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Composition and modification of the lung microbiome in patients with idiopathic pulmonary fibrosis

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ABSTRACT

Lungs have long been considered sterile, but recent research has shown that a large number of microbiological organisms exist in the lungs of healthy subjects (including bacteria, fungi, and viruses), collectively known as the microbiome. It undergoes changes in patients with respiratory pathology. Studies in idiopathic pulmonary fibrosis show that a large number of bacteria or the abundant presence of potentially pathogenic bacteria can cause disease progression and exacerbation, and can implicitly increase mortality. There seems to be a quantitative balance, a well-established proportion between the components of this microbiome, which is disturbed during a disease and can reach a "state of pulmonary dysbiosis". Evidence suggests that the microbiome may be used as a prognostic biomarker and may also explain the pathogenesis of interstitial fibrosis.

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Introduction

The microbiome consists of microorganisms (and their products) that are physiologically found in the human body [1]. The human microbiota is made up of trillions of symbiotic microbial cells, mainly bacteria in the intestine; in addition to this, viruses (including bacteriophages), fungi, and archaea (archaebacteria, part of the oral, intestinal and vaginal microbiome) are also identified [1,2]. Projects on the microbiome, from all over the world, have been launched in order to understand the roles that these germs play in the appearance and evolution of different pathologies, as well as their impact on human health. The human body and microorganisms have coexisted for millions of years, so the immune system of the human body and the microbiome are closely linked [1,3]. Apparently, each compartment of the human body has a unique microbiome made up of specific microorganisms, and consequently the respiratory tree has its own microbiome [1].

The purpose of the current review is to analyze idiopathic pulmonary fibrosis, a disease with fatal impact, from the point of view of the pulmonary microbiome and the benefits it may bring to our knowledge in the evolution of the disease.

Discussions

The experimental part

Studies that aimed to determine the composition of the microbiome in the healthy man, in the patient with diagnosed idiopathic pulmonary fibrosis, and the modification of the microbiome in the context of the evolution of the disease and exacerbations (where the patients' condition allowed) were analyzed.

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Most of the studies that investigated the human microbiome insisted on the intestinal one, considering the wide range of bacteria found at this level. Also, in the oropharynx, skin, vagina, and nasal cavity, the microbiome was easier studied in comparison with the lung, due not only to greater accessibility, but also to the large number of present microorganisms [4].

The microbiome controversy is about the physiological colonization of the respiratory tree. Lungs have long been considered sterile because standard microbiological culture techniques consistently give negative results [5], only 1% of bacteria can be grown in normal laboratories [6]. In the last decade, however, the use of molecular techniques has shown that this theory is incorrect and that a large number of microbiological organisms (including bacteria, fungi, and viruses), collectively known as the microbiome, coexist in the lungs not only of healthy subjects, but also in patients with respiratory diseases[5].

The challenges for describing the respiratory microbiome are increasing and take into account the difficulty obtaining samples from the lower respiratory tract as well as their contamination in the upper respiratory tract [1].

The respiratory mucosa is host to numerous micro-organisms. The lung microbiome evaluation studies were based on the analysis of bronchoalveolar lavage obtained by bronchoscopy [1]. This technique is widely used to study and highlight the pathogenetic mechanisms and inflammatory response through the influx of pro-inflammatory cells [7]. Although sputum examination is a less invasive method compared to bronchoscopy, it presents an increased risk of contamination when passing through the oropharyngeal cavity [1].

The most representative bacteria in the respiratory tract have so far proved to be: Bacteroidetes (including Prevotella sp.), Firmicutes (including Streptococcus sp. and Veillonella sp.) and, in lower quantities, Proteobacteria and Actinobacteria [8–10]. It is worth mentioning that despite the difference in pH, temperature, and oxygen concentration, the microbiome in healthy subjects is relatively constant.

The composition of the microbiome is influenced by mucociliary clearance and host defense mechanisms, local growth factors contributing very little to the composition of the microbiome in healthy lungs [9]. Smoking can directly affect the composition of the microbiome, which can lead to changes in the structure of microorganisms [11].

Various types of bacteria promote inflammation in the respiratory tract. Although they are less common (Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Haemophilus parainfluenzae, Staphylococcus aureus, and Pseudomonas aeruginosa), their persistence in the respiratory tract favors chronic respiratory diseases and chronic inflammation [12–14]. These microorganisms become particular in the case of certain pathologies, and the overlap and agglomeration of bacterial species decrease the physiological species richness of the pulmonary microbiota and are associated with the evolution of some diseases [15].

All of these bacterial species carry a unique imprint, a distinct complement of genomic DNA. Since it is impossible to completely sequence each genome in each cell, microbial ecology has defined a series of molecular markers that express a DNA sequence which identifies that particular genome [16].

Several markers have been evaluated over time, including ribosomal protein subunits, growth factors, and RNA polymerase subunits. The most present and historically significant is the small or 16S ribosomal RNA subunit gene, commonly referred to as 16s rRNA [16,17]. This subunit is present in the genome of all bacteria, making it easy to identify bacteriological sequences and is the target site for amplification or sequencing [18]. The rRNA-16s sequencing identifies bacterial DNA in 97% of the cases that have benefited from bronchial lavage, compared to the conventional culture technique, which detects bacteria in 39.1% of the lavage sample [8].

Pulmonary microbiome and idiopathic pulmonary fibrosis

Interstitial lung disease (ILD) is a heterogeneous group of conditions with similar clinical, imaging, and histopathological features. While some are associated with immune impairment, others are allergic or even idiopathic. The pathogenesis of these diseases is not fully elucidated; the exaggeration of the host's immune response, which acts as a continuous stimulant to the alveolar epithelium, may be a triggering mechanism of the disease [19]. The pathogenesis of pulmonary fibrosis is complex and may be specific for different agents, with the destruction of pulmonary architecture [20]

Few studies have been carried out on the microbiome in interstitial lung disease. However, more recent studies suggest the importance and connection of the lung microbiome with respiratory pathologies, including interstitial pulmonary fibrosis (IPF) [1]. Recent studies have described the microbiome from stable idiopathic pulmonary fibrosis and correlated the microbial composition and bacterial load with the evolution of pulmonary fibrosis [21]. The lung microbiome was evaluated in patients with IPF in the (COMET) -IPF (Correlating Outcomes with biochemical Markers to Estimate Time-progression) study, and the most prevalent identified bacteria were Prevotella, Veillonella and Cronobacter species (spp.) [22].

As research into the composition of the microbiome in IPF is in its early stages, correlations with intensively studied pathologies (such as COPD) and with healthy

subjects were analyzed through PCR studies from bronchoalveolar lavage. Patients with IPF had a significantly higher bacterial load compared to the COPD group and the healthy group. The most abundant species present in these groups were Streptococcus, Prevotella, and Veillonella spp. and patients with IPF showed lower bacterial diversity compared to the control group, harboring potentially pathogenic bacteria such as Haemophilus, Neisseria and Streptococcus spp. [23]. Among patients with IPF, high bacterial load was associated with a significant decrease in survival time compared to those with lower bacterial load, an effect that is independent of age and tobacco use [23-25].

Interestingly, patients with IPF who have the MUC5B minor allele genotype, previously at increased risk of developing IPF and playing an important role in mucociliary clearance [26,27], had significantly lower bacterial burden compared with patients with IPFs that do not have this genotype [23].

Different studies have evaluated gene expression in blood samples and polymorphisms of MUC5B genes in patients with IPF, compared to healthy control groups. Overexpression of a gene in the IPF group was associated with death and disease progression, with a high level of neutrophils in both peripheral blood and bronchoalveolar lavage, a high bacterial load in bronchoalveolar lavage, and a relatively low amount of Neisseria sp [28].

After adjusting the parameters (age, gender, smoking status, presence of gastroesophageal reflux, baseline lung function, and 6-minute walk test), the presence of Streptococcus or Staphylococcus over a certain limit was associated with a significant clinical reduction in survival. This finding shows that potentially pathogenic microorganisms (Streptococcus and Staphylococcus sp.) are associated with an increased risk of disease progression in humans, coinciding with exacerbation of pulmonary fibrosis in 2 specimens of mice infected with Streptococcus pneumonia [22].

We must not forget that there are a number of limitations in the evaluation of the lung microbiome in the context of an exacerbation. The worsening of the respiratory condition, with significant desaturations can prevent the obtaining of bronchoalveolar lavage. Also, the administration of the antibiotic before the harvest influences the obtained material.

An acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF)) has been defined as an acute clinical worsening of dyspnea, which develops in less than 1 month without an alternative etiology.

While AE-IPF is increasingly recognized and perceived as a severe event with high mortality, there is only a limited amount of clinical data on exacerbation of other interstitial lung pathologies [29]. Precisely from this more accurate identification of IPF, the pulmonary microbiome was further studied in the context of exacerbation of IPF, which does not yet have a definite conclusion. Some studies incriminate Staphylococcus and Streptococcus in aggravating the underlying disease [19].

Molecular techniques have identified several common pathogenic viruses that appear in exacerbation, including respiratory syncytial virus and cytomegalvirus [30]. Described as molecular techniques applied to sputum cultures of patients with acute exacerbation, 89% of gram-negative bacteria with pathogenic potential were found: Klebsiella pneumonia (26%), Mycobacterium tuberculosis (21%), and Acinetobacter baumannii (10%) were the dominant sputum samples [31].

PF progression includes exacerbations like other respiratory pathologies. Acute exacerbations have been associated with poor prognosis. Patients who died by exacerbation had a higher percentage of neutrophils in the bronchoalveolar fluid and a lower number of lymphocytes in the same sample, compared to those who survived an exacerbation [32]. The correlation of the neutrophils with the change in the composition of the lung microbiome could be a key for future studies.

Also, the role of acute phase PCR protein has been studied, which has been shown to be an independent predictor of survival, and a viral or bacterial infection and/ or inflammation may be one of the pathogenic mechanisms of exacerbation [32,33]. Patients with IPF who experienced an exacerbation were found to have four times greater bacterial load compared to patients with stable IPF from the control group, who met the same criteria for age, gender, smoking status, and lung volume.

Exacerbated patients had a higher colony of Proteobacteria sp and potentially pathogens such as Campylobacter and Stenotrophomonas spp. [34]; Campylobacter is known to be a pathogen that originates from the gastrointestinal tract. This finding supports the idea that gastroesophageal reflux would contribute to exacerbations in IPF [35,36].

Evaluation of the microbiome from the sputum of patients with idiopathic pulmonary fibrosis showed an increase in bacterial load with Campylobacter sp. and Stenotrophomonas sp., together with a significant decrease of Veillonella sp. [34]

The contribution of viral infections, including viral hepatitis C, transfused viruses, and herpes virus has been intensively studied, but with conflicting results regarding their importance in the pathogenesis of the disease [37]. Herpetic viruses (including Epstein-Barr virus, cytomegalovirus, herpes simplex virus, and human herpes virus-7 and -8) have been identified in a large proportion in the lung tissue of IPF patients, compared to the control group [38] and similar viruses have developed fibrosis in experimental animals [37]. These data are influenced by the immunosuppressive treatment given to patients with

IPF, but may suggest that viruses are co-factors in the progression of pulmonary fibrosis [21].

Lowering of respiratory infections is a benefit on overall mortality in patients with IPF who have received antibiotic therapy with cotrimoxazole [39]. The standard treatment of idiopathic pulmonary fibrosis with the main antifibrotic agents (Pirfenidone and Nintedanib) has as a secondary effect the modification of the intestinal motility [39], through the transit modifications that it favors (diarrhea and constipation).

Modification of the intestinal microbiome favors respiratory infections [40], with all the implications described above, practically proving that in patients with IPF, they are very difficult to control. In this context, an ideal therapeutic agent should combine the antimicrobial, antiviral, and antifibrotic effects [41,42], although it remains to be discussed whether alternative therapies would have antiviral, antimicrobial, anti-inflammatory and antifibrotic effects [43,44]

Herbal remedies such as Black Elder (Sambucus nigra L.) have been identified so far which associates antiviral (herpes simplex virus type 1, influenza virus) and antimicrobial (Streptococcus pneumoniae, Haemophilus influenza, Streptococcus pyogenes, group C and G Streptococci, Branhamella caterrhalis, and Haemophilus influenza) [45] effects, with demonstrated myocardial antifibrotic effects [46] and renal tissue [47].

Analyzing the previously described aspects, we find that the progression of idiopathic pulmonary fibrosis is correlated with the modification of the respiratory microbiome. There is also some evidence to suggest that viral infection may be responsible for a proportion of acute exacerbations of IPF. The role of the bacteria in the pathogenesis of IPF is less clear. Studies of other respiratory diseases suggest that changes in the lung microbiome are associated with the disease and that these changes influence the behavior of the disease [37].

Conclusions

Interest in the lung microbiome has grown steadily over the past decade, proving through various studies that respiratory microbiota microorganisms play an important role in maintaining the health-disease balance.

Respiratory tract dysbiosis causes an irregular immune response, which in turn can promote host susceptibility to infections, with the deepening microbial imbalance.

In the evolution of chronic lung disease, the microbiome could help select appropriate, targeted, and more personalized antibiotics during the disease, especially in exacerbations of idiopathic pulmonary fibrosis.

In this context, more advanced metagenomic analyses are needed to elucidate the functional role of individual genes and bacterial communities in the progression of idiopathic pulmonary fibrosis.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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All authors have equal rights as the first author of this paper.

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