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

Laura Michalick[1], Sarah Weidenfeld[1], Benjamin Grimmer[1], Diana Fatykhova[2], Philip Daniel Solymosi[1], Felix Behrens[1,5], Melanie Dohmen[2,11], Markus C. Brack[2], Sabrina Schulz[1], Elisa Thomasch[1], Szandor Simmons[1], Holger Müller-Redetzky[2], Norbert Suttorp[2,6], Florian Kurth[2], Jens Neudecker[3], Mario Toennies[12], Torsten T. Bauer[12], Stephan Eggeling[13], Victor Max Corman[4,7], Andreas C. Hocke[2,6], Martin Witzenrath[2,6*], Stefan Hippenstiel[2,6*], Wolfgang M. Kuebler[1,6,8-10*]

[1]Institute of Physiology, [2]Department of Infectious Diseases and Respiratory Medicine, [3]Dept of General, Visceral, Vascular and Thoracic Surgery, [4]Institute of Viroloy, all Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health (BIH), Berlin, Germany; [5]Berlin Institute of Health (BIH), Berlin, Germany; [6]German Center for Lung Research (DZL); [7]German Centre for Infection Research (DZIF); [8]The Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Toronto, ON; [9]Depts. of Surgery and Physiology, University of Toronto, ON; [10]German Centre for Cardiovascular Research (DZHK), [11]Max Delbrück Center for Molecular Medicine, Berlin, [12]Lungenklinik Heckeshorn, HELIOS Klinikum Emil von Behring, Walterhöferstrasse 11, 14165 Berlin, Germany, [13]Vivantes Netzwerk für Gesundheit, Klinikum Neukölln, Klinik für Thoraxchirurgie, Berlin, Rudower Straße 48, 12351 Berlin, Germany

[*]These authors contributed equally to this study

Address for Correspondence: Wolfgang M. Kuebler, MD; Institute of Physiology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany; Phone: +49 (0)30 450 528 501; Fax: +49 (0)30 450 528 920; Email: wolfgang.kuebler@charite.de

Author Contributions: LM, ACH, MW, SH, and WMK conceived study; LM, SW, BG, DF, PDS, MD, MCB, VMC and SaS performed experiments and analyzed the data; HMR, FK, NS, FB, ET, SzS, JN, MT, TTB and SE provided and collected samples, FK, MW led the clinical study, LM, ACH, MW, SH, and WMK wrote manuscript, all authors revised and approved manuscript.

Funding Sources: LES is supported by DFG (SFB-TR84), BMBF (PROVID), Berlin University Alliance (BUA, PreEP_Corona); ACH and SH are supported by Berlin University Alliance GC2 Global Health (Corona Virus Pre Exploration Project), BMBF (RAPID, alvBarriere-COVID-19, PROVID), DFG (SFB-TR 84), BIH, Charite 3[R], and Charité-Zeiss MultiDim; MW is supported by DFG (SFB-TR84) and BMBF (CAPSyS-FKZ, Phage4Cure-FKZ, SYMPATH, PROVID); WMK is supported by grants-in-aid from the Ministry of Education and Research (BMBF) (SYMPATH, PROVID), the German Research Foundation (DFG) (DFG 1218/9-1, 1218/11-1, SFB-TR84), the German Centre for Cardiovascular Research (DZHK), and the Canadian Institutes for Health Resarch (CIHR).

Running Head: Lung endothelial barrier failure in COVID-19

Word Count: 993

Most important finding/message: Plasma of COVID-19 patients induces pulmonary microvascular barrier failure which increases with disease severity. Here, we report a versatile high-throughput screening platform to test for involved plasma mediators and the therapeutic potential of barrier stabilizing compounds.

To the Editor

Approximately 20% of symptomatic patients with SARS-CoV-2 infection progress to severe coronavirus disease (COVID-19) with critical hypoxemia fulfilling the criteria of acute respiratory distress syndrome (ARDS). Consistent with the classic features of ARDS, severe COVID-19 is characterized by ground glass opacities in CT imaging and diffuse alveolar damage *post mortem* (5) suggesting permeability-type lung edema as driver of respiratory failure. Consistent with this concept, autopsy findings show severe lung endothelial injury in patients who succumbed to COVID-19 (1)**.**

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 (1), endothelial cells are not thought to be infected directly in SARS (4). 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 utilized to i) identify pathologic mediators and ii) screen for the therapeutic potential of barrier stabilizing 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 (hospitalized, 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 (i) electrical cell-substrate impedance sensing (ECIS® Z-Theta, Applied BioPhysics), (ii) measurement of trans-endothelial electrical resistance (TEER) using a REMS Auto Sampler (World Precision Instruments), and (iii) 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 24h. Data are presented as means±SEMs. 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 1-2h) and sustained (>6h) increase in endothelial permeability of HPMEC monolayers (Fig. 1A, B). As shown by two methods, ECIS® and TEER, the decrease in monolayer resistance after 6h 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 µMol/L platelet-activating factor (PAF), 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, formation of actin stress fibers, and inter-endothelial gap formation in response to plasma from severe COVID-19 patients as compared to healthy controls (Fig. 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 (Fig. 1D). All plasma samples, as well as cell lysates and supernatants from plasma-exposed HPMECs tested negative for SARS-CoV2-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 (3) . While this barrier-disruptive effect is *per se* likely not unique to COVID-19 but 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 (3) suggests the involvement of specific mediators or pathways in COVID-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 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 i) endothelial injury and barrier failure are characteristic features of severe COVID-19 that ii) are caused by endogenous plasma factors, rather than direct injury by viral infection and iii) may drive the progression from mild disease to critical ARDS (1, 2). 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 (3) 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 signaling mechanisms. Of equal relevance, this platform allows to screen for the prophylactic or therapeutic effectiveness of drugs with demonstrated barrier-protective action (3). 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-stabilizing adjunctive therapies. We therefore propose that pharmacological stabilization of the endothelial barrier should be considered as a third pillar for the treatment of COVID-19 in addition to anti-virals and immunomodulators.

Acknowledgements

The authors thank the Advanced Medical Bioimaging Core Facility (AMBIO) of Charité—Universitätsmedizin Berlin (Berlin, Germany) for the support in acquisition of the imaging data.

References

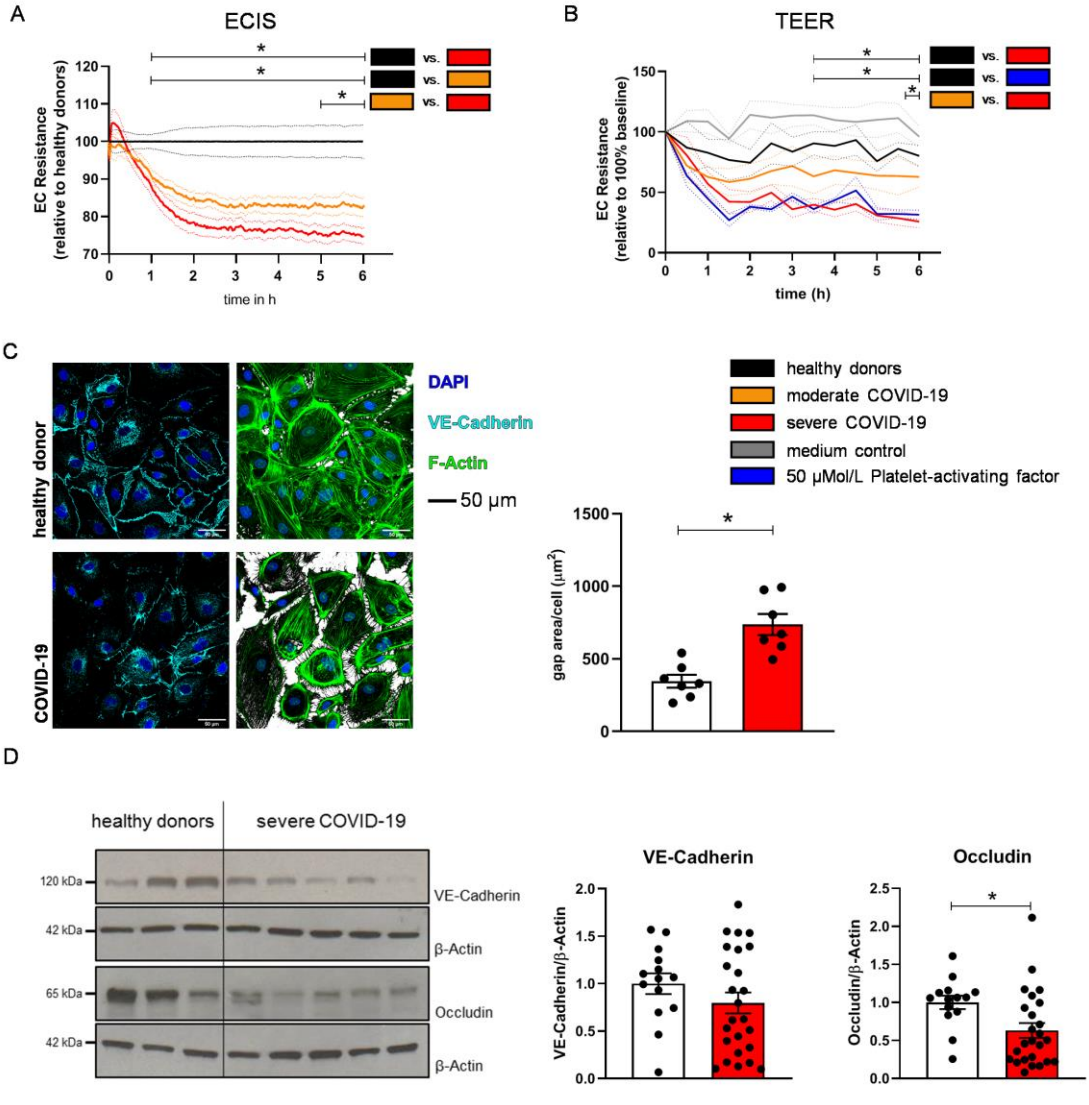
1. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, Li WW, Li VW, Mentzer SJ, Jonigk D. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med*. 2020 May 21. doi: 10.1056/NEJMoa2015432.

2. 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 May 13. doi: 10.1152/ajplung.00161.2020.

3. Messner CB, Demichev V, Wendisch D, Michalick L, White M, Freiwald A, Textoris-Taube K, Vernardis SI, Egger A-S, Kreidl M, Ludwig D, Kilian C, Agostini F, Zelezniak A, Thibeault C, Pfeiffer M, Hippenstiel S, Hocke A, von Kalle C, Campbell A, Hayward C, Porteous DJ, Marioni RE, Langenberg C, Lilley KS, Kuebler WM, Mülleder M, Drosten C, Witzenrath M, Kurth F, Sander LE, Ralser M. Ultra-high-throughput clinical proteomics reveals classifiers of COVID-19 infection. *Cell Syst*. 2020 June 02. doi:10.1016/j.cels.2020.05.012.

4. 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* 203: 740–743, 2004. doi: 10.1002/path.1597.

5. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang F-S. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 8: 420–422, 2020. doi: 10.1016/S2213-2600(20)30076-X.


A
ECIS
EC Resistance (relative to healthy donors)
time in h
vs.
B
TEER
EC Resistance (relative to 100% baseline)
time (h)
C
healthy donor
COVID-19
DAPI
VE-Cadherin
F-Actin
50 µm
healthy donors
moderate COVID-19
severe COVID-19
medium control
50 µMol/L Platelet-activating factor
gap area/cell (µm²)
D
healthy donors
severe COVID-19
120 kDa
VE-Cadherin
42 kDa
β-Actin
65 kDa
Occludin
VE-Cadherin/β-Actin
Occludin/β-Actin