Plasma mediators in patients with severe COVID-19 cause lung endothelial barrier failure

To the Editor:

Approximately 20% of symptomatic patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection progress to severe coronavirus disease 2019 (COVID-19) with critical hypoxaemia fulfilling the criteria of acute respiratory distress syndrome (ARDS). Consistent with the classic features of ARDS, severe COVID-19 is characterised by ground glass opacities on computed tomography imaging and diffuse alveolar damage *post mortem* [1], suggesting permeability-type lung oedema as driver of respiratory failure. Consistent with this concept, autopsy findings show severe lung endothelial injury in patients who succumbed to COVID-19 [2].

At present, the extent of endothelial barrier failure and its underlying mechanisms in COVID-19 remain unclear. While the occasional presence of viral particles in lung endothelial cells has been reported in autoptic studies [2], endothelial cells are not thought to be infected directly in SARS [3]. Alternatively, endothelial barrier failure may be caused by barrier-disruptive mediators released from the infected airspace epithelium and the consecutive immune response. If so, these mediators and their barrier-disruptive effect should be recoverable from circulating blood plasma. Here, we provide proof-of-principle for this concept and report a screening platform for endothelial barrier regulation, that can be utilised to 1) identify pathological mediators and 2) screen for the therapeutic potential of barrier-stabilising compounds in COVID-19.

Citrate plasma was sampled as part of the prospective observational Pa-COVID-19 cohort study (ethics approval EA2/066/20) in 14 patients with moderate (hospitalised, no invasive ventilation; WHO severity score 3–4) and 19 with severe (high flow O_2 or intubated and mechanically ventilated; WHO severity score 5–7) COVID-19. Plasma samples were diluted to 10% (v/v) in cell culture medium without FCS and tested for their ability to disrupt barrier integrity of primary human pulmonary microvascular endothelial cells (HPMEC, passage 4–7) monolayers by 1) electrical cell-substrate impedance sensing (ECIS Z-Theta, Applied BioPhysics), 2) measurement of trans-endothelial electrical resistance (TEER) using a REMS Auto Sampler (World Precision Instruments), and 3) immunofluorescence for endothelial VE-cadherin and F-actin. Plasma from 15 healthy donors (ethics approval EA2/075/15) served as control. Samples were probed for SARS-CoV-2 RNA by real-time RT-PCR. Human lung tissue from non-COVID-19 patients (ethics approval EA2/079/13) was probed for protein levels of VE-cadherin and occludin following stimulation with plasma for 24 h. Data are presented as mean±SEM. Different treatment groups were compared by Mann–Whitney U-test or two-way ANOVA followed by Tukey's *post hoc* test. Statistical significance was assumed at $p<0.05$.

In contrast to healthy donor plasma, plasma from COVID-19 patients induced a rapid (within 1–2 h) and sustained (>6 h) increase in endothelial permeability of HPMEC monolayers (figure 1a and b). As shown by two methods, ECIS and TEER, the decrease in monolayer resistance after 6 h was more pronounced in plasma from patients with severe as compared to moderate disease. The barrier-disruptive effect of 10% severe COVID-19 plasma was comparable to that of 50 $\mu mol \cdot L^{-1}$ platelet-activating factor, an established biological disruptor of the lung microvascular barrier. HPMEC monolayer disruption was similarly evident by immunofluorescence microscopy, demonstrating loss of junctional VE-cadherin and cortical actin,

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The plasma of COVID-19 patients induces pulmonary microvascular barrier failure, which increases with disease severity. Here, a screening platform to test for plasma mediators and the therapeutic potential of barrier stabilising compounds is reported. https://bit.ly/3k4C0tB

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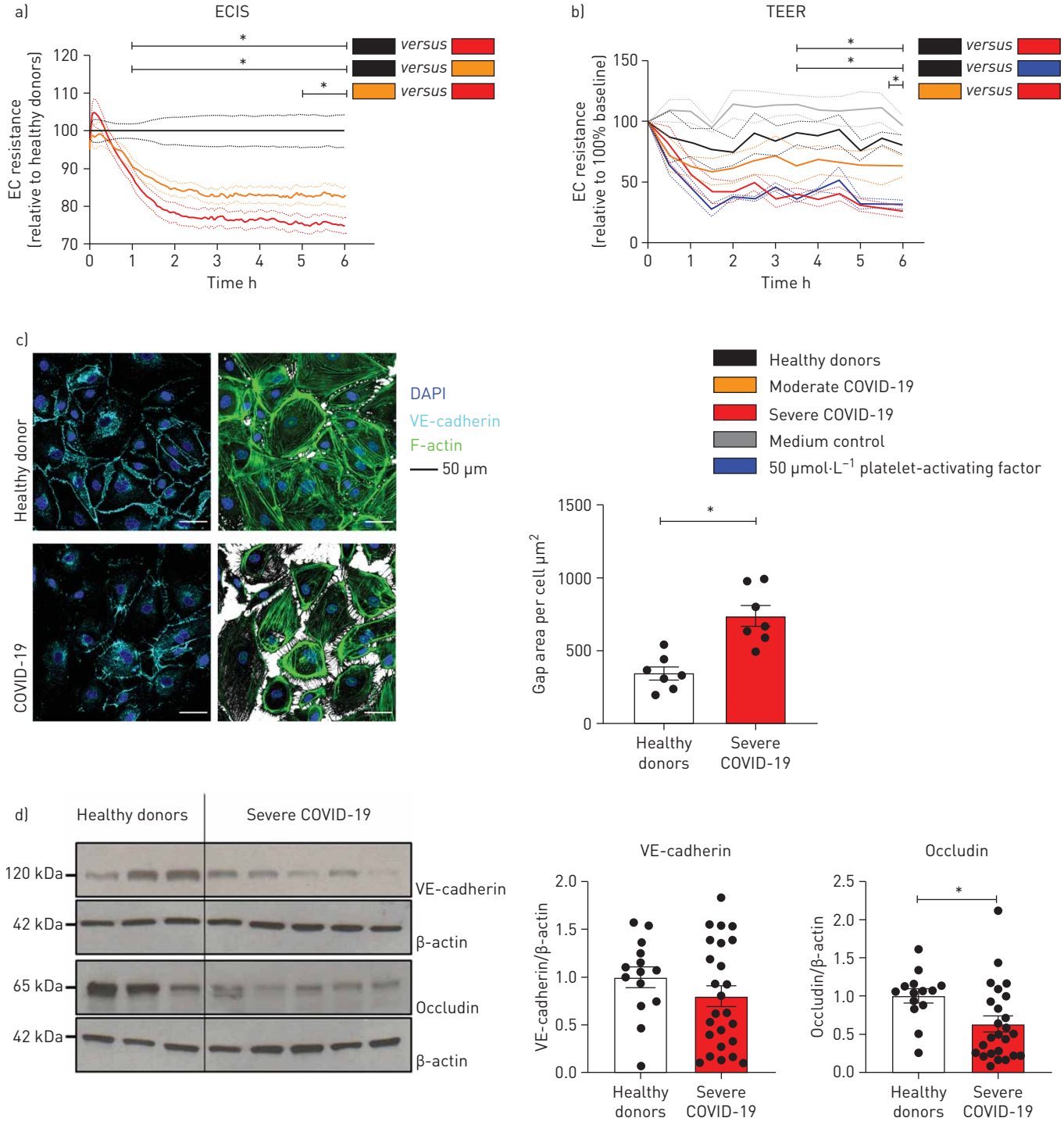

FIGURE 1 Plasma of coronavirus disease 2019 (COVID-19) patients induces endothelial barrier failure. a) Barrier integrity of pulmonary microvascular endothelial cell monolayers assessed by electric cell impedance sensing (ECIS) in response to stimulation with 10% (v/v) plasma (at t=0 h) of healthy controls (number of samples/donors: 17/14), and moderate (25/14) or severe (22/16) COVID-19 patients. In cases of multiple samples from the same patient these were obtained at different time-points. Data are normalised to mean endothelial cell (EC) resistance in healthy donors. b) Trans-endothelial electrical resistance (TEER) response of EC monolayers to stimulation with 10% (v/v) plasma (at t=0 h) of healthy controls (7/7), and moderate (4/4), or severe (8/7) COVID-19 patients, with medium control and platelet activating factor as negative and positive controls. c) Representative immunofluorescence images of EC monolayers treated for 6 h with 10% (v/v) plasma of either healthy donors or severe COVID-19 patients stained for (left) VE-cadherin (cyan) or (centre) F-actin (green). Nuclei are shown in blue (DAPI). Gap area per cell (white in F-actin images) was identified by an automated segmentation algorithm (selecting pixels with zero values in both the F-Actin and VE-cadherin channel) using Fiji/MorpholibJ-Plugin, and was quantified for EC monolayers treated with plasma from healthy donors (7/7) or severe COVID-19 patients (7/7) (right). d) Representative western blots and quantification of VE-cadherin and occludin protein levels (normalised to healthy donors) in human lung samples from five donors treated for 24 h with plasma of three healthy donors or five severe COVID-19 patients. *: p≤0.05.

formation of actin stress fibres, and inter-endothelial gap formation in response to plasma from severe COVID-19 patients as compared to healthy controls (figure 1c). Exposure of human lung tissue to severe COVID-19 plasma caused loss of the junctional molecule occludin while changes in VE-cadherin protein levels did not reach statistical significance (figure 1d). All plasma samples, as well as cell lysates and supernatants from plasma-exposed HPMECs tested negative for SARS-CoV-2 RNA.

Here, we demonstrate that plasma from COVID-19 patients induces robust barrier failure of the lung microvascular endothelium. This barrier-disruptive potential of plasma increased from moderate to severe disease, a finding that is in line with proteomic analyses demonstrating changes in inflammatory plasma biomarkers as a function of disease severity [4]. While this barrier-disruptive effect is *per se* likely not unique to COVID-19 and may be equally evident in other critically ill patients, the recent identification of a distinct biomarker signature in the plasma of COVID-19 patients, as compared to *e.g.* severe influenza infection [4], suggests the involvement of specific mediators or pathways in COVID-19-related endothelial barrier failure.

Treatment of healthy endothelial monolayers with plasma of COVID-19 patients was associated with significant endothelial gap formation and loss of junctional VE-cadherin in endothelial monolayers. Loss of junctional proteins was also evident in human lung tissue, although large scatters in expression levels resulted in considerable overlap between healthy and COVID-19-plasma treated human lung tissue. In line with the clinical course of patients following SARS-CoV-2 infection, these findings indicate substantial biological variability of the host tissue both at baseline and in response to stimulation with COVID-19 plasma. Notably, these effects were not attributable to a direct action of SARS-CoV-2 on the endothelium, as demonstrated by the absence of viral RNA. Our findings provide proof-of-principle for the concepts that 1) endothelial injury and barrier failure are characteristic features of severe COVID-19, 2) are caused by endogenous plasma factors, rather than direct injury by viral infection, and 3) may drive the progression from mild disease to critical ARDS [2, 5]. Notably, endothelial activation may not be restricted to the regulation of vascular permeability, but could also contribute to other clinical manifestations of COVID-19 associated vasculopathies, such as microthrombosis and vascular inflammation.

The present work in conjunction with our recent proteome analyses in COVID-19 plasma [4] provides for a versatile platform to screen for the relevance of individual plasma mediators of endothelial permeability in high throughput mode. To this end, correlative changes in plasma mediators and barrier failure as a function of disease severity may guide the identification of key signalling mechanisms. Of equal relevance, this platform allows to screen for the prophylactic or therapeutic effectiveness of drugs with demonstrated barrier-protective action [4]. Importantly, the unique time profile of COVID-19 with a slow progression from symptom onset to mild, moderate, and ultimately severe disease provides a model scenario for the application of barrier-stabilising adjunctive therapies. We therefore propose that pharmacological stabilisation of the endothelial barrier should be considered as a third pillar for the treatment of COVID-19 in addition to anti-virals and immunomodulators.

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References

1 Xu Z, Shi L, Wang Y, *et al.* Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; 8: 420–422.
2 Ackermann M, Verleden SE, Kuehnel M, *et al.* Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020; 383: 120–128.
3 To KF, Lo AWI. Exploring the pathogenesis of severe acute respiratory syndrome (SARS): the tissue distribution of the coronavirus (SARS-CoV) and its putative receptor, angiotensin-converting enzyme 2 (ACE2). *J Pathol* 2004; 203: 740–743.
4 Messner CB, Demichev V, Wendisch D, *et al.* Ultra-high-throughput clinical proteomics reveals classifiers of COVID-19 infection. *Cell Syst* 2020; 11: 11–24.e4.
5 Kuebler WM, Jordt S-E, Liedtke WB. Urgent reconsideration of lung edema as a preventable outcome in COVID-19: inhibition of TRPV4 represents a promising and feasible approach. *Am J Physiol Lung Cell Mol Physiol* 2020; 318: L1239–L1243.