

**COVID-19–associated Acute Respiratory Distress Syndrome Clarified: A Vascular Endotype?***To the Editor:*

Coronavirus disease (COVID-19), the pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, can lead to sepsis and acute respiratory distress syndrome (ARDS), resulting in an extraordinary level of ICU use and considerable mortality. Several pathophysiological features of COVID-19–associated ARDS appear to be overrepresented in comparison with non-COVID etiologies. Whether COVID-19–induced lung injury is truly unique or represents one end of the ARDS spectrum remains unclear at this time. With the caveat that studies are ongoing, and appropriately powered studies are needed, the observations discussed here implicate vascular dysfunction in the pathogenesis of COVID-19–induced ARDS, leading to the hypothesis that COVID-ARDS is a distinct vascular endotype of ARDS (Figure 1).

In recently published reports, ARDS related to COVID-19 often presents with relatively preserved compliance (1). In these cases, the static compliance has been 30–40 ml/cm H₂O despite $FiO_2 > 70\%$ and positive end-expiratory pressure > 15 cm H₂O. Thus, although the compliance is abnormal and consistent with the ARDS conceptual model articulated in the Berlin definition (2), compliance is less impacted than oxygenation and dead space in COVID-ARDS.

Early reports of COVID-19 patient cohorts from China and Italy identified several risk factors for severe illness and death. Some of these risk factors are unsurprising as they are also risk factors for non-COVID ARDS, including age and obesity. However, others appear to be overrepresented in patients who develop COVID-ARDS, suggesting a distinct ARDS endotype. Strikingly, diabetes is a risk factor for COVID-ARDS whereas it is a negative predictor in the Lung Injury Prediction Score for non-COVID ARDS (3). Cardiovascular disease (including hypertension and hyperlipidemia) are common among critically ill patients with COVID-19 ARDS, although they have not previously been reported as ARDS risk factors, highlighting what may be a prominent role for underlying vascular dysfunction in this subtype.

ABO blood group antigens are expressed on vascular endothelium. Blood type A has been associated with increased risk of vascular disease and possibly ARDS. Recently, the first published genome-wide association study of COVID-19 identified an association between genetic variation that determines ABO blood type and severe COVID-19 disease (4). Specifically, ABO blood type A was overrepresented and blood type O was underrepresented in patients with COVID-19 relative to blood donors. Findings were inconclusive for types B and AB given the smaller population

prevalence of these blood types. This is consistent with reports of an association between blood type A and increased risk of infection with SARS-CoV-1 (5). Although the mechanism underlying this association is unknown, ABO blood type A is also associated with a higher risk of multiple thrombotic diseases including myocardial infarction, stroke, and venous thromboembolism, as well as higher plasma concentrations of endothelial-derived proteins important in microvascular coagulation and cell adhesion. Collectively, these observations suggest that blood type A and chronic conditions such as diabetes and cardiovascular disease may prime the endothelium for injury when faced with SARS-CoV-2, thereby lowering the threshold for infection to progress to organ failure including ARDS, kidney injury, and shock.

In addition to facilitating gas exchange and performing critical barrier functions, the endothelium regulates leukocyte trafficking, hemostasis, and vascular tone. The pulmonary microvascular endothelium is unique in that it filters the entire systemic circulation and is routinely exposed to noxious stimuli including bloodborne pathogens, toxins, and endogenous inflammatory mediators. Maintenance of endothelial quiescence under basal conditions is essential to lung homeostasis, and endothelial protective mechanisms promote this antiinflammatory phenotype. Although most respiratory viruses do not infect endothelial cells directly, the inflammatory response induced by these pathogens can cause significant injury to the vasculature. Inflammation-induced disruption of homeostatic endothelial functions can result in impaired diffusion, disrupted barrier function, aberrant coagulation, and increased permeability. Perturbation of endothelial homeostasis in patients with chronic diseases may predispose these susceptible populations to organ failure in response to vascular injury induced by SARS-CoV-2. This is consistent with the finding that thrombosis and kidney injury are predominant features of COVID-19 in susceptible populations. Multiple autopsy studies now confirm the involvement of the endothelium in COVID-ARDS, demonstrating microvascular thrombi, vascular complement deposition, possible direct endothelial infection, and endothelial cell death (Table 1) (6, 7). Furthermore, aberrant endothelial cell death and dysregulated angiogenesis are observed in COVID-ARDS when compared with influenza-associated ARDS (8).

One possible contributing factor to this vascular ARDS phenotype may be the SARS-CoV and CoV-2 receptor, ACE2 (angiotensin-converting enzyme 2). ACE2 is a key player in the renin-angiotensin system responsible for regulating vascular tone. Angiotensin II acts on a variety of target cells to produce acute and long-term physiological effects, including vasoconstriction, sympathetic nervous stimulation, smooth muscle and fibroblast proliferation, and inflammation. ACE2 counteracts angiotensin II activity by catalyzing its proteolytic cleavage into angiotensin (1–7), which counteracts acute lung injury.

As the viral receptor, it might be expected that higher levels of ACE2 would result in more severe disease. However, studies after the original SARS outbreak indicate the opposite, as ACE2 knockout mice exhibit much more severe lung injury after acid aspiration, whereas administration of recombinant ACE2 is protective. Moreover, binding of SARS-CoV Spike protein to ACE2 resulted in a loss of ACE2 protein, and administration of recombinant Spike-Fc protein worsened lung injury by increasing angiotensin II activity (9), presumably owing to competition for available ACE2. These

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Supported by NHLBI 126788 (N.S.M.), NHLBI 131817 (A.E.V.), NHLBI 137006 and NHLBI 137915 (N.J.M.), and NHLBI 125723 (J.P.R.).

Originally Published in Press as DOI: 10.1164/rccm.202006-2598LE on July 6, 2020

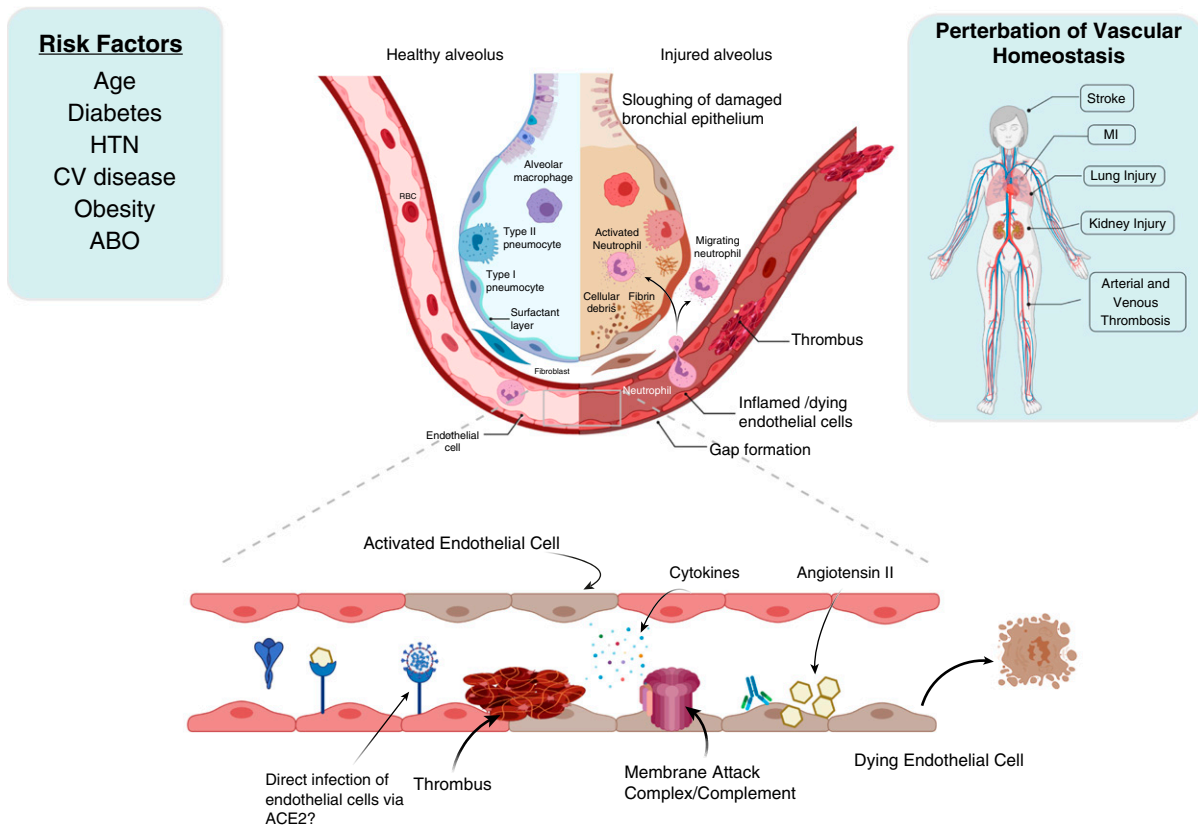


Figure 1. Risk factors and disease manifestations of coronavirus disease (COVID-19) suggest the central involvement of the pulmonary vasculature. Although some risk factors (age and obesity) are common for undifferentiated acute respiratory distress syndrome, others, including cardiovascular disease and diabetes, are overrepresented with regard to severe COVID-19. Regarding viral pathogenesis at the tissue level, the epithelium represents the primary cell type affected as with most respiratory viruses. However, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is notable for the induction of microthrombi. Multiple mechanisms are likely to promote endothelial injury, including viral competition for ACE2 (angiotensin-converting enzyme 2) binding (thus increasing angiotensin II) and direct activation of complement by SARS-CoV-2 structural proteins, in addition to elevated cytokines and complement activation and cell death observed in acute respiratory distress syndrome and sepsis. These vascular perturbations likely contribute to systemic thrombosis and organ injury in susceptible hosts. CV = cardiovascular; HTN = hypertension; MI = myocardial infarction; RBC = red blood cell.

studies were performed with the original SARS-CoV Spike protein, so it is not certain whether CoV-2 Spike would have similar effects. Nonetheless, they provide significant rationale for some of the pathophysiological differences observed with COVID-19 ARDS, and clinical trials using recombinant ACE2 and angiotensin (1–7) to treat COVID-19 are ongoing. Loss of ACE2 repression of angiotensin II activity promotes microvascular thrombosis through direct and indirect means (10), and prolonged vasoconstriction and hypertension are well known to induce endothelial injury. Recent autopsy reports demonstrating direct endothelial injury may be mediated by this dysregulation of the renin–angiotensin system.

Another potential mechanism of vascular injury contributing to ARDS and kidney injury involves dysregulated complement activation. The complement system serves as a first-line defense against pathogens and is essential for the removal of dead cells. Although the effector functions of opsonization, inflammation, chemotaxis, and cytolysis promote pathogen clearance, dysregulated or excessive complement activation can lead to tissue injury and organ failure, one of the clearest examples being the prothrombotic and anaphylatoxic effects of activated complement component 5.

Cytokine release and complement activation have long been implicated in organ failure and ARDS in sepsis (11). Although cytokine levels are comparable with non-COVID ARDS (medRxiv preprint DOI: <https://doi.org/10.1101/2020.05.15.20103549>), complement-mediated damage to the lung microvascular endothelial cells appears to be a predominant feature of COVID-ARDS, whereas direct comparisons with non-COVID ARDS have not been published as of this writing (6). Preclinical studies demonstrate that the nucleocapsid protein of several coronaviruses, including SARS-CoV-2, binds directly to and activates MASP-2, a key protease in the lectin pathway of complement. In murine studies of SARS-CoV-induced lung injury, mice deficient in C3 were relatively protected from lung injury following SARS-CoV infection and exhibited less lung neutrophil recruitment and lower levels of cytokines in the lungs and circulation (12).

The alternative pathway of complement activation is always “on,” requiring tight regulation by soluble and membrane-bound complement regulatory proteins to protect the endothelium. Medical conditions such as diabetes, among others identified as risk factors for SARS-CoV-2 mortality, leads to dysfunctional

Table 1. Potential Vascular Complications in Critically Ill Patients with COVID-19

	Complication	Organ Affected
Macrovascular	Venous	Deep vein thrombosis
		Pulmonary embolism
Arterial		<i>In situ</i> pulmonary thrombosis*
		Stroke
		Myocardial infarction
		Mesenteric ischemia
		Limb Ischemia
Microvascular	Thrombosis	Lung, heart, intestines, kidneys, and skin
Extracorporeal	ECMO oxygenator clotting	N/A
	Renal replacement filter clotting	N/A

Definition of abbreviations: CNS=central nervous system; COVID-19=coronavirus disease; ECMO=extracorporeal membrane oxygenation; N/A=not applicable.

Vascular complications in patients with COVID-19 that have been reported in the literature and organs involved (both reported and extrapolated).

*Postulated mechanism that is not reported in the literature as of this writing.

endothelial complement regulatory proteins, thereby increasing susceptibility to complement-induced endothelial damage. Complement activation and dysregulation of the renin-angiotensin system may be most severe within viral damaged lung vasculature but may also contribute to the pathogenesis of strokes, myocardial and mesenteric ischemia, and cutaneous lesions owing to limb ischemia. Given the atypical vascular-centric risk factors for COVID-ARDS, it is plausible that complement activation and dysregulated ACE2-angiotensin repression in susceptible hosts might lead to widespread endothelial dysfunction.

Despite the best care and implementation of lung protective strategies, the mortality for COVID-ARDS remains high. Given the many indications pointing toward vascular involvement, vascular-centric, endothelial protective therapies should be considered as adjuncts in the treatment of COVID-ARDS. Although no previous medical therapy has improved sepsis or ARDS mortality, there is reason to believe COVID-ARDS may be unique. Unlike most sepsis-associated ARDS, both the timing and pathogen are known in COVID-ARDS. Additionally, the higher incidence of vascular manifestations should justify consideration of COVID-ARDS as a distinct endotype with prominent vascular dysfunction (13). Specific vascular targeting may present a unique opportunity to intervene. In view of the potential for targeted therapies of the complement pathway, supplements, or alternatives to heparin as an antithrombotic, and endothelial protective therapies such as nitric oxide, corticosteroids, and statins to restore endothelial homeostasis, a comprehensive molecular understanding of vascular endothelial dysfunction in COVID-ARDS is urgently needed. Although we still do not know enough to definitively classify COVID-ARDS as a vascular endotype, COVID-ARDS may be an extreme example of a phenotype present in the more general population of ARDS, and investigations into the dysregulated immune response in the vasculature may advance the understanding and treatment of all forms of ARDS.

Some of the results of these studies have been previously reported in the form of a preprint (OSFPrePrints, 24 April 2020 <https://osf.io/ckdpe/>). ■

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Rhinovirus Infections in Individuals with Asthma Increase ACE2 Expression and Cytokine Pathways Implicated in COVID-19

To the Editor:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel virus first identified in December 2019 in Wuhan, China, as causing coronavirus disease (COVID-19), with more than 7.5 million cases currently reported worldwide (1). ACE2 (angiotensin-converting enzyme 2) is the receptor for SARS-CoV-2 and has recently been identified as an IFN-stimulated gene (2). Rhinovirus (RV) infections are potent inducers of IFN-stimulated genes and subsequent cytokine production. RV infections are the most frequent virus identified in the common cold and are responsible for the majority of asthma exacerbations in children and adults (3). Young people with asthma have higher rates of COVID-19, accounting for 27% of hospitalized patients in the United States in the 18- to 49-year-old age group (4). We hypothesized that RV infections could increase expression of ACE2 and subsequently activate cytokine pathways associated with severe COVID-19 infections.

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Supported in part by The University of Arizona Health Sciences Center for Biomedical Informatics and Biostatistics, the BIO5 Institute, and the NIH (HL132532, HL147187, and AI146131). The University of Arizona Health Sciences Center for Biomedical Informatics and Biostatistics supported in part the salaries of N.P. and Y.A.L. This article did not receive sponsorship for publication.

Author Contributions: Conception and design: E.H.C., Y.A.L., and F.D.M. Analysis and interpretation: E.H.C., A.L.W., C.E.R., D.A.C., N.P., J.L., Y.A.L., and F.D.M. Drafting the manuscript for important intellectual content: E.H.C., A.L.W., C.E.R., D.A.C., N.P., J.L., Y.A.L., and F.D.M.

Originally Published in Press as DOI: 10.1164/rccm.202004-1343LE on July 10, 2020

We developed air–liquid interface (ALI) cultures from nasal tissues biopsied from 30 adults with physician-diagnosed asthma. Subjects averaged 35 years of age, 60% were non-Hispanic white individuals, and subjects were evenly divided by sex. We infected ALI cultures with common RV strains RV-A16 (1×10^5 RNA copies/well), RV-C15 (1×10^5 RNA copies/well), or Dulbecco's modified Eagle medium/F12 media (control) for 4 hours at 34°C, 5% CO₂. RNA was then extracted from whole-cell lysates, sequenced using KAPA Stranded RNA-Seq libraries on an Illumina HiSeq 3000 for a 1×50 run, demultiplexed with Illumina Bcl2fastq2 (v2.17), and then mapped to the UCSC transcript set using Bowtie2 (v2.1.0). We processed the discovery ($n = 22$) and validation ($n = 8$) cohorts separately through the NOISeq library (5) to filter out genes with low counts (counts per million < 30), resulting in 7,474 and 7,905 unique genes in the discovery and validation cohorts. We then used the function “ARSyNseq” followed by “voomWithQualityWeights” (6) to process RNA counts for downstream statistical analysis with the linear model implemented in the LIMMA R library. We used the moderate *t* test for paired samples for statistical analyses to prioritize 402 differentially expressed genes (DEGs) adjusted by false discovery rate < 1% and absolute log₂ fold change > 0.5.

When compared with controls, both RV-A16- and RV-C15-infected ALI cultures resulted in a greater than threefold increase in ACE2 expression in the discovery and validation cohorts (Figure 1). Interestingly, levels of TMPRSS2 (transmembrane serine protease 2), a protease that primes the SARS-CoV-2 virus for cellular entry, were not increased after either RV-A16 or RV-C15 infections. How could RV infections induce ACE2 expression? Ziegler and colleagues determined that stimulation of primary nasal epithelial cells with IFN increased ACE2 expression. They also identified four potential ACE2 transcription factors located within 2 kbp of the ACE2 start site: STAT1, STAT3, IRF8, and IRF1 (2). Of these four transcription factors, only IRF1 was reproducibly differentially expressed in our data set and showed a significant threefold increase in expression after RV-A and RV-C infections.

Next, we sought to determine if the patterns observed in nasal cells among patients with asthma were also observed for other viruses in human bronchial epithelial cells unselected for asthma. We analyzed microarray data (GSE32140) to quantify gene expression changes after exposure to influenza A and respiratory syncytial virus in ALI cultures of human bronchial epithelial cells. Two hours after infection with influenza A or respiratory syncytial virus, ACE2 expression levels were sixfold higher whereas TMPRSS2 levels were not altered compared with control uninfected cells (data not shown).

The role of ACE2 overexpression on the cytokine surge, which has been shown to be clinically relevant in the severity of COVID-19, is unknown. Huang and colleagues recently reported that critically ill patients with COVID-19 had high serum levels of IL-1β, IL-1RA, IL-2, IL-4, IL-7, IL-8, IL-9, IL-10, IL-13, IL-17, G-CSF, IFN-γ, IP-10, MCP-1, MIP-1A, and TNF-α (SARS-CoV-2-associated cytokine surge) (7). Using our *in vitro* model, we sought to identify DEGs associated with RV-induced ACE2 overexpression and with SARS-CoV-2 cytokine regulation. Sixty-three DEGs were correlated to RV-induced ACE2 overexpression and overrepresented in the “Regulation of cytokine production” gene ontology (GO) set (GO:0001817). We then identified 34 GO annotations correlated to the regulation and production of the SARS-CoV-2-associated cytokine surge (8, 9). Twenty-nine of these 63 DEGs were annotated in 7 GO annotations,