Could the Sputum Microbiota Be a Biomarker That Predicts Mortality after Acute Exacerbations of Chronic Obstructive Pulmonary Disease?

Our understanding of chronic obstructive pulmonary disease (COPD) is shifting to a personalized approach in which we have a better appreciation of the multiple factors involved in its pathogenesis. The technological advances made in the last two decades have revealed a breadth of different biomarkers and mechanisms involved in this disease. In this new "omic" era, the implementation of bioinformatic approaches has allowed us to digest multidimensional datasets to create interpretable results and embrace the existence of multiple noncanonical pathways that contribute to the development and clinical course of complex diseases such as COPD. One of such omic approach is the use of molecular methods that, by measuring microbial genes, allow for a comprehensive characterization of complex microbial communities that we call the microbiome. This advancement from our previous culture-dependent view of the microbial world invites us to reexplore the role of bacteria in COPD.

For many years, we have recognized the effects of microbes on the natural history of COPD. In stable COPD, nonpotential pathogenic microorganisms such as many of our oropharyngeal commensals are isolated more frequently than potential pathogenic microorganisms such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* (1). During acute exacerbations of COPD (AECOPD), culture of respiratory secretions frequently identifies increase in bacterial loads and/or acquisition of a new strain (2–8). AECOPD are associated with increased mobility and mortality, and thus, understanding the complex microbial landscape around AECOPD may reveal novel insights. With the implementation of culture-independent methods, we now know that potential pathogenic microorganisms are frequently found in culture-negative respiratory specimens (3, 9). During AECOPD, the sputum microbiota has decreased diversity and increased proportion of Proteobacteria, whereas other distinct microbiota signatures have been associated with positive bacterial cultures and with elevated eosinophils (10). However, the clinical relevance of these microbiota signatures in airway samples has not been elucidated.

In this issue of the *Journal*, Leitao Filho and colleagues (pp. 1205–1213) used sputum samples obtained at the time of hospital admission for AECOPD in 102 subjects to examine for associations between sputum microbiota and 1-year follow-up mortality (11). In total, there were 19/102 deaths within that period. The nonsurvivor group had a lower alpha diversity (intrasample diversity, or how many different types of bacteria are present in a sample) compared with the survivor group. A decrease in alpha diversity usually identifies microbial communities in which a small number of bacteria bloom and dominate. However, lower alpha diversity may also be the result of microbial pressures, such as antibiotics, that may not have been fully controlled by the investigators (as acknowledged by the authors). Differences in beta diversity (intergroup diversity or a measure of how similar samples from different individuals are) and in taxonomic composition were also noted between survivors and nonsurvivors. At the genus level, the sputum microbiota of survivors was enriched with *Rothia*, *Prevotella*, *Veillonella*, *Fusobacterium*, and *Actinomyces* (genera frequently identified as oral commensals), whereas the sputum microbiota of nonsurvivors was enriched with *Staphylococcus* and *Escherichia-Shigella*. The presence of *Staphylococcus* in sputum samples was associated with prolonged hospital stay (an extra 1.5 d) and 7.3 times increased mortality compared with subjects without this genus in their sputum. Even more impressive, the absence of *Veillonella* genus in a sputum sample was associated with 13.5 times increased mortality during the study period. Importantly, Cox regression models were adjusted for age, sex, smoking status, ethnicity, home oxygen therapy, and use of antibiotics during hospitalization. These provocative results suggest that microbial signatures present in sputum microbiota may be used as a predictor of poor outcome for patients with AECOPD.

Although this study generates some provocative results, it has also raised many unanswered questions. First, we must acknowledge that when dealing with high-dimensionality data, statistically significant associations identified need to be cautiously interpreted, even when adjusted for multiple comparisons. As outlined here, the acceptance of microbiota signatures as biomarkers will require extensive validation in separate cohorts. Second, as the authors have acknowledged, many possible confounders were difficult to be fully assessed. An example of this is the use of antibiotics before sampling, a variable that is challenging to control for in the setting of AECOPD and that likely affects the airway microbiota. From a mechanistic point of view, it would be important to determine whether the microbiota signatures identified in this study are representative of changes occurring in the upper or in the lower airway microbiota. There is now increasing evidence that the sputum microbiota is a better reflection of the oral microbiota than of the lower airway microbiota (12, 13), and thus, the signatures identified in sputum in the current study should not be assumed to represent changes of the lower airway microbiota in AECOPD.

We are at the very early stages of airway microbiome discovery, and at an even earlier time for its use as a biomarker for diagnosis or prognosis. Most biomarkers in use have required large cohorts for discovery phase, validation phase, and in some, prospective clinical trials. It is well accepted that biomarker

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development requires certain standards for analytical validity (meaning that the biomarker needs to be accurate, reproducible, and reliable), clinical validity (ability to separate groups with distinct clinical/biological outcomes or differences), and clinical utility (the use of the biomarker should improve measurable clinical outcomes) (14). When studying the airway microbiota, we are still frequently faced with analytical validity challenges, in part related to the low biomass and risk for reagent contamination (most important for lower airway samples), as well as the lack of uniformity of sequencing techniques and analytical approaches. Further, unlike gut microbiome studies, airway microbiome studies have been small and frequently limited to few centers, even when noninvasive samples, such as sputum, are used. Thus, for the most part, the clinical validity is limited by the single discovery cohort design (such as the one described in this study) and the lack of validation. And finally, as promising biomarkers arise, we need effective strategies to test whether the use of microbiome data can affect clinical outcomes. Thus, the current study is an important initial step in biomarker discovery. The road ahead will require larger cohorts and different designs so we can have a personalized approach in which noninvasive microbial signatures may have clinical implications for patients with COPD. ■

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Building Strong Neighborhoods in the Lung with a Little Help from My Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can be isolated from numerous tissues, with the most studied sources being the bone marrow, skeletal muscle, amniotic fluid, and adipose tissue (1–3). By definition, MSCs must meet the following requirements: *1*) adherence to plastic; *2*) trilineage differentiation into adipocytes, chondrocytes, and osteoblasts; and *3*) expression of cell-surface mesenchymal markers (CD105, CD90, CD73, CD13, CD166, CD44, and CD29) and a lack of expression of hematopoietic and endothelial surface markers (CD45, CD31, and CD34) (4).

Furthermore, key unique features of MSCs are their ability to repair tissue through paracrine support of injured cells, partially due to their transfer of mitochondria into damaged cells (i.e., alveolar epithelium), and their ability to modulate the immune response

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