *in vivo*.

Viral replication has been described in the infected cultures of lung tissue-derived microvascular endothelial cells, albeit its intensity is appreciably lower compared to that observed in the paired cultures of lung tissue-derived epithelial cells (2). In both types of infected cells, generalized aberrations of the proteome have been described (2). Even though SARS-CoV-2 virions and S-protein are only barely detectable in endothelial cells, there is a marked expression of nucleocapsid protein (NP) with the accompanying alterations of certain ultra-structures, including the Golgi apparatus.

NP of SARS-CoV-2 – but not that of other coronaviruses, that is, SARS-CoV, MERS-CoV, HUB1-CoV, and/or H1N1 – can strongly activate human endothelial cells via the engagement of TLR2/NFKB and MAPK signaling pathways. SARS-CoV-2 NP potentiates the expression of ICAM1 and VCAM1, thus promoting monocyte adhesion to endothelial surfaces, as well as such proinflammatory

cytokines as SELE, MCP-1/CCL2, IL1B (mRNA), and TNF – a potent inducer of endothelial activation; it is notable that the nature and the magnitude of SARS-CoV-2 NP's effects on the endothelium are comparable to those of TNF ([25], [26]). TNF and various selectins (of endothelial as well as platelet origin) are known to be potent stimulators of von Willebrand factor (vWF) and coagulation factor F8/(F)VIII secretion from the endothelium; in COVID-19, a strong elevation of vWF and F8 secretion from the endothelium has been reported ([26], [27]).

Tissue factor and COVID-19

Evidence continues to accumulate that COVID-19-associated coagulopathies are associated with tissue factor (TF)-driven thrombosis. Under physiological conditions, TF is absent from the circulation and triggers clotting by forming a 1:1 complex with the serine protease coagulation Factor 7/FVII (a) upon tissue and/or vessel wall injury; however, blood-borne TF-bearing microvesicles (MVs) can contribute to thrombosis in various pathological states, including COVID-19 (reviewed in Mackman *et al.*) ([28]). TF+ MVs may transfer enzymatically active TF to the surface of endothelial cells as well as platelets, rendering them more thrombogenic. Two seminal observations documented the presence of TF+ MVs in the blood of COVID-19 patients ([29], [30]). Most recently, TF protein was found to be significantly elevated in the blood of COVID-19 patients with thrombosis compared to COVID-19 patients without thrombosis; complement factor C2 was proposed as a possible contributor to the increased TF expression ([31]). The cell sources of TF in the circulation of COVID-19 patients are thought to be primarily lung epithelial cells and monocytes ([28]); however, it cannot be excluded that activated endothelial cells may also release TF+ MVs in COVID-19 patients.

Encephalopathy and COVID-19

Encephalopathies (brain fog) comprise a frequent, clinically pronounced manifestation of COVID-19 – during the acute stage, throughout convalescence, as well as post-COVID. Its presentation is rather distinct in patients with mild/moderate vs severe disease; in the latter group, it is influenced by many pathogenic factors including hypoxia, cytokine-induced damage, direct neuronal invasion by the virus, drug toxicity, and macrovascular (thromboembolic) events, for example, strokes, the occurrence of which is

significantly higher even among younger patients ([32]). Patient age and comorbidities are equally important. It is very likely that, in older patients, COVID-19 is exacerbated by such age-related conditions as cerebrovascular disease, encephalopathies of hypertonic and/or metabolic origin, and Alzheimer's disease. It is all but certain that COVID-19 fuels the progression of such encephalopathies ([33]), particularly because this infection worsens the already-present neurodegeneration.

With that being said, the frequency of encephalopathy-type symptoms among younger patients (16–30 years old) with moderate COVID-19 is remarkable, and even more so – their persistence for many months in over a half of such patients ([34]). This suggests that pre-morbidity neurodegeneration factors, comorbidities, macrovascular events/strokes, and/or other major cerebral abnormalities do not play a major role in such patients; rather, the root cause(s) may lie in organic neurotoxic insults or, equally possibly, micro(vascular) focal brain injury. Furthermore, even mild COVID-19 may lead to brain atrophy, increased cerebrospinal fluid volume, and decreased ability to perform complex tasks ([35]).

In support of this tenet, Qin *et al.* ([36]) demonstrated that, in post-COVID-19, the odds of micro-focal brain injury/microcirculatory-type deficiencies are high even in the absence of overt neurological symptoms. We note that these deficiencies are likely related to inflammatory factors, as well as endothelial damage. In COVID-19, cerebral endotheliitis with micro-thrombosis and hemorrhage (micro-focal manifestation) has been demonstrated via clinical observations, as well as histologically using various visualization techniques ([20], [37], [38]). Micro-emboli in the cerebral vascular bed can also be deemed a proven phenomenon in COVID-19 ([39]).

SARS-CoV-2 entry into target cells: mechanisms and uncertainties

It is remarkable that even though SARS-CoV-2 and its RNA have been detected in mature human platelets and vascular endothelial cells, these cells typically do not express functional components of the classical stepwise mechanism of SARS-CoV-2's entry into the cell that comprises the cell surface receptor/protease ACE2 and the transmembrane protease serine-2 (TMPRSS2). A few isolated reports notwithstanding ([14], [23]) the expression and/or biosynthesis of these two key peptidases has been detected in endothelial cells and/or platelets at extremely low levels ([11], [12], [40], [41], [42]).

Then how, in the absence of the canonical mechanisms, does SARS-CoV-2 virus manage to infect target cells-effectors of hemostasis, that is, platelets and endothelial cells? Several possibilities can be proposed. First, low-level peptidase expression may be sufficient to yield viral infection, yet it may elude detection using conventional approaches/testing conditions. For instance, Kaneko *et al.* (43) demonstrated that pressure shifts in the pulsating circulation are required for the expression of ACE2 in endothelial cells to become manifest; other experimental conditions favoring ACE2 expression have been described (22). We note that high viral load and inflammation likely serve as a direct trigger of this mechanism (42). Secondly, protease expression may depend on vascular bed-specific features of the endothelium, for example, the type of the vessel and its diameter, the vessel's presence in a particular tissue and/or organ, etc. One example of this comprises the prevalence of ACE2/TMPRSS2 expression in the coronary capillaries above arterioles/venules and, even more so, above coronary arteries (1, 23). Thirdly, other, yet-to-be-described mechanisms of viral entry may exist: matrix metalloprotease BSG/CD147 (basigin) is able to functionally substitute ACE2 (44, 45, 46), whereas the function of TMPRSS2 can be substituted by that of several cellular proteases, for example, proprotein convertase furin (47) and cysteine proteases cathepsin B and L (48). The $\alpha_v\beta_3$ integrin-mediated endocytosis may also facilitate SARS-CoV-2's entry into endothelial cells (49). Finally, another potential route of SARS-CoV-2's entry into cells and the ensuing infection should be pointed out, which may be critically important.

Stem cells as a SARS-CoV-2 gateway

It has been reported that ACE2 and TMPRSS2 – the two peptidases that play key roles in facilitating the canonical entry of SARS-CoV-2 into cells – are expressed at high levels in hematopoietic stem cells (HSCs) and endothelial progenitor cells (EPCs). Moreover, the expression of these peptidases is at the highest level in their precursors – CD45-positive very small embryonic-like stem cells, which may correspond to hemangioblasts (50, 51). At the same time, the proportion of cells expressing ACE2 decreases significantly during their differentiation and conversion into mature blood cells and vascular endothelial cells (50). Concomitantly, an alternative mechanism of viral entry into cells may come into play: recent studies demonstrated that, in COVID-19, the above-mentioned metalloprotease

BSG/CD147 facilitates SARS-CoV-2's entry into megakaryocytes which results in specific transcriptome-level aberrations that can be passed on to mature platelets with pathological consequences (52). In agreement with this, studies using HSCs and EPCs demonstrated that SARS-CoV-2-induced transcriptome-level aberrations can be passed on to mature cells during the differentiation process (50); the possible mechanisms that lead to SARS-CoV-2-induced pathological changes in HSCs are discussed in detail elsewhere (53).

Among the aberrations that were detected in infected endothelial cells at the RNA and protein levels, some of the most notable comprise inflammatory hyperactivation, diminution of the regenerative potential and pathological transformation, elicitation of pyroptosis, enhanced release of cytosolic components (e.g. cytokines and TF) into the intercellular space, and peripheral cell thrombosis (22, 50, 51, 54, 55, 56).

Infection of stem/progenitor cells vis-à-vis viral and thrombotic dissemination

Viremia is one of the cardinal determinants of outcomes in severe COVID-19; it is observed frequently and can persist for weeks and even months. Concomitantly, heightened levels of infection-prone endothelial precursors are present in the circulation; precursor levels positively associate with viremia (57, 58). Infection by SARS-CoV-2 damages endothelial cells as well as platelets in ways that may contribute to the development of thrombotic micro-vasculitis, thrombosis of large veins and arteries, and embolisms – all characteristic for and prevalent in the course of severe acute COVID-19 and long COVID: we note that precursor cell infection likely contributes not only to multi-organ and thrombo-vascular complications but also to the development of long COVID (Fig. 1). We note that endothelial dysfunction/damage and thrombotic complications increasingly appear to be frequent and prolonged sequelae of COVID-19 (59, 60, 61, 62). Interestingly, there is evidence that vasculitis comprises a locus minoris resistentiae: judging from [18F]FDG-PET/CT data, the hallmark of inflammation associated with long COVID is its predominance in the bone marrow and in the vasculature (63). The elegant recent work by Pretorius and colleagues demonstrated that platelets are hyperactivated in patients with long COVID (64), which may explain the fact that d-dimer levels can remain elevated up to several months post-acute phase of the disease (65). Virus-induced
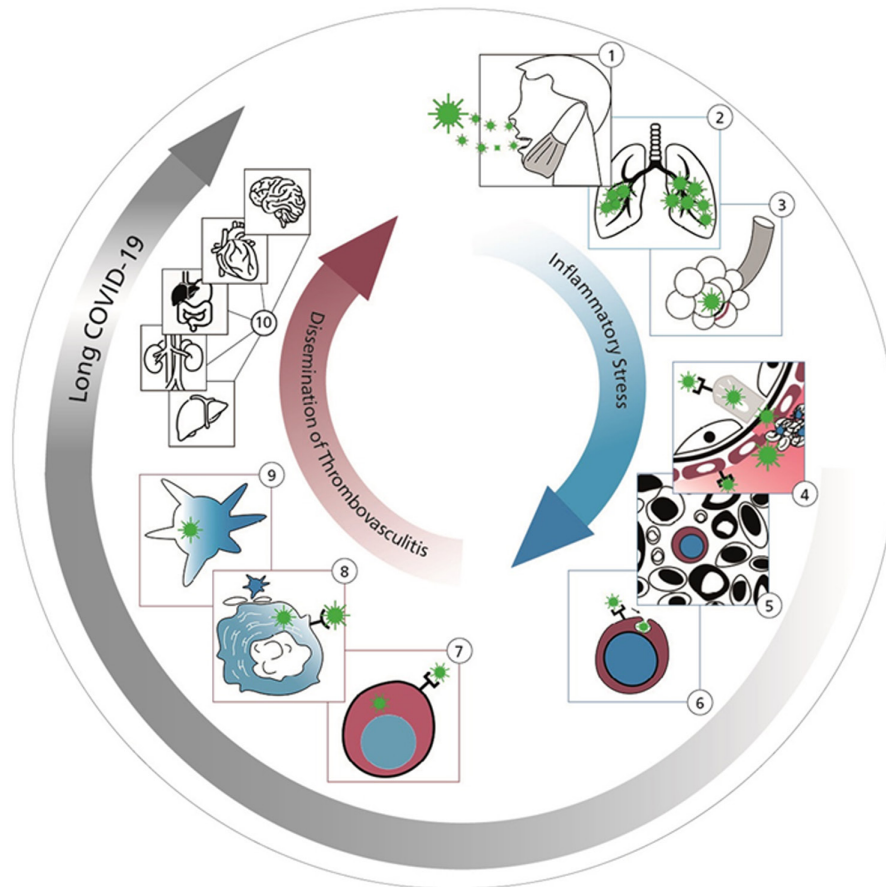
Figure 1

Pathophysiological hallmarks of acute SARS-CoV-2 infection and its chronic consequences (long COVID). 1. Infection by SARS-CoV-2 occurs via inhalation of virus-containing respiratory droplets. 2 and 3. SARS-CoV-2 infects alveolar cells. 4. In alveoli, ACE2-expressing alveolar type II cells comprise SARS-CoV-2's target (ACE2 is depicted in the drawing with a viral particle bound to it:). ACE2 facilitates viral entry into the cells; subsequently, viral replication leads to the development of alveolitis and necrotic inflammation accompanied by basal membrane disruption whereby viral particles come in contact with endothelial cells of alveolar capillaries. Endothelial cells are progressively damaged by inflammation and, at high viral titers, become infected even though the levels of ACE2 expressed on their surfaces are quite low. Viral replication does not seem to occur in endothelial cells; however, their damage leads to a pro-thrombotic phenotype, exemplified by the release of von Willebrand factor. The resultant pathological changes comprise thrombotic microvascultis of the lung, which is accompanied by a pro-thrombotic shift in the plasma proteome. 5. SARS-CoV-2 originating from lung microvascular endothelium enters the bloodstream, which comprises the onset of the septic phase during which – aside from the systemic viral dissemination and inflammation, vasculitis, and hyper-coagulation – infection of stem/progenitor cells in the bone marrow and other tissues is a significant pathological hallmark. 7. ACE2 is also expressed in several types of other progenitor cells, including hematopoietic and endothelial progenitors, which renders them vulnerable to SARS-CoV-2. 8 and 9. Megakarvocvtes express their own alternative gateways for coronavirus entry, for example, BSG/CD147 (depicted in the drawing with a viral particle bound to it). Viral replication does not seem to occur in megakaryocytes; however, aberrations of the transcriptome have been described in megakaryocytes as well as platelets produced by them, likely with pro-thrombotic consequences. 10. Damage to the lung endothelium caused by the infection of endothelial cells, hematogenous viral dissemination, infection of the cellular precursors of endothelial cells/platelets, and transcriptome-level thrombogenic aberrations caused by the virus in infected cells significantly contribute to a) the severity of early SARS-CoV-2 infection; b) the emergence of SARS-CoV-2 infection's septic multi-organ and thrombo-vascular complications; c) the development of long COVID.

cellular senescence may be of particular relevance to platelet pathology in long COVID: supernatants from virus-infected senescent human fibroblasts – but not from non-infected and/or senescence-incapable cells – prompted platelet activation, shortened clotting times, and induced the formation of neutrophil extracellular traps in vitro (66).

Alpha, beta, gamma, delta, omicron... and how far beyond? Treating and preventing (long) COVID

As we write this review, a highly transmissible omicron variant of SARS-CoV-2 (B.1.1.529) has become the predominant strain in the United States. Omicron's spike

protein sequence has 30+ mutations that render it not only more transmissible and invasive than all previous variants (67) but also resistant to most MAB therapies as was presciently predicted by Chen and colleagues (68). However, Omicron also seems to cause less severe disease (presumably due to a less severe viral burden in the lower respiratory tract) and, to date, reports about thrombotic complications have been extremely rare (69, 70). We note that this echoes the observations in murine and hamster models of B.1.1.529 infection (71). While it is too early to know whether Omicron causes long COVID and, if so, whether it is less severe, we posit that the ability of SARS-CoV-2 to mutate so rapidly heightens the need for inexpensive therapies whose efficacy is less likely to be impacted by viral adaptations in its genome; in this light, Paxlovid holds promise (72). Equally important is the clinicians' ability to predict COVID-19 severity; one promising technology comprises testing for early non-neutralizing, afucosylated IgG antibodies specific to SARS-CoV-2 – in a recent study, they were found to be associated with progression from mild to more severe COVID-19 (73).

Concluding remarks

As we await SARS-CoV-2 to turn endemic, the numbers of those with long-term consequences of COVID-19 continue to rise. In this brief overview, we aimed to convey the notion that the interactions between the virus, endothelial cells, and platelets likely comprise a pathophysiological ménage à trois with many systemic ramifications and, possibly, additional cell (sub)types participating. Thus, the development of approaches to effectively manage long COVID is critical, and improving our understanding of its cellular and molecular underpinnings is essential to the success of this effort.

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References

1 Magro CM, Mulvey J, Kubiak J, Mikhail S, Suster D, Crowson AN, Laurence J & Nuovo G. Severe COVID-19: a multifaceted viral vasculopathy syndrome. *Annals of Diagnostic Pathology* 2021 **50** 151645. (https://doi.org/10.1016/j.anndiagpath.2020.151645)

2 Wang P, Luo R, Zhang M, Wang Y, Song T, Tao T, Li Z, Jin L, Zheng H, Chen W, *et al*. A cross-talk between epithelium and endothelium mediates human alveolar-capillary injury during SARS-CoV-2 infection. *Cell Death and Disease* 2020 **11** 1042. (https://doi.org/10.1038/s41419-020-03252-9)

3 Suh YJ, Hong H, Ohana M, Bompard F, Revel MP, Valle C, Gervaise A, Poissy J, Susen S, Hékimian G, *et al*. Pulmonary embolism and deep vein thrombosis in COVID-19: a systematic review and meta-analysis. *Radiology* 2021 **298** E70–E80. (https://doi.org/10.1148/radiol.2020203557)

4 Nicosia RF, Ligresti G, Caporarello N, Akilesh S & Ribatti D. COVID-19 vasculopathy: mounting evidence for an indirect mechanism of endothelial injury. *American Journal of Pathology* 2021 **191** 1374–1384. (https://doi.org/10.1016/j.ajpath.2021.05.007)

5 Bonaventura A, Vecchié A, Dagna L, Martinod K, Dixon DL, Van Tassell BW, Dentali F, Montecucco F, Massberg S, Levi M, *et al*. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. *Nature Reviews: Immunology* 2021 **21** 319–329. (https://doi.org/10.1038/s41577-021-00536-9)

6 Page EM & Ariëns RAS. Mechanisms of thrombosis and cardiovascular complications in COVID-19. *Thrombosis Research* 2021 **200** 1–8. (https://doi.org/10.1016/j.thromres.2021.01.005)

7 Perico L, Benigni A, Casiraghi F, Ng LFP, Renia L & Remuzzi G. Immunity, endothelial injury and complement-induced coagulopathy in COVID-19. *Nature Reviews: Nephrology* 2021 **17** 46–64. (https://doi.org/10.1038/s41581-020-00357-4)

8 Stenmark KR, Frid MG, Gerasimovskaya E, Zhang H, McCarthy MK, Thurman JM & Morrison TE. Mechanisms of SARS-CoV-2-induced lung vascular disease: potential role of complement. *Pulmonary Circulation* 2021 **11** 20458940211015799. (https://doi.org/10.1177/20458940211015799)

9 Wagner JUG, Bojkova D, Shumliakivska M, Luxán G, Nicin L, Aslan GS, Milting H, Kandler JD, Dendorfer A, Heumueller AW, *et al*. Increased susceptibility of human endothelial cells to infections by SARS-CoV-2 variants. *Basic Research in Cardiology* 2021 **116** 42. (https://doi.org/10.1007/s00395-021-00882-8)

10 Dolci M, Signorini L, D'Alessandro S, Perego F, Parapini S, Sommariva M, Taramelli D, Ferrante P, Basilico N & Delbue S. In vitro SARS-CoV-2 infection of microvascular endothelial cells: effect on pro-inflammatory cytokine and chemokine release. *International Journal of Molecular Sciences* 2022 **23** 4063. (https://doi.org/10.3390/ijms23074063)

11 Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, Stubben C, Petrey AC, Tolley ND, Guo L, Cody M, *et al*. Platelet gene expression and function in patients with COVID-19. *Blood* 2020 **136** 1317–1329. (https://doi.org/10.1182/blood.2020007214)

12 Zaid Y, Puhm F, Allaeys I, Naya A, Oudghiri M, Khalki L, Limami Y, Zaid N, Sadki K, Ben El Haj R, *et al*. Platelets can associate with SARS-Cov-2 RNA and are hyperactivated in COVID-19. *Circulation Research* 2020 **127** 1404–1418. (https://doi.org/10.1161/CIRCRESAHA.120.317703)

13 Bakogiannis C, Sachse M, Stamatelopoulos K & Stellos K. Platelet-derived chemokines in inflammation and atherosclerosis. *Cytokine* 2019 **122** 154157. (https://doi.org/10.1016/j.cyto.2017.09.013)

14 Zhang S, Liu Y, Wang X, Yang L, Li H, Wang Y, Liu M, Zhao X, Xie Y, Yang Y, *et al*. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. *Journal of Hematology and Oncology* 2020 **13** 120. (https://doi.org/10.1186/s13045-020-00954-7)

15 Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, *et al*. Pulmonary

vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *New England Journal of Medicine* 2020 **383** 120–128. (https://doi.org/10.1056/NEJMoa2015432)

16 Escher F, Pietsch H, Aleshcheva G, Bock T, Baumeier C, Elsaesser A, Wenzel P, Hamm C, Westenfeld R, Schultheiss M, *et al*. Detection of viral SARS-CoV-2 genomes and histopathological changes in endomyocardial biopsies. *ESC Heart Failure* 2020 **7** 2440–2447. (https://doi.org/10.1002/ehf2.12805)

17 Puelles VG, Lütgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, Chilla S, Heinemann A, Wanner N, Liu S, *et al*. Multiorgan and renal tropism of SARS-CoV-2. *New England Journal of Medicine* 2020 **383** 590–592. (https://doi.org/10.1056/NEJMc2011400)

18 Stahl K, Bräsen JH, Hoeper MM & David S. Direct evidence of SARS-CoV-2 in gut endothelium. *Intensive Care Medicine* 2020 **46** 2081–2082. (https://doi.org/10.1007/s00134-020-06237-6)

19 Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR, Schuepbach RA, Ruschitzka F & Moch H. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020 **395** 1417–1418. (https://doi.org/10.1016/S0140-6736(20)30937-5)

20 Kirschenbaum D, Imbach LL, Rushing EJ, Frauenknecht KBM, Gascho D, Ineichen BV, Keller E, Kohler S, Lichtblau M, Reimann RR, *et al*. Intracerebral endotheliitis and microbleeds are neuropathological features of COVID-19. *Neuropathology and Applied Neurobiology* 2021 **47** 454–459. (https://doi.org/10.1111/nan.12677)

21 Ko CJ, Harigopal M, Gehlhausen JR, Bosenberg M, McNiff JM & Damsky W. Discordant anti-SARS-CoV-2 spike protein and RNA staining in cutaneous perniotic lesions suggests endothelial deposition of cleaved spike protein. *Journal of Cutaneous Pathology* 2021 **48** 47–52. (https://doi.org/10.1111/cup.13866)

22 Liu F, Han K, Blair R, Kenst K, Qin Z, Upcin B, Wörsdörfer P, Midkiff CC, Mudd J, Belyaeva E, *et al*. SARS-CoV-2 infects endothelial cells in vivo and in vitro. *Frontiers in Cellular and Infection Microbiology* 2021 **11** 701278. (https://doi.org/10.3389/fcimb.2021.701278)

23 Maccio U, Zinkernagel AS, Shambat SM, Zeng X, Cathomas G, Ruschitzka F, Schuepbach RA, Moch H & Varga Z. SARS-CoV-2 leads to a small vessel endotheliitis in the heart. *EBiomedicine* 2021 **63** 103182. (https://doi.org/10.1016/j.ebiom.2020.103182)

24 Bermejo-Martin JF, González-Rivera M, Almansa R, Micheloud D, Tedim AP, Domínguez-Gil M, Resino S, Martín-Fernández M, Ryan Murua P, Pérez-García F, *et al*. Viral RNA load in plasma is associated with critical illness and a dysregulated host response in COVID-19. *Critical Care* 2020 **24** 691. (https://doi.org/10.1186/s13054-020-03398-0)

25 Qian Y, Lei T, Patel PS, Lee CH, Monaghan-Nichols P, Xin HB, Qiu J & Fu M. Direct activation of endothelial cells by SARS-CoV-2 nucleocapsid protein is blocked by simvastatin. *Journal of Virology* 2021 **95** e0139621. (https://doi.org/10.1128/JVI.01396-21)

26 Rossouw TM, Anderson R, Manga P & Feldman C. Emerging role of platelet-endothelium interactions in the pathogenesis of severe SARS-CoV-2 infection-associated myocardial injury. *Frontiers in Immunology* 2022 **13** 776861. (https://doi.org/10.3389/fimmu.2022.776861)

27 De Cristofaro R, Liuzzo G, Sacco M, Lancellotti S, Pedicino D & Andreotti F. Marked von Willebrand factor and factor VIII elevations in severe acute respiratory syndrome coronavirus-2-positive, but not severe acute respiratory syndrome coronavirus-2-negative, pneumonia: a case-control study. *Blood Coagulation and Fibrinolysis* 2021 **32** 285–289. (https://doi.org/10.1097/MBC.0000000000000998)

28 Mackman N, Grover SP & Antoniak S. Tissue factor expression, extracellular vesicles, and thrombosis after infection with the respiratory viruses influenza a virus and coronavirus. *Journal of Thrombosis and Haemostasis* 2021 **19** 2652–2658. (https://doi.org/10.1111/jth.15509)

29 Rosell A, Havervall S, von Meijenfeldt F, Hisada Y, Aguilera K, Grover SP, Lisman T, Mackman N & Thålin C. Patients with COVID-19 have elevated levels of circulating extracellular vesicle tissue factor activity that is associated with severity and mortality-brief report.

Arteriosclerosis, Thrombosis, and Vascular Biology 2021 **41** 878–882. (https://doi.org/10.1161/ATVBAHA.120.315547)

30 Guervilly C, Bonifay A, Burtey S, Sabatier F, Cauchois R, Abdili E, Arnaud L, Lano G, Pietri L, Robert T, *et al*. Dissemination of extreme levels of extracellular vesicles: tissue factor activity in patients with severe COVID-19. *Blood Advances* 2021 **5** 628–634. (https://doi.org/10.1182/bloodadvances.2020003308)

31 Madeeva DV, Borges K, Shallow M, Juthani PV, Wang SY, Gupta A, Chun HJ, Lee AI & Pine AB. Proteomic profiles in patients with thrombosis due to COVID-19 are distinct from non-COVID-19 thrombosis. *Blood* 2021 **138** (Supplement 1) 777. (https://doi.org/10.1182/blood-2021-153959)

32 Oxley TJ, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh IP, De Leacy RA, Shigematsu T, Ladner TR, Yaeger KA, *et al*. Large-vessel stroke as a presenting feature of Covid-19 in the young. *New England Journal of Medicine* 2020 **382** e60. (https://doi.org/10.1056/NEJMc2009787)

33 Xia X, Wang Y & Zheng J. COVID-19 and Alzheimer's disease: how one crisis worsens the other. *Translational Neurodegeneration* 2021 **10** 15. (https://doi.org/10.1186/s40035-021-00237-2)

34 Blomberg B, Mohn KG, Brokstad KA, Zhou F, Linchausen DW, Hansen BA, Lartey S, Onyango TB, Kuwelker K, Sævik M, *et al*. Long COVID in a prospective cohort of home-isolated patients. *Nature Medicine* 2021 **27** 1607–1613. (https://doi.org/10.1038/s41591-021-01433-3)

35 Abbasi J. Even mild COVID-19 may change the brain. *JAMA* 2022 **327** 1321–1322. (https://doi.org/10.1001/jama.2022.4507)

36 Qin Y, Wu J, Chen T, Li J, Zhang G, Wu D, Zhou Y, Zheng N, Cai A, Ning Q, *et al*. Long-term microstructure and cerebral blood flow changes in patients recovered from COVID-19 without neurological manifestations. *Journal of Clinical Investigation* 2021 **131** e147329. (https://doi.org/10.1172/JCI147329)

37 Keller E, Brandi G, Winklhofer S, Imbach LL, Kirschenbaum D, Frontzek K, Steiger P, Dietler S, Haeberlin M, Willms J, *et al*. Large and small cerebral vessel involvement in severe COVID-19: detailed clinical workup of a case series. *Stroke* 2020 **51** 3719–3722. (https://doi.org/10.1161/STROKEAHA.120.031224)

38 Sim R, Cheung G, Ting D, Wong E, Wong TY, Yeo I & Wong CW. Retinal microvascular signs in COVID-19. *British Journal of Ophthalmology* 2021. (https://doi.org/10.1136/bjophthalmol-2020-318236)

39 Batra A, Clark JR, LaHaye K, Shlobin NA, Hoffman SC, Orban ZS, Colton K, Dematte JE, Sorond FA, Koralnik IJ, *et al*. Transcranial Doppler ultrasound evidence of active cerebral embolization in COVID-19. *Journal of Stroke and Cerebrovascular Diseases* 2021 **30** 105542. (https://doi.org/10.1016/j.jstrokecerebrovasdis.2020.105542)

40 Nascimento Conde J, Schutt WR, Gorbunova EE & Mackow ER. Recombinant ACE2 expression is required for SARS-CoV-2 to infect primary human endothelial cells and induce inflammatory and procoagulative responses. *mBio* 2020 **11** e03185-20. (https://doi.org/10.1128/mBio.03185-20)

41 McCracken IR, Saginc G, He L, Huseynov A, Daniels A, Fletcher S, Peghaire C, Kalna V, Andaloussi-Mäe M, Muhl L, *et al*. Lack of evidence of angiotensin-converting enzyme 2 expression and replicative infection by SARS-CoV-2 in human endothelial cells. *Circulation* 2021 **143** 865–868. (https://doi.org/10.1161/CIRCULATIONAHA.120.052824)

42 Schimmel L, Chew KY, Stocks CJ, Yordanov TE, Essebier P, Kulasinghe A, Monkman J, Dos Santos Miggiolaro AFR, Cooper C, de Noronha L, *et al*. Endothelial cells are not productively infected by SARS-CoV-2. *Clinical and Translational Immunology* 2021 **10** e1350. (https://doi.org/10.1002/cti2.1350)

43 Kaneko N, Satta S, Komuro Y, Muthukrishnan SD, Kakarla V, Guo L, An J, Elahi F, Kornblum HI, Liebeskind DS, *et al*. Flow-mediated susceptibility and molecular response of cerebral endothelia to SARS-CoV-2 infection. *Stroke* 2021 **52** 260–270. (https://doi.org/10.1161/STROKEAHA.120.032764)

44 Ahmetaj-Shala B, Vaja R, Atanur SS, George PM, Kirkby NS & Mitchell JA. Cardiorenal tissues express SARS-CoV-2 entry genes and basigin (BSG/CD147) increases with age in endothelial cells. *JACC: Basic to Translational Science* 2020 **5** 1111–1123. (https://doi.org/10.1016/j.jacbts.2020.09.010)

45 Guney C & Akar F. Epithelial and endothelial expressions of ACE2: SARS-CoV-2 entry routes. *Journal of Pharmacy and Pharmaceutical Sciences* 2021 **24** 84–93. (https://doi.org/10.18433/jpps31455)

46 Maugeri N, De Lorenzo R, Clementi N, Antonia Diotti R, Criscuolo E, Godino C, Tresoldi C, Angels For Covid-BioB Study Group B, Bonini C, Clementi M, *et al*. Unconventional CD147-dependent platelet activation elicited by SARS-CoV-2 in COVID-19. *Journal of Thrombosis and Haemostasis* 2022 **20** 434–448. (https://doi.org/10.1111/jth.15575)

47 Bestle D, Heindl MR, Limburg H, Van Lam van T, Pilgram O, Moulton H, Stein DA, Hardes K, Eickmann M, Dolnik O, *et al*. TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. *Life Science Alliance* 2020 **3** e202000786. (https://doi.org/10.26508/lsa.202000786)

48 Wazny V, Siau A, Wu KX & Cheung C. Vascular underpinning of COVID-19. *Open Biology* 2020 **10** 200208. (https://doi.org/10.1098/rsob.200208)

49 Bugatti A, Filippini F, Bardelli M, Zani A, Chiodelli P, Messali S, Caruso A & Caccuri F. SARS-CoV-2 infects human ACE2-negative endothelial cells through an $\alpha_v\beta_3$ integrin-mediated endocytosis even in the presence of vaccine-elicited neutralizing antibodies. *Viruses* 2022 **14** 705. (https://doi.org/10.3390/v14040705)

50 Ropa J, Cooper S, Capitano ML, Van't Hof W & Broxmeyer HE. Human hematopoietic stem, progenitor, and immune cells respond ex vivo to SARS-CoV-2 spike protein. *Stem Cell Reviews and Reports* 2021 **17** 253–265. (https://doi.org/10.1007/s12015-020-10056-z)

51 Ratajczak MZ, Bujko K, Ciechanowicz A, Sielatycka K, Cymer M, Marlicz W & Kucia M. SARS-CoV-2 entry receptor ACE2 is expressed on very small CD45- precursors of hematopoietic and endothelial cells and in response to virus spike protein activates the Nlrp3 inflammasome. *Stem Cell Reviews and Reports* 2021 **17** 266–277. (https://doi.org/10.1007/s12015-020-10010-z)

52 Barrett TJ, Bilaloglu S, Cornwell M, Burgess HM, Virginio VW, Drenkova K, Ibrahim H, Yuriditsky E, Aphinyanaphongs Y, Lifshitz M, *et al*. Platelets contribute to disease severity in COVID-19. *Journal of Thrombosis and Haemostasis* 2021 **19** 3139–3153. (https://doi.org/10.1111/jth.15534)

53 Balzanelli MG, Distratis P, Dipalma G, Vimercati L, Inchingolo AD, Lazzaro R, Aityan SK, Maggiore ME, Mancini A, Laforgia R, *et al*. Sars-CoV-2 virus infection may interfere CD34+ hematopoietic stem cells and megakaryocyte-erythroid progenitors differentiation contributing to platelet defection towards insurgence of thrombocytopenia and thrombophilia. *Microorganisms* 2021 **9** 1632. (https://doi.org/10.3390/microorganisms9081632)

54 Kucia M, Ratajczak J, Bujko K, Adamiak M, Ciechanowicz A, Chumak V, Brzezniakiewicz-Janus K & Ratajczak MZ. An evidence that SARS-Cov-2/COVID-19 spike protein (SP) damages hematopoietic stem/progenitor cells in the mechanism of pyroptosis in Nlrp3 inflammasome-dependent manner. *Leukemia* 2021 **35** 3026–3029. (https://doi.org/10.1038/s41375-021-01332-z)

55 Nader D, Fletcher N, Curley GF & Kerrigan SW. SARS-CoV-2 uses major endothelial integrin αvβ3 to cause vascular dysregulation in-vitro during COVID-19. *PLoS ONE* 2021 **16** e0253347. (https://doi.org/10.1371/journal.pone.0253347)

56 Shao Y, Saredy J, Xu K, Sun Y, Saaoud F, Drummer 4th C, Lu Y, Luo JJ, Lopez-Pastrana J, Choi ET, *et al*. Endothelial immunity trained by coronavirus infections, DAMP stimulations and regulated by anti-oxidant NRF2 may contribute to inflammations, myelopoiesis, COVID-19 cytokine storms and thromboembolism. *Frontiers in Immunology* 2021 **12** 653110. (https://doi.org/10.3389/fimmu.2021.653110)

57 Mancuso P, Gidaro A, Gregato G, Raveane A, Cremonesi P, Quarna J, Caccia S, Gusso L, Rusconi S, Giacomelli A, *et al*. Circulating endothelial progenitors are increased in COVID-19 patients and correlate with SARS-CoV-2 RNA in severe cases. *Journal of Thrombosis and Haemostasis* 2020 **18** 2744–2750. (https://doi.org/10.1111/jth.15044)

58 Poyatos P, Luque N, Eizaguirre S, Sabater G, Sebastián L, Francisco-Albesa Í, Peracaula M, Boixadé M, Orriols R & Tura-Ceide O. Post-COVID-19 patients show an increased endothelial progenitor cell production. *Translational Research* 2022 **243** 14–20. (https://doi.org/10.1016/j.trsl.2022.01.004)

59 Nishijima Y, Hader SN, Hanson AJ, Zhang DX, Sparapani R, Gutterman DD & Beyer AM. Prolonged endothelial-dysfunction in human arterioles following infection with SARS-CoV-2. *Cardiovascular Research* 2022 **118** 18–19. (https://doi.org/10.1093/cvr/cvab339)

60 Haffke M, Freitag H, Rudolf G, Seifert M, Doehner W, Scherbakov N, Hanitsch L, Wittke K, Bauer S, Konietschke F, *et al*. Endothelial dysfunction and altered endothelial biomarkers in patients with post-COVID-19 syndrome and chronic fatigue syndrome (ME/CFS). *Journal of Translational Medicine* 2022 **20** 138. (https://doi.org/10.1186/s12967-022-03346-2)

61 Al-Aly Z, Xie Y & Bowe B. High-dimensional characterization of post-acute sequelae of COVID-19. *Nature* 2021 **594** 259–264. (https://doi.org/10.1038/s41586-021-03553-9)

62 Xie Y, Xu E, Bowe B & Al-Aly Z. Long-term cardiovascular outcomes of COVID-19. *Nature Medicine* 2022 **28** 583–590. (https://doi.org/10.1038/s41591-022-01689-3)

63 Sollini M, Ciccarelli M, Cecconi M, Aghemo A, Morelli P, Gelardi F & Chiti A. Vasculitis changes in COVID-19 survivors with persistent symptoms: an [18F]FDG-PET/CT study. *European Journal of Nuclear Medicine and Molecular Imaging* 2021 **48** 1460–1466. (https://doi.org/10.1007/s00259-020-05084-3)

64 Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J & Kell DB. Persistent clotting protein pathology in long COVID/post-acute sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. *Cardiovascular Diabetology* 2021 **20** 172. (https://doi.org/10.1186/s12933-021-01359-7)

65 Townsend L, Fogarty H, Dyer A, Martin-Loeches I, Bannan C, Nadarajan P, Bergin C, O'Farrelly C, Conlon N, Bourke NM, *et al*. Prolonged elevation of D-dimer levels in convalescent COVID-19 patients is independent of the acute phase response. *Journal of Thrombosis and Haemostasis* 2021 **19** 1064–1070. (https://doi.org/10.1111/jth.15267)

66 Lee S, Yu Y, Trimpert J, Benthani F, Mairhofer M, Richter-Pechanska P, Wyler E, Belenki D, Kaltenbrunner S, Pammer M, *et al*. Virus-induced senescence is a driver and therapeutic target in COVID-19. *Nature* 2021 **599** 283–289. (https://doi.org/10.1038/s41586-021-03995-1)

67 Pawłowski P. SARS-CoV-2 variant Omicron (B.1.1.529) is in a rising trend of mutations increasing the positive electric charge in crucial regions of the spike protein S. *Acta Biochimica Polonica* 2021 **69** 263–264. (https://doi.org/10.18388/abp.2020_6072)

68 Chen J, Gao K, Wang R & Wei GW. Revealing the threat of emerging SARS-CoV-2 mutations to antibody therapies. *Journal of Molecular Biology* 2021 **433** 167155. (https://doi.org/10.1016/j.jmb.2021.167155)

69 Tiecco G, Storti S, Degli Antoni M, Focà E, Castelli F & Quiros-Roldan E. Omicron genetic and clinical peculiarities that may overturn SARS-CoV-2 pandemic: a literature review. *International Journal of Molecular Sciences* 2022 **23** 1987. (https://doi.org/10.3390/ijms23041987)

70 Vallejo S, Mendez-Echevarria A, Del Rosal T, Falces-Romero I, Muñoz-Caro JM, Buitrago Sánchez NM, Aragón-Ramos P & Calvo C. Omicron and thrombosis in children: cause for concern? *Pediatric Infectious Disease Journal* 2022 **41** e252–e254. (https://doi.org/10.1097/INF.0000000000003501)

71 Halfmann PJ, Iida S, Iwatsuki-Horimoto K, Maemura T, Kiso M, Scheaffer SM, Darling TL, Joshi A, Loeber S, Singh G, *et al*. SARS-CoV-2 Omicron virus causes attenuated disease in mice and hamsters. *NAture* 2022 **603** 687–692. (https://doi.org/10.1038/s41586-022-04441-6).

72 Fernando K, Menon S, Jansen K, Naik P, Nucci G, Roberts J, Wu SS & Dolsten M. Achieving end-to-end success in the clinic: Pfizer's learnings on R&D productivity. *Drug Discovery Today* 2021 **27** 697–704. (https://doi.org/10.1016/j.drudis.2021.12.010)

73 Chakraborty S, Gonzalez JC, Sievers BL, Mallajosyula V, Chakraborty S, Dubey M, Ashraf U, Cheng BY, Kathale N, Tran KQT, *et al*. Early non-neutralizing, afucosylated antibody responses are associated with COVID-19 severity. *Science Translational Medicine* 2022 **14** eabm7853. (https://doi.org/10.1126/scitranslmed.abm7853)