The Upper Airway Microbiome and Lung Injury in COVID-19

The coronavirus disease (COVID-19) pandemic has just surpassed the estimated 675,000 deaths of the 1918 H1N1 influenza pandemic in the United States. We understand that a substantial driver of the high death toll of the 1918 influenza pandemic was secondary bacterial pneumonia. But how do we know this? A meta-analysis done by Morens and colleagues of 8,398 autopsies from 1918 to 1919 found that the vast majority had evidence of bacterial pneumonia (1) and concluded that most were from "common upper respiratory-tract bacteria." Even as this was from the preantibiotic era, the findings have been foundational knowledge for bacterial–viral copathogenesis. In contrast to general agreement on the importance of bacterial superinfection in influenza infection, the role of bacterial copathogenesis in COVID-19 is less clear (2–5) and interactions may operate through indirect pathways.

In this issue of the *Journal*, Ren and colleagues (pp. 1379–1390) conducted a microbiome analysis of 192 patients in the LOTUS (Lopinavir-Ritonavir Trial for Suppression of SARS-CoV-2 in China) randomized clinical trial (6). These patients had severe COVID-19 with a mortality rate of 22.1%. We believe two design features deserve mention.

First, why swab the oropharynx of patients with COVID-19 when the major driver of morbidity and mortality is severe lower respiratory infection that leads to acute respiratory distress syndrome, respiratory failure, and potentially death? This pragmatic choice allowed for dense, longitudinal sampling that provided 588 patient samples. A common limitation in microbiome research has been cross-sectional design and this sampling strategy allowed for more sophisticated models delving into microbiome structure. We also know that the upper respiratory tract is the major source of lung microbes in health via microaspiration (7, 8). In disease, this relationship becomes more variable with changing local conditions in the lung (9), and the upper respiratory tract importantly contributes to lung immune tone (10). Although this study densely sampled the oropharynx, there remains an opportunity through paired sample analysis to better dissect the oral-lung axis's direct or indirect impacts on lung injury (Figure 1).

Second, the metatranscriptomic approach deserves mention. Here, total RNA is isolated from the patient swab and converted to complementary DNA, which is then sequenced. Although this allows parallel sequencing of human and microbial RNA, challenges include potential for rapid *ex vivo* changes in microbial transcriptomes after harvest and complex analysis. The upsides are the ability to profile both taxonomy (including not just bacteria, but also fungi, viruses, and microeukaryotes) and directly quantify gene expression, which can provide insight into the function of both microbes and host response genes. A recent metatranscriptomic study found that this methodology better captured microbial community function and dynamics than that imputed through 16S amplicon taxonomic or shotgun metagenomic sequencing (11). Despite the RNA-based approach deployed here, this study did not exploit the full capacity to explore either microbial or host gene expression, which remains an exciting area of future analysis.

Major findings of this manuscript are that patients with severe COVID-19 who had a *Streptococcus*-enriched oral microbiome (particularly *S. parasanguinis*) on admission were more likely to survive to discharge. This might serve as a prognostic biomarker but needs external validation. Second, from an ecological perspective, the oropharyngeal microbiomes of patients who died deviated more from their own staring point than those who survived. This may reflect an association with lung injury propagation. In addition, the oropharyngeal microbiome was associated with systemic inflammatory markers. These findings are consistent with another study reported recently showing destabilization of the oropharyngeal microbiome in COVID-19 and associations between the oropharyngeal microbiome at entry and clinical outcomes as well as with systemic immune parameters (2). The authors of this study also describe enrichment of potential lung pathogens in the oropharynx, though the significance is unclear as *Candida* and *Enterococcus* are not generally considered respiratory pathogens and the lungs were not directly sampled.

Given the observational nature of the study, confounding is a potential hazard. Healthy control subjects used for comparison were from the same geographical area but not matched for other features. There were also important differences in the COVID-19 cohorts: deceased patients were older, had more comorbidities, and were more likely to be on high-flow oxygen or mechanical ventilation, receive corticosteroids, and receive "high-grade antibiotics." These imbalances, although controlled for analytically where possible, might directly impact the microbiome and therefore influence the relationship clinical outcomes, given the strong associations between demographic and clinical factors and COVID-19–related mortality (12). Therefore, it is uncertain whether microbiome differences are linked to COVID-19 *per se* or reflect underlying demographic or comorbidity differences.

Nevertheless, a key question is whether the association between oropharyngeal microbiome features and COVID-19 outcome might reflect a causal role. What of the biological plausibility of a protective effect of oral *Streptococcus* or deleterious effects of a depleted community type against COVID-19–related mortality? On its face it may seem implausible that the relative abundance of an oral microbe is causally linked to the severe lung injury induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, the high-biomass upper respiratory tract system has been shown to constrain pathogens via metabolic exclusion and direct antimicrobial activity (13). In addition, oropharyngeal microbes influence immune

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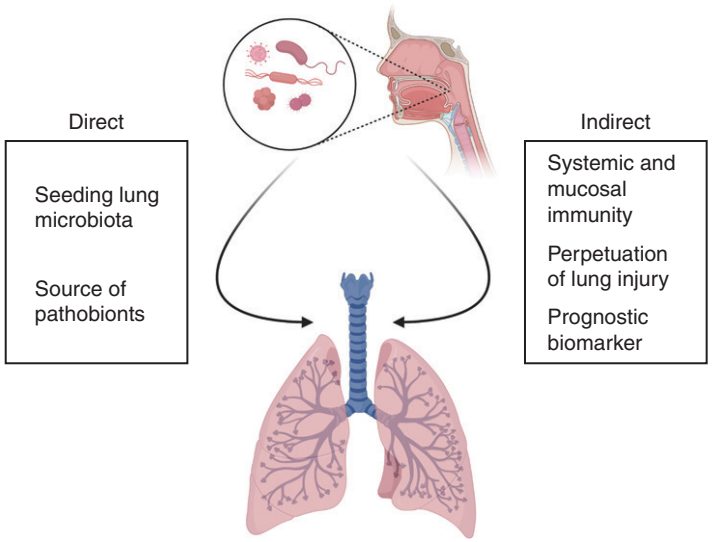

Figure 1. The upper airway microbiome influences lung health and disease through direct and indirect mechanisms. Previous respiratory viral pandemics have elucidated the importance of the upper airways as a source of potential pathogens. Increasingly, investigations of the upper airway microbiome have highlighted its additional influences on immunity and host response, as well as its potential prognostic value. Figure created with BioRender.com.

tone during viral infections through such mechanisms as induction of alternatively activated (M2)-type alveolar macrophages (14), enhancing adaptive influenza $CD4^+$ (cluster of differentiation 4–positive) and $CD8^+$ cellular and antibody responses (15). Indeed, associations reported between the upper respiratory microbiome and outcomes from influenza or respiratory syncytial virus (16, 17) might reflect such mechanisms. Finally, given that the upper respiratory track seeds the lower respiratory track through microaspiration, these indirect effects may operate within the oropharyngeal and lung compartments.

How might this study enrich our understanding of viral–bacterial relationships in relation to the foundational knowledge gleaned from previous viral pandemics? The authors should be commended on conducting a complex study during a pandemic that helps direct the field to future causal questions. Their findings expand on the paradigm of viral-induced injury leading to secondary bacterial infection driving mortality to include broader mechanisms of indirect regulators of outcome. As we have learned during this pandemic, immunomodulatory therapies help patients with COVID-19, and the ability to harness the immunomodulatory functions of the microbiome in lung health is an exciting frontier. ■

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Reducing Ventilator-associated Brain Injury by Diaphragm Neurostimulation

Racking the Diaphragm to Protect the Brain?

Mechanical ventilation is of paramount importance in improving the survival of patients suffering from respiratory failure, as most recently confirmed by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Notwithstanding, it is well established that mechanical ventilation has unfavorable effects, some of them being likely to worsen the prognosis of the primary disease, for which mechanical ventilation was indicated. Among the harmful effects associated with the use of mechanical ventilation, it has become evident that ventilation in critically ill patients can augment or cause lung injury, leading to ventilator-induced lung injury (1). Therefore, providing a lung-protective ventilation—limiting stress and strain—is currently the basis of good clinical practice in critical care settings. However, this approach requires putting the respiratory muscles at rest, which may lead to ventilator-induced diaphragmatic dysfunction (2). Besides ventilator-induced lung injury and ventilator-induced diaphragmatic dysfunction, studies have also reported a strong association between the use of mechanical ventilation and delirium in the ICU (3) and long-term cognitive impairment in acute respiratory distress syndrome survivors who are ventilated for prolonged periods (4, 5). The causal association between mechanical ventilation and neurocognitive dysfunction is extremely difficult to investigate and multifactorial, encompassing nonmodifiable factors such as pre-ICU cognitive impairment, sepsis, and acute illness severity and modifiable factors such as use of opiates, benzodiazepines, and anticholinergic drugs. In addition, mechanical ventilation generates cyclic alveolar collapse and overstretching, causing local and systemic inflammation (6), potentially leading to neurological injury and ventilator-associated brain injury. The concept of ventilator-associated brain injury is supported by several findings confirming hippocampal neuronal cell apoptosis (7), but the long-term impact is still not fully elucidated, considering the plasticity and regenerative capacity of specific hippocampal regions, such as the dentate gyrus (8). Whether ventilator-associated brain injury is mediated though a systemic (hyper)inflammatory or a neural pathway is unclear, and further investigations will have to address this point.

In this issue of the *Journal*, Bassi and colleagues (pp. 1391–1402) provided thought-provoking insights on ventilator-associated brain injury and a novel preventive intervention (9). They used a porcine model to investigate a hybrid strategy of 50-hour mechanical ventilation, including synchronized diaphragmatic neurostimulation. Neurostimulation of the diaphragm was provided through a catheter advanced up to the superior vena cava through the left subclavian, which stimulated the phrenic nerve to reduce ventilator pressure–time product by 15–20%. The intriguing hypothesis was that a "physiological" mechanical ventilation, generated by the contraction of the diaphragm and a preserved ventilation homogeneity, would reduce inflammation and modulate the pulmonary afferent signal, leading to mitigation of cellular apoptosis in the hippocampus. Four interventions were investigated: lung-protective mechanical ventilation, diaphragm neurostimulation either every other breath or every breath in synchrony with lung-protective mechanical ventilation, or no ventilation. During the experimental protocol, the investigators applied consistent sedation protocols among all ventilated groups and therapeutic regimens to control hemodynamics, temperature, and gas exchanges. Interestingly, the heart rate variability was used as a surrogate of autonomic nervous system activity. Significantly greater apoptotic indices, microglia percentages, and reactive astrocyte percentages were found in the mechanical ventilation group in comparison with the other groups, suggesting a protective effect of diaphragm neurostimulation on hippocampal injury. In addition, blood biomarkers of brain injury were significantly lower in the group that had every breath in synchrony with lung-protective mechanical ventilation. Yet, systemic markers of inflammation and lung injury scores were similar between the groups, which may imply that hippocampal apoptosis, rather than being triggered by the inflammatory pathway, was possibly caused by a neuropathway of injury.

This study brings to light important new knowledge about pathophysiology of ventilator-associated brain injury and a promising

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