Microbiome in chronic obstructive pulmonary disease

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Abstract: The introduction of culture-independent techniques for the microbiological analysis of respiratory samples has confirmed that the respiratory system hosts a large number of microorganisms, which include a wide range of bacteria. The regular exposure to tobacco smoke changes the microbiome in healthy smokers, first in the oropharynx, increasing the presence of a restricted number of genera which attain high relative abundance, a pattern that may be considered as dysbiosis. In chronic obstructive pulmonary disease (COPD), microbiome analyses of sputum samples have demonstrated an important decline in bacterial diversity, with a change to a restricted flora with an overrepresentation of the Proteobacteria phylum, which include most of the bacteria commonly considered as potentially pathogenic microorganisms, paralleled by a decline in the relative abundance of microorganisms part of the Firmicutes phylum. In exacerbations, specific bacteria overrepresented in microbiome analyses and potentially causal of the acute episode may not be recovered by sputum culture, while colonizing microorganisms grow easily, in spite that their relative abundance have not changed from previous stability. This situation has been described in patients showing chronic colonization by *Pseudomonas aeruginosa*, who suffer from exacerbations that in most cases are due to other PPMs, in spite of the persistence of positive cultures for the colonizing *Pseudomonas* strains. Interaction between different microorganisms can be addressed through microbiome analyses, and functional metagenomics, that describes the genomic potential of the community, has shown that, in spite that the bronchial microbiome as a whole may not change significantly, clear changes in carbohydrate metabolism, cancer, cell growth and death, transport and catabolism pathways often appear during exacerbations. These functional changes may be important because through them the resident community as a whole show its power to modify important metabolic patterns.

Keywords: Chronic obstructive pulmonary disease (COPD); respiratory microbiome; exacerbation; bronchial colonization; proteobacteria; microbial interaction

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The respiratory microbiome

The bronchial tree and the lung have been considered sterile in healthy subjects till the last decade in front of the negativity of cultures obtained from these sites (1,2). The introduction of culture-independent techniques for the microbiological analysis of respiratory samples, however, has confirmed that the respiratory system hosts a large number of microorganisms, which include a wide range bacteria, viruses and fungi. The analysis of the bacterial composition of bronchial samples has been based on the gene encoding 16S ribosomal RNA (*16S rRNA*), and has shown that only 1% of the bacteria part of the respiratory microbiome are recovered from cultures. The ribosome is essential for the transcription of messenger RNA and the *16S rRNA* gene codes a component of the ribosome that has remained unchanged in prokaryotes over centuries, since any mutation in this gene would limit microbial viability. Sequencing of the *16S rRNA* gene from respiratory samples allows a taxonomic classification of each bacteria identified and is used to describe the composition of the microbial

ecosystem as a whole (3). Reference databases of the *16S rRNA* gene allow to classify the sequences in the sample from the highest taxonomic levels (phylum), to the lower (genus) ones, in most cases reaching the species level. With this purpose bacterial identification uses the term "Operational Taxonomic Unit" (OTU), considering as such each taxonomic unit similar to a specific reference. An OTU will be considered to be equivalent to this reference at gender level when the sequence is at least 94% coincident, and to species when this coincidence is over 97%. The composition of the microbiome is in most cases expressed as relative abundance, meaning the proportion of copies of the *16S rRNA* gene corresponding to each identified OTU from the whole, a value that has shown to be correlated with absolute microbial counts recovered at culture, at least for *Pseudomonas aeruginosa* (4). *16S rRNA* gene analysis is unable to obtain virus and fungal information, however, and these microorganisms need to be targeted with other sequencing approaches.

In the normal subject the microbial flora of the respiratory tree is phylogenetically diverse (5,6), with Firmicutes, Bacteroidetes and Proteobacteria as their most frequent phyla, and a low frequency of OTUs corresponding to potentially pathogenic microorganisms such as those of the genus *Haemophilus* (7). The microbiome harbored by the bronchial tree and the oropharynx have a close similarity in the healthy subject, mainly due to aspiration of oropharyngeal secretions during sleep (8,9). The local environment of the bronchial tree and the lung is able to modify the microbiome after the aspiration of secretions, however, as shown by the higher relative abundance of specific OTUs part of the *Prevotella* genus in respiratory samples (9).

The regular exposure to tobacco smoke changes the microbiome of the oropharynx in healthy smokers, increasing the presence of a restricted number of genera which attain high relative abundance, a pattern that may be considered as dysbiosis (10). These smoking-induced changes of the oropharyngeal flora are not extensive to the bronchial tree in absence of respiratory disease, because the analyses of respiratory flora have not found differences between smokers and subjects who have never smoked, when they have a normal lung function (11). These findings suggest that the response to smoke components is magnified in the oropharynx, and would be found in the bronchial tree only when chronic symptoms and/or an abnormal lung function appear.

16S rRNA pyrosequencing allow not only to assess the bacterial community structure of COPD patients, but also may obtain information on its metabolic functionality, using shotgun metagenomics with MetaGenomics RAST server (MG-RAST), or the PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) programme, which predict metagenomes from 16S data. With the first approach millions of fragments of short DNA reads are created, and the fragments obtained are mapped to databases of orthologous gene groups such as KEGG (Kyoto Encyclopedia of Genes and Genomes) (12), to identify matches to genes with previously described functions (13). This approach depends on the isolation of sufficient quantities of bacterial DNA, however, and a second option is to use evolutionary modelling through PICRUSt, to predict metagenomes from 16S data and a reference genome database (14), useful for detecting microbial functions and their variability, when the quantity of bacterial DNA present is low (15).

Chronic obstructive pulmonary disease (COPD)

The appearance of COPD is associated to the colonization of the bronchial tree by potentially pathogenic microorganisms from its early stages, which are found easily at culture (1). *16S rRNA* gene pyrosequencing of sputum samples from these patients has demonstrated additionally an important decline in bacterial diversity when the disease attains the level of advanced disease. In this clinical situation the microbiome changes to a restricted flora with an overrepresentation of the Proteobacteria phylum, which include most of the bacteria commonly considered as PPMs, paralleled by a decline in the relative abundance of Firmicutes (16). This change disrupts deeply the continuity of the microbiome pattern observed from the oropharynx to the bronchial tree, paralleling the severity of the disease, as is demonstrated by this inverse correlation between the relative abundances of Firmicutes and Proteobacteria, that attains its maximum in advanced disease. This change is associated with an increase in the regional heterogeneity of the respiratory microbiome, identifiable through microbiome analyses of bronchial biopsies (5). These severity-related changes of the respiratory microbiome found in COPD patients has been also reported in cystic fibrosis, where the respiratory flora shows similarly an overrepresentation of genera which included PPMs (17-19).

Most microbiome studies on COPD have used sputum as the sample representing the respiratory system, due to its easy recovery and standardized processing procedure

(20,21). In analyses of the respiratory microbiome, however, it must be considered that sputum originates mainly from the in the proximal bronchi, which harbor a flora that have shown clear-cut differences with the microbial pattern found in distal bronchi and the alveolar space (22,23), that is targeted through bronchial biopsies, the protected specimen brush or bronchoalveolar lavage, samples that have been shown as microbiologically equivalent (5), while in COPD the microbial diversity pattern has been demonstrated to be different in sputum and distal samples (24), a finding confirming the differences between the proximal bronchial flora and the microorganisms lodged in distal bronchi and the lung.

Exacerbation

Microbial cultures have related the appearance of respiratory symptoms in COPD exacerbations to the incorporation of new strains to the bronchial flora (25), but this change in the bacterial flora justifies only part of the exacerbations. Microbiome analyses show high relative abundances of specific genera, which may be considered etiologic, for most of the exacerbations, while the remaining flora do not change significantly (26-29). Furthermore, exacerbations are not only related to overrepresentations of isolated genera, but also associated with collateral changes of microbiome composition as a whole, not always identifiable through the measurement of relative abundances (30,31).

An increase in the relative abundance of a specific genus may be considered causal in a COPD exacerbation, but it is not always paralleled by the results of cultures. Different studies have demonstrated that bacteria clearly overrepresented in analyses of bronchial secretions may not be recovered by culture, while colonizing microorganisms grow easily from the analyzed sample, in spite that their relative abundance have not changed from previous stability. This situation has been described mainly for patients showing chronic colonization by *Pseudomonas aeruginosa*, who suffer from exacerbations that in most cases are due to other PPMs, in spite of the persistence of positive cultures for *Pseudomonas* (4,29). Thus, the examination of the respiratory microbiome confirms that a causal bacterial pathogen may keep unnoticed by conventional microbiology in some exacerbations, while the culture identifies microorganisms that are only colonizers. This equivocal information of conventional microbiology has been reported in up to twenty percent of patients chronically colonized by *Pseudomonas aeruginosa* (4,27).

Microbiome analyses have demonstrated a different pattern in infectious and eosinophilic exacerbations, with a clear overrepresentation of Firmicutes in the second situation, in front of the predominance of Proteobacteria in exacerbations showing positive cultures for bacteria (32). This finding supports the clinical characterization of exacerbations in these two categories, considering that their microbial pattern is fully different, and will require different therapeutic approaches.

Treatment during exacerbations influences the respiratory microbiome differently if based on antibiotics, that reduced bacterial abundance, mainly of Proteobacteria, or on oral steroids, that when administered systemically as a single treatment does not influence bacterial richness but favours an overrepresentation of specific taxa from the Proteobacteria phylum (29,33).

Microbial interaction

Interaction between different microorganisms can be addressed through microbiome analyses, and in a clinical model an effect of viral infection on the microbiome composition has been demonstrated in COPD patients. An induced rhinovirus infection has shown no effect on the microbiome of bronchial secretions in healthy subjects, but was associated with an increase in the relative abundance of Proteobacteria in COPD patients, two weeks after the viral infection, a change that returns to the baseline some weeks later (28). This observation confirms the role of rhinovirus infection as inducer of changes of the respiratory flora with overrepresentation of Proteobacteria, and justifies the finding of coinfections virus-bacteria in one quarter of COPD exacerbations (34).

In spite of the appearance of changes in specific microorganisms in most COPD exacerbations, the bronchial microbiome as a whole do not change significantly in some patients. However, its analysis using functional metagenomics, an approach that describes the genomic potential of the community (35), has shown that clear differences may be found in carbohydrate metabolism, cancer, cell growth and death, transport and catabolism pathways during exacerbations (36). Huang *et al.* (33) have also reported increases in various metabolic pathways during viral and bacterial infections. involving apoptosis and biosynthesis. Any modifications of the carbohydrate metabolism pathway in these patients is probably related to the fact that these molecules are a main energy source for bacteria. Functional changes during acute episodes may

be important because the resident community as a whole appears as able to modify its metabolic patterns during the exacerbation, in spite that there were only one specific bacteria which increase their relative abundance.

Conclusions

Although the microbial composition of the respiratory flora in COPD has been known through microbiome analyses, the involvement of the respiratory flora in the pathogenesis of the disease, and especially the role microorganisms considered non-pathogenic by culture-based microbiology, is practically unknown. Bacterial diversity loss, often related to an increase in the relative abundance of Proteobacteria, is associated with greater severity in COPD, and may be one of the determinants that influence its progression, as has been previously demonstrated in idiopathic pulmonary fibrosis (37). Metagenomics and the analysis of bacterial RNA will be able to provide functional information on the respiratory microbiome, detailing interactions between viruses, fungi and bacteria, and, potentially, to facilitating the design of intervention studies aimed at conserving the flora that acts as positive mutualist, as opposed to the respiratory pathogens that progressively replace it when COPD progresses to advanced disease.

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Footnote

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References

1. Rosell A, Monsó E, Soler N, et al. Microbiologic determinants of exacerbation in chronic obstructive pulmonary disease. Arch Intern Med 2005;165:891-7.
2. Laurenzi GA, Potter RT, Kass EH. Bacteriologic flora of the lower respiratory tract. N Engl J Med 1961;265:1273-8.
3. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A 1990;87:4576-9.
4. Garcia-Nuñez M, Marti S, Puig C, et al. Bronchial microbiome, PA biofilm-forming capacity and exacerbation in severe COPD patients colonized by P. aeruginosa. Future Microbiol 2017;12:379-92.
5. Erb-Downward JR, Thompson DL, Han MK, et al. Analysis of the lung microbiome in the "healthy" smoker and in COPD. PLoS One 2011;6:e16384.
6. Charlson ES, Bittinger K, Chen J, et al. Assessing bacterial populations in the lung by replicate analysis of samples from the upper and lower respiratory tracts. PLoS One 2012;7:e42786.
7. Charlson ES, Bittinger K, Haas AR, et al. Topographical continuity of bacterial populations in the healthy human respiratory tract. Am J Respir Crit Care Med 2011;184:957-63.
8. Dickson RP, Erb-Downward JR, Freeman CM, et al. Spatial Variation in the Healthy Human Lung Microbiome and the Adapted Island Model of Lung Biogeography. Ann Am Thorac Soc 2015;12:821-30.
9. Bassis CM, Erb-Downward JR, Dickson RP, et al. Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. MBio 2015;6:e00037.
10. Wu J, Peters BA, Dominianni C, et al. Cigarette smoking and the oral microbiome in a large study of American adults. Isme J 2016;10:2435-46.
11. Munck C, Helby J, Westergaard CG, et al. Smoking Cessation and the Microbiome in Induced Sputum Samples from Cigarette Smoking Asthma Patients. PLoS One 2016;11:e0158622.
12. Kanehisa M, Goto S, Kawashima S, et al. The KEGG resource for deciphering the genome. Nucleic Acids Res 2004;32: D277-280.
13. Carr R, Borenstein E. Comparative analysis of functional metagenomic annotation and the mappability of short reads. PloS One 2014;9:e105776.
14. Langille MG, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013;31:814-21.
15. Davenport M, Poles J, Leung JM, et al. Metabolic alterations to the mucosal microbiota in inflammatory bowel disease. Inflamm Bowel Dis 2014;20: 723-31.
16. Garcia-Nuñez M, Millares L, Pomares X, et al. Severity-related changes of bronchial microbiome in chronic obstructive pulmonary disease. J Clin Microbiol 2014;52:4217-23.
17. Cox MJ, Allgaier M, Taylor B, et al. Airway microbiota

and pathogen abundance in age-stratified cystic fibrosis patients. PLoS One 2010;5:e11044,
18. Fodor AA, Klem ER, Gilpin DF, et al. The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. PLoS One 2012;7:e45001.
19. Zhao J, Schloss PD, Kalikin LM, et al. Decade-long bacterial community dynamics in cystic fibrosis airways. Proc Natl Acad Sci USA 2012;109:5809-14.
20. Murray PR, Washington JA. Microscopic and bacteriologic analysis of expectorated sputum. Mayo Clin. Proc 1975;50:339-44.
21. Van Scoy RE. Bacterial sputum cultures: a clinician's viewpoint. Mayo Clin. Proc 1977 52:39-41.
22. Millares L, Bermudo G, Pérez-Brocal V, et al. The respiratory microbiome in bronchial mucosa and secretions from severe IgE-mediated asthma patients. BMC Microbiol 2017;17:20.
23. Cabrera-Rubio R, Garcia-Nunez M, Seto L, et al. Microbiome diversity in the bronchial tracts of patients with chronic obstructive pulmonary disease. J Clin Microbiol 2012;50:3562-8.
24. Pragman AA, Kim HB, Reilly CS, et al. The lung microbiome in moderate and severe chronic obstructive pulmonary disease. PLoS One 2012;7:e47305.
25. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med 2008;359:2355-65.
26. Huang YJ, Kim E, Cox MJ, et al. A persistent and diverse airway microbiota present during chronic obstructive pulmonary disease exacerbations. OMICS 2010;14:9-59.
27. Millares L, Ferrari R, Gallego M, et al. Bronchial microbiome of severe COPD patients colonised by Pseudomonas aeruginosa. Eur J Clin Microbiol Infect Dis 2014;33:1101-11.
28. Molyneaux PL, Mallia P, Cox MJ, et al. Outgrowth of the bacterial airway microbiome after rhinovirus exacerbation of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2013;188:1224-31.
29. Dy R, Sethi S. The lung microbiome and exacerbations of COPD. Curr Opin Pulm Med 2016;22:196-202.
30. Sethi S, Maloney J, Grove L, et al. Airway inflammation and bronchial bacterial colonization in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006;173:991-8.
31. Chalmers JD, Smith MP, McHugh BJ, et al. Short and long-term antibiotic treatment reduces airway and systemic inflammation in non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med 2012:186:657-65.
32. Wang Z, Bafadhel M, Haldar K, et al. Lung microbiome dynamics in COPD exacerbations. Eur Respir J 2016;47:1082-92.
33. Huang YJ, Sethi S, Murphy T, et al. Airway microbiome dynamics in exacerbations of chronic obstructive pulmonary disease. J Clin Microbiol 2014;52:2813-23.
34. Papi A, Bellettato CM, Braccioni F, et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med 2006;173:1114-21.
35. Iwai S, Huang D, Fong S, et al. The lung microbiome of Ugandan HIV-infected pneumonia patients is compositionally and functionally distinct from that of San Franciscan patients. PloS One 2014;9:e95726.
36. Millares L, Pérez-Brocal V, Ferrari R, et al. Functional Metagenomics of the Bronchial Microbiome in COPD. PLoS One 2015;10:e0144448.
37. Molyneaux PL, Maher TM. The role of infection in the pathogenesis of idiopathic pulmonary fibrosis. Eur Respir Rev 2013;22:376-81.

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