Targeting Chronic Obstructive Pulmonary Disease Phenotypes, Endotypes, and Biomarkers

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Abstract

Chronic obstructive pulmonary disease (COPD) is now well recognized to be a phenotypically heterogeneous disease, and this heterogeneity is underpinned by biological heterogeneity. An “endotype” is a group of patients who share the same observed characteristic(s) because of shared underlying biology, and the “endotype” concept has emerged as one way of bringing order to this phenotypic heterogeneity by focusing on its biological underpinnings. In principle, biomarkers can help identify endotypes and mark these specific groups of patients as suitable candidates for targeted biological therapies. Among the better-described endotypes of COPD are alpha-1 antitrypsin deficiency and eosinophilic COPD. Both of these endotypes have biomarkers and at least some evidence of preferential benefit from targeted therapy. Other biological pathways that may define endotypes of

COPD include more general pathways of type 2 inflammation, IL-17–driven inflammation (due to autoimmunity or deposition of nanoparticulate carbon black), bacterial colonization, pathological alterations of the airway mucus gel, and others that are beyond the scope of this review. Whether these biological pathways ultimately are found to segregate patients into very distinct endotypes or subsets (like alpha-1 antitrypsin deficiency) or, instead, are present as “treatable traits” in various combinations is uncertain. However imperfect, the endotype concept forces a focus on heterogeneity at a biological level, and the development of biomarkers of biological heterogeneity should help advance the goal of developing new therapies for COPD.

Keywords: alpha-1 antitrypsin deficiency; biomarker; chronic obstructive pulmonary disease; endotype; phenotype

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Chronic obstructive pulmonary disease (COPD) is now well recognized to be a heterogeneous disease, and this clinical heterogeneity is underpinned by biological heterogeneity (1). The concepts of phenotypes and endotypes capture this relationship in that phenotypes are observed characteristics (often clinical characteristics) and endotypes are groups of patients who share observed characteristics because of shared underlying biology (2). The formal identification of an endotype implies the recognition of several shared disease features including clinical characteristics, genetics, physiology, histopathology, epidemiology, and treatment response (2).

This formal definition is useful in guiding the types of multilevel biological and clinical analyses that may be performed in the investigation of an endotype, but current descriptions of endotypes rarely fulfil every one of these criteria. This may reflect inadequate depth of knowledge in one or more areas of investigation. Alternatively, the formal definition of an endotype may be overly idealized and difficult to fulfil in the same way that Koch’s postulates are difficult to fulfil when investigating the causative relationship between a microorganism and a disease. Biomarkers may be useful in the study of endotypes in several ways: 1) by providing insight into underlying biology in

human studies, 2) by marking endotypes when they become better defined, and 3) by directing personalized treatment approaches that target those endotypes. The rationale for understanding the interrelationships between phenotypes, endotypes, and biomarkers is that treatment of COPD based on clinical phenotypes alone has inherent limitations. Using information about the underlying biology may help in efforts to develop new therapies for COPD and in treatment decisions related to new and existing therapies. The concept of endotyping maintains a focus on underlying biology in studies aimed at subtyping COPD.

Recognized Endotypes in COPD

Alpha-1 antitrypsin (A1AT) deficiency is perhaps the best example of an endotype in COPD. It has a defined genetic underpinning: mutations in the *SERPINA1* gene. It has distinct histopathology: predominant lower lobe, panacinar emphysema. Its epidemiology is similar to other causes of COPD in that cigarette smoking is an important cofactor, but its epidemiology is distinct in that its onset is earlier, reflecting increased susceptibility to cigarette smoke. The obstructive lung physiology observed in A1AT deficiency mimics that of other forms of COPD, and consequently A1AT can go unrecognized, leading to delays in diagnosis. Therefore, biomarkers are critical for diagnosis and proper treatment. Biomarkers used for the diagnosis of this disease include measurement of A1AT levels, proteinase inhibitor (Pi) typing and A1AT genotyping (3). Diagnosis based on use of these biomarkers is essential for monitoring, personalized counseling, and, in some instances, targeted replacement therapy (3).

Eosinophilic COPD is another emerging endotype of COPD. Sputum eosinophils are associated with short-term response to systemic (4, 5) and inhaled

corticosteroids (6, 7). Elevated blood eosinophils are common in COPD. For example, persistent blood eosinophilia (>2%) was present in 37% of subjects with COPD in ECLIPSE (8). Elevated blood eosinophils have been associated with higher rates of COPD exacerbations (9). Importantly, several clinical studies to date have studied whether higher blood eosinophils levels are associated with better clinical response to a widely used COPD therapy, inhaled corticosteroids (ICS) (summarized in Table 1). In a *post hoc* analysis of two randomized controlled trials (RCTs) of more than 3,000 patients, reductions in exacerbations with fluticasone furoate and vilanterol, compared with vilanterol alone, were greater when blood eosinophil counts were higher (10). In a *post hoc* analysis of an RCT of beclomethasone plus formoterol fumarate versus formoterol fumarate alone, higher blood eosinophils were associated with higher exacerbation rate in the control group and better exacerbation reduction, better improvement in lung function, and greater improvement in the St. George's Respiratory Questionnaire with ICS (11). Two other *post hoc* analyses of pooled RCTs have confirmed greater reduction in COPD exacerbations with ICS in participants with higher blood eosinophils as compared with those with

lower blood eosinophil levels (12, 13). Similar results have been observed in an ICS withdrawal trial. In a secondary analysis of the WISDOM study, the moderate or severe exacerbation rate was higher in the ICS-withdrawal group as compared with the ICS-continuation group in patients with blood eosinophil counts of at least 2% (14). However, the literature is not monolithic, with at least one equivocal study. Specifically, a *post hoc* analysis of the ISOLDE study found a lower rate of FEV₁ decline in patients with blood eosinophils more than 2% but also found that exacerbation rate reduction on ICS versus placebo was higher in the low blood eosinophil (<2%) group compared with the high blood eosinophil (≥2%) group (the opposite of expected) (15).

Current data do not support the use of new biological therapies that target IL-5 in patients with COPD with higher blood eosinophil counts. *Post hoc* analyses of an RCT of benralizumab (anti-IL-5 receptor) in COPD with sputum eosinophilia suggested a response in a subgroup with elevated blood eosinophil levels (either ≥200 or ≥300 cells/μl) (16). However, based on press releases, two pivotal phase 3 RCTs of benralizumab as add-on therapy in patients with moderate to very severe COPD with a history of exacerbations across a

Table 1. *Post hoc* analyses of randomized controlled trials that investigate a relationship between higher blood eosinophil level and better clinical response to inhaled corticosteroids

Reference	PMID	Study	Finding
Studies Supporting a Relationship between Higher Blood Eosinophil Level and Better Clinical Response to Inhaled Corticosteroids			
Siddiqui <i>et al.</i> (2015) (11)	26051430	NCT00929851 (FORWARD)	AECOPD rate reduction, improvement in FEV ₁ , SGRQ
Pavord <i>et al.</i> (2016) (12)	26585525	NCT00361959 (INSPIRE)	AECOPD rate reduction in INSPIRE and TRISTAN
		No NCT (TRISTAN)	
		No NCT (SCO30002)	
Bafadhel <i>et al.</i> (2018) (13)	29331313	NCT00206167, NCT00206154, NCT00419744	AECOPD rate reduction, improvement in FEV ₁ , SGRQ in pooled data
Pascoe <i>et al.</i> (2015) (10)	25878028	NCT01009463 (H2C102871)	AECOPD rate reduction in pooled data
		H2C102871 (H2C102970)	
Watz <i>et al.</i> (2016) (14)	27066739	NCT00975195 (WISDOM)	Increased moderate/severe exacerbations after ICS withdrawal
Studies with Equivocal Results			
Barnes <i>et al.</i> (2016) (15)	26917606	No NCT (ISOLDE)	AECOPD reduction with ICS was greater in the low blood Eos group (<2%) 3-yr rate of decline in FEV ₁ was lower in the high blood Eos group (>2%)

Definition of abbreviations: AECOPD = acute exacerbation of chronic obstructive pulmonary disease; Eos = eosinophil; FEV₁ = forced expiratory volume in 1 second; FORWARD = Foster 48-Week Trial to Reduce Exacerbations in COPD; ICS = inhaled corticosteroids; INSPIRE = Investigating New Standards for Prophylaxis in Reduction of Exacerbations; ISOLDE = Inhaled Steroids in Obstructive Lung Disease in Europe; PMID = PubMed identifier; SGRQ = St. George's Respiratory Questionnaire; TRISTAN = Trial of Inhaled Steroids and Long-Acting β₂-Agonists; WISDOM = Withdrawal of Inhaled Steroids during Optimized Bronchodilator Management.

range of baseline blood eosinophil levels (the GALATHEA and TERRANOVA studies) did not meet their primary endpoints of exacerbation reduction. Full presentation of these data, in a peer-reviewed form, is still pending. Two phase 3 RCTs, the METREX and the METREO studies, evaluated mepolizumab (anti-IL-5) for the prevention of COPD exacerbations (17). The METREX study enrolled patients with COPD with a range of blood eosinophil levels and found, among patients with COPD and elevated blood eosinophils, that the mepolizumab group had a lower annual rate of moderate to severe exacerbations than the placebo group. The METREO study enrolled patients with COPD with elevated blood eosinophils and randomized them to placebo or one of two doses of mepolizumab (100 or 300 mg). This study found that neither the 100- nor 300-mg mepolizumab group had significantly lower rates of COPD exacerbations than the placebo group after adjustment for multiple comparisons. After reviewing these data, the U.S. Food and Drug Administration Pulmonary Allergy Drugs Advisory Committee voted 16 to 3 against recommending mepolizumab as add-on therapy to reduce exacerbations in patients with COPD. Among the factors cited in this decision were the following: 1) the primary endpoint was achieved in only one of the two pivotal trials; and 2) uncertainty still exists regarding accurate defining criteria for eosinophilic COPD (18).

Taken together, this relatively large body of human study data suggests that eosinophilic COPD may comprise an endotype of COPD that has distinct clinical characteristics and histopathology. Patients with eosinophilic COPD may have a better response to treatment with ICS, based on largely retrospective studies thus far. However, criteria for the definition of eosinophilic COPD have not yet been standardized, and randomized trials of biologics that target IL-5 have not demonstrated sufficient efficacy to recommend treatment with these biologics.

Emerging Endotypes in COPD

Eosinophilia in COPD may reflect the more general presence of type 2 inflammation in the lung, which can be manifested by other markers of inflammation. Type 2 inflammation is an immune response mediated by adaptive immune cells that

include helper T type 2 cells and innate immune cells that include type 2 innate lymphoid cells, basophils, eosinophils, and mast cells. The canonical cytokines that mediate type 2 inflammation include IL-4, IL-5, and IL-13. Type 2 immunity is protective against helminthic parasites, but dysregulated in atopic disease (19). A key endotype of asthma is marked by type 2 inflammation ("type 2 high") and characterized by airway hyperresponsiveness, airway and blood eosinophilia, and preferential response to ICS (20). Airway responses to type 2 inflammation may mark a distinct endotype in COPD, similar to the well-established type 2 asthma paradigm. In a gene expression study characterizing type 2 inflammation in COPD, asthma-associated transcripts were upregulated in the large and small airways of a subset of participants with COPD and associated with distinct clinical features (21). In a *post hoc* analysis of an RCT of fluticasone with or without salmeterol versus placebo for 30 months, a genomic signature of type 2 inflammation was associated with increased airway biopsy eosinophil count and bronchodilator responsiveness at baseline. After ICS treatment, higher type 2 gene expression was associated with preferential improvement in hyperinflation (21). Importantly, type 2 gene expression was not associated with a clinical history of asthma, suggesting that this inflammation can be occult. Furthermore, a potential "type 2 high" endotype in COPD may represent more than eosinophilic COPD, as eosinophilia is a distal event in the type 2 inflammatory cascade that may incompletely reflect the breadth of type 2 responses in the airway.

Other pathways of adaptive immunity can be engaged in some patients with COPD. The relevance of T-helper cell type 1 (Th1)-driven inflammation in the pathogenesis of COPD is well described (22). However, emerging evidence suggests that type 17 helper T cells (Th17 cells) and their principal cytokine, IL-17A (which we will refer to as "IL-17"), may play key roles in the pathogenesis of COPD in some individuals. Multiple studies have demonstrated elevations of IL-17 in COPD (23–25). Mechanistic data suggest that IL-17 may be particularly important in the pathogenesis of emphysema. In a study examining the immunological underpinnings of emphysema, dendritic cells isolated from emphysematous

individuals demonstrated enhanced potential for stimulating both Th1 and Th17 responses compared with dendritic cells from healthy participants (26). Participants with emphysema had higher levels of Th17 cells in their lungs, and these cells were responsive to elastin fragment stimulation *in vitro*. This suggests that adaptive immune responses against elastin or its fragments can drive emphysematous changes. Furthermore, the fold change in IL-17 secretion in response to elastin stimulation was significantly associated with the percentage of emphysema found on computed tomographic (CT) imaging (26), underscoring the potential clinical importance of Th17 mediated autoimmunity in COPD. More recently, the same investigators found that insoluble nanoparticulate carbon black, a long-lived product of cigarette combustion, accumulates in human myeloid dendritic cells from emphysematous lung and can cause IL-17-associated emphysema in mice (27). Thus IL-17-driven inflammation in COPD may have several, potentially overlapping, initiating causes. Ultimately, robust biomarkers of IL-17 inflammation in COPD may be necessary to guide targeted anti-IL-17 therapy in the appropriate subset of patients with COPD.

Perturbations to the airway microbiota may also be relevant in COPD. The role of infection is well recognized in COPD exacerbations; however, microbial colonization may also play a role in stable disease (28). Bacterial colonization in the sputum (29, 30) and bronchoalveolar lavage (31–33) has been described in COPD. Importantly, colonization is associated with enhanced inflammation, suggesting that these microbes are pathogenic. Sequencing-based techniques to detect microbes suggest that shifts in microbial composition are associated with COPD (34, 35); however, these studies are limited by small sample sizes. Comprehensive profiling of the airway microbiome across a broader range of COPD phenotypes and levels of severity may help identify endotypes that could benefit from antimicrobial therapy. Novel work suggests that shifts in airway microbial composition can drive specific inflammatory responses, such as Th17-mediated inflammation, which is known to function in antibacterial immunity (36, 37). Studies integrating perturbations in microbial composition with host inflammatory responses will be necessary to

develop a mechanistic link between the airway microbiome and chronic pulmonary inflammation in COPD.

Chronic bronchitis is a hallmark feature of COPD (38). Pathological mucus abnormalities, which are potentially treatable, may play a key role in the pathogenesis of chronic bronchitis. Some patients with COPD who have chronic cough and sputum have elevated sputum mucus concentration (39). This elevated sputum mucus concentration is associated with greater airflow obstruction and increased risk for COPD exacerbation. Some smokers who do not have spirometric obstruction that is severe enough to qualify as having COPD nonetheless have symptoms, exacerbation-like events, and airway wall thickening on CT scan (40). These “smokers with symptoms despite preserved spirometry” also have increased sputum mucin concentration as compared with those without symptoms. Beyond perturbations in mucin concentration, biochemical properties of mucus may also be clinically relevant in COPD. The two main secreted airway mucins are MUC5AC and MUC5B. *In vitro*, MUC5AC appears to be tethered to the airway epithelial layer, impeding normal mucociliary transport (41), suggesting that it has pathological biophysical properties. In contrast, the MUC5B-deficient mouse develops bacterial colonization and chronic lung disease, suggesting that MUC5B has important homeostatic properties (42). Although both MUC5AC and MUC5B sputum concentrations increase with increasing airflow obstruction in COPD, the concentration of MUC5AC increases to a

greater degree. Therefore, the MUC5AC:MUC5B ratio is higher in more severe obstruction (39), which may contribute to a pathological mucus gel. In cystic fibrosis, oxidative stress can lead to mucus gel stiffening that can be decreased *in vitro* by reducing agents (43). Oxidative stress is increased in COPD (44), and it is therefore plausible that an oxidative environment in the lung may contribute to pathologically altered mucus in COPD. If we can devise therapies that improve the hydration of mucus, reduce mucin concentration, alter the MUC5AC:MUC5B ratio, reduce oxidative cross-linking, or otherwise interfere with cross-linking of mucin glycoproteins, we may be able to improve airway health and homeostasis in subsets of patients with COPD who have pathological mucus gels.

Treatable Traits

Although this review is organized around the concept of treating groups of patients on the basis of their endotype, another concept related to targeted therapy that has emerged in the literature is the concept of “treatable traits” (45). “Treatable traits” refer to patient characteristics that can form the rational basis for a targeted therapeutic intervention, and this concept does not require the grouping of patients into distinct subsets. In principle, these traits could be physiological, histological/radiographic, or even a biological marker. Indeed, the concept of treatable traits can be useful, especially when all the features of an endotype are not yet clear. For example, we do not need to know

why some patients have predominantly upper lobe emphysema to use that information when deciding on their suitability for lung volume reduction surgery. The treatable trait concept also allows more explicitly that any individual patient might fit into more than one category (i.e., they could have both upper lobe emphysema and eosinophilia). Ultimately both concepts, endotype and treatable trait, are likely to be useful in the development and application of novel therapies for COPD, although the concept of endotype implies a more integrated understanding of how biological and clinical features in any given patient are related.

Summary

COPD is phenotypically heterogeneous, and an improved understanding of the biological underpinnings of this heterogeneity can help define endotypes of COPD. Clinical and biological studies of COPD endotypes benefit from biomarkers that can mark underlying biological pathways in clinical studies and hold promise for defining endotypes and guiding targeted therapy. We have made progress in the identification of a small number of endotypes in COPD (e.g., A1AT deficiency and eosinophilic COPD); however, further progress is needed in the definition of additional endotypes, the refinement of biomarkers, and the study of the stability of these endotypes over time. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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