Precision Endotyping in Bronchiectasis

Significant heterogeneous pulmonary diseases, such as asthma and pulmonary hypertension, have been categorized into unique molecular and inflammatory endotypes (1, 2). These endotypes correlate with clinical features of disease that allow targeted therapies to address specific pathophysiologic mechanisms, often referred to as "precision medicine" (3, 4). In contrast, bronchiectasis, which is the archetypal heterogeneous respiratory disease because of its inflammatory, microbial, and mucociliary components (5, 6), has not yet been similarly categorized. This lack of endotype categorization has limited the understanding of the natural history of bronchiectasis and has impeded the development of precision medicine for bronchiectasis patients. This void has now been diminished by Choi and colleagues (pp. 1166–1176) in their original research article titled "Inflammatory Molecular Endotypes in Bronchiectasis: A European Multicenter Cohort Study" in this issue of the *Journal* (7).

The aim of Choi and colleagues (7) was to reveal inflammatory molecular endotypes of patients with bronchiectasis through the identification of mutually exclusive clusters of inflammation and further analyze each inflammatory cluster for relationships with the microbiome and clinical outcomes. The study cohort included patients with stable but symptomatic bronchiectasis from within the BRIDGE (Bronchiectasis Research Involving Databases, Genomics, and Endotyping) study, a research effort specifically created to determine molecular endotypes of bronchiectasis with the goal of guiding response to treatment. Three European bronchiectasis centers (in Milan, Italy; Barcelona, Spain; and Dundee, United Kingdom) contributed to the total of 199 analyzed subjects in the study. The patients were mostly White ($n = 197$ [99%]) and of typical bronchiectasis age (69 yr [interquartile range, 61–77 yr]), and 54.8% were women. Mild, moderate, and severe disease, as measured using the bronchiectasis severity index, accounted for 27.6%, 35.7%, and 36.7% of the cohort, respectively.

To identify molecular endotypes, a total of 33 inflammatory markers were selected on the basis of previous evidence of relevance, obtained from a combination of sputum and serum, and grouped into four algorithmically predefined, mutually exclusive clusters. In parallel, the sputum microbiome was analyzed using 16S microbiome analysis. Univariate and multivariate linear regression was performed to identify relationships among six sputum inflammatory markers (IL-1β, TNF-α [tumor necrosis factor-α], neutrophil extracellular traps, IL-5, VEGF [vascular endothelial growth factor], and GRO-α [growth-regulated oncogene-α]) and the top five most abundant bacteria (at the genus level) in all microbial populations (*Streptococcus*, *Pseudomonas*, *Haemophilus*, *Veillonella*, and *Prevotella*). The model was adjusted for age, sex, inhaled corticosteroids, oral antibiotics, and inhaled antibiotic use. Exacerbation frequency over a 12-month follow-up period was also compared among the clusters.

The authors found that the four inflammatory clusters manifested mutually exclusive, varying predominancies of neutrophil and/or type 2 inflammation as follows: cluster 1, milder neutrophilic inflammation; cluster 2, mixed neutrophil and type 2 inflammation; cluster 3, most severe neutrophilic inflammation; and cluster 4, mixed epithelial and type 2 inflammation.

Each individual inflammatory cluster manifested uniquely defining characteristics at the microbiome level. β diversity differed significantly among the clusters. Cluster 3 (most severe neutrophilic inflammation) contained the lowest α diversity; cluster 1 (milder neutrophilic inflammation) manifested the highest α diversity. The relative abundance of bacteria at the genus level correlated with the extent of neutrophilic inflammation. *Pseudomonas* was most abundant in cluster 3 (most severe neutrophilic inflammation) but declined progressively with decreasing neutrophilic inflammation and was lowest in cluster 4 (mixed epithelial and type 2 inflammation). Conversely, the highest abundance of *Streptococcus* aligned with the lowest neutrophilic inflammation (cluster 4) and progressively declined as neutrophilic inflammation increased. By linear regression, the authors further demonstrated associations between chronic infection and inflammation. Among these, *Pseudomonas* was associated with decreased VEGF concentration.

To align the molecular endotypes with clinical outcomes, Choi and colleagues (7) analyzed exacerbation frequency over a 12-month follow-up period to provide rate ratios of exacerbations among the clusters. The highest risk for exacerbations, including severe exacerbations, was among the clusters with the highest neutrophilic inflammation, thereby revealing at-risk populations on the basis of inflammatory profile.

Choi and colleagues (7) thoroughly reviewed the limitations of their study, namely, the use of a relatively small number of patients in two of the defined clusters and the use of 16S ribosomal RNA (which excludes the contribution of viral and fungal communities). Sputum was used for microbiome analysis. This was proposed as an additional limitation because of its potential for oral contamination. However, it can be argued that real-world patients with bronchiectasis are continually exposed to such oral contamination, as the two sources are in physical communication with each other and should be considered together.

The galvanizing strength of this research is its novel integration of inflammatory clusters with the microbial community and clinical outcomes. The association of worse disease with high neutrophilic inflammation (8) and low microbiome diversity (9) have previously been independent findings. But Choi and colleagues (7) have now demonstrated the interconnectedness between inflammation and the microbiome. They demonstrated that the four unique inflammatory clusters were consistent among patients regardless of country of origin, suggesting the durability of their findings. Such consistency

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will provide a framework on which to develop new therapeutic interventions, build future multicenter trials, and help ensure the reliability of results.

Moreover, the study presents a paradigm shift in our understanding of the significance of certain chronically infecting pathogens. Thus far, *Pseudomonas* has received the most attention for its role in influencing bronchiectasis disease severity (10, 11). Choi and colleagues (7) revealed a high abundance of *Haemophilus* in the clusters that manifested the highest rate ratio of exacerbations and in which microbial diversity was lowest. Thus, they have demonstrated that exacerbation risk is more accurately predicted by the combined inflammatory–microbial profile rather than an isolated airway microbe. In addition, they have provided a sophisticated guide for developing clinically available biomarkers that may be used to identify at-risk patients more accurately than identifying patients by microbial profiles alone.

Hopefully, Choi and colleagues (7) will continue to build on this work by expanding their research to include additional validation cohorts in more diverse geographic locations and by analyzing the cluster behavior during clinical events, such as exacerbations. Regardless, Choi and colleagues have taken phenotyping of bronchiectasis patients to a higher level. By integrating the inflammatory and microbiome profiles, they have ushered in a new era of classifying patients with bronchiectasis, "precision endotyping," and created a pathway to provide patients with bronchiectasis with precision medicine. ■

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Bad Neighbors or Bad Neighborhoods: Pathogenic Residency of T Cells in Chronic Obstructive Pulmonary Disease

The lung is an incredibly diverse cellular neighborhood, composed of structural cell types present since embryonic specification along with circulating cell types that take up residency later in life. The lung's capacity to host circulating immune cells allows a rapid response to pathogens at the site of entry, particularly adaptive immune cells such as T cells that can take up residency in the organ and impart immunologic memory to foreign antigens (1). However, hosting resident immune cells comes with a cost because these cells provoke inflammatory responses, both adaptive and maladaptive, in the lung.

In this issue of the *Journal*, Villaseñor-Altamirano and colleagues (pp. 1177–1195) performed in-depth profiling of tissue-resident $CD8^+$ T cells in mild-moderate chronic obstructive pulmonary disease (COPD) to uncover putative drivers of pathogenic alterations in the lung (2). The presence of $CD8^+$ T cells in COPD has been well described in histologic analyses of airways derived from patients (3). These $CD8^+$ T cells are mostly localized within lymphoid follicles surrounding the airways that accumulate with disease progression in patients with COPD (4). Preclinical studies have also shown that $CD8^+$ T cells are required for the pathologic phenotypes seen in mouse models of emphysema (5). However, these studies

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