

- Qin Z, Liu F, Blair R, Wang C, Yang H, Mudd J, *et al.* Endothelial cell infection and dysfunction, immune activation in severe COVID-19. *Theranostics* 2021;11:8076–8091.
- Liu F, Han K, Blair R, Kenst K, Qin Z, Upcin B, *et al.* SARS-CoV-2 infects endothelial cells *in vivo* and *in vitro*. *Front Cell Infect Microbiol* 2021;11:701278.
- Uhrin P, Perkmann T, Binder B, Schabbauer G. ISG12 is a critical modulator of innate immune responses in murine models of sepsis. *Immunobiology* 2013;218:1207–1216.
- Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, *et al.* IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* 2005;434:772–777.

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## Reply to Khatun *et al.*

From the Authors:

We thank Khatun and colleagues for their insightful comments on our recent manuscript describing the *in vitro* effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and of coronavirus disease (COVID-19) patient sera on human endothelial cells (1). We are pleased that the authors found our proposed model of COVID-19–associated endotheliopathy to be consistent with their published data (2, 3). We apologize for not citing these important publications. In our study, we detected SARS-CoV-2 RNA in lysates of primary human lung microvascular endothelial cells (HMVEC) treated with live SARS-CoV-2 and observed that exposure to live virus induced an increase in permeability and activation of HMVEC. We also found increased permeability of HMVEC treated with sera from COVID-19 patients that correlated with disease severity. We postulated that COVID-19–associated endotheliopathy results from a combination of the actions of systemic and localized inflammatory mediators and cells on the endothelium, and by SARS-CoV-2 infection of the endothelium. Using a model of severe COVID-19 in K18-hACE2 mice, the authors previously reported SARS-CoV-2 in lung capillary endothelial cells in both the early and delayed stages of severe COVID-19, as well as lung edema, perivascular inflammation, upregulated adhesion molecule expression, and decreased vascular endothelial–cadherin expression (2, 3). Similarly, they observed co-localization of SARS-CoV-2 viral proteins with the endothelial marker CD31 in nonhuman primates with severe COVID-19, and also in autopsy samples from a patient that died of COVID-19. In their comment, the authors present additional single-cell RNAseq data on samples from SARS-CoV-2–infected mice. Their data confirm the presence of viral RNA within endothelial cells and demonstrate increased expression of *Ifi2712a* and *Irf7*, and decreased expression of *Cldn5* by infected endothelial cells. These new data further support the concept that SARS-CoV-2 infection of endothelial

cells may directly promote endothelial barrier dysfunction, and they suggest that viral infection of endothelial cells contributes to the COVID-19–associated endotheliopathy. An interesting aspect of the authors’ studies is that K18-hACE2 mice have very low ACE2 expression by endothelial cells. This suggests that ACE2 may not be required for SARS-CoV-2 entry into endothelial cells and supports a model of viral transduction of endothelial cells described by the authors.

We believe that the results of our clinical-translational study and the authors’ basic-translational work support our proposed multicomponent model of COVID-19–associated endotheliopathy. Furthermore, there is encouraging consistency in the effects of SARS-CoV-2 on cultured human endothelial cells (1) and *in vivo* on mouse endothelial cells (2, 3). The concordance in endothelial infection and dysfunction between the humans and mice suggests shared mechanisms of COVID-19–associated endotheliopathy. It also indicates that mouse models may lead to insights into processes that cannot be studied longitudinally in humans, such as how COVID-19 affects fixed cells in organs and tissue, including endothelial cells, tissue leukocytes and neurons, which are inaccessible in real-time in humans. Also, the apparent concordance between human and mouse endothelial cells may facilitate complementary preclinical testing of potential therapies using cultured human endothelial cells and mouse models, and even facilitate studies on effects of COVID-19 on endothelium that might predispose to the chronic effects of SARS-CoV-2 infection on the lung and other organs.

The available data suggest that the etiology of COVID-19–associated endotheliopathy involves viral infection of the endothelium, as well as the actions of systemic and localized mediators and cells on the endothelium. More work is needed to understand the mechanisms, and the relative importance of endothelial infection and of systemic and localized inflammatory cells and mediators in COVID-19. Although the specific roles of these factors in driving COVID-19–associated endotheliopathy and respiratory failure remain to be determined, collectively the data suggest that the endothelium may represent an important but understudied therapeutic target in COVID-19. ■

**Author disclosures** are available with the text of this letter at [www.atsjournals.org](http://www.atsjournals.org).

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## References

- Joffre J, Rodriguez L, Matthay ZA, Lloyd E, Fields AT, Bainton RJ, *et al.*; COMET consortium and the Co-ACIT Study Group. COVID-19–associated lung microvascular endotheliopathy: a “from the bench” perspective. *Am J Respir Crit Care Med* 2022;206:961–972.

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2. Liu F, Han K, Blair R, Kenst K, Qin Z, Upcin B, et al. SARS-CoV-2 infects endothelial cells *in vivo* and *in vitro*. *Front Cell Infect Microbiol* 2021;11:701278.
3. Qin Z, Liu F, Blair R, Wang C, Yang H, Mudd J, et al. Endothelial cell infection and dysfunction, immune activation in severe COVID-19. *Theranostics* 2021;11:8076–8091.

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## Heart Rate Response in OSA: A Clue to Reveal Cardiovascular Benefit from CPAP?

To the Editor:

Obstructive sleep apnea (OSA) is associated with a cluster of serious adverse outcomes, including cardiovascular (CV) morbidity and mortality. However, the protective effect of the first-line therapy of OSA—continuous positive airway pressure (CPAP) against adverse CV outcomes remains controversial (1–3). It is increasingly recognized that the prognostic significance of CPAP on CV events may not be uniform across the entire spectrum of OSA populations. Understanding which phenotypes may experience benefit from CPAP will be essential to provide constructive guidance in clinical practice.

In a recent issue of the *Journal*, Azarbarzin and colleagues (pp. 766–773) conducted a *post hoc* analysis of the Randomized Intervention with Continuous Positive Airway Pressure in Coronary Artery Disease and Obstructive Sleep Apnea (RICCADSA) trial (4). They first revealed an optimistic CPAP effect on adverse CV outcomes in selected nonsleepy OSA patients with coronary artery disease (CAD) who exhibited exaggerated pulse rate response to respiratory events ( $\Delta$ HR) (4). Nevertheless, critical questions remained to be addressed.

Emerging evidence has recognized a low prognostic value of the traditional measure of OSA–apnea–hypopnea index for CV events (3). Instead, a novel metric, “hypoxic burden” proposed by Azarbarzin and colleagues was more consistently correlated with CV disease–related mortality in the general population (5). The rationale for the current study (4) was also based on the authors’ prior work in which a subgroup of patients with OSA presenting a higher  $\Delta$ HR was at an increased risk of CV events, particularly in those with substantial hypoxic burden (6). However, in patients with CAD, the latest study did not engage the impact of hypoxic burden *per se* and its combination with  $\Delta$ HR on the long-term CV risk, and did not elucidate whether the CPAP effect would be moderated by hypoxic burden (4).

Moreover, the authors used the pulse rate derived from a pulse oximetry sensor to estimate the heart rate (HR). Pulse oximetry may be chosen over electrocardiogram due to its convenient accessibility and widespread application. However, a simple measure of pulse rate or HR may not fully depict the complex process of autonomous regulatory mechanisms. A high  $\Delta$ HR may represent a pronounced vagally induced bradycardia during an event and sympathetic response to hypoxemia, and/or a combination of both. Notwithstanding, the actual contribution of sympathetic or parasympathetic activity to  $\Delta$ HR was not systemically examined. Heart rate variability (HRV) derived from electrocardiogram is generally considered as a reliable and noninvasive measure of autonomic modulation response and adaptation to multiple stimuli in healthy or pathogenic conditions. With more analytical approaches and techniques developing, HRV indices specific to respiratory events could provide additional information on impaired CV alteration related to subclinical CV outcomes.

Another critical issue that requires further clarification lies in the analysis of  $\Delta$ HR when an arrhythmic heartbeat occurs within the same timescale of respiratory signals. The occurrence rate of concomitant arrhythmias may be high in patients with acute myocardial infarction (accounting for 49.6% of the study population) (4). Although patients with chronic atrial fibrillation were excluded in the sensitivity analysis, the measurement of  $\Delta$ HR might remain unreliable when other overt arrhythmias coincide with apneas or hypopneas. Furthermore, the baseline levels of diurnal and nocturnal HR were not delineated in the study, so one may suspect that  $\Delta$ HR appears to merely reflect higher night-to-day variability of HR in general rather than HR responses to obstructive events.

We sincerely recognize that the work of Azarbarzin and colleagues is a valuable contribution to demonstrating the CV benefit of CPAP in the nonsleepy CAD patients exhibiting a higher  $\Delta$ HR in OSA. However, further studies should provide greater insight into the HRV metrics in response to respiratory events to allow better CV risk stratification, and to clarify whether CPAP therapy would benefit selected patients with both greater  $\Delta$ HR and hypoxic burden. ■

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