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Review

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Influence of the Lung Microbiota Dysbiosis in Chronic Obstructive Pulmonary Disease Exacerbations: The Controversial Use of Corticosteroid and Antibiotic Treatments and the Role of Eosinophils as a Disease Marker

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Abstract

Chronic obstructive pulmonary disease (COPD) is a debilitating lung disease associated with loss of lung function, poorer quality of life, co-morbidities, significant mortality, and higher health care costs. Frequent acute exacerbations of COPD are sudden worsening of symptoms, the nature of which is associated with bacterial or viral infections. However, one-third of exacerbations remain of undetermined origin. Although it is largely discussed and controversial, current guidelines recommend treatment of exacerbations with bronchodilators, antibiotics, and systemic corticosteroids; this is despite being associated with limited benefits in term of reducing mortality, side effects and without paying attention to the heterogeneity of these exacerbations. Increasing evidence suggests that the lung microbiota plays an important role in COPD and numerous studies have reported differences in the microbiota between healthy and disease states, as well as between exacerbations and stable COPD, leading to the hypothesis that frequent acute exacerbation is more likely to experience significant changes in lung microbiota composition. These findings will need further examination to explain the causes of lung dysbiosis, namely microbial composition, the host response, including the recruitment of eosinophils, lifestyle, diet, cigarette smoking and the use of antibiotics and corticosteroids. It is now important to assess: 1) Whether alterations in the lung microbiota contribute to disease pathogenesis, especially in exacerbations of unknown origin; 2) The role of eosinophils; and 3) Whether the microbiota of the lung can be manipulated therapeutically to improve COPD exacerbation event and disease progression. In summary, we hypothesize that the alterations of the lung microbiota may explain the undetermined origins of exacerbations and that there is an urgent need to facilitate the design of intervention studies that aim at conserving the lung microbial flora.

Keywords: Chronic obstructive pulmonary disease; Lung microbiota dysbiosis; Corticosteroids; Antibiotics; Eosinophils; Probiotics

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Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease affecting 200 million people worldwide and causes three million deaths each year [1]. COPD includes chronic bronchitis and emphysema, characterized by reduced lung function and prolonged airflow obstruction. Cigarette smoking is the major recognized cause; however, other lung irritants are known, such as air pollution, dust, chemical fumes and genetic predisposition [2]. Although 80% of patients are smokers, only 15-50% of smokers develop COPD [3], suggesting other genetic or environmental factors involved.

One of the difficulties in the management of this disease is its heterogeneity in terms of progression, severity, exercise tolerance and symptoms [4, 5]. This severity is also evident during frequent acute exacerbation of COPD that is a transient period of increased COPD symptoms requiring additional medical treatment and often, hospitalization [6, 7]. Known subtypes of exacerbations include bacterial or viral infections (54.7% and 48.4% respectively) [8], associated or not associated with high eosinophil levels, and these molecular events are typically treated with a combination of antibiotics and corticosteroids in a non-specific manner [6, 9]. Importantly, at least one-third of exacerbations are of undetermined origin [10-12].

The pathogenesis of the progressive airflow limitation in patients with COPD has been related to the innate and adaptive inflammatory immune response of the host [13-15]. However, antigens that drive these responses remain poorly understood. In 2002, Sethi et al have shown that acute COPD exacerba-

tions were associated with new bacterial strains isolated from sputum and bronchial brushings, and that lungs exhibited extraluminal interactions between bacteria and leukocytes, in particular, alveolar macrophages, suggesting a possible role of bacteria in the pathogenesis of the disease [16, 17]. More recently, bacterial diversity loss was often associated with greater severity of COPD and may be one of the major determinants that influence disease progression and exacerbations.

We hypothesized that change in the lung microbiota due to lifestyle, diet, cigarette smoking and the use of antibiotics and corticosteroids may explain exacerbation cases of undetermined origin and can contribute to examination of COPD complexity and severity. Understanding the causes of lung microbiota alterations may also influence the choice of adequate treatment.

Phenotypes in Acute Exacerbations of COPD

COPD can no longer be considered as a disease that only involves the lungs, but rather a disease associated with a systemic inflammatory component, which links the disease with other extrapulmonary co-morbidities, such as cardiovascular disorders, skeletal muscle alterations, diabetes, osteoporosis, anxiety, and depression [18, 19]. The great heterogeneity of COPD exacerbations has been widely used to describe and classify the characteristics among patients, with the purpose of identifying different subgroups of patients with a prognostic value that may be screened earlier or that may better respond to therapy. Recently, exacerbation phenotypes were classified according to etiology, inflammatory biomarkers, co-morbidity, clinical manifestation and frequency [19].

The first phenotypical group is based on the etiology of the disease and may have an important therapeutic implication for the appropriate choice of antimicrobial treatment. In particular, some studies highlighted differences in the clinical symptoms, prognosis, and treatment between bacterial infections and viral infections [20]; this latter group commonly shows sore throat, cough, dyspnea [20], higher interleukin 6 (IL-6) levels [21], lower levels of C-reactive protein [22] and longer duration of hospitalization, compared to bacterial infections [23]. On the contrary, a typical symptom in bacterial exacerbations is the purulent sputum due to neutrophil inflammation in both blood and airway [19]. The guideline published in 2011 by the European Respiratory Society recommends avoiding empiric antiviral treatment, except when associated with typical influenza symptoms [24]. The same guideline recommends the use of antibiotics in infections due to bacteria [25].

The second group of exacerbation phenotypes regards inflammation. Patients who manifest exacerbation commonly display heterogeneous inflammatory cells and heterogeneous inflammatory markers. Typical systematic inflammatory markers mainly include C-reactive protein, procalcitonin, serum amyloid A, fibrinogen, and others [19]. Inflammatory cells rather divide patients into four groups: eosinophilic predominant, neutrophilic predominant, paucigranulocytic predominant and mixed granulocytic predominant [26], with different pathological and physiological characteristics [19]. Notably, neutrophilic group poorly responds to standard therapy; whereas eosinophilic group has a better response to corticosteroids [9].

The third phenotype group is based on clinical symptoms that divide exacerbations into three groups [27]. Type 1 includes dyspnea, sputum volume, and purulence; type 2 involves any two of the latter symptoms and type 3 includes one of the latter symptoms combined with cough, wheeze, or others. Antibiotics are recommended for type 1 patients.

The fourth and last phenotype group is based on the frequency of exacerbations. This group includes patients who manifest dynamic lung hyperinflation, increased susceptibility to viral infection, increased extrapulmonary co-morbidities and changes in lower airway bacterial colonization, which is the major argument of this manuscript. Patients who undergo frequent exacerbations have increased systemic and airway inflammation [28, 29], the rapid decline in lung function, worse quality of life [30] and higher mortality [31, 32], compared to patients with no frequent exacerbation. Recommended therapy is long-term inhalation of corticosteroids; anti-inflammatory and anti-infection treatments may be non-therapeutic [19]. However, recommended therapy for frequent exacerbations is bronchial relaxation combined with inhaled corticosteroids [33], in spite of being associated with limited benefits in term of reducing mortality, side effects and without paying attention to the heterogeneity of these exacerbations. In summary, the use of inhaled corticosteroids is very controversial and should be tested with large-scale, randomized, double-blind and controlled studies in order to assess the real effectiveness.

Lung Microbiota and COPD

The microbiota is defined as the "ecological community of commensal, symbiotic and pathogenic organisms that share our body space" [34]. The bacterial communities that inhabit our environments such as the gut, skin or lung are now appreciated for their role in maintaining tissue, organ and immune homeostasis. Alterations of the resident microbiota have been associated with numerous disorders including cancer, respiratory and cardiovascular diseases as well as obesity [35-39].

Compared to the gut microbiota that is easily accessible by the probe, the lungs and the bronchial tree have been considered sterile in healthy subjects till the last decade, due to the negative cultures obtained from these anatomical sites [40, 41]. Over time, despite the difficulties in the application of these techniques, the new field of culture-independent technique application to the identification of bacteria in the community has shown that lungs are not sterile, in contrast with this historical long-held view [42]. The healthy lung microbiota contains lots of commensal bacteria coming from and shared with the upper respiratory tract and these new results led the nowadays-accepted knowledge that lower airways contain a consortium that can vary among individuals and across regions [43-45].

The composition of the lung microbiota is determined by the balance of migration of the bacteria from inhalation, mucosal dispersion or micro-aspiration and the microbial elimination by innate and adaptive host defences, cough and mucocili-

ary clearance [16]. Unlike the gut, the lung is oxygen-rich and it contains lots of lipid-rich surfactants that have bacteriostatic effects against selected bacterial species [46]. In contrast, during disease, the lung environment changes dramatically, creating permissive niches for selective microbial reproduction, leading to the growth of species well-adapted to the injured lung conditions [16]. Alterations in the taxonomic composition are known as dysbiosis and have been associated with the pathogenesis of the lung. Literature reports present differences in the microbiota composition between healthy and COPD states: dominant healthy microbiota is composed of *Bacteroidetes phylum* that under certain condition, such as disease, shifts towards *Proteobacteria*, the phylum that contains putative pathogen gram-negative bacteria [16, 47]. This shift included, besides a significant increase in *Hemophilus influenzae* (*Proteobacteria*), a decrease in *Veillonella* (*Firmicutes*) and *Prevotella* (*Bacteroidetes*).

The lung microbiota also changes during COPD exacerbation conditions compared to stable disease sample [48-50], where the microbial composition shifts toward an abundance of *Proteobacteria* and decrease in *Firmicutes* [51]. In particular, *Hemophilus influenzae* increased [6] whereas the relative abundance of *Streptococcus pneumoniae* species decreased. In addition, a significant increase of *Moraxella catarrhalis* was seen between exacerbation versus non-exacerbation samples [51]. Notably, there was a significant positive correlation between *Moraxella catarrhalis* and the percentage of sputum neutrophils [51], suggesting the possible implication of the host immune response. Other reports have determined colonization by *Pseudomonas aeruginosa* during exacerbations [47, 52].

Cigarette smoking also contributes to impaired the lung innate immunity through the alterations in ciliary function, mucus, cell phagocytosis and increasing bacterial virulence (e.g. enhanced biofilm formation) [53, 54]. Notably, the regular exposure to tobacco smoking, which is one of the major causes of COPD, provokes changes of the microbiota in healthy smokers leading to dysbiosis [46]. Smoking damages airway epithelia and epithelial tight junctions causing bronchitis [55]. In addition, smoking is also positively associated with Chron's disease, suggesting further toxic effect on the intestine [56].

Corticosteroids and the Lung Microbiota

Current guidelines recommend bronchodilators, antibiotics and systemic corticosteroid therapy for acute exacerbations of COPD [57, 58]; this is despite being associated with limited benefits in term of reducing mortality and side effects concerning about the use of corticosteroids for a long term [58, 59]. There is also a lack of concern about the biological heterogeneity of these exacerbations.

Changes in lung microbiota were also seen during antibiotics and steroids treatment [51, 60]. These reports assessed a decreased microbial diversity with an increment of *Proteobacteria* over *Firmicutes* in patients treated with corticosteroids alone, which corresponded to an increment of *Hemophilus influenzae* and *Moraxella catarrhalis* and a decrement of *Streptococcus pneumonia* [51]. An opposite trend was observed in patients treated with antibiotics (with or without steroids) [51]. Although this latter study and others reported a beneficial role of antibiotics for preventing COPD exacerbations [30, 61-63], a group of researchers recently found an increasing recurrence of *Clostridium difficile* infections after treating primary infections with antibiotics [64]. All these considerations suggest that it is controversial whether the recurrent use of antibiotics during exacerbations can have adverse consequences on the lung microbiota by driving the loss of diversity that may trigger to a higher risk of other exacerbations or disease progression.

The Role of Eosinophils

Eosinophils are immune cells from the innate response. These cells were first described by Paul Ehrlich in 1879 [65]. Eosinophils represent less than 5% of total leucocytes and are innate immune cells formed in the bone marrow. They normally reside in the thymus and gastrointestinal tract [66, 67], spending less than 18 h in circulation [68]. Under certain condition, eosinophils can migrate to tissues acting as inflammatory leading cells, promoting the release of their cytotoxic granular products, chemokines, and cytokines. The role of eosinophilic inflammation is still incompletely understood, although it is widely accepted that their role influences allergic conditions and helminth infection.

Eosinophils are able to reach the lungs, both in healthy and disease states, with incompletely understood role. Increased number of eosinophils has been reported in both central and peripheral airways of COPD patients, detected in the bronchial biopsy, sputum and bronchoalveolar lavage [69]. In the lungs, eosinophils are responsible for augmented endothelial adhesiveness [70] and promotion of humoral immunity, stimulating B cells and type 2 immunity [71]. In addition, the lipid bodies in eosinophils can produce cysteinyl leukotrienes, key mediators in the contractility of bronchial smooth muscle [72]. Interestingly, the cysteinyl leukotriene receptor numbers of the airway mucosal tissue were increased in patients with stable COPD and even more greatly during an exacerbation [73].

Sivapalan et al [74] determined whether blood eosinophil counts could reduce systemic corticosteroid exposure of COPD patients admitted to hospital. They found the non-inferiority of eosinophil-guided therapy compared to standard care, with reduction of corticosteroids exposure. Other researchers [75] positively used blood eosinophil levels for predicting response to glucocorticoid treatment and the risk of severe acute exacerbations of COPD patients. Recently, Petite et al [76] evaluated the hospital length of stay (LOS) in hospitalized COPD patients with exacerbations treated with systemic corticosteroid and antibiotic therapy. They assessed that hospital LOS was shorter for patients prescribed standard-dose systemic corticosteroids and who use guideline-recommended systemic corticosteroid and antibiotic therapy is recommended for hospitalized patients with COPD exacerbation.

Differential expression of tool-like receptors on eosinophils and their association with pathogen molecular patterns suggest different regulatory eosinophil phenotypes, which can vary according to the stimulus applied. Linch et al [77] showed

that mouse eosinophils possess antibacterial properties against *Pseudomonas aeruginosa* bacterial infection. Furthermore, Eltboli et al [78] assessed that COPD exacerbation frequency and severity are associated with impaired macrophage efferocytosis of eosinophils.

In COPD exacerbations, a microbiota analysis has demonstrated overrepresentation of *Firmicutes* associated with eosinophilic exacerbation, compared to the predominance of *Proteobacteria* in exacerbations showing positive cultures for bacteria [51]. The *Proteobacteria*/*Firmicutes* ratio notably decreased in eosinophilic groups during exacerbation [51]. Interestingly, Kolsum et al showed an inverse relationship between eosinophil counts in COPD and bacterial infection of *Hemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae*, which are potentially pathogenic microorganisms [79]. The different expression of airway microbiota between eosinophilic and non-eosinophilic patients with both stable and exacerbation COPD further suggests the implication of these mechanisms in the pathogenesis of the disease. The decrement in blood eosinophils during COPD exacerbation with bacterial presence may also be an important determinant of individual response to corticosteroids [79]. As mentioned before, the use of inhaled corticosteroids for exacerbation COPD treatment is greatly discussed and controversial. The first association between corticosteroid administration and airway eosinophil response to treatment was seen in chronic bronchitis in 1978 [80]. Other clinical trials in COPD have shown that patients with increased eosinophil cells had a better response to corticosteroid treatment compared to those with limited numbers [81-83]; in particular, this finding was seen when airway eosinophil levels were greater than 2% in exacerbation [84]. Since it seems that inhaled corticosteroids have not shown benefit in COPD exacerbation patients and because of safety concerns on the long-term use of inhaled corticosteroids, elucidation of the eosinophil role in exacerbation of COPD should be largely assessed. Several studies showed no effects of this therapy in reducing lung decline, exacerbation, and mortality [85-89]. In addition, the WISDOM study showed no reduction in the time of exacerbation between patients treated with inhaled corticosteroids and patients treated without it [90], although the group treated with inhaled corticosteroids was associated with lung function decline at the end of the study. Furthermore, low eosinophil levels were predictive of worsened outcomes in COPD exacerbation [91, 92]. Sputum eosinophilia is associated with a positive response to stable COPD treated with corticosteroids [80, 81, 93] and the count of these immune cells can be used as a marker to monitor the reduction of COPD exacerbation during corticosteroid treatment.

These findings led to assessing that the circulating eosinophils in stable diseases can be used in predicting the risk of exacerbation and those counted in exacerbation can be used in predicting worsened outcomes and response to corticosteroids [94].

Possible Role of Probiotics in Augmenting Treatment Response

There is an urgent need to assess whether the manipulation of the lung microbiota may correct the dysbiosis associated with poor clinical outcomes. A possible change in microbiota can be the eradication of key pathogens, the modification of the lifestyle, including diet, cigarette smoking, drugs and/or the use of probiotics.

Evidence reveals that influenza infection and the use of antibiotics can disrupt gut microbiota leading to a reduction in *Lactobacillus* and *Lactococcus*, and an outgrowth of *Enterobacteriaceae.* This injury was induced by Th17 cells and IL-17 neutralization [53, 95]. In addition, the prolonged use of corticosteroids alters the microbiota equilibrium, leading to an inflammation process and gut atherosclerosis [96, 97].

It is possible that the same mechanisms that cause inflammation and atherosclerosis in the intestine intervene in the lung. Notably, intestinal symptoms of COPD include malabsorption and inflammation that are further exacerbated with disease progression and severity [98, 99].

We also know that modulation of gut microbiota using probiotics is a usual and accepted method for restoring intestinal flora. This latter medical procedure has been shown to increase both the abundance of B cells expressing IgA in the lymph nodes and colon and the lymph node T follicular helper (TFH) cells. In addition, it increases IL-23-expressing dendritic cells. All changes are likely to improve host defence [100].

Lactobacillus is often overlooked in the lung microbiota, associated with anti-inflammatory effects in COPD [101] and protection against viral infections [102]. Sequencing-based studies of tissue from patients with COPD have demonstrated increasing *Firmicutes* community in severe disease linked with an increase in the *Lactobacillus* genus [103]. Interestingly, human macrophages can phagocytose *Lactobacillus* species, reducing cigarette smoking-related inflammation, suggesting that these species have a beneficial effect on smoking-related lung disease, such as COPD [101]. Tomosada et al have modulated the anti-viral response within the lungs of animal models infected with a respiratory syncytial virus (RSV) by the administration of *Lactobacillus rhamnosus* species prior to infection [104]. Thus, changes in the microbiota represent a way forward to protect the lung from respiratory viral infection.

Conclusions and Future Directions

The recent important advance in immunology research has been the recognition of the presence of millions of commensal bacteria, called "microbiota", not only in known tissue districts such as vaginal environment, skin, and gut, but also in the lungs. This microbial community is important for metabolism, nutrition, epithelial homeostasis and defence against foreign pathogens [39, 105]. Dysbiosis of the resident microbiota has been associated with numerous disorders including cancer, respiratory and cardiovascular diseases as well as obesity [35-39]. There could be different variables that affect the lung microbiota, such as bacterial composition, host immune response, lifestyle, diet, cigarette smoking and the use of antibiotics and corticosteroids that are the standard therapy for COPD exacerbations.

The microbial flora of the respiratory tract in healthy sub-

jects contains phylogenetically diverse species such as *Firmicutes*, *Bacteroidetes,* and *Proteobacteria* as most frequent phyla [44] and low presence of potentially pathogenic micro-organisms, such as *Hemophilus influenzae*. During lung disease, the microbiota changes to a restricted flora that shows the prevalence of the *Proteobacteria* species, which include most of the potential pathogen micro-organisms, paralleled with a decline of *Firmicutes* [47]. These changes greatly disrupt the continuity of the bacterial pattern observed from the oropharynx to the bronchial tree, concurrently with the severity of the disease, as is demonstrated by the inverse correlation between *Firmicutes* and *Proteobacteria* relative abundance.

This situation is quite different in exacerbations, a condition that seems to be associated with lower microbial diversity, with an increment of phylum *Proteobacteria* [60, 106] and an increased proliferation of *Moraxella catarrhalis.* This latter species was positively correlated with the percentage of sputum neutrophils [51], consistently with its presence found in COPD exacerbation, which enhances airway inflammation by triggering neutrophils [107].

Hemophilus influenzae is known to produce biofilms protecting itself from the host immune system and antibiotics. This behaviour may explain its persistence after antibiotic treatments and may suggest the importance of adapting a new therapeutic approach. *Moraxella catarrhalis* and *Pseudomonas aeruginosa* can also form a biofilm that confers them the antibiotic resistance. This knowledge may also support the hypothesis to use vaccines or targeted antibacterial drug against these pathogens in order to prevent lung microbiota disruption.

A reduction of microbiota diversity and an increment of *Proteobacteria*/*Firmicutes* ratio in subjects treated with corticosteroids alone and the reversed trend seen in patients who received antibiotics support that the current standard of care therapy for COPD exacerbations can alter the lung microbiota. In agreement with previous reports on the limited efficacy and the higher side-effects of steroids [108, 109], it is also probable that corticosteroid treatment alone could potentially affect the lung microbiota. Furthermore, treatment with steroids may negatively influence the eosinophilic inflammation in COPD exacerbation that could be considered as a potential biomarker for identifying subgroups of patients who may respond to therapy, blocking what is called precision medicine (i.e. blood eosinophil-guided prednisolone therapy) [51, 84].

The possibilities for grouping patients by specific phenotypes could yield distinct microbiota population and the probabilities of repeating that type of COPD exacerbation [6].

In summary, treatment during exacerbations influences the lung microbiota differentially if based on antibiotic use, which reduces the bacterial abundance, especially that of *Proteobacteria*, or use or corticosteroids, which when administered systematically as a single treatment do not influence microbial richness but trigger the predominance of specific taxa from the *Proteobacteria* species [52, 60].

Understanding the causes and consequences of lung dysbiosis may influence the clinician's choice of adequate therapy, especially for exacerbations. All these results suggest that the lung microbiota is critical to COPD disease and that manipulation of these microbial patterns may be an attractive new tool for future treatments in preventing exacerbations. This manipulation may be achieved with the use of probiotics, such as *Lactobacillus* or *Bifidobacterium* species, or by directly influencing the diet.

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Conflict of Interest

The authors declare that they do not have a conflict of interest.

Author Contributions

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