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ANEXO 2: PUBLICACIONES PROYECTO “Respiratory Microbiome and COPD exacerbations”

Ramón Pla Armengol

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Collaborative research

The microbiome in respiratory medicine: current challenges and future perspectives

Rosa Faner[1,2,18], Oriol Sibila[3,18], Alvar Agustí[1,2], Eric Bernasconi[4], James D. Chalmers[5], Gary B. Huffnagle[6], Chaysavanh Manichanh[7,8], Philip L. Molyneaux[9], Roger Paredes[10], Vicente Pérez Brocal[11,12], Julia Ponomarenko[13,14], Sanjay Sethi[15], Jordi Dorca[16,19] and Eduard Monsó[2,17,19]

Affiliations: [1]Hospital Clinic, IDIBAPS, Universitat de Barcelona, Barcelona, Spain. [2]CIBER de Enfermedades Respiratorias - CIBERES, Madrid, Spain. [3]Hospital Universitari de la Santa Creu i Sant Pau, Universitat Autónoma Barcelona, Barcelona, Spain. [4]Service de Pneumologie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland. [5]University of Dundee, Dundee, UK. [6]University of Michigan, Ann Arbor, MI, USA. [7]Dept of Gastroenterology, Vall d'Hebron Research Institute, Barcelona, Spain. [8]CIBER de Enfermedades Hepáticas y Digestivas (CIBEREHD), Madrid, Spain. [9]Royal Brompton Hospital, London, UK. [10]Hospital Universitari Germans Trias i Pujol, Universitat Autónoma Barcelona, Barcelona, Spain. [11]CIBER en Epidemiología y Salud Pública (CIBERESP), Madrid, Spain. [12]Joint Research Unit on Genomics and Health, Foundation for the Promotion of Health and Biomedical Research of Valencia Region (FISABIO-Public Health) and Cavanilles Institute for Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain. [13]Centro de Regulación Genómica, Barcelona, Spain. [14]Universitat Pompeu Fabra (UPF), Barcelona, Spain. [15]University of Buffalo, Buffalo, NY, USA. [16]Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Hospitalet del Llobregat, Barcelona, Spain. [17]Hospital Universitari Parc Taulí, Universitat Autònoma de Barcelona, Barcelona, Spain. [18]These co-primary authors contributed equally to this work. [19]These co-senior authors contributed equally to this work.

Correspondence: Eduard Monsó, Servei de Pneumologia, Hospital Universitari Parc Taulí, Parc Taulí 1, 08208 Sabadell, Barcelona, Spain. E-mail: emonso@tauli.cat

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ABSTRACT The healthy lung has previously been considered to be a sterile organ because standard microbiological culture techniques consistently yield negative results. However, culture-independent techniques report that large numbers of microorganisms coexist in the lung. There are many unknown aspects in the field, but available reports show that the lower respiratory tract microbiota: 1) is similar in healthy subjects to the oropharyngeal microbiota and dominated by members of the Firmicutes, Bacteroidetes and Proteobacteria phyla; 2) shows changes in smokers and well-defined differences in chronic respiratory diseases, although the temporal and spatial kinetics of these changes are only partially known; and 3) shows relatively abundant non-cultivable bacteria in chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, cystic fibrosis and bronchiectasis, with specific patterns for each disease. In all of these diseases, a loss of diversity, paralleled by an over-representation of Proteobacteria (dysbiosis), has been related to disease severity and exacerbations. However, it is unknown whether dysbiosis is a cause or a consequence of the damage to bronchoalveolar surfaces.

Finally, little is known about bacterial functionality and the interactions between viruses, fungi and bacteria. It is expected that future research in bacterial gene expressions, metagenomics longitudinal analysis and host–microbiome animal models will help to move towards targeted microbiome interventions in respiratory diseases.

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Introduction

Healthy lungs have been traditionally considered to be a sterile organ because standard microbiological culture techniques consistently yield negative results [1]. In the last decade, however, the use of culture-independent molecular techniques has demonstrated that this dogma is wrong, and that large numbers of microbiological organisms, including bacteria, fungi and viruses, collectively known as the microbiome, coexist in the lungs of healthy subjects and patients with respiratory diseases [2, 3], challenging our understanding of the microbiology in respiratory medicine [3]. Indeed, addressing the nature of the relationships between the lung microbiota and respiratory epithelial surfaces appears to be one of the most promising research fields in respiratory medicine [1]. For instance, a large body of evidence now supports the concept that abnormal regulation of host–microbiota crosstalk in different organs and at different body surfaces may play an important pathogenic role in several chronic inflammatory disorders [4–7]. As a consequence, there is growing interest in determining the potential value of the characterisation of airway microbiome composition as a prognostic marker or as an element capable of guiding therapy in several respiratory diseases [3]. This manuscript reflects the current level of knowledge on the respiratory microbiome (see Box 1 for the current terminology), and its unspecificities can be intrinsically related to the heterogeneity of the clinical stratification of respiratory diseases that is currently in use. With these considerations in mind, the Barcelona Respiratory Network organised an international, multidisciplinary workshop on June 3rd, 2016, to discuss and identify research challenges, priorities and gaps in the field, as well as to examine future directions and implications both for patients and healthcare systems. The discussions that took place there, as well as the main conclusions of the workshop, are summarised below. Full presentations were video-recorded and are freely available online at the Barcelona Respiratory Network website (www.brn.cat/microbiome2016).

Challenges for different scientific disciplines

The bioinformatics view

The 16S rRNA gene has several variable regions that can be used for bacterial and archaea classification (*i.e.* taxonomy) [8–10]. Further, because its sequencing is fast and relatively inexpensive [11], it is often used to determine the composition, abundance and diversity of bacteria and archaea harboured in different ecosystems, such as the human respiratory tract [12–14]. However, this method has some important limitations. Firstly, as in any research activity, researchers must identify the right question and select the appropriate workflow from a range of available bioinformatics tools to address the question properly [15], because too many analyses can generate confusion and lead to loss of study focus. Secondly, appropriate control of the potential sources of variation in the study, including patient diversity, sampling methods, DNA extraction procedures, amplification and sequencing batches, is essential in microbiome research because they can all easily introduce unwanted variability and unexpected biases [16] (table 1). As discussed below, trying to keep these sources of variation as low as possible is the best strategy to overcome these hurdles. Thirdly, 16S rRNA gene sequencing does not provide information about viruses and fungi, or

Box 1 General terminology

Microbiota: microbial community membership associated with a defined habitat, such as the human body.

Microbiome: the genetic information (genomes) and inferred physico-chemical properties of the gene products of a microbiota.

Human microbiome: microbiome collectively found in internal and external habitats of the human body.

Metagenomics: shotgun random sequencing of total DNA in a sample, including DNA from host and microbe origin, which is analysed, organised and identified using sequence databases and computational tools.

16S ribosomal RNA (16S rRNA) gene: component of the 30S small subunit of prokaryotic ribosomes. It is used in reconstructing phylogenies owing to the extremely slow rate of evolution of this gene and the presence of both variable and constant regions allowing amplification.

Hypervariable region of the 16S rRNA gene: a DNA sequence that demonstrates diversity among different bacterial species.

16S rRNA gene analyses (or gene sequencing): a common amplicon sequencing method used to identify and compare bacteria present within a given sample. 16S rRNA gene sequencing is a well-established method for studying the phylogeny and taxonomy of samples from complex microbiomes or environments that are difficult or impossible to study.

Amplicon: DNA product of DNA amplification *via* PCR.

Shotgun sequencing: method for DNA sequencing in which DNA is fragmented into segments that are sequenced.

Dysbiosis: alteration of microbiota composition linked to perturbation of local ecological conditions, generally associated with impaired host–microbe interactions.

TABLE 1 Major sources of variability in microbiome studies

Sampling	DNA extraction	16s amplification and sequencing	Bioinformatics
Processing biases	Species bias due to different wall composition	Selection of regions to amplify	Thresholds for abundance
Constraints associated with type of sample	Batch effect[#]	Polymerase chain reaction and sequencing errors	Alignment of sequences to databases
		Adapter addition[¶]	Classification of sequences
		Batch effect	

[#]: batch effect refers to the bias introduced if not all samples are processed at the same time, in a single batch; [¶]: adapters are oligonucleotides that are ligated to the amplified DNA in order to do the sequencing. The efficiency of the ligation process can influence the sequencing results.

about their interactions with the bacterial microbiota, which need to be investigated using alternative approaches such as metagenomics and/or internal transcribed spacer sequencing. Finally, from a purely bioinformatics point of view, a number of issues related to the incompleteness of databases and methodological constraints discussed below (see Box 2 for current terminology) also need to be considered.

Database constraints

The existing 16S rRNA gene databases currently provide (partial or complete) gene sequences for more than 1.7 million bacteria and archaea [17], and are detailed enough to classify bacteria at different taxonomic levels, from phylum (high taxonomic level) to genus (low taxonomic level) (figure 1). Yet, these databases contain unresolved information for some sequences, so species-level identification is not attainable for some microorganisms [18]. It is also possible, owing to high levels of 16S sequence homology between species, that a sequence gives more than one hit with the same score in two or more different records in the database, indicating an inability to differentiate them. To resolve this situation, the "lowest common ancestor concept" is generally used [19]. Following this approach, the assignment of taxa is not given at the level of species, and reaches only the genus level for some bacteria. For example, this is the case for the *Streptococcus* genus, which is prevalent in the respiratory system and includes pathogenic bacterial species such as *S. pneumoniae* and commensals such as the viridans streptococci group. This limitation of species assignment obviously restrains the scope for identifying microorganisms ascribed to these genera. For all these reasons, bioinformatics tools used in microbiome research generally use a common approach to cluster sequencing reads at some level of similarity under the general term operational taxonomic units (OTUs). Thus, sequence similarities of at least 97% with the reference database of 16S rRNA sequences are generally acceptable to consider the identified OTUs as equivalent to the species level, or to the genus level when the similarity only attains 94%.

Methodological issues

As indicated above, the 16S rRNA gene has several variable regions (V1–V3 or V3–V5) that can be used for bacterial taxonomy purposes [3, 8, 9]. However, it is unclear which of them provides the best assessment of

Box 2 Bioinformatic terminology

OTU (operational taxonomic unit): cluster of microorganisms, grouped by DNA sequence similarity of a specific taxonomic marker gene, *e.g.* 16S rRNA. OTUs are used as proxies for microbial "species" at different taxonomic levels: phylum, class, order, family, genus and species. Sequence similarity is defined based on the similarity criteria; *e.g.* the sequencing reads with 97% similarity can be clustered together and represent a single OTU, and for some bacteria can attain the equivalence of the species level.

Diversity: the number and distribution of distinct OTUs in a sample or in the originating population. Thus, so-called alpha-diversity estimates describe the number of species (or similar metrics) in a single sample, while beta-diversity estimates describe the differences in species diversity between samples. A widely used diversity index is the Shannon–Wiener diversity index.

Relative abundance: how common or rare an OTU is relative to other OTUs in a community, measured as a percentage of the total number of OTUs in the population. Thus, OTU abundance is treated as a surrogate measure of bacterial species abundance.

Evenness: measure of the similarity of the relative abundances of the different OTUs in the population.

Taxon: group of one or more populations of an organism or organisms considered to form a unit.

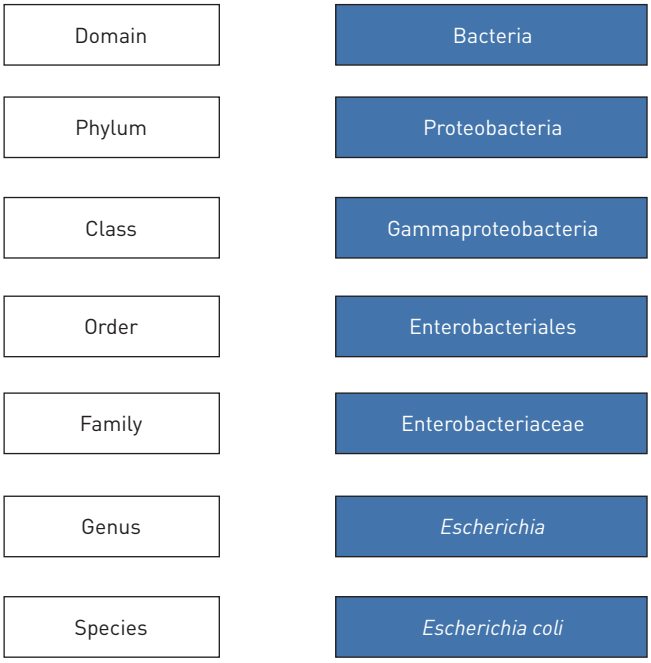

FIGURE 1 Taxonomic classification of *Escherichia coli*.

the respiratory microbiome. Moreover, it has been demonstrated that different sequencing platforms, including 454, Illumina HiSeq and MiSeq, can produce different results [16]. This is partially due to the specific variable region of the 16S gene used, the primers employed and the length of the amplicons produced by the different platforms. To reduce sequencing errors, longer reads are preferred [16]. An additional methodological problem is that the use of different algorithms, assumptions and parameters can lead to different results [13, 19, 20]. Therefore, it is important to be aware of these limitations and, if possible, use different sequencing and bioinformatics tools (*e.g.* marker gene, shotgun genome or transcriptome sequencing) to compare results obtained with different methods. Finally, it is worth saying that 16S sequencing provides qualitative but not quantitative microbiome information, and complementary methods such as quantitative PCR or digital PCR are recommended to complete the information attained through the analysis of the 16S rRNA gene.

Other bioinformatics challenges

Other bioinformatics challenges to consider include the following. First, most of the studies performed until now have estimated per taxon relative abundances based on the number of copies of 16S rRNA genes recovered in a sequence library [21]. Yet, variation in gene abundance can result from differences in the actual bacterial load or from the genomic copy number that a specific bacterial taxa is able to attain during the analytical procedure. The relative weight of these two factors on estimates of microbial community structure is unknown, but can be a source of systematic bias in studies using 16S rRNA sequencing. There are methods that correct for the copy number of 16S rRNA genes, but this correction is available for <5% of known bacterial species [22]. It is also worth noting that other genes like *cpn06* can also be used to infer bacterial community diversity [23]; thus, the possibility of using more than one gene should also be considered. Second, to compare results between studies performed in different laboratories it is recommended that mock communities are used, created *in vitro* with a predefined content of bacterial operons specific for the lung microbiome [24], but it may be more convenient to create consortia that would perform all the analyses in a single centre. Third, differences in DNA extraction [25] and PCR amplification methods can also introduce methodology-related variability [26].

The view from respiratory medicine

The microbiome in the healthy lung

The study of the normal human lung microbiome is still in its infancy, but it is clear now that healthy lungs harbour a phylogenetically diverse microbial community [2, 3, 27–31]. Results of published studies are somewhat limited by their small size and lack of longitudinal sampling but show that, in healthy subjects, Firmicutes, Bacteroidetes and Proteobacteria are the most frequently identified bacteria at the phylum level [32]. At the genus level, *Prevotella, Veillonella* and *Streptococcus* are the predominant microorganisms, with a minimal contribution from common pathogenic Proteobacteria including *Haemophilus* [32]. Healthy airways are challenging to sample because healthy subjects do not produce spontaneous sputum, so

sampling requires bronchoscopy, and repeating the endoscopic procedure in healthy individuals is cumbersome, limiting the possibility of having longitudinal data. However, recent studies that included bronchoscopic sampling of the proximal and distal bronchial tree have reported that the microbiota of the oropharynx, the bronchial tree and the alveolar surfaces have a similar composition in healthy individuals [29]. This similarity has been attributed to aspiration of oropharyngeal secretions during sleep [33–35]. This scenario may be altered in respiratory diseases, where perturbation of growth conditions in the bronchial tree and lung parenchyma promotes a shift in microbial community composition, with potentially pathogenic bacteria able to persist for longer periods of time [3, 29–31] (figure 2).

In any microbiome study contamination is a concern, and the potential contamination of lower airway samples by the oropharynx microbiota is a major issue to be specifically addressed in respiratory diseases [3]. The respiratory system lodges lower amounts of microorganisms than other human body surfaces, and low biomass samples such as those obtained by a protected specimen brush (PSB) or bronchoalveolar lavage (BAL) may not provide sufficient DNA, while the background signal from reagents may be misinterpreted as a real signal [36]. Thus, in order to discriminate signal from noise, proper technical controls are critically needed in sequence-based analyses of samples, particularly in respiratory samples, which may suffer from a dilution effect.

Information on the long-term effects of smoking on the respiratory microbiome of healthy subjects is scarce, and clearly needs research. Initial studies of the oropharynx microbiota in smokers have reported modifications in the microbial composition, affecting mainly the Firmicutes phylum and *Neisseria* species, that are important enough to be considered as dysbiosis [37], and a decrease in the relative abundance of Proteobacteria; these modifications do not revert after giving up smoking [38]. By contrast, studies of the respiratory microbiota in bronchial secretions have not identified significant differences between smokers and non-smokers [37], nor relevant changes in bacterial diversity after smoking cessation [39], suggesting that exposure to smoke results in proximal microbiome changes that are not reflected by corresponding downstream alterations in the bronchial tree, at least in the absence of respiratory disease. Differences in the oral microbiome of current *versus* former smokers with and without respiratory disease have not been properly assessed, however, and it is not currently possible to properly discern temporary dysbiosis caused by the exposure to irritants and acute injury from dysbiosis associated with chronic disease.

Chronic obstructive pulmonary disease

Bronchial colonisation by potentially pathogenic microorganisms has been well established in chronic obstructive pulmonary disease (COPD) by several previous studies [40, 41], but the direction of causality between this colonisation and airway inflammation, airflow limitation, and bronchial and lung parenchyma destruction remains unsettled. There is evidence of a relationship between the appearance of symptoms of exacerbation and the acquisition of new bacterial strains [40], but this change in the bacterial flora only partially justifies the appearance of exacerbations.

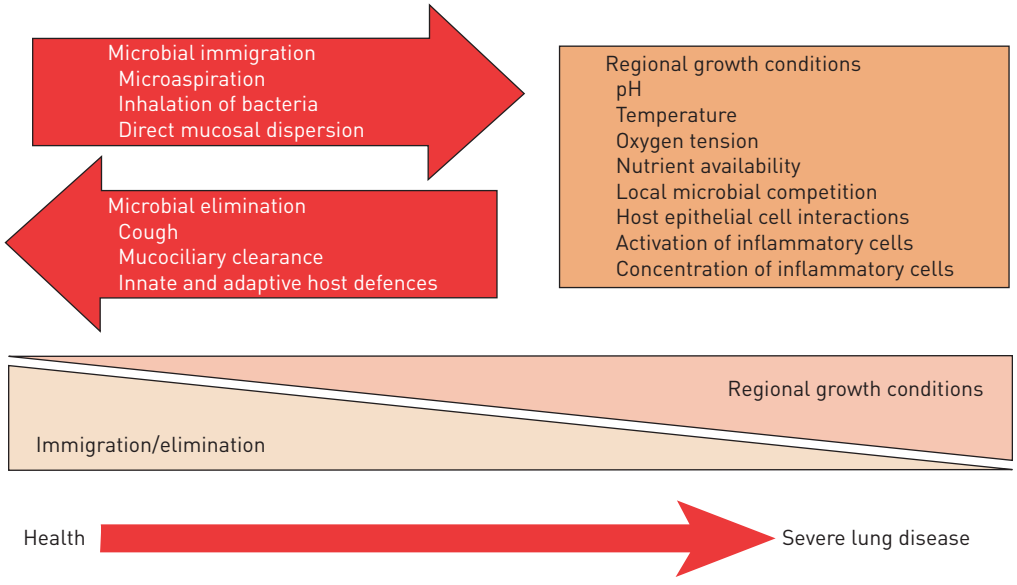

FIGURE 2 Key factors determining the respiratory microbiome: microbial immigration, microbial elimination and the relative reproduction rates of its members. In healthy subjects, the microbiome is determined mainly by immigration and elimination. In severe lung diseases, however, regional growth conditions are a main determinant of microbiome composition. Reproduced from [102] with permission.

In patients with clinically stable COPD, several studies have now reported a rich lung microbiome that is clearly different from that seen in healthy controls [2, 27, 30, 31, 42–46]. Common phyla in these patients are Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes, with *Pseudomonas*, *Streptococcus*, *Prevotella* and *Haemophilus* being common genera in these patients [2, 27].

Most data available from COPD come from samples obtained from biopsies [46], lung tissue explants [30], BAL or PSB [2, 27, 31, 46], and sputum [43–47]. Different sampling procedures target different regions of the respiratory system, however, and results have shown that sputum harbours microbial communities that are different from those in bronchoalveolar samples [46], and have confirmed that, in fact, bronchi and alveoli of patients with COPD contain a distinct microbiome [3] (see figure 3 in Dickson *et al.* [3]).

During exacerbations, some genera increase their relative abundance whereas others do not significantly change [42, 44, 48, 49]. In addition, exacerbations seem to be associated not only with over-representation of isolated genera, but also with collateral changes in microbiome composition as a whole, which in turn appear to be associated with increases in inflammation-related markers in BAL [41, 50]. Additionally, there seem to be interactions between viral infections and bacterial community composition, with increases in the relative abundance of Proteobacteria after experimental rhinovirus infection [48]. Similar interactions have been proposed between fungi and bacteria [51]. Furthermore, treatment during exacerbations influences the respiratory microbiome differently when based on antibiotics, which reduce bacterial abundance, mainly of Proteobacteria, *versus* oral steroids, which when administered systemically do not influence bacterial richness but favour an over-representation of specific taxa [40, 52].

Finally, several challenges need to be tackled before benefits from microbiome research in COPD can be meaningfully incorporated into clinical practice: 1) with regards the reported differences in the respiratory microbiome of distal and proximal bronchi, targeted by BAL and sputum respectively [46], meaningful thresholds need to be determined to identify clinically significant bacterial over-representations for all sample types; 2) the role of non-cultivable but potentially pathogenic microbes identified by microbiome studies is unclear and needs to be investigated; and 3) interactions between bacteria, viruses and fungi with the host need to be targeted.

All in all, despite these important hurdles, lung microbiome research has the potential to unravel new and relevant insights into COPD pathogenesis that may lead to better clinical management of COPD. Specifically, there is a clear need to understand the impact of current standard COPD treatments, particularly of inhaled corticosteroids, on the COPD airway microbiome, because these agents have been shown to reduce the frequency of exacerbations but, at the same time, to increase the risk of pneumonia, possibly through direct modulation of the airway microbiome. Eventually, changes in the microbiome may become important mechanisms (*i.e.* endotypes) underlying the different clinical presentations (*i.e.* phenotypes) of COPD.

Cystic fibrosis and bronchiectasis

Airway bacterial infection is central to our understanding of the pathophysiology of cystic fibrosis (CF) and (non-CF) bronchiectasis. Traditional culture-based microbiology techniques have revealed the importance of well-known pathogens such as *H. influenzae*, *P. aeruginosa* and *Moraxella catarrhalis* in bronchiectasis [53], and additionally *Staphylococcus aureus* and *Burkholderia cepacia* in CF [54]. Microbiome studies are moving our understanding of these two diseases forwards. For instance, previously unrecognised organisms are abundant in some patients, both in CF [55, 56] and in bronchiectasis [57, 58]. In addition, studies characterising the airway microbiome following antibiotic treatment have shown a remarkable resistance of bacterial communities to change over time in these patients [57, 59, 60]; antibiotic treatments primarily result in a reduction in bacterial diversity, but this effect disappears after some weeks, with the recovery of the previous microbial composition [57]. Overall bacterial diversity, measured using composite indices such as the Shannon–Wiener diversity index, has been linked to the level of airflow limitation present and other markers of disease severity both in CF and bronchiectasis. Additionally, an Australian randomised clinical trial in patients with non-CF bronchiectasis has shown that the relative abundance of potentially pathogenic microorganisms from the *Pseudomonas* genus increases in patients receiving chronic treatment with macrolides [60], but the extent to which the microbiome changes are attributable to the antibiotic regime is not known. The role of fungi, viruses and *Mycobacteria* (which are not identified by standard bacterial 16S rRNA sequencing) is unclear in both CF and bronchiectasis, and requires future research [61]. Likewise, other important questions that need to be examined in this clinical setting include the extent to which 16S rRNA gene sequencing provides useful clinical information beyond culture, the interactions with the host, the possibility to select antibiotic treatment based on microbiome profiles, the usefulness of microbiome results to evaluate therapeutic responses, the prognostic implications of microbiome analyses and the effect of antibiotics on the emergence of new pathogens. The ease with which sputum can be obtained in these patient populations facilitates large-scale studies in the coming years.

Interstitial lung diseases

Traditionally, interstitial lung diseases (ILD) have been considered to be non-infectious parenchymal lung diseases. However, the recent characterisation of the respiratory microbiome in idiopathic pulmonary fibrosis (IPF) has shown an over-representation of specific organisms such as *Streptococcus*, *Prevotella* and *Staphylococcus* in these patients as compared to healthy controls [62, 63] (figure 3). Whether or not they can drive disease progression is a hypothesis that merits future research [63].

The existence of acute exacerbations of IPF has been increasingly recognised as a major cause of mortality in these patients [64]. The exact pathogenesis of these episodes remains unclear, and current diagnostic criteria specifically require the exclusion of any infective trigger [65]. Despite this, there is evidence supporting an infectious hypothesis of IPF exacerbations: 1) a randomised controlled trial showed reduced mortality in patients who received prophylactic cotrimoxazole [66], 2) immunosuppression is associated with an increased rate of acute exacerbations [67], 3) a higher proportion of exacerbations occurs during the winter months, and 4) infectious episodes confer an identical mortality to non-infective exacerbations [63]. There is therefore great interest in using culture-independent molecular techniques to explore the role of infection in acute exacerbations of IPF, although the unpredictable nature of these events and difficulty in sampling have been limiting factors in addressing this topic.

Microbiome research in the entire range of different ILDs should establish 1) if there is any role at all of lung microbial composition in their occurrence and evolution; and 2) what the optimal sampling modality is in these patients, given that these parenchymal diseases may not be appropriately represented by bronchial samples such as sputum.

Lung transplantation

Owing to the long-term use of prophylactic and/or therapeutic immunosuppressive drugs and antibiotics, the lower airways of lung transplant recipients offer a special niche for the resident microbiota [68, 69]. In fact, alterations in local conditions during the first months post-transplant facilitate lower airway infections due to

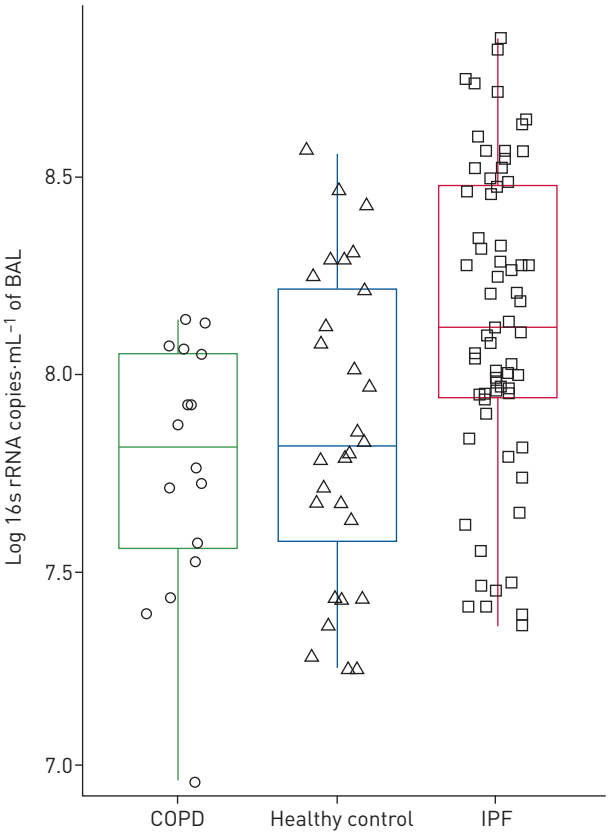

FIGURE 3 Bacterial load (16S copy number·mL^{-1} of bronchoalveolar lavage (BAL)) in patients with idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD) and healthy controls. Patients with IPF (red; n=64) had a significantly higher bacterial burden than subjects with COPD (green; n=17) and the healthy control subjects (blue; n=27) (p=0.006 and p=0.0007, respectively). The box signifies the 25th and 75th percentiles, and the median is represented by a short line within the box. Reproduced from [53] with permission.

opportunistic bacterial pathogens. A blunted inflammatory status commonly prevails between 6 and 12 months post-transplant, in association with a strong predominance of bacteria typically found in the oropharyngeal microbiota [70]. Modifications in the respiratory microbiota composition in the lung transplantation setting are strong enough to be considered as dysbiosis, and are manifested through the over-representation of specific OTUs, including those listed below, that have been related to the persistence of abnormal underlying host inflammatory profiles [70, 71]. Furthermore, the onset of bronchiolitis obliterans syndrome following transplantation has also been linked to host–microbe interactions, through pathogen-driven inflammatory triggers and/or impaired host innate responses affecting bacterial clearance [72, 73].

Studies using culture-independent techniques that identified microbiota dysbiosis in patients with lung transplants reported a frequent clear-cut predominance of Proteobacteria and/or Firmicutes, linked to microorganisms from the *Pseudomonas* and *Staphylococcus* genera [68, 74], and Burkholderiaceae family [75]. These bacteria, which may represent over 70% of the BAL microbial community, are typically associated with a pro-inflammatory response, whereas an over-representation of similar magnitude of Bacteroidetes, mostly due to the abundance of *Prevotella*, was instead linked to a remodelling host gene expression profile [70]. These findings suggest that microbiome–host interactions influence innate immune processes within the transplanted lung. Future research should try to relate these patterns to long-term allograft outcome and the risk of transplant rejection occurrence.

Lessons from other human organ systems: the gut

Gut microbiome research has pioneered the field of microbiome research and is far more advanced than that of respiratory microbiome. First, it is now using next-generation sequencing techniques, which allow the understanding of microbial communities in greater depth through the study of microbial genes or full genomes [76], and metatranscriptomics, which include RNA sequencing (see the terminology in Box 3). Second, initiatives like the Human Microbiome [77] and the MetaHIT [78] projects, sponsored by the National Institutes of Health (USA) and the European Commission, respectively, have allowed a deep characterisation of the human gut microbiome in health and disease states. As a result, we now know that the human gastrointestinal (GI) tract harbours one of the most complex and abundant existing microbial communities of more than 100 trillion microorganisms, with the number of microbial genes exceeding by about 100-fold the number of human GI cells. Although stable across ages, the composition and functions of the intestinal microbiome is influenced by a number of factors, including genetics and exposures at birth related to delivery, age, geographic location, diet, smoking and medical treatments [79]. Third, while there are also many potential sources of variability that can significantly impact the results of GI microbiota studies, a global effort has been made to define best practices and protocols to compare different GI microbiota studies, meta-analyse them and extract new knowledge. The protocols of this effort, the International Human Microbiome Standards Project, are available online (www.microbiome-standards.org). Fourth, the gut microbiota not only influences the GI tract, it can also affect many functions of the body, ranging from processing and harvesting of nutrients from our diets, to the shaping of innate and adaptive immune system responses [80, 81]. Hence, GI microbiota changes can favour the development of GI as well as non-GI diseases. For example, a vast body of literature now links functional and metabolic GI disorders, such as inflammatory bowel disease, irritable bowel syndrome or obesity, with gut microbiome alterations [82–85], but there also reports of a relationship between changes in the gut microbiome and neurological disorders (*e.g.* autism) [86–89] and respiratory diseases (such as the acute respiratory distress syndrome occurring in patients with septic shock [90]). Fifth, the HIV epidemic has taught us that homosexual men often have a distinct composition in their faecal microbiota, with increased microbial richness and diversity, as well as enrichment in the *Prevotella* enterotype, independent of their HIV status [91]. HIV-1 infection is associated with reduced bacterial richness, particularly in subjects with suboptimal CD4+ T cell counts under antiretroviral therapy [91]. Finally, interventions designed to modify the composition of the gut microbiome have been successful in specific GI diseases. Faecal microbiota transplantation is becoming increasingly accepted as an effective and safe intervention in patients with *Clostridium difficile* infection, and different centres have reported success rates >90% with this treatment [92]. This approach is much more complicated in inflammatory bowel disease, where faecal transplant has success rates of around 13% [93]. The effects of the bacterial modifications of the gut microbiota on the respiratory tract microbiome of

Box 3 Other systems terminology

International Human Microbiome Standards: standard operating procedures designed to optimise data quality and comparability in the human microbiome field.

Faecal transplantation: process of transplantation of faecal bacteria from a healthy individual into a recipient.

healthy subjects and patients with varied respiratory diseases, as well as potential indirect effects *via* alterations in the host immune response (and their response to faecal transplant) to date have not been properly addressed. Current knowledge, including early-life beneficial and detrimental alterations of the gut microbiota, and its relationships with allergic respiratory diseases, have been recently reviewed [94], and the gut–lung axis now offers a wide range of research possibilities, as discussed below.

Workshop limitations and further reading

The present manuscript is a report of a workshop on which some aspects that deserve comment were not covered. Several investigators have addressed the role of the microbiome in asthma and paediatric diseases other than CF, a research field that has recently been reviewed [95–98]. These reviews interestingly describe mouse and human data of the lung–gut axis on asthma development. Similarly, the role of anaerobic bacteria in respiratory disease has been only marginally addressed in diseases such as CF and bronchiectasis to date [57, 99–101] and needs focused research.

Future respiratory microbiome research

From the above discussion, participants in the workshop agreed on the following nine specific aspects that need to be specifically addressed by future respiratory microbiome research:

1. Normality patterns: Studies performed in healthy subjects so far have clearly demonstrated that there is a rich microbiota in the respiratory system that includes microorganisms from the Firmicutes, Bacteroidetes and Proteobacteria phyla, and displays a close similarity to that of the oropharyngeal microbiota. Normality patterns for viruses and fungi still need to be defined, however. The microbial composition of the respiratory microbiota changes in chronic respiratory diseases, but the timing and the distribution of these changes are only partially known.
2. Diversity in sampling procedures: There is a wide consensus that the best sample procedure depends on the question being addressed. Sputum may be an appropriate approach for the study of respiratory diseases that have a significant bronchial component, considering that it can be obtained from a wide range of patients and does not require invasive procedures, but more reliable information on the peripheral bronchial tree and alveolar surfaces requires invasive samples (*i.e.* BAL, PSB, bronchial or lung biopsies). Similarly, in GI tract research, faeces are now collected for large studies and local biopsies are used to answer specific questions in a restricted number of patients. In any case, these measurements still need to be paralleled by conventional microbiological studies because, although sequencing provides a general picture of the composition of the bacterial community, microbiological cultures provide clinically meaningful information on the role of respiratory pathogens such as *Haemophilus* and *Pseudomonas* in disease, which still do not have an equivalent in microbiome analyses.
3. Standardisation: There is a pressing need to standardise protocols to be used to analyse the respiratory microbiome, including sampling, processing and bioinformatics methodologies. The creation of consortia and networks for research on this topic would facilitate this standardisation and, as a result, the possibility of sharing results from different cohorts.
4. Non-cultivable and/or non-pathogenic bacteria: 16S rRNA gene analyses have shown high relative abundance and specific patterns of non-cultivable microorganisms (with a general over-representation of Proteobacteria) in bronchial and lung samples obtained from patients with COPD, IPF, CF and bronchiectasis. The role of specific species previously considered non-pathogenic needs to be addressed in these different clinical conditions.
5. Loss of diversity: Loss of diversity has been related to disease severity in COPD, IPF and CF, and it has also been described during exacerbations of these diseases. A similar observation has been reported in the gut, suggesting that a general pattern of a decrease in the diversity of the microbial composition associated with the over-representation of specific OTUs may occur in human diseases, but the temporal dynamics of these microbial changes are widely unknown. What drives this loss of bacterial diversity, including the impact of interspecies competition, antibiotic exposure and host immune responses, must be defined.
6. Interactions with the host: Data on microbiome–host interactions is incomplete in gut diseases and almost non-existent in respiratory diseases. Future studies should address both the local and systemic impact of microbial communities, because important remote effects can be exerted through the release of mediators in the bloodstream. Hence, dissecting the intricate interplay of host–microbe interactions in different body sites, such as the lung, gut and skin, represents a major challenge in future microbiome research but has the potential to help clarify the determinants of progression in several chronic respiratory diseases. To properly assess this point, new studies should include research on the diversity of the microbiome in the same host at several sites; have a longitudinal dimension; assess the local and systemic immunity of the host; and, finally should prove the effects of microbiome patterns on the pathogenesis of respiratory diseases through microbiome transplantation in animal models.

7. Bacterial RNA and metagenomics: After 16S rRNA gene analysis, a new stage in the study of the microbiome is beginning with DNA shotgun sequencing and RNA analysis. These techniques need to be implemented in the study of the respiratory microbiome because they will provide functional information, which is absent in 16S rRNA gene analyses. Furthermore, 16S rRNA gene cannot differentiate between living and dead bacteria, and how long DNA from dead bacteria persists in respiratory samples is not known.
8. Viruses and fungi: The role of viruses, including the vast number of phages that infect bacteria, and fungi in respiratory health and disease cannot be targeted through 16S rRNA gene analyses, and needs investigation. Interactions between viruses, fungi and bacteria have been only marginally assessed so far, but preliminary results have shown well-defined effects of non-bacterial microbiota on Proteobacteria abundance.
9. Interventions: Bacterial supplementation and modulation of the microbiota through probiotics and equivalents has not yet been explored in respiratory diseases, but it is a potentially fruitful research field. Whether probiotics directly targeting the lung parenchyma, or restoring normal upper airway or gut microbiota, can produce beneficial effects in respiratory diseases remains to be determined.

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RESEARCH ARTICLE Open Access

Relationship between the respiratory microbiome and the severity of airflow limitation, history of exacerbations and circulating eosinophils in COPD patients

Laura Millares[1,2,3], Sergi Pascual[4], Concepción Montón[3,5,6,7], Marian García-Núñez[1,2,3], Cristina Lalmolda[1,2], Rosa Faner[2,7], Carme Casadevall[2,4,8], Laia Setó[1,2], Silvia Capilla[9], Amàlia Moreno[5], Ady Angélica Castro-Acosta[2,10], Carlos José Alvarez-Martinez[2,10], Oriol Sibila[7,11], Germán Peces-Barba[2,12], Borja G. Cosio[2,13], Alvar Agustí[2,7,14], Joaquim Gea[2,4,7,8] and Eduard Monsó[2,5,7,15*]

Abstract

Background: The respiratory microbiome is altered in COPD patients but its relationship with core components of the disease, such as the severity of airflow limitation, the frequency of exacerbations or the circulating levels of eosinophils, is unclear.

Methods: Cross-sectional study comprising 72 clinically stable COPD patients (mean age 68 [SD 7.9] years; FEV1 48.7 [SD 20.1]% of reference) who provided spontaneous sputum samples for 16S rRNA gene amplification and sequencing. The microbiome composition was analysed with QIIME.

Results: We observed that: (1) more severe airflow limitation was associated with reduced relative abundance (RA) of *Treponema* and an increase in *Pseudomonas*; (2) patients with ≥2 exacerbations the previous year showed a significantly different bacterial community with respect to non-exacerbators ($p = 0.014$), with changes in 13 genera, including an increase of *Pseudomonas,* and finally, (3) peripheral eosinophils levels ≥2% were associated with more diverse microbiome [Chao1 224.51 (74.88) vs 277.39 (78.92) $p = 0.006$; Shannon 3.94 (1.05) vs 4.54 (1.06) $p = 0.020$], and a significant increase in the RAs of 20 genera.

Conclusion: The respiratory microbiome in clinically stable COPD patients varies significantly according to the severity of airflow limitation, previous history of exacerbations and circulating eosinophils levels.

Keywords: Bacterial community, Diversity, Eosinophils, Exacerbations, Sputum, Stable COPD

Summary at a glance

Core components of COPD such as airflow limitation, history of previous exacerbations and level of circulating eosinophils have an impact in the bronchial respiratory microbiome of clinically stable COPD patients.

Background

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease [1–3]. The study of the respiratory microbiome in COPD has revealed a specific bacterial community composition in these patients [4, 5]. However, the relationship between this microbiome and core components of the disease, such as, the severity of the airflow limitation and the type of treatment received remains unclear. In addition, changes in the microbiome have been described in COPD exacerbations [6, 7], but it is not known if differences between patients who suffer two or more exacerbations per year, who are considered frequent exacerbators [8, 9], and non-exacerbators can

* Correspondence: emonso@tauli.cat
[2]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[5]Department of Respiratory Medicine, Hospital Universitari Parc Taulí, Parc Taulí 1, 08208, Sabadell, Barcelona, Spain
Full list of author information is available at the end of the article

be detected during clinical stability. Likewise, levels of circulating eosinophils ≥2% in clinically stable patients identifies a subgroup of COPD patients who are prone to recurrent exacerbations and are more responsive to treatment [10–12], but it is not clear if this is associated with a different respiratory microbiome. This work sought to investigate these questions.

Methods

Methods are detailed in the Additional file 1 and summarized below.

Study design and ethics

This is a cross-sectional, prospective, uncontrolled, multicentre, observational study. The study protocol was approved by the Ethics Committees of the participating hospitals (IMIM-Hospital del Mar, Hospital Universitari Parc Taulí, Hospital Clinic, Hospital 12 Octubre, Fundación Jimenez Díaz and Hospital Son Espases), and all patients included signed their informed consent.

Population

Current or former smokers (≥ 10 pack-year) with stable COPD, attending the outpatients' clinics of five Spanish hospitals between 2014 and 2016 were included in this study. The diagnosis and severity staging of COPD was established in accordance with GOLD criteria [8]. Exclusion criteria were: age less than 40 years; a lifetime diagnosis of asthma, cystic fibrosis, bronchiectasis or cancer; patients receiving long-term treatment with oral corticosteroids or immunosuppressants; any comorbidity limiting cognitive capabilities and ≥ 3 admissions or 1 episode severe enough to require more than 30 days in hospital the previous year. Patients who had been treated with short-term antibiotics and/or corticosteroids at any time during the previous three months were considered unstable and not considered for the study.

Variables and measurements

Sociodemographic data were recorded by specific questionnaires. Lung function values during stability were obtained from the most recently available forced spirometry with reversibility testing performed according to standard techniques the previous year [13]. Peripheral blood cell counts were obtained at enrolment and used to identify patients with ≥2% circulating eosinophils [14]. Episodes of increased dyspnoea, sputum production and/or purulence during the previous year were identified and considered as exacerbations when treated with antibiotics and/or corticosteroids [15, 16]. Participants were considered as frequent exacerbators (FE) when they reported ≥2 exacerbations the previous year.

Sample collection, DNA extraction, PCR amplification and 16S sequencing

Spontaneous sputum samples were collected and processed within 60 min on the day of the visit. Sputum quality was assessed according to Murray-Washington criteria [17] and only samples with > 25 leucocytes per field (M-W ≥ 3) were considered for the study. Sputum samples were frozen until processing, which was carried out in a certified BSL2 hood with appropriate laminar flow.16S rRNA gene was amplified following the 16S Metagenomic Sequencing Library Preparation Illumina protocol (Part # 15044223 Rev. A, Illumina, CA, USA). Details are provided in the Additional file 1.

Sequence analysis

The Quantitative Insights Into Microbial Ecology (QIIME) pipeline 1.9.0 [18] was used for sequence processing to obtain taxonomic information. Further technical details are provided in the Additional file 1.

Statistical analyses

Details are provided in the Additional file 1. In brief, categorical variables are expressed as absolute and relative frequencies, and continuous variables as means and standard deviations (SD) when the distribution was normal, or as medians and interquartile range (IQR) otherwise. Linear discriminant analysis Effect Size (LEfSe) was used to identify the differentially abundant taxa that explained the differences between the groups of participants. The threshold value of the logarithmic LDA score for discriminative features was 2.0. Bacterial α-diversity was assessed through the Chao1 estimator [19] and the Shannon index [20], calculating both indexes after subsampling with QIIME so as to avoid sequencing effort bias. Principal Coordinates Analysis (PCoA) with Bray-Curtis dissimilarity index [21] was used to study community composition, assessing the statistical significance of the differences in sample groupings through Adonis testing. Interaction between independent variables was assessed through stratification and multivariate analyses with α-diversity as dependent variable. Statistical tests used in the study were two-sided, and a p value of 0.05 or less was reported as statistically significant. Statistical analyses were performed using the SPSS statistical software package version 18 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Table 1 summarizes the main demographic and clinical characteristics of the 72 patients included. They were mostly men (88.9%), with a mean age of 68 (SD 7.9) years and FEV1 of 48.7 (SD 20.1)% of reference.

Table 1 Demographic and clinical characteristics of the patients

N	All patients	Exacerbations previous year			*p*
		0	1	≥2	
	72	31	18	23	
Age, mean (SD)	68 (7.9)	66 (9)	69 (7)	68 (7)	0.387
Sex (male), *n* (%)	64 (88.9)	27 (87.1)	16 (88.9)	21 (91.3)	0.888
Cumulative smoking (pack-year), median (IQR)	60 (45–80)	60 (44–76)	50 (41–85)	60 (49–97)	0.383
Postbronchodilator FEV1%, median (IQR)	44 (33–60)	52 (42–70)	35 (32–52)	35 (28–49)	0.001
BMI, median (IQR)	27 (24–30)	28 (25–29)	28 (23–30)	26 (23–30)	0.669
Blood eosinophils ($\times 10^9$/L), median (IQR)	200 (100–270)	200 (130–3009	185 (92–255)	200 (100–300)	0.414
Blood eosinophils (%), median (IQR)	2.4 (1.4–3.4)	2.8 (1.7–3.6)	2 (1.3–2.8)	1.8 (1.1–3.4)	0.156
Blood leucocytes ($\times 10^9$/L), median (IQR)	7845 (6635–9180)	7210 (6520–8940)	7915 (6505–8510)	8110 (7030–10,170)	0.481
Exacerbations last year, median (IQR)	1 (0–2)	0	1	3 (2–4)	
Airflow limitation severity (GOLD), *n* (%)					
GOLD 1	6 (8.3)	5 (16.1)	0 (0)	1 (4.3)	0.013
GOLD 2	19 (26.4)	11 (35.5)	4 (22.2)	4 (17.4)	
GOLD 3	36 (50)	14 (45.2)	12 (66.7)	10 (11.1)	
GOLD 4	11 (15.3)	1 (3.2)	2 (18.2)	8 (34.8)	

16S rRNA analysis

At phylum level, 13 different phyla were identified, six of them with median relative abundance (RA) above 0.1% (Additional file 1: Table S1). At genus level, 190 different genera were identified and, after removing the genera present in only one sample, 171 remained for subsequent analyses, 26 of them with RA above 0.1% (Table 2).

Age

Alpha-diversity parameters showed a negative relationship with age ($R^2 = 0.075$ $p = 0.020$ and $R^2 = 0.074$ $p = 0.020$ respectively), but β-diversity analysis did not show significant differences in relation with this variable ($p = 0.389$).

Airflow limitation

We found a significant progressive increase in the RA of *Pseudomonas* genus and a decrease in the RA of *Treponema* in patients with more severe airflow limitation (Fig. 1). Regarding bacterial diversity, neither α-diversity parameters nor β-diversity analysis showed significant differences between GOLD grades of airflow limitation. Of note, airflow limitation severity was not related to age ($p = 0.245$).

Pharmacological treatment

Forty-nine COPD patients had not modified their inhaled maintenance treatment during the previous year; thirty-six of them (73.5%) used a combination of LAB/ICS, 9 (18.4%) were treated with LAB as monotherapy and 4 (8.2%) were not receiving COPD treatment. LAB/ICS treatment did not have any effect on either α-diversity ($p = 0.365$) or bacterial community composition in the patients studied ($p = 0.963$), when compared with patients not receiving this treatment. Similarly, the continuous use of LAB as monotherapy was not associated with significant changes in the respiratory microbiome ($p = 0.854$).

Exacerbation frequency

In the previous year, 31 patients (43.1%) did not report any acute episodes, 18 (25%) referred only one and 23 suffered two or more (31.9%), and were considered FE. Demographic and clinical characteristics of these three groups only showed statistically significant differences in lung function, with lower values in COPD patients reporting one or more exacerbations the previous year (Table 1) Comparisons between their respiratory microbiomes were made in pairs using patients without exacerbations as the reference. Patients with one exacerbation had significantly lower RA of the phylum TM7 (Additional file 1: Figure S1) and lower RAs of 13 different genera (Additional file 1: Figure S2). However, α-diversity parameters did not show significant differences between the groups, and β-diversity analysis did not demonstrate bacterial communities with a different composition ($p = 0.081$). FE showed a significant decrease in the RA of TM7 and Spirochaetes at phylum level (Additional file 1: Figure S3). At genus level, the RAs of *Pseudomonas*, *Selenomonas* and *Anaerococcus* increased, while 10 different genera decreased (Fig. 2). Alpha-diversity analysis did not show significant differences between groups, but β-diversity analysis demonstrated that the bacterial communities of COPD patients with frequent exacerbations differed significantly ($p = 0.014$).

Table 2 Relative abundance of the genera detected. Only genera appearing in more than one sample and with median relative abundances over 0.1% are shown

Genera	Relative abundance, median (IQR)
Rothia	18.65 (9.37–30.33)
*Gemellaceae*_g	7.32 (2.24–13.51)
Prevotella	6.87 (2.33–15.05)
Granulicatella	4.43 (2.16–6.58)
Fusobacterium	2.23 (0.26–3.93)
Porphyromonas	1.97 (0.13–8.22)
Actinomyces	1.80 (0.58–4.41)
Streptococcus	1.92 (1.23–3.44)
Pseudomonas	1.39 (0.40–6.36)
Veillonella	1.00 (0.50–1.44)
Atopobium	0.69 (0.30–1.56)
Oribacterium	0.62 (0.15–1.26)
Leptotrichia	0.51 (0.09–1.90)
*Lachnospiraceae*_g	0.50 (0.08–1.09)
[*Prevotella*]	0.44 (0.04–1.87)
Moryella	0.35 (0.06–0.94)
Campylobacter	0.35 (0.10–0.77)
Capnocytophaga	0.29 (0.01–0.99)
TM7-3_o_f_g	0.28 (0.04–1.45)
Megasphaera	0.25 (0.02–1.17)
Bulleidia	0.20 (0.05–0.85)
Haemophilus	0.19 (0.05–1.32)
Selenomonas	0.18 (0.04–0.62)
Parvimonas	0.11 (0.01–0.54)
Lactobacillus	0.12 (0.01–1.07)
*Lactobacillales*_Other_Other	0.11 (0.04–0.24)

Circulating eosinophils

Forty-two of the participants (58.3%) had ≥2% blood eosinophils. There were no significant differences in age ($p = 0.368$), sex ($p = 1.00$) and number of exacerbations the previous year ($p = 0.080$) between patients with ≥2% circulating eosinophils or less. The bacterial community in the former had significantly higher RAs of the phyla Bacteroidetes and Spirochaetes (Additional file 1: Figure S4). At genus level, 20 genera showed significantly higher RA and one genus, *Peptostreptococcus,* had lower RA in these patients (Fig. 3). Alpha-diversity was significantly higher in patients with ≥2% circulating eosinophils [Chao1 index: 224.51 (74.88) vs 277.39 (78.92), $p = 0.006$; and Shannon index: 3.94 (1.05) vs 4.54 (1.06), $p = 0.020$] (Fig. 4). Pearson's correlation coefficients were r = 0.282 ($p = 0.016$) for Chao1 and r = 0.231 ($p = 0.051$) for Shannon. β-diversity analysis showed a trend towards different bacterial communities ($p = 0.072$).

Multivariate analyses were performed with α-diversity as dependent variable and eosinophils levels as predictive factor, including age and lung function as covariates. Eosinophils in blood, expressed as percentage, kept a statistically significant relationship with Chao1 in this analysis ($p = 0.026$) and a borderline significance for Shannon ($p = 0.051$), a finding confirming that the bronchial microbiome was related to blood eosinophils independently of the functional limitations suffered by the patient.

To explore potential interactions between the previous history of exacerbations and eosinophils levels, we compared the microbiome in COPD patients with and without circulating eosinophils ≥2% stratified by the frequency of exacerbations. We found that the significant differences in the microbial composition related to patients with eosinophils ≥2% were maintained in the subsample of patients with no exacerbations or only one episode ($p = 0.033$), but this effect disappeared in FE ($p = 0.995$).

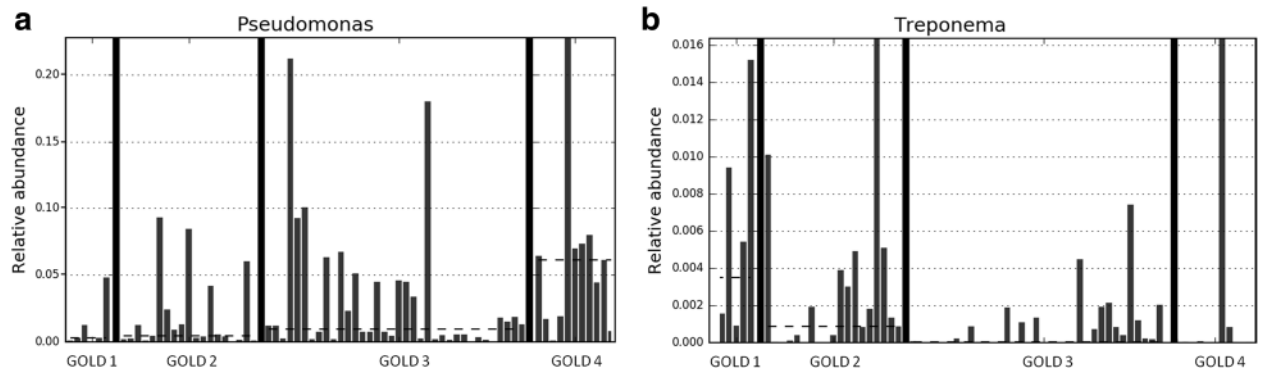

Fig. 1 Genera showing significant differences in their relative abundance according to GOLD severity level, with higher figures for *Treponema* in GOLD 1 (**b**) and for *Pseudomonas* in GOLD 4 (**a**) (dotted line = median)

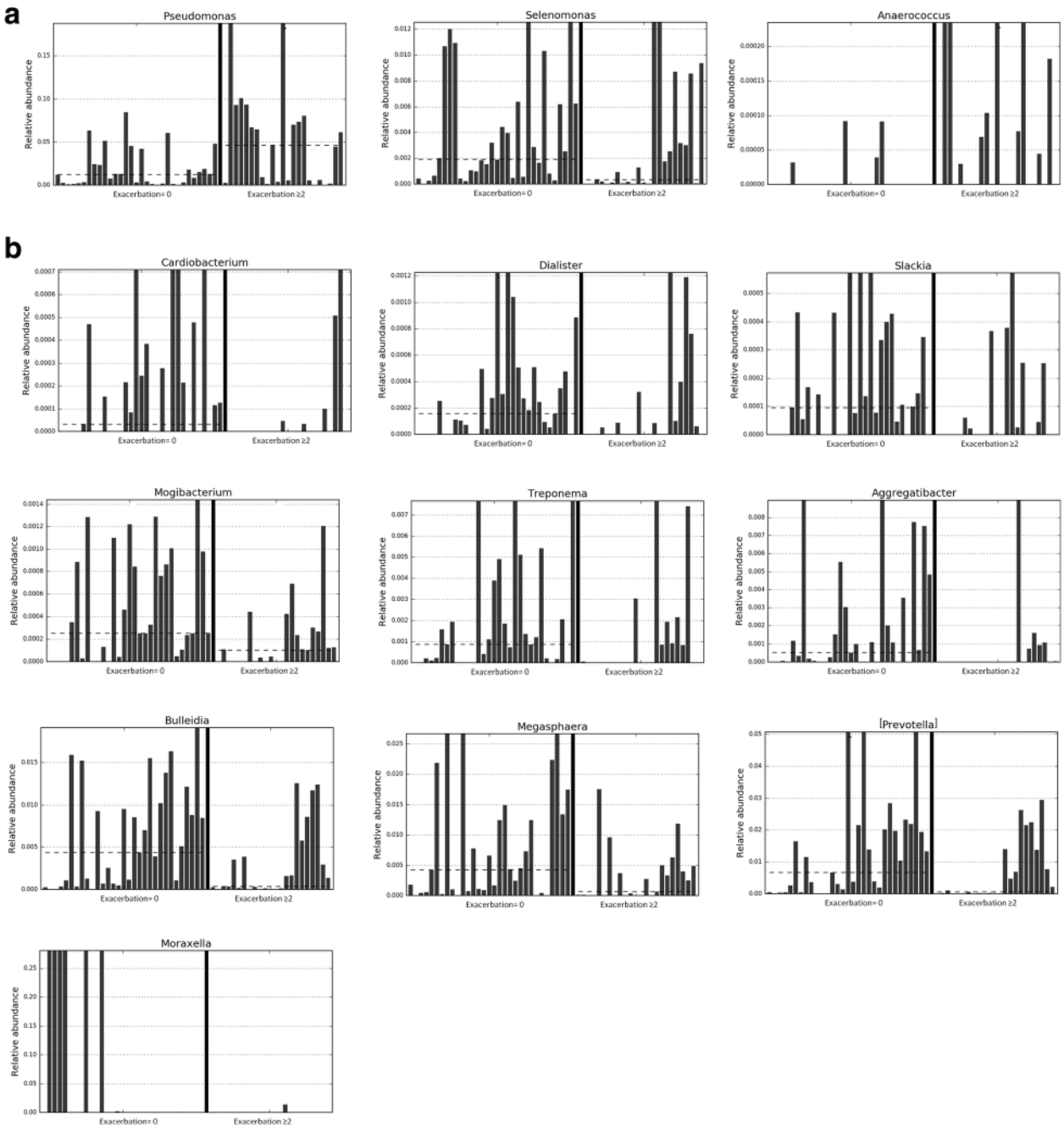

Fig. 2 The RA of three genera significantly increased (**a**) and 10 genera decreased (**b**) in patients with ≥2 exacerbations the previous year compared with non-exacerbators (dotted line = median)

Discussion

The main findings of this study were that the diversity and composition of the respiratory microbiome in clinically stable COPD patients change in relation to age, the severity of airflow limitation, exacerbation frequency and eosinophils in peripheral blood.

In our study, older age was significantly associated with a loss of diversity, which has been also found in the gut microbiome [22]. Besides, in patients with severe asthma, an inverse correlation between α-diversity and age has been also reported [23]. A previous work has shown less microbial diversity of the respiratory microbiome in younger COPD patients using bronchoalveolar lavage [24], but this sample targets the peripheral airway of the lung and it is not representative of the bronchial tree mainly sampled by sputum [25].

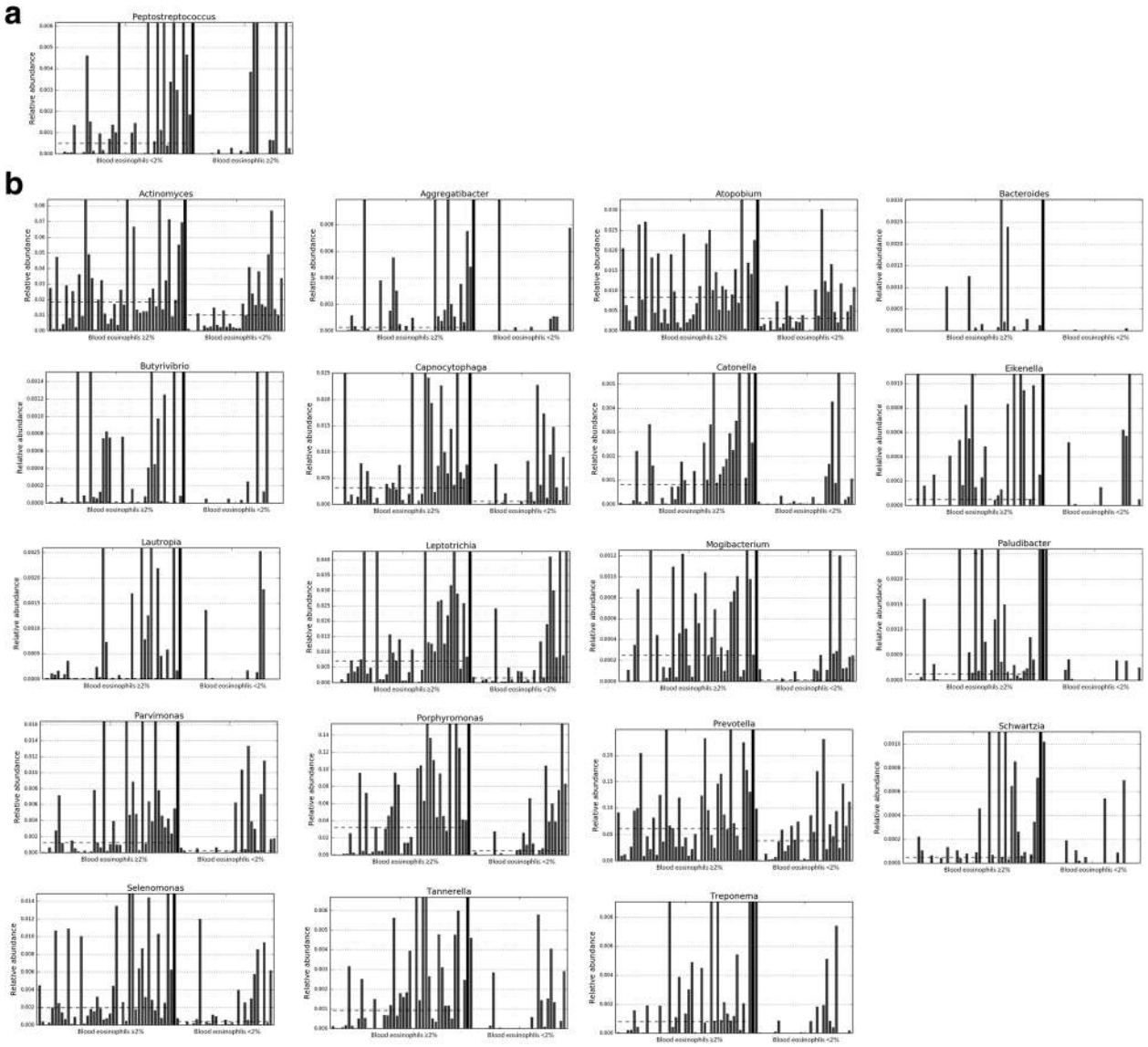

Fig. 3 Genera with significantly higher ($n = 20$) (**b**) and lower (**a**) RAs ($n = 1$) in patients with circulating eosinophils ≥2% (dotted line = median)

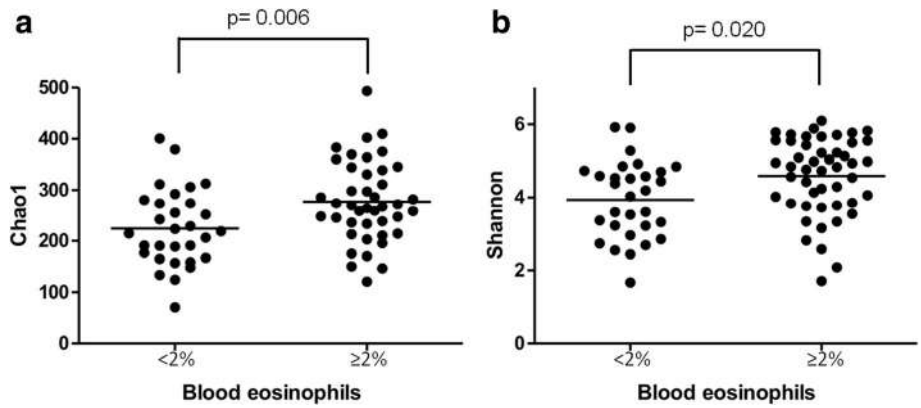

Fig. 4 α-diversity parameters, Chao1 (**a**) and Shannon (**b**), in patients classified according to circulating eosinophils ≥2%

We found that patients with more severe airflow limitation had a significant decrease in the RA of *Treponema* and a progressive increase in the RA of *Pseudomonas*. These results suggest that severity-related changes in the respiratory microbiome are based on a decrease in specific genera, which are partially substituted by *Pseudomonas*. This change may be partly related to recurrent antibiotic exposure in previous years, considering the antibiotic sensitivity of the microorganisms part of *Treponema* genus. Previous cross-sectional studies evaluating the relation between bacterial diversity and more severe airway limitation have mostly showed a decline in advanced stages [26–28], associated with changes in the RAs of specific genera such as *Haemophilus* [28, 29]. These partly discordant results may be due to patient selection, considering that most of the previous studies have focused on a restricted number of patients with moderate or severe disease [26, 27] or an overrepresentation of patients with moderate disease [28] whereas we studied a wider range of disease severity (GOLD 1–4). Our results, therefore, support a significant role for *Pseudomonas* as the severity of the disease increases to higher lung function impairment.

We also found that the respiratory microbiome was significantly different in FE. Previous studies have investigated the characteristics of the respiratory microbiome during exacerbations [7, 30], and recently, like we do in this study, Mayhew and cols. [28] reported specific characteristics in the bronchial microbiome recovered from FE patients during clinical stability. Both studies show that FE have a different respiratory microbiome during clinical stability, suggesting that the microbial changes during exacerbations in FE may be a mixture of the dysbiosis found in stability and specific exacerbation-related perturbations of the lung bacteria community composition [28, 31].

Circulating eosinophils ≥2% were associated with higher microbial diversity in the population studied. Patients with ≥2% blood eosinophils have been reported to have more frequent exacerbations and a better response to ICS preventive therapy [32] Previous studies have demonstrated a different bronchial microbiome in eosinophilic COPD exacerbations [7, 31], which seems related to Th2 inflammation in both COPD and asthma [33]. In our study we observed that in patients with ≥2% blood eosinophils higher microbial diversity is already present in stability, with an increase in the RA of 20 genera. Similar results have been reported in stable asthmatic patients, who showed a good correlation between the percentage of eosinophils in bronchoalveolar lavage and bacterial diversity [34]. Higher bacterial diversity may have a protective role in patients with ≥2% blood eosinophils avoiding the presence of pathogenic bacteria such as *Haemophilus influenzae* and *Streptococcus pneumonia* which has been reported to be overrepresented in patients with eosinophils counts below 2% treated with ICS [12]. Yet, when we stratified the COPD patients included according to both the level of circulating eosinophils and the frequency of exacerbations, we observed that the differences related to blood eosinophils disappeared in FE, likely highlighting a higher impact of frequent exacerbations on the respiratory microbiome in these patients.

This study has some potential limitations. First, we do not have a wide representation of the respiratory microbiome, which has been shown to be heterogeneous throughout the airway, because only sputum samples were analysed. Second, although the patients included had not taken antibiotics three months before their inclusion, we lack information on previous antibiotic treatments, which may have had an effect on their microbial communities. Finally, we analysed only bacterial communities, fungi and virus may also have an effect on these patients, either directly or through interactions with other microorganisms and the host.

Conclusions

This study shows that the respiratory microbiome in clinically stable COPD patients changes in relation to age, severity of airflow limitation, history of previous exacerbations and level of circulating eosinophils. These factors need to be considered when interpreting respiratory microbiome changes in patients with COPD.

Additional file

Additional file 1: Table S1. Relative abundances of the phyla detected. **Figure S1.** The TM7 phylum had significantly lower relative abundance in patients with one exacerbation than patients without exacerbations the previous year (dotted line = median). **Figure S2.** Thirteen genera with significantly lower relative abundances in COPD patients with one exacerbation the previous year compared to non-exacerbators. **Figure S3.** A significant reduction in the RA of phyla TM7 and Spirochaetes in patients with ≥2 exacerbations the previous year, using patients without exacerbations as the reference (dotted line = median). **Figure S4.** Phyla with significantly higher relative abundances in COPD patients showing circulating eosinophils ≥2%. (DOCX 725 kb)

Abbreviations

COPD: Chronic obstructive pulmonary disease; DNA: Deoxyribonucleic acid; FE: Frequent exacerbators; FEV1: Forced expiratory volume the first second; GOLD: Global Initiative for Chronic Obstructive Lung Disease; ICS: Inhaled corticosteroids; IQR: Interquartile range; LAB: long-acting bronchodilator; LDA: Linear discriminant analysis; LEfSe: Linear discriminant analysis Effect Size; PCoA: Principal Coordinates Analysis; PCR: Polymerase chain reaction; QIIME: Quantitative Insights Into Microbial Ecology; RA: Relative abundance; rRNA: Ribosomal ribonucleic acid; SD: Standard deviations; vs: Versus

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Authors' contributions

CM, CC, AM, AC, CA, OS, GPB, BGC, AA, JG and EM recruited the patients and obtained the samples. EM, JG, BGC, OS and AA obtained funding for the project. LM, SP, MGN, CL, LS, RF and SC analyzed the samples and obtained the clinical information. LM, SP and EM interpreted the data. LM and EM wrote the paper. AA and JG contributed in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Bacterial 16SrRNA datasets from this study are accessible in the European Nucleotide Archive under the study PRJEB26773 with the sample numbers ERS2486515–609.

Ethics approval and consent to participate

This is a cross-sectional, prospective, uncontrolled, multicentre, observational study. The study protocol was approved by the Ethics committees of the participating hospitals (IMIM-Hospital del Mar, Hospital Universitari Parc Taulí, Hospital Clinic, Hospital 12 Octubre, Fundación Jimenez Díaz and Hospital Son Espases), and all patients included signed their informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Fundació Parc Taulí- Institut d' Investigació i Innovació Parc Taulí (I3PT), Barcelona, Spain. [2]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III (ISCIII), Madrid, Spain. [3]Universitat Autònoma de Barcelona, Esfera UAB, Barcelona, Spain. [4]Respiratory Medicine Department, Hospital del Mar – IMIM, Barcelona, Spain. [5]Department of Respiratory Medicine, Hospital Universitari Parc Taulí, Parc Taulí 1, 08208, Sabadell, Barcelona, Spain. [6]Health Services Research on Chronic Diseases Network- REDISSEC, Galdakao, Spain. [7]Barcelona Respiratory Network, Barcelona, Spain. [8]DCEXS, Universitat Pompeu Fabra, Barcelona, Spain. [9]Department of Microbiology, Hospital Universitari Parc Taulí, Sabadell, Spain. [10]Department of Respiratory Medicine, Hospital Universitario 12 de Octubre, Madrid, Spain. [11]Respiratory Department, Biomedical Research Institute Sant Pau (IIB-Sant Pau), Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. [12]Department of Respiratory Medicine, Fundación Jiménez Díaz, Madrid, Spain. [13]Department of Respiratory Medicine, Hospital Universitari Son Espases-IdISBa, Mallorca, Spain. [14]Institut Respiratori, Hospital Clinic, IDIBAPS, Universidad de Barcelona, Barcelona, Spain. [15]Department of Medicine, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain.

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Reduced airway levels of fatty-acid binding protein 4 in COPD: relationship with airway infection and disease severity

Lídia Perea[1], Ana Rodrigo-Troyano[2], Elisabet Cantó[1], Marisol Domínguez-Álvarez[3,4], Jordi Giner[2], Ferran Sanchez-Reus[5], Judit Villar-García[6], Sara Quero[4,7], Marian García-Núñez[4,7,8], Alicia Marín[4,8], Eduard Monsó[4,7], Rosa Faner[4,9], Alvar Agustí[4,9], Silvia Vidal[1†] and Oriol Sibila[2*†]

Abstract

Background: For still unclear reasons, chronic airway infection often occurs in patients with Chronic Obstructive Pulmonary Disease (COPD), particularly in those with more severe airflow limitation. Fatty-acid binding protein 4 (FABP4) is an adipokine involved in the innate immune response against infection produced by alveolar macrophages (Mϕ). We hypothesized that airway levels of FABP4 may be altered in COPD patients with chronic airway infection.

Methods: In this prospective and controlled study we: (1) compared airway FABP4 levels (ELISA) in induced sputum, bronchoalveolar lavage fluid (BALF) and plasma samples in 52 clinically stable COPD patients (65.2 ± 7.9 years, FEV_1 59 ± 16% predicted) and 29 healthy volunteers (55.0 ± 12.3 years, FEV_1 97 ± 16% predicted); (2) explored their relationship with the presence of bacterial airway infection, defined by the presence of potentially pathogenic bacteria (PPB) at $\geq 10^3$ colony-forming units/ml in BALF; (3) investigated their relationship with the quantity and proportion of Mϕ in BALF (flow cytometry); and, (4) studied their relationship with the severity of airflow limitation (FEV_1), GOLD grade and level of symptoms (CAT questionnaire).

Results: We found that: (1) airway levels of FABP4 (but not plasma ones) were reduced in COPD patients vs. controls [219.2 (96.0–319.6) vs. 273.4 (203.1–426.7) (pg/ml)/protein, $p = 0.03$ in BALF]; (2) COPD patients with airway infection had lower sputum FABP4 levels [0.73 (0.35–15.3) vs. 15.6 (2.0–29.4) ng/ml, $p = 0.02$]; (3) in COPD patients, the number and proportion of Mϕ were positively related with FABP4 levels in BALF; (4) BALF and sputum FABP4 levels were positively related with FEV_1, negatively with the CAT score, and lowest in GOLD grade D patients.

Conclusions: Airway FABP4 levels are reduced in COPD patients, especially in those with airway infection and more severe disease. The relationship observed between Mϕ and airway FABP4 levels supports a role for FABP4 in the pathogenesis of airway infection and disease severity in COPD.

Keywords: FABP4, Chronic obstructive pulmonary disease, Macrophages, Bronchoalveolar lavage fluid

* Correspondence: osibila@santpau.cat
†Silvia Vidal and Oriol Sibila share the senior authorship
[2]Respiratory Department, Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau (IIB Sant Pau), Autonomous University of Barcelona, Barcelona, Spain
Full list of author information is available at the end of the article

Introduction

Chronic Obstructive Pulmonary Disease (COPD) currently is the third leading cause of mortality worldwide [1]. Chronic airway bacterial infection often occurs in COPD patients, particularly in those with more severe airflow limitation [2, 3]. The presence of airway infection increases the economic impact of the disease and worsens clinical outcomes including increased mortality [4]. Several studies have demonstrated that innate immunity alterations favor airway infection in COPD [5, 6]. However, the underlying biological mechanisms leading to chronic airway infection in COPD have not been yet fully elucidated.

Alveolar macrophages (Mϕ) are a central component of the innate immune response against airway infection. Several alterations of Mϕ in marker expression and functions have been previously described in COPD [7, 8]. Among many other functions, Mϕ produce fatty acid-binding protein 4 (FABP4), also known as adipocyte A-FABP or aP2. FABP4 is a member of the FABP family of small-molecular weight intracellular lipid chaperones that functions as a secreted adipokine and plays a role in airway defense against infection [9, 10]. For instance, in an experimental model of *Pseudomonas aeruginosa* infection in mice, the presence of FABP4 protected against airway infection [11]. However, limited data on COPD and its relationship with disease severity and airway infections are available.

We hypothesized that airway FABP4 levels may be reduced in COPD patients, especially in those with the more severe disease and with potentially more dysfunctional Mϕ. Accordingly, this study sought to: (1) compare airway (and plasma) FABP4 levels in COPD and healthy volunteers and, (2) study their relationship with presence of airway infection, quantity and proportion of Mϕ and several clinical markers of disease severity, such as the severity of airflow limitation (FEV_1), the GOLD classification and the level of symptoms (CAT questionnaire).

Methods

Study design and ethics

This was a prospective, multicenter, cross-sectional study that included clinically stable COPD patients and healthy volunteers with normal lung function, who served as controls. Participants were recruited from five university tertiary hospitals [Hospital de la Santa Creu i Sant Pau (Barcelona, Spain), Hospital del Mar (Barcelona, Spain), Hospital Universitari Parc Taulí (Sabadell, Spain), Hospital Germans Trias i Pujol (Barcelona, Spain) and Hospital Clínic (Barcelona Spain)]. The study protocol was approved by the local institutional review board (IIBSP-MIC-2015-57) and all subjects gave signed informed consent.

Participants

The diagnosis of COPD was established according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines [12]. The inclusion criteria were: 8 weeks of clinical stability (defined by the absence of an exacerbation that required oral corticosteroids and/or antibiotic treatment), age between 40 and 75 years, and FEV_1 between 20 and 70% predicted. All patients underwent a computerized tomography scan, and those with bronchiectasis, lung cancer, pneumonia, and/or interstitial lung diseases were excluded. Other exclusion criteria were patients with active malignant disease and/or any type of immunosuppression, drug addiction or alcohol abuse. As controls, we included adult volunteers without respiratory diseases and with normal spirometry recruited in these same centers [13].

Clinical assessment

Demographic data, the number of exacerbations in the previous year, the time from last exacerbation, relevant comorbid conditions and current treatments were recorded at inclusion using standardized questionnaires. All patients underwent spirometry (Datospir-600; Sibelmed SA, Barcelona, Spain) following international recommendations. Reference values were those of Mediterranean populations [14].

Samples collection and processing

Bronchoalveolar lavage fluid (BALF), induced sputum and plasma were obtained from all participants and were processed immediately. BALF samples were recovered using 150 ml saline lavage with the bronchoscope wedged in the right middle lobe. BALF samples were centrifuged at 800 *xg* for 10 min to obtain the cellular pellet and the supernatant. Induced sputum was collected just before the bronchoscopy as previously described [15]. Sputum samples were disaggregated using dithiothreitol (Oxoid Ltd., Hampshire, United Kingdom) for 15 min and were centrifuged at 600 *xg* for 6 min to obtain the supernatant. Proteases inhibitors (Calbiochem, San Diego, CA) were added to the supernatants during thawing. Plasma samples were obtained from blood collected in ethylenediamine tetra-acetic acid (EDTA) tube and centrifuged at 850 *xg* for 10 min. BALF and sputum supernatants and plasma were stored immediately at −80 °C until analysis.

Microbiological study

Samples were processed for qualitative and quantitative bacteriology, as previously described [16]. Airway infection was defined as the presence of potentially pathogenic bacteria (PPB) at $\geq 10^3$ colony-forming units/ml in BALF [17] in clinically stable patients.

FABP4 measurement

FABP4 levels were measured by validated, commercially available ELISA kit (RayBiotech, Peachtree Corners, GA) according to the manufacturer's instructions. The limit of kit detection was 38 pg/ml. The dilutions used were 1/7 for BALF supernatants, 1/5 for sputum supernatants and 1/75 for plasma. BALF FABP4 levels were adjusted to the total protein content quantified using Qubit fluorometer (Invitrogen, Carlsbad, CA).

BALF flow cytometry

In COPD patients, cellular pellet obtained from BALF samples was lysed to avoid red blood cells contamination (RBC lysing solution; BioLegend, San Diego, CA). Cells were resuspended in one ml of PBS supplemented with 2% bovine serum albumin (BSA; Roche Diagnostics GmbH, Mannheim, Germany) to quantify the number of total cells in a MACSQuant cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were adjusted to $1x10^6$cells/ml and stained for 15 min at room temperature in dark with viability dye (Zombie NIR; BioLegend), CD45-FITC, CD14-APC (Immunotools GmbH, Friesoythe, Germany) and CD15-PE (Biolegend, San Diego, CA). Aggregated and non-viable cells were excluded from the analysis. Mϕ were gated according to CD45 positive population, CD15 negative, CD14 positive and high side scatter (SSC) parameter.

Statistical analyses

Results of continuous variables are presented as mean and standard deviation (SD) or median and interquartile range [25th – 75th percentile IQR], according to the Kolmogorov-Smirnov test of normality distribution, whereas categorical variables are presented as frequencies. Groups were compared using Student t-test, ANOVA test or their corresponding non-parametrical test when required. Correlations were analyzed using Spearman's Rho due to the variables did not present a normal distribution. A p-value < 0.05 was considered significant. Statistical analyses were performed using SPSS version 22 and Graph Pad Prism 7 software.

Results

Characteristics of participants

Fifty-two COPD patients and 29 controls were included. Table 1 shows their demographic and clinical characteristics. COPD patients were older (65.2 ± 7.9 vs. 55.0 ± 12.3, $p = 0.0002$) than controls but gender (75.9 vs. 65.4% males, $p = 0.6$) and BMI (26.3 ± 4.7 vs. 28.7 ± 9.7, $p = 0.7$) were similar in both groups. Airflow limitation in patients with COPD ranged from mild to severe (FEV_1 of 59 ± 15% of predicted) whereas spirometry was normal in controls by design. Twenty-four patients (46%) were classified as GOLD grade C and D, and 21 patients (40%) were frequent exacerbators, defined as those patients who suffered from 2 or more exacerbations during the previous year to the inclusion.

Table 1 Demographics and clinical characteristics among controls and COPD patients

	Controls ($n = 29$)	COPD ($n = 52$)	P value
Age	55.0 ± 12.3	65.2 ± 7.9	0.0002
Male, n (%)	22 (75.9)	34 (65.4)	0.6
BMI (kg/m^2)	28.7 ± 9.7	26.3 ± 4.7	0.7
Smoking status, n (%)			
Never	5 (17.2)	0 (0.0)	< 0.0001
Former	10 (34.5)	41 (78.8)	
Current	14 (48.3)	11 (21.1)	
Pack-years	26.0 ± 19.0	43.5 ± 19.2	0.002
FEV_1 (% pred)	97 ± 16	59 ± 16	< 0.0001
FVC (% pred)	92 ± 15	85 ± 16	0.07
FEV_1/FVC	0.79 ± 0.05	0.52 ± 0.14	< 0.0001

Data is presented as mean ± SD unless otherwise indicated

Twelve COPD patients (23%) had airway infection. Patients with airway infection were predominantly males (92%) and older than non-infected ones (Table 2). *Haemophilus influenzae* was the most common PPB isolated ($n = 9$, 75%), followed by *Streptococcus pneumoniae* ($n = 2$, 17%) and *Moraxella catharralis* ($n = 1$, 8%).

Airway and systemic FABP4 levels in patients and controls

Airway FABP4 levels were lower in COPD patients than in controls, reaching statistical significant differences in BALF [219.2 (96.0–319.6) vs. 273.4 (203.1–426.7) (pg/ml)/protein, $p = 0.03$], but not in sputum [12.2 (0.7–27.5) vs. 14.5 (0.5–45.6) ng/ml, $p = 0.4$] (Fig. 1A, C). Yet, we observed a positive significant correlation between BALF and sputum FABP4 levels in the entire population (rho = 0.31, $p = 0.01$) and in COPD patients (rho = 0.37, $p = 0.01$). On the other hand, plasma FABP4 levels were similar in patients and controls [26.6 (19.3–39.5) vs. 25.2 (18.3–36.5) ng/ml, $p = 0.4$)] and no significant correlation was observed between systemic and airway FABP4 levels.

FABP4 levels and airway infection in COPD patients

Airway FABP4 levels were lower in COPD patients with airway infection vs. those without it, reaching statistical significant differences in sputum [0.73 (0.35–15.3) vs. 15.6 (2.0–29.4) ng/ml, $p = 0.02$] but not in BALF [181.5 (28.7–395.5) vs. 228.5 (106.3–319.1) (pg/ml)/protein, $p = 0.7$) (Fig. 1B, D). No differences in plasma FABP4 levels were observed between COPD patients with and without airway infection [29.8 (20.4–45.0) vs. 26.1 (19.3–37.9) ng/ml, $p = 0.6$].

Table 2 Patient demographics, clinical characteristics and prior treatments among non-infected and infected patients

	Non-infected ($n = 40$)	Infected ($n = 12$)	P value
Age	63.7 ± 7.6	69.9 ± 6.9	0.02
Male, *n* (%)	23 (57.5)	11 (91.7)	0.04
Smoking status, *n* (%)			
Never	0 (0)	0 (0)	0.7
Former	31 (77.5)	10 (83.3)	
Current	9 (22.5)	2 (16.7)	
Pack-years	42.3 ± 20.0	47.8 ± 16.1	0.2
Comorbid conditions, *n* (%)			
Cardiovascular	13 (32.5)	6 (50)	0.3
Hypertension	15 (37.5)	6 (50)	0.4
Diabetes	0 (0)	0 (0)	1
Gastroesophageal reflux	14 (35)	6 (50)	0.3
Treatment, *n* (%)			
ICS	22 (55)	6 (50)	0.5
LABA	32 (80)	8 (66.7)	0.3
LAMA	32 (80)	11 (91.7)	0.3
FEV_1 (% pred)	58 ± 169	61 ± 17	0.6
FVC (% pred)	87 ± 16	76 ± 17	0.04
BMI (kg/m^2)	26.4 ± 4.8	26.0 ± 4.4	0.8
GOLD stage, *n* (%)			
A	13 (32.5)	6 (50)	0.4
B	8 (20)	1 (8.3)	
C	9 (22.5)	1 (8.3)	
D	10 (25)	4 (33.4)	
Prior exacerbations, *n* (%)			
0	16 (40)	4 (33.3)	0.9
1	8 (20)	3 (25)	
≥ 2	16 (40)	5 (41.7)	
Weeks from last exacerbation	26.2 ± 18.9	27.3 ± 19.1	0.6
CAT questionnaire	11.2 ± 6.5	10.5 ± 7.6	0.6

Data is presented as mean ± SD unless otherwise indicated

FABP4 levels and disease severity

Airway (but not plasma) FABP4 levels were related with several measures of disease severity, including GOLD stage, airway limitation and quality of life. Patient with GOLD D had the lowest values both in BALF (Fig. 2A) and sputum (Fig. 2B). In addition, a positive correlation among airway FABP4 levels and FEV_1 (% predicted) (Fig. 3A, B) and a negative correlation with levels of symptoms measured in CAT questionnaire (Fig. 3C, D) were observed.

No differences in airway FABP4 levels were observed regarding age and smoking status. Patients using inhaled corticosteroid (ICS) had lower values in BAL compared with those without ICS [(pg/ml)/protein, 123.6 (12.6–306.4) vs 250 (171.6–344.7), $p = 0.03$)].

FABP4 and alveolar Mϕ

Alveolar Mϕ represented 49.9 (26.4–76.8) % of cells in BALF of COPD patients, corresponding to an absolute number of 140 (48.9–311) Mϕ per μl. Both proportion and absolute number of alveolar Mϕ were significantly related with BALF FABP4 levels [proportion (rho = 0.54, $p = 0.0003$) and absolute number (rho = 0.52, $p = 0.0006$)] (Fig. 4). No significant differences among infected and non-infected patients and related to GOLD stage were found.

Discussion

The main results of this study show that, as hypothesized: (1) airway (but not plasma) levels of FABP4 are reduced in COPD patients, particularly in those with airway infection and more severe disease; and, (2) BALF

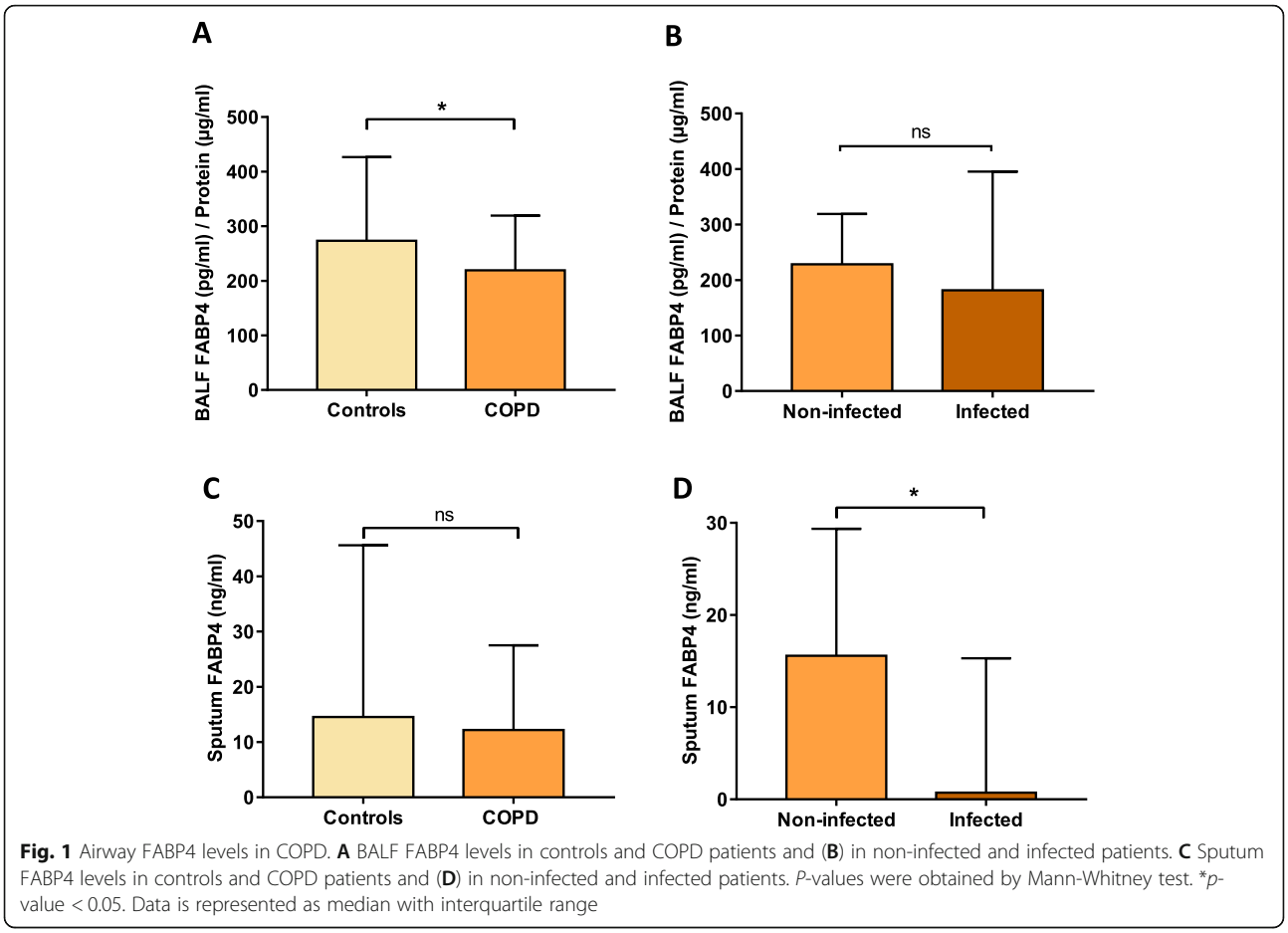

Fig. 1 Airway FABP4 levels in COPD. **A** BALF FABP4 levels in controls and COPD patients and (**B**) in non-infected and infected patients. **C** Sputum FABP4 levels in controls and COPD patients and (**D**) in non-infected and infected patients. *P*-values were obtained by Mann-Whitney test. *p-value < 0.05. Data is represented as median with interquartile range

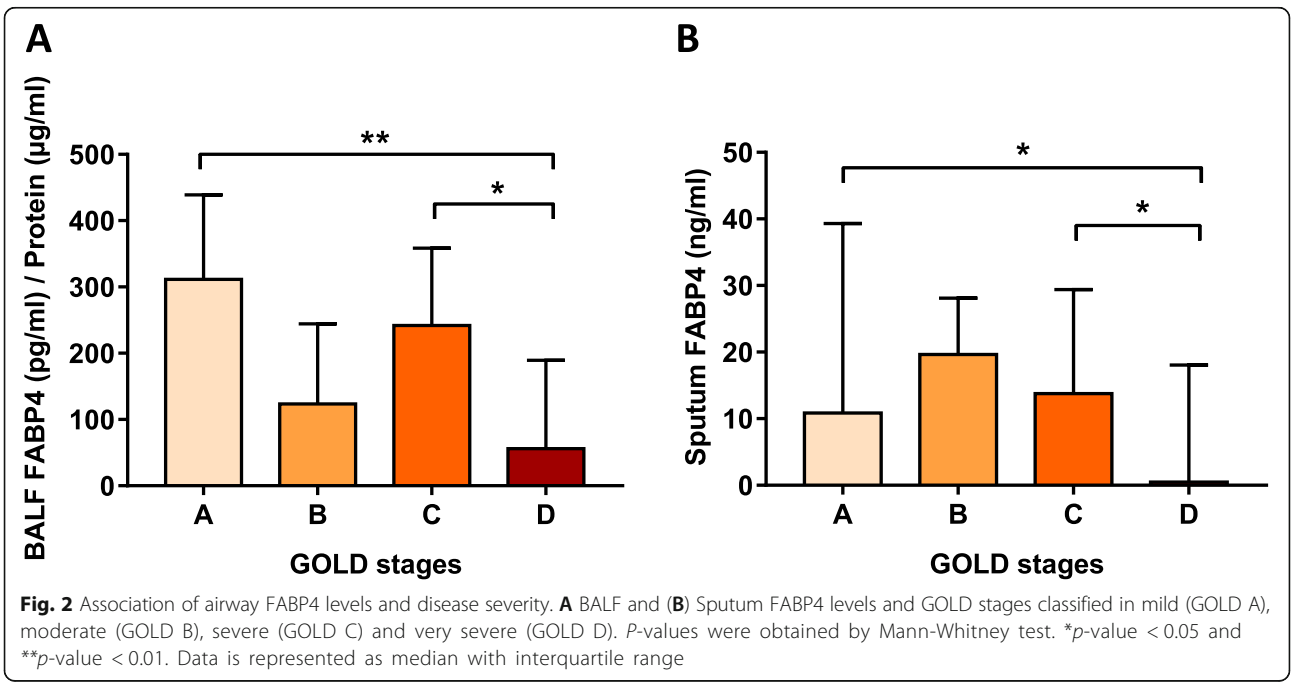

Fig. 2 Association of airway FABP4 levels and disease severity. **A** BALF and (**B**) Sputum FABP4 levels and GOLD stages classified in mild (GOLD A), moderate (GOLD B), severe (GOLD C) and very severe (GOLD D). *P*-values were obtained by Mann-Whitney test. *p-value < 0.05 and **p-value < 0.01. Data is represented as median with interquartile range

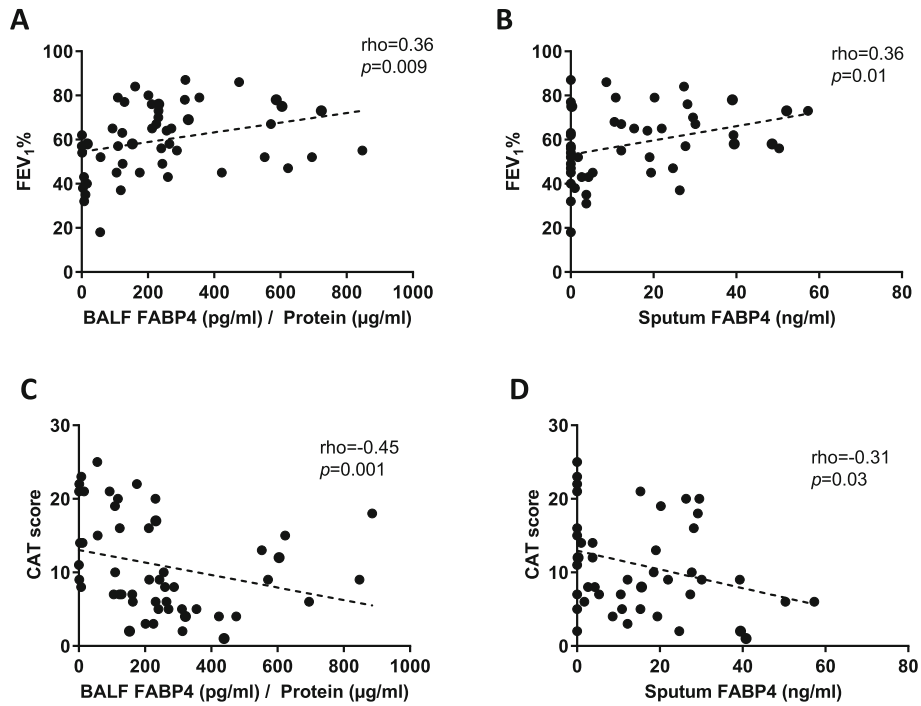

Fig. 3 Relationship between FEV_1 and (**A**) BALF FABP4 and (**B**) sputum FABP4. Relationship between CAT score and (**C**) BALF FABP4 and (**D**) sputum FABP4. Data was obtained using Spearman correlation

FABP4 levels are related to the number and proportion of alveolar Mϕ. Collectively, these results suggest that reduced production of FABP4 by alveolar Mϕ is associated with the presence of airway infection in COPD.

Previous studies

FABP4 is an adipokine widely studied in metabolic and cardiovascular diseases [18]. Its role in chronic respiratory diseases is poorly understood, although it has been recognized that FABP4 is associated with airway inflammation [19]. Likewise, a dysregulation of airway FABP4 has been previously described in asthma [20] where it has been shown in experimental models that FABP4 participates in the recruitment and activation of eosinophils [21]. In patients with COPD, a dysregulation of systemic FABP4 compared with controls has been suggested [22], which is at variance with our findings here. Differences may be related to distinct inclusion criteria. Whereas Zhang et al excluded patients with metabolic and vascular

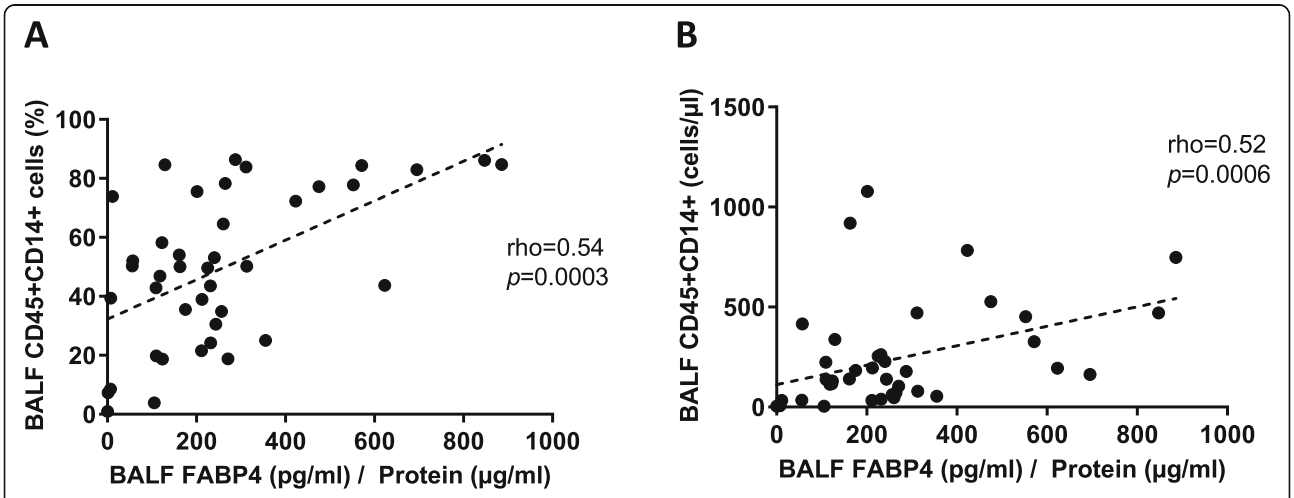

Fig. 4 Relationship between BALF FABP4 levels and (**A**) proportion and (**B**) absolute number of BALF Mϕ. Data was obtained using Spearman correlation

comorbidities [22], known to be associated with elevated systemic FABP4 levels [23, 24], we did not because cardiovascular comorbidities are highly prevalent in patients with COPD (74% in our cohort) and we did not want to bias our study population. In any case, to our knowledge, our study is the first to investigate airway FABP4 levels, and their relationship with airway infection and disease severity in COPD.

Interpretation of novel findings

Bacterial airway infections are relevant in the natural history of COPD [3] because it worsens clinical outcomes, including mortality and costs [4]. The molecular and cellular mechanisms that favor bacterial infection in some COPD patients are, however, not yet fully elucidated. We observed that airway FABP4 levels were reduced in COPD patients, particularly in those with evidence of airway infection, and we found a relationship between BALF Mϕ (absolute number and proportion) and FABP4 levels in COPD. Given that alveolar Mϕ produce FABP4 [9], these observations support a pathogenic role for a locally defective Mϕ production of FABP4. In support of this interpretation is the fact that FABP4 facilitates the interaction between Mϕ and neutrophils through the regulation of CXCL1, a chemokine secreted by Mϕ to recruit neutrophils to the site of infection [11, 25]. Further works studying Mϕ subpopulations would help to better understand the immunological mechanism related to FABP4 production by Mϕ.

On the other hand, we found that patients with severe COPD showed the lowest airway FABP4 levels. We also found that there was a direct relationship between airway FABP4 concentration and FEV_1 levels and an inverse relationship between the former and CAT score (i.e. worse health status). In keeping with these observations, some previous studies had demonstrated that patients with severe COPD have altered airway innate immunity [16, 26, 27] that leads to chronic airway inflammation and dysregulation of normal alveolar Mϕ function [28]. Yet, because our study is cross-sectional, we cannot infer what is the cause and what is the consequence, this is, what comes first.

Potential limitations

Our study has some limitations that deserve comment. First, controls were younger than COPD patients, but we did not observe any relationship between airway FABP4 levels and the age of the participants. Second, induced sputum was collected just before the bronchoscopy so we cannot discard any interaction with BALF samples, although this procedure had been applied to all the participants. Third, we did not perform flow cytometry in BALF from controls, but it is well reported in the literature that COPD alveolar Mϕ differ from healthy controls in phenotype [29] and in the ability to phagocytose bacteria and apoptotic cells [30, 31]. Forth, we have not determined FABP4 in infected patients without COPD and it would be of great interest to better understand its role in the pathogenesis or airway infection. Finally, we did not obtain follow-up samples, so we cannot infer if any therapeutic intervention (e.g., treatment with low dose azithromycin) can restore airway FABP4 levels and what clinical consequences that may have.

Conclusions

FABP4 airway levels (but not plasma ones) are reduced in COPD patients, especially in those with chronic airway infection and more severe disease, in relation to a reduced number of alveolar Mϕ. These observations may be relevant for a better understanding of the pathogenesis (and eventual prevention or treatment) of chronic airway infection in these patients.

Abbreviations

APC: Allophycocyanin; BALF: Bronchoalveolar lavage fluid; BSA: Bovine serum albumin; CAT: COPD assessment test; COPD: Chronic obstructive pulmonary disease; EDTA: Ethylenediamine tetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; FABP4: Fatty-acid binding protein 4; FEV_1: Forced expiratory volume in one second; FITC: Fluorescein isothiocyanate; GOLD: Global Initiative for chronic obstructive lung disease; IQR: Interquartile range; PE: Phycoerythrin; PPB: Potentially pathogenic bacteria; SD: Standard deviation; SSC: Side scatter

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Authors' contributions

Study design: OS, SV, AA, RF and EM. Patient recruitment and data collection: OS, AR, MD, JG, FS, JV, SQ, MG, AM, RF. Performed experiments and sample processing: LP, AR, EC, SV. Writing the manuscript: LP, OS, SV, AA, EM. Revising of the manuscript and approval of submission: all authors. Responsible for the overall content as guarantor: OS.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The study protocol was approved by the local institutional review board (IIBSP-MIC-2015-57) and all subjects gave signed informed consent.

Consent for publication

Obtained.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Inflammatory Diseases, Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau (IIB Sant Pau), Barcelona, Spain. [2]Respiratory Department, Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau (IIB Sant Pau), Autonomous University of Barcelona, Barcelona, Spain. [3]Pneumology Department, Hospital del Mar,

Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Autonomous University of Barcelona, Barcelona, Spain. [4]Centro de Investigación en red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III (ISC III), Barcelona, Spain. [5]Microbiology Department, Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau (IIB Sant Pau), Autonomous University of Barcelona, Barcelona, Spain. [6]Department of Infectious Diseases, Hospital del Mar, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain. [7]Department of Respiratory Medicine, Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí, I3PT, Sabadell, Spain. [8]Respiratory Department, Hospital Universitari Germans Trias i Pujol, Fundació Institut d'Investigació Germans Trias I Pujol, Badalona, Spain. [9]Institut Respiratori, Hospital Clinic, Institut de Recerca Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain.

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Publisher's Note

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Sociedad Española de Neumología y Cirugía Torácica SEPAR

The microbiome in respiratory medicine

June, 2nd 2016
WORKSHOP
MARIE CURIE ROOM
(attendance by invitation)

June, 3rd 2016
SYMPOSIUM
AUDITORI
(attendance by registration)

Barcelona Biomedical Research Park (PRBB)
c/ Aiguader 88, Barcelona

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Welcome

The microbiome in respiratory medicine

Dear Guests and Participants,

It is our pleasure to welcome you to the meeting “The Microbiome in Respiratory Medicine”, co-organized by the Barcelona Respiratory Network (BRN) and the Center for Genomic Regulation (CRG), and endorsed by the European Respiratory Society (ERS).
In recent years the use of culture-independent microbiologial techniques has enabled tremendous growth in understanding how large amounts of microbiological organisms: bacteria, fungi and viruses, collectively known as the microbiome, coexist in intimate contact with different body surfaces, both in health and disease. The lung is not an exception to this phenomenon, and this fact has challenged the previous belief that the healthy lung was sterile. Understanding the nature of the relationship between the lung microbiome and the respiratory epithelial surfaces that are in close contact with it, appears as one of the more promising research fields in respiratory medicine.

Today, a large body of evidence supports the concept that dysregulation of host-microbiota crosstalk at body surfaces may underlie chronic inflammatory disorders. As a consequence, from the clinical point of view, there is a growing interest in determining the potential value of the airway microbiome composition as a prognostic marker, or even as an indicator for monitoring airway disease progression that eventually could prompt specific therapeutic interventions. However, before this can be implemented, several challenges have to be considered such as: 1) the harmonization of methodologies for airway sampling and sample processing, 2) the understanding of the broader interactions of the microbiome components and how they impact the lung disease pathogenesis and 3) the functional characterization of the respiratory microbiome using proteomic, transcriptomic, metabolomic and animal models. These and other relevant questions about this exciting field have to be addressed in this meeting.

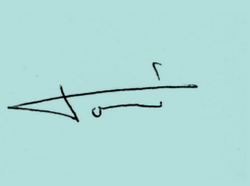
We would like to thank you for joining us at this meeting dedicated to this highly important topic and we hope that you will find it exciting and interesting. We encourage you to actively participate in the scientific discussions.

Yours faithfully,

Jordi Dorca,
President,
Fundació BRN

Eduard Monsó
Chairman,
Fundació BRN

Toni Gabaldón
Group Leader,
Centre for Genomic Regulation



Scientific agenda

June, 2nd 2016

WORKSHOP

MARIE CURIE ROOM

Certainties and uncertainties in the respiratory microbiome

14:00h
Welcome and introduction

Welcome, Goals and Round Presentations.
Organizing committee.

Introduction:
The respiratory microbiome: a new frontier in medicine.
Eduard Monsó, Sabadell, Spain.

14:30h
Block 1: Bioinformatic challenges

Bioinformatic challenges: 16S rRNA analyses.
Vicente Pérez Brocal, Valencia, Spain.

"Sala La Lengua": Study of human mouth microbiome as a large citizen science project.
Julia Ponomarenko, Barcelona, Spain.

General discussion.

15:15h
Block 2: Respiratory microbiome

The Promises and Challenges of the Study of the Lung Microbiome.
Gary Huffnagle, Ann Arbor, USA.

Microbiome in COPD: Pitfalls and Progress.
Sanjay Sethi, Buffalo, USA.

Microbial dysbiosis in bronchiectasis and cystic fibrosis.
James Chalmers, Dundee, UK.

Respiratory microbiome in IPF: Pathogenesis, Progression and Exacerbations.
Philip Molyneaux, London, UK.

Plasticity of the pulmonary microbiota over the spectrum of inflammation to immunosuppression.
Eric Bernasconi, Lausanne, Switzerland.

17:00h
Coffee Break

17:30h
Block 3: Lessons from other human systems

Meta-omics used to study the gut microbiota: from the bench to the computer.
Chaysavanh Manichanh, Barcelona, Spain.

The gut microbiome in HIV infection.
Roger Paredes, Barcelona, Spain.

General discussion.

18:15h
Round discussion: Specific challenges on lung microbiome research
Eduard Monsó, Sabadell, Spain.

19:15h
Closing Remarks

Scientific agenda

June, 3rd 2016

SYMPOSIUM

AUDITORI

The microbiome in respiratory medicine

8:30h
Welcome and introduction

Welcome to the Symposium.
Jordi Dorca, L'Hospitalet de Llobregat, Spain.

The respiratory microbiome: a new frontier in medicine.
Eduard Monsó, Sabadell, Spain.

9:00h
Block 1: Understanding the microbiome
Chairs: Oriol Sibila, Pilar Francino

Understanding the microbiome: How can we determine/analyse it? Technical issues.
Vicente Pérez Brocal, Valencia, Spain.

"Sala La Lengua": Study of human mouth microbiome as a large citizen science project.
Julia Ponomarenko, Barcelona, Spain.

10:00h
Block 2: Lung microbiome: were we are - airway diseases
Chairs: Rosa Faner, Marian Garcia-Núñez

The Dynamics of the Lung Microbiome during Health and Disease.
Gary Huffnagle, Ann Arbor, USA.

Microbiome in COPD: Pitfalls and Progress.
Sanjay Sethi, Buffalo, USA.

Microbial dysbiosis in bronchiectasis and cystic fibrosis.
James Chalmers, Dundee, UK.

11:30h
Coffee Break

12:00h
Block 3: Lung microbiome: were we are - airway diseases
Chairs: Julia Ponomarenko, Jordi Dorca

Respiratory microbiome in IPF: Pathogenesis, Progression and Exacerbations.
Philip Molyneaux, London, UK.

Host-microbe interplay sets the lower airway microenvironment in lung transplantation.
Eric Bernasconi, Lausanne, Switzerland.

13:00h
Block 4: Lessons from other microbiomes
Chairs: Vicente Pérez Brocal, Eduard Monsó.

Lesson from the human gut microbiome.
Chaysavanh Manichanh, Barcelona, Spain.

The gut microbiome in HIV infection.
Roger Paredes, Barcelona, Spain.

14:00h
General discussion, wrap-up and next steps.

Jordi Dorca, Eduard Monsó

Invited Speakers, Scientific Committee & Chairs

Eduard Monsó

Scientific Leader of the Meeting & Invited Speaker

Hospital Universitari del Parc Taulí, Sabadell, Spain.

Dr. Eduard Monsó graduated in Medicine from the Universitat de Barcelona in 1981, and attained his Ph.D. Degree in Medicine at the Universitat Autònoma de Barcelona in 1987. He finished his training in Respiratory Medicine in 1985, and worked after his degree at the Institut Català de la Salud. He is currently Head of the Department of Respiratory Diseases at the Hospital Universitari del Parc Taulí de Sabadell and professor at the Universitat Autónoma de Barcelona. His research interests have focused on COPD, bronchial infections, lung cancer, endoscopy techniques, respiratory epidemiology, occupational lung diseases and telemedicine. He is currently Head of the Research Group in Respiratory Diseases Metropolitana Nord de Barcelona (RESPINORD-BCN), part of Centro de Investigación Biomédica en Red de Enfermedades Respiratorias – Ciberes - Instituto de Salud Carlos III. Dr. Eduard Monsó is author of 150 published research papers.

Àlvar Agustí

Member Scientific Committee

Hospital Clínic, Barcelona, Spain.

Prof. Alvar Agustí is currently Director of the Respiratory Institute at Hospital Clinic in Barcelona (www.hospitalclinic.org), and Associate Professor of Medicine at the University of Barcelona. His main research interests include COPD and sleep disorders. He has published more than 400 papers in peer-reviewed journals (H-Index 62) and has made over 40 contributions to books. He is regularly invited to speak at international conferences and symposia. He is a member of several professional societies, including the American Thoracic Society, and the European Respiratory Society (ERS), in which he has been a Member of its Executive Committee. He has a seat at the Royal Academy of Medicine of the Balearic Islands, he is an Honorary Fellow of the Royal College of Physicians of Edinburgh (FRCP), a Fellow of the European Respiratory Society (FERS) and a member of the Scientific Committee and the Board of Directors of GOLD (www.goldcopd.org).

Eric Bernasconi

Invited Speaker

University Hospital of Lausanne, Lausanne, Switzerland.

Holding a PhD in molecular virology, I have been trained since 1999 as a mucosal immunologist in the Division of Immunology of the University Hospital of Lausanne, Switzerland, where I studied the ability of gut-derived micro-organisms to modulate epithelial permeability and allergic responses. I then joined, in 2005, the Division of Gastroenterology, where my focus was on innate cell activation during colitis, and the critical role of macrophages in wound healing. Since 2011, my studies as Research associate in the Division of Respiratory Medicine directed by LP Nicod (Head of Division) and BJ Marsland (Head of Research), also at the University Hospital of Lausanne, have allowed me to bring these different topics together. Specifically, we assess the extent to which variations in the pulmonary microbiota composition are associated with underlying immunological changes, and how such variations may impact upon lung function within the transplantation context.

Jerónimo Carnés

Member Scientific Committee

Laboratoris LETI, SL,
Barcelona, Spain.

Dr Jerónimo Carnés, PhD is a Doctor in Animal Physiology. He qualified in Biological Sciences in Madrid, Spain in 1994. He started to work on Immunology and Allergy in 1995. From that point onwards, he has collaborated, designed and directed research projects in different fields, including Immunology, Allergy and, more recently, Vaccines for parasitic diseases. Currently he is the R&D Director of the Immunology and Allergy Business Unit at Laboratorios LETI S.L, Spain. In this period he has developed and launched the best-selling product of the company for the treatment of allergenic diseases and he has participated in the design, development and registration of different molecules in Germany and recently in the EMA. He has published more than 90 peer-review articles in different scientific journals, is the author of 2 patents and has been speaker in more than 50 national and international conferences. He is currently involved in different research projects for the development of Immunotherapy for the treatment of allergic diseases and the research and development of vaccines for parasitic infectious diseases.

James Chalmers

Invited Speaker

University of Dundee,
Dundee, UK.

Dr James Chalmers is Senior Lecturer and Honorary Consultant at the University of Dundee. His research is focused on understanding interactions between bacteria and neutrophilic inflammation in the lung, with a particular focus on bronchiectasis, COPD and cystic fibrosis. He is chair of the European Bronchiectasis Registry (EMBARC), an initiative of the European Union and European Respiratory Society to accelerate research and clinical trials in non-CF bronchiectasis. He is associate editor of the European Respiratory Journal and a member of the international advisory board of the Lancet Respiratory Medicine. He has published more than 120 papers with an H index of 30. He is chair of the European Bronchiectasis guidelines group, which will produce clinical practice guidelines for bronchiectasis towards the end of 2016.
He is also involved in continuing medical education and is the secretary of the European Board for Accreditation in Pneumology, and is active in the European Respiratory Society and American Thoracic Society.

Jordi Dorca

Member Scientific Committee & Chair

Hospital Universitari de Bellvitge,
L'Hospitalet de Llobregat, Spain.

Jordi Dorca is currently Chief of the Respiratory Department at the Hospital Universitari de Bellvitge, located in the Barcelona area. He is Professor of Medicine at the University of Barcelona and Coordinator of the Respiratory Diseases Research Group at the Institut d'Investigació Biomèdica de Bellvitge (IDIBELL). His main research interests are focused on the epidemiology, diagnosis and treatment of different kinds of respiratory infections. He has published more than 110 papers in peer-reviewed journals with a combined IF of 511 and a h-index of 29. He is also a member of several scientific societies. Currently, he is the president of Barcelona Respiratory Network.

Invited Speakers, Scientific Committee & Chairs

Rosa Faner

Member Scientific Committee & Chair

CIBER Enfermedades Respiratorias, Barcelona, Spain.

Maria Rosa Faner is a Post-Doctoral researcher at the CIBER Respiratory Diseases group 10 (Hospital Clínic, Barcelona). She has a degree in Biological Sciences (Universitat Autònoma de Barcelona, 2001) and a PhD in Immunology (Universitat Autònoma de Barcelona, 2006).
She has been PI of 5 projects funded by competitive agencies, author of 26 papers in international peer-reviewed journals with a total IF of 196.7, H index of 12, 351 citations and author of 3 patents.
Her main contributions to the research in the respiratory diseases described the pulmonary immune response deregulation observed patients with COPD using unbiased omics and network medicine methods, the genetic basis of observed multimorbidity, and the gender differences in the systemic response to smoke.

Pilar Francino

Chair

Fundación para el Fomento de la Investigación Sanitaria y Biomédica, Valencia, Spain.

M. P. Francino studied Biology at the National University of Mexico and then pursued graduate studies at the University of Rochester (New York), where she obtained her Ph.D. degree working on analyses of rates and patterns of DNA sequence evolution in bacteria and primates. Next, she conducted postdoctoral research in bacterial genetics as an EMBO Fellow at the University of Paris. After that, she served as a Research Scientist at the U.S. Department of Energy Joint Genome Institute for five years, and was Head of their Evolutionary Genomics Program from 2007 to 2009. Since 2009, she has been a Senior Scientist at the Genomics and Health Department of FISABIO-Public Health in Valencia, and the Head of the Department since 2012. Her current research focuses on the metagenomic analysis of human microbiome communities, in particular on understanding the development of the gut microbiota in infants. Work in her research group studies this process by analyzing the taxonomic composition, coding capabilities and gene expression patterns of the gut microbial community at different stages during infancy, as well as the relationships of these features with infant health.

Toni Gabaldón

Member Scientific Committee

Centre for Genomic Regulation, Barcelona, Spain.

A biochemist by training (University of Valencia, Spain, 1997), Toni Gabaldón carried out a PhD in comparative genomics at The Radboud university (Nijmegen, The Netherlands) in 2005, and an EMBO-funded postdoc at the CIPF center (Valencia, Spain). In 2008 he started his own group at the Centre for Genomic Regulation (Barcelona, Spain). Gabaldón has always used an evolutionary perspective to address different biological questions. His research is not only focused on understanding how complex biological systems work, but also how they have came to be as they are. Over his career he has been awarded prestigious grants and awards such as the ICREA professorship and the ERC Starting Grant.

Marian Garcia-Nuñez

Chair

CIBER Enfermedades Respiratorias,
Sabadell, Spain.

PhD in Biology. Postdoctoral Researcher of the CIBER in Respiratory Diseases for the Instituto de Salud Carlos III (chief researcher: Dr. Eduard Monsó). Lead researcher of the "Lung Microbiome and Respiratory Community-acquired Infections Group" from the Institut d'Investigació i Innovació Parc Taulí (I3PT), Barcelona. Member of RESPINORD group (2014SGR801) financed by the Generalitat de Catalunya (Catalan Regional Government).
Her main research areas are focused on the role of lung microbiome in COPD and cystic fibrosis diseases; development of new tools and screening strategies of bacterial molecular typing; and improvement in the diagnosis of respiratory diseases, particularly in the field of Legionnaires' disease.

Gary B. Huffnagle

Invited Speaker

University of Michigan,
Ann Arbor, USA.

Gary B. Huffnagle, PhD received his PhD in immunology from the University of Texas Southwestern Medical School. He holds faculty appointments (Professor) in Internal Medicine, Microbiology & Immunology, and Molecular Cellular & Developmental Biology, as well as an endowed professorship in the Mary H. Weiser Food Allergy Center at the University of Michigan. He was elected to the American Academy of Microbiology of the American Society for Microbiology in 2013 and has been a frequent reviewer for the NIH (USA). The overall goals of his current research are to identify and delineate the interactions between the microbiome (lung and gut) and the immune system. Using animal models, clinical samples and in vitro assays, his laboratory is investigating host-microbiome interactions in the control of pulmonary inflammation, allergic responses and infectious disease. Over the past decade, his laboratory has developed expertise in applying high-throughput sequencing and gene expression technologies to biological processes and disease, including bacterial genomics and the microbiome.

Chaysavanh Manichanh

Invited Speaker

Vall d'Hebron Research Insitute,
Barcelona, Spain.

Chaysavanh Manichanh, PhD (University Pierre et Marie-Curie, Paris, 2001) is a research scientist and head of the Metagenomics Lab in the Department of Physiology and Physiopathology (Vall d'Hebron Research Institute, Barcelona). Since 2002, she has been using meta-omics approaches to study the human microbiome associated with human diseases. She has collaborated with the European MetaHIT consortium in building a comprehensive gene catalogue from the human gut microbiome and has participated in the International Human Microbiome Standards (IHMS) project that seeks to coordinate the development of standard operating procedures (SOPs) and protocols to optimize data comparisons in the human microbiome field. With her group, she develops molecular as well as bioinformatics tools to characterize the human microbiome associated with different human disorders (https://sites.google.com/site/manichanhlab/).

Invited Speakers, Scientific Committee & Chairs

Philip Molyneaux

Invited Speaker

Royal Brompton Hospital
London, UK.

Dr Philip Molyneaux qualified from Guy's, King's and St Thomas' School of Medicine in 2004, where he completed an intercalated BSc. in Molecular Genetics. He undertook his clinical training at Guy's and St Thomas' and went on to attain an NIHR Academic Clinical Fellow position in Respiratory medicine at Imperial College. He spent the next two years training at St Mary's Hospital and working with Professors Cookson, Moffatt and Johnston studying the respiratory microbiome in COPD.
Moving to the Royal Brompton Hospital he went on to complete a PhD examining the host response and microbial flora in Idiopathic Pulmonary Fibrosis (IPF) as part of the Prospective Study of Fibrosis In the Lung Endpoints (PROFILE) study with Dr Toby Maher. His ongoing work concentrates on delineating the host microbe interaction in fibrotic lung disease.

Roger Paredes

Invited Speaker

IRSICaixa AIDS Research Institute,
Barcelona, Spain.

Dr. Roger Paredes, MD, PhD, leads the Microbial Genomics Group at the irsiCaixa AIDS Research Institute and is attending HIV physician at the HIV Unit, Hospital Universitari Germans Trias i Pujol in Barcelona, Catalonia, Spain. He's assistant professor in infectious diseases at the Universitat Autònoma de Barcelona (UAB) and Lecturer in the Chair on AIDS and Related Diseases, UVIC-UCC. His team has made seminal contributions to the current understanding of the clinical relevance of minority drug-resistant HIV and X4 viruses on antiretroviral treatment outcomes and HIV disease progression. He's now using next-generation genomics to characterize the role of the human microbiome on HIV disease, chronic inflammation and aging. Dr. Paredes is clinical virologist for several European HIV cohorts (EuroSIDA), advisor to the WHO HIV ResNet group and member of the organizing committee of the first International Workshop on Microbiome in HIV Pathogenesis, Prevention and Treatment, held annually in Bethesda, MD.

Vicente Pérez Brocal

Invited Speaker
& Chair

Fundación para el Fomento
de la Investigación Sanitaria y Biomédica,
Valencia, Spain.

I got my degree in Biology, at the University of Valencia in 2001, where I did my PhD research project in the program 'Biodiversity and Evolutionary Biology' from 2002 to 2006. I worked on bacterial genome reduction and minimal genomes. I sequenced the smallest bacterial genome at that time, that of Buchnera aphidicola, the primary endosymbiont from the cedar aphid. Next, I worked as a postdoctoral researcher at the London School of Hygiene and Tropical Medicine at the University of London, from 2006 to 2009. My topic there was the genetic evolution of mitochondrial and related organelles during the transition from an aerobic to a strictly anaerobic lifestyle working on Blastocystis.
Next, I held a 'Sara Borrell' contract at the 'Genomics and Health Area' at the, FISABIO (Valencia), from 2010 to 2014 to work on Metagenomics of microbial and viral communities associated to inflammatory bowel disease and other pathologies affecting the gastrointestinal tract. Since then, I have also collaborated with groups of Barcelona (respiratory microbiome and virome), Madrid (celiac disease virome), Italy (fermenting bacteria), Girona (gut microbiota-brain relationship) etc., with a contract at the FISABIO.

Julia Ponomarenko

Invited Speaker
& Chair

Centre for Genomic Regulation,
Barcelona, Spain.

Dr. Julia Ponomarenko is Head of the Bioinformatics Unit at the CRG, Barcelona, Spain. After obtaining in 2002 the PhD in computational biology, Julia moved to the San Diego Supercomputer Center, University of California San Diego (UCSD), where until 2015 she was the US NIH Principal Investigator. Dr. Ponomarenko is one of the primary developers of the Immune Epitope Database (IEDB. org), a $35M NIAID/NIH contract. In 2014, she received the US National Science Foundation RAPID award for studies of the immunity against the Ebola virus epitope. Dr. Ponomarenko's research interests include NGS data analysis, immunoinformatics, structural genomics, transcriptomics, systems biology, development of biological databases and bioinformatics software.

Sanjay Sethi

Invited Speaker

University of Buffalo,
Buffalo, USA.

Sanjay Sethi, MD, FACP, is a Professor in the Department of Medicine at the University of Buffalo at the State University of New York (SUNY) in Buffalo, NY. He is Chief of the Division of Pulmonary/Critical Care/Sleep Medicine, Assistant Vice President for Health Sciences and Director of the Clinical Research office at the University at Buffalo. Dr Sethi's main research interests include chronic obstructive pulmonary disease (COPD) and respiratory infections, focused on the specific areas of exacerbations, new therapeutics and innate lung defense in COPD. Dr Sethi has co-authored more than a 180 research articles, reviews and book chapters in many peer-reviewed medical journals. He is a member of the editorial board for several pulmonary journals. He was a member of the lung cellular, molecular, and immunobiology study section of the National Institutes of Health (NIH) and the Pulmonary study section of the VA, and is an ad hoc reviewer for several North American and European research funding agencies. Dr. Sethi is currently active in several professional organizations including the American Thoracic Society where he has chaired the Clinical Problems program committee, and is chair for the Clinical Problems Assembly.

Oriol Sibila

Member Scientific
Committee & Chair

Hospital de la Santa Creu i Sant Pau,
Barcelona, Spain.

Medical Doctor. Pulmonary Disease Specialist since 2004. PhD Cum Laude at University of Barcelona (UB) in 2008. Research Fellow at University of Texas Health Science Center at San Antonio (UTHSCSA), San Antonio, TX (2011-2012). Pulmonologist staff at Hospital de la Santa Creu i Sant Pau, Barcelona since 2009. Clinical Associate Professor of Autonoma University of Barcelona (UAB).

Honors: Best young Investigator Award by Spanish Society of Pneumology (SEPAR) at 2009. Early-Career Peer Recognition Award by Assembly of Microbiology, Tuberculosis and Pulmonary Infections (MTPI) of the American Thoracic Society at 2016. Principal investigator of one active grant supported by Spanish Government focused on the study of microbiome in COPD. More than 30 international publications in the last 5 years.

WORKSHOP

The respiratory microbiome: a new frontier in medicine

Eduard Monsó
Sabadell, Spain.

Bronchial colonization by potentially pathogenic bacteria is common in stable chronic obstructive pulmonary disease (COPD), and in about a half of patients positive cultures for Haemophilus influenzae, Streptococcus pneumoniae and Moraxella catarrhalis are found during stability periods. However available culture techniques are not suitable for the identification of an important part of the bacterial flora inhabitant of the respiratory mucosa. Most bacteria present do not grow adequately on commonly used selective cultures, and are masked by the presence of other faster-growing bacteria.
Through independent culture techniques, such as amplification and sequencing, it is possible to determine the composition of bronchial microbiome, both bronchial and viral, defining the relationships between the colonizing flora and MPOC. The use of these techniques in bronchial secretions has shown the existence of a diverse microbiome in COPD, and a predominant presence of bacteria from the phylum Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes.
The bacterial flora observed in patients with COPD alters the pattern of continuity observed in the oropharynx and the bronchial tree of normal subjects, through a partial disappearance of microorganisms common in the healthy population. The magnitude of these changes parallels the severity of the disease, with a relative increase in the proportion of Proteobacteria in severe patients, inversely correlated with the decrease of Firmicutes in advanced COPD. Thus, the bacterial diversity in the respiratory tract appears to be clearly higher in the healthy subject and the patient with mild-moderate COPD, to be replaced in severe COPD by a narrow range of microorganisms, with a high proportion of potentially pathogenic bacteria. In patients with severe COPD, frequent colonization by Pseudomonas aeruginosa does not determine a change in the bronchial flora as a whole, which has a similar spectrum in all patients with advanced disease, independently of their colonizing patterns.

The study of the bacterial flora in COPD exacerbations have shown increases in specific bacterial genera in most patients, which include, in most cases, one or more potentially pathogenic species. These increases in many cases are not identified by culture, a finding that has confirmed the insufficient sensitivity of conventional techniques for identifying causative pathogens in COPD. This approach have shown that the bacterial pattern in exacerbations from patients colonized by Pseudomonas aeruginosa, when identified from independent culture techniques, are similar to patterns found in non-colonized patients. This supports the hypothesis that microorganisms causing exacerbations in Pseudomonas-colonized COPD patients are not different from the pathogens related to bronchial infections in non-colonized patients, and the colonizing role of Pseudomonas aeruginosa continues, both in stability periods and in exacerbations. These findings from microbiome analysis have potential implications for recommendations in therapeutic guidelines.
One additional advantage of the identification of the composition of respiratory microbiome by culture-independent techniques in respiratory samples is the characterization of the functional features of the bacterial genome, through metagenomics. This approach has shown that in COPD exacerbations the functional activity of the background flora may change, in spite of the absence of changes in their composition, with increases in the abundance of genes involved in carbohydrate metabolism and carcinogenesis, and parallel decreases in genes related to cell growth and catabolism.
Thus, the introduction of microbiological identification techniques not based on culture has demonstrated that the diversity of bacterial flora is reduced with advanced COPD, with an overrepresentation of potentially pathogenic microorganisms. In exacerbations, 16S rRNA gene sequencing have identified causal pathogenic bacteria not recovered through cultures, and functional changes in bacterial flora with potential clinical significance.

Certainties and uncertainties in the respiratory microbiome

Bioinformatic challenges: 16S rRNA analyses

Vicente Pérez Brocal
Valencia, Spain.

In order to answer our questions regarding the diversity of the microbiome harboured in complex ecosystems such as that of the human respiratory tract, we require the development of a series of computational software to help us untangle its composition, abundance and diversity, based on the 16S rRNA. However, bioinformatic tools alone are not enough to tackle the challenges arising from the increasingly demanding biomedical research. Thus, researchers must contemplate an appropriate conceptual framework in order to achieve a proper use of those tools. In summary, being able to pose the right questions and accordingly, carrying out the appropriate workflow using the available bioinformatic tools may be as challenging and is, at least, as important as the tools we use themselves. Therefore, features such as the awareness of the relevance that the detection of shortcomings has in currently existing 16S rRNA-based databases, the presence of chimeras and other bioinformatic challenges, rather than the automatic application of a particular software or even pipeline, represent aspects that must be emphasized.

"Sala La Lengua": Study of human mouth microbiome as a large citizen science project

Julia Ponomarenko
Barcelona, Spain.

Sala La Lengua is a citizen science project studying the human mouth microbiome. Bacterial profiles of over 1500 school children across Spain were obtained using 16S rRNA sequencing and analyzed in relation to their diet, mouth hygiene, geographic area and other environmental characteristics and lifestyle. In my talk I will discuss the results of the project and also bioinformatics challenges we have encountered undertaking the project.

WORKSHOP

The Promises and Challenges of the Study of the Lung Microbiome

Gary Huffnagle
Ann Arbor, USA.

The identification and confirmation that the lungs are not sterile during health has begun to change views of the role of indigenous bacteria in lung health and disease. However, the airways are a challenging site to sample and repeated sampling by bronchoscopy is not ethically feasible in a healthy population or in most diseased individuals. Despite the theoretical possibility of bronchoscope contamination by nasal or oral microbiome during insertion, this has not proven to be an issue. Contamination is usually below the limits of detection for culture-independent sequencing-based assays. Rather, the issue that has emerged is that sequencing of low biomass samples can generate spurious signals. This underscores the critical need for proper technical controls in sequence-based analyses of the low bacterial biomass of the airways during health (and even some diseases) in order to separate "signal from noise." The emerging concept of the lung microbiome is of that of a tidal ecosystem in which immigration, elimination and regional growth conditions are the primary factors that determine the composition of the lung microbiome.

Microbiome in COPD: Pitfalls and Progress

Sanjay Sethi
Buffalo, USA.

Microbiome studies of respiratory secretions have provided exciting new observations regarding the bacterial causation of exacerbations of COPD, and the impact of treatment of the exacerbation on the airway microbiome. Bacterial exacerbations likely represent abrupt major changes in the microbiome with resultant large increases in airway and systemic inflammation. Microbiome studies could lead to discovery of new pathogens that have been difficult to obtain with standard culture techniques. The Vicious Circle Hypothesis embodies the likely contribution of an altered microbiome to COPD progression, with an unhealthy microbiome driving the inflammatory process in stable COPD. While studies with conventional microbiology found potential pathogenic bacteria to be virtually absent in bronchoscopic samples in healthy controls, smoking and development of COPD was associated with 35–50% incidence of isolation of pathogenic bacteria. In contrast, recent microbiome studies have found no or minor differences from controls in smokers and COPD. These contradictory findings could be explained by the extreme sensitivity of the microbiome technique to upper airway contamination. Several challenges need to be tackled to fully utilize the benefits of microbiome research in COPD. Paramount is the issue of upper airway and environmental contamination of lower airway samples. Significant concentration thresholds in microbiome data need to be defined. The microbiome differs in sputum, bronchoalveolar lavage and lung parenchyma samples obtained from patients with COPD. Determination as to which of the various new microbes that will be identified in microbiome studies are pathogenic is still unclear, especially for unculturable pathogens. Though various obstacles need to be surmounted, ultimately lung microbiome studies will provide new insights in to how infection contributes to COPD.

Certainties and uncertainties in the respiratory microbiome

Microbial dysbiosis in bronchiectasis and cystic fibrosis.

James Chalmers
Dundee, UK.

Bacterial infection is central to our understanding of the pathophysiology of bronchiectasis and cystic fibrosis. Traditional culture based microbiology techniques have revealed the importance of well characterised pathogens such as Haemophilus influenzae, Pseudomonas aeruginosa and Moraxella catarrhalis in bronchiectasis, and Staphylococcus aureus and Burkholderia cepacia in cystic fibrosis among many others. The management of these diseases and drug development efforts have been largely devoted to antimicrobial therapies targeting these pathogens. P. aeruginosa represents a special case, having been shown to impact prognosis in both diseases and being both frequently multidrug resistant and difficult to eradicate.

The availability of next generation sequencing technologies and particularly characterisation of the bacterial lung microbiome are causing an evolution in our understanding of the disease. Previously unrecognised organisms are found to dominate the microbiome in some patients, while studies characterising the airway microbiome following antibiotic treatment show remarkable resistance of bacterial communities to change. The addition of fungi, viruses and Mycobacteria adds to the complexity of understanding the host/pathogen interaction is these polymicrobial lung diseases.

Key questions still need to be addressed with regard to the microbiome in bronchiectasis and CF, including the extent to which sequencing provides clinical information beyond that provided by culture, whether antibiotic treatment can be targeted based on microbiota profiles, and whether prognostic information can be gained. Finally, it is important to determine if the microbiome can be used to evaluate therapeutic response or give insights into adverse effects of antibiotics such as emerging of new pathogens or loss of bacterial diversity. I will discuss all of these issues during the meeting.

Respiratory microbiome in IPF: Pathogenesis, Progression and Exacerbations

Philip Molyneaux
London, UK.

The recent characterization of the respiratory microbiome in idiopathic pulmonary fibrosis (IPF) has suggested that an increased bacterial burden, and presence of specific organisms, could drive disease progression. This strengthens the epidemiological argument that environmental factors may be integral to the pathogenesis of IPF in genetically susceptible individuals. However, there remain a number of unanswered questions regarding the role of the microbiome in IPF including the effect of the MUC5B polymorphism and the optimal sampling modality to study a parenchymal disease process.

We will review the current understanding of the microbiome in fibrotic lung disease, and explore the evidence supporting the role of the microbiome in the pathogenesis and progression of IPF. We will then discuss how an understanding of the microbiome may help us to gain further insights into currently “non-infective” acute exacerbations of IPF.

WORKSHOP

Plasticity of the pulmonary microbiota over the spectrum of inflammation to immunosuppression

Eric Bernasconi
Lausanne, Switzerland.

The composition of a « core » pulmonary microbiota community in healthy subjects starts to emerge, with interactions between community members and functional redundancy believed to reinforce resistance to disturbance or support a return to community equilibrium, after being disturbed. However, in a range of disease states and treatment regimens, the magnitude and/or duration of disturbance in lower airways microenvironment is sufficient to trigger profound changes in community composition. Considering that the lower airways represent an ecosystem shared by the microbiota and host immune cells, e.g. alveolar macrophages, substantial disturbance in local conditions may be expected to impact upon both of these components in parallel. Accordingly, acute pneumonia or exacerbation in COPD, both eliciting strong type 1 host inflammatory responses, were reported to be typically associated with the outgrowth of Firmicutes or Gammaproteobacteria (e.g. Staphylococcus aureus and Pseudomonas aeruginosa, respectively).

In addition, the relative abundance of the Bacteroidetes genus Prevotella, associated with the healthy state in combination with Streptococcus and Veillonella, was strongly reduced in inflammation, suggesting these latter taxa do not adapt to the same microenvironmental conditions as the aforementioned pathogens. In a marked contrast, we found Prevotella, Streptococcus and Veillonella co-occurring and dominating in a large set of lung transplant recipients, reaching levels that exceeded the reported values for healthy subjects. In this setting, characterized by an abnormally low baseline inflammatory status due to constant exposure to immunosuppressive drugs aiming at controlling immune responses to the allograft, Staphylococcus and Pseudomonas were poorly represented, except in overt infection. Overall, the composition of the pulmonary microbiota appears to vary in concert with host inflammatory status over a range of clinical conditions, that reflect contrasting alterations in lower airways microenvironment.

Certainties and uncertainties in the respiratory microbiome

Meta-omics used to study the gut microbiota: from the bench to the computer

Chaysavanh Manichanh
Barcelona, Spain.

Functions of the gut microbiota affects many aspects of our systems physiology, ranging from processing and harvesting of nutrients from our diets, to shaping the features of our innate and adaptive immune system. Any factors that disturb this mutualism could result in diseases. Over the last decade, the limitations of culture-based methods have been overcome thanks to Next Generation Sequencing techniques, allowing us to understand the microbial gut community in greater depth through the study of microbial genes or full genomes, called metagenomics. To catalyse the field, the NIH and the European Commission launched, in 2008, the Human Microbiome and the MetaHIT Projects, respectively. These initiatives have allowed a deep characterization of the human gut microbiome in health and disease states. The human GI-tract harbours one of the most complex and abundant microbial ecosystems colonised by more than 100 trillion microorganisms, and the number of microbial genes is about 100 times higher than that of our own genes. Although stable across ages, the composition and functions of the microbiome may be influenced by a number of factors including genetics, mode of delivery, age, diet, geographic location and medical treatments. Alteration in the microbiome structure and function has been linked to inflammatory, functional and metabolic disorders such as IBD, IBS and obesity.

The gut microbiome in HIV infection

Roger Paredes
Barcelona, Spain.

The human intestinal microbiota is essential for human health and well-being and is driven by genetic, lifestyle and environmental factors. The precise effects of HIV-1 on the gut microbiome are unclear. Initial cross-sectional studies provided contradictory associations between microbial richness and HIV serostatus and suggested shifts from Bacteroides to Prevotella predominance following HIV-1 infection, which have not been found in animal models or in studies matched for HIV-1 transmission groups. We demonstrate in two independent cohorts of HIV-1-infected subjects and HIV-1-negative controls in Europe that gay men often have a distinct composition of the human fecal microbiota, with increased microbial richness and diversity and enrichment in the Prevotella enterotype. This is independent of HIV-1 status, and with only a limited contribution of diet effects. After accounting for sexual orientation, however, HIV-1 infection remains associated with reduced bacterial richness, more so in subjects with suboptimal CD4+ T-cell count recovery under antiretroviral therapy. Our findings indicate that all studies of HIV-microbiota relationships should carefully investigate possible confounding or effect modification by sexual orientation, injection drug use, and demographics. They also suggest interventions on gut bacterial richness as possible novel avenues to improve HIV-1-associated immune dysfunction.

SYMPOSIUM

The respiratory microbiome: a new frontier in medicine

Eduard Monsó
Sabadell, Spain.

Bronchial colonization by potentially pathogenic bacteria is common in stable chronic obstructive pulmonary disease (COPD), and in about a half of patients positive cultures for Haemophilus influenzae, Streptococcus pneumoniae and Moraxella catarrhalis are found during stability periods. However available culture techniques are not suitable for the identification of an important part of the bacterial flora inhabitant of the respiratory mucosa. Most bacteria present do not grow adequately on commonly used selective cultures, and are masked by the presence of other faster-growing bacteria.
Through independent culture techniques, such as amplification and sequencing, it is possible to determine the composition of bronchial microbiome, both bronchial and viral, defining the relationships between the colonizing flora and MPOC. The use of these techniques in bronchial secretions has shown the existence of a diverse microbiome in COPD, and a predominant presence of bacteria from the phylum Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes.
The bacterial flora observed in patients with COPD alters the pattern of continuity observed in the oropharynx and the bronchial tree of normal subjects, through a partial disappearance of microorganisms common in the healthy population. The magnitude of these changes parallels the severity of the disease, with a relative increase in the proportion of Proteobacteria in severe patients, inversely correlated with the decrease of Firmicutes in advanced COPD. Thus, the bacterial diversity in the respiratory tract appears to be clearly higher in the healthy subject and the patient with mild-moderate COPD, to be replaced in severe COPD by a narrow range of microorganisms, with a high proportion of potentially pathogenic bacteria. In patients with severe COPD, frequent colonization by Pseudomonas aeruginosa does not determine a change in the bronchial flora as a whole, which has a similar spectrum in all patients with advanced disease, independently of their colonizing patterns.

The study of the bacterial flora in COPD exacerbations have shown increases in specific bacterial genera in most patients, which include, in most cases, one or more potentially pathogenic species. These increases in many cases are not identified by culture, a finding that has confirmed the insufficient sensitivity of conventional techniques for identifying causative pathogens in COPD. This approach have shown that the bacterial pattern in exacerbations from patients colonized by Pseudomonas aeruginosa, when identified from independent culture techniques, are similar to patterns found in non-colonized patients. This supports the hypothesis that microorganisms causing exacerbations in Pseudomonas-colonized COPD patients are not different from the pathogens related to bronchial infections in non-colonized patients, and the colonizing role of Pseudomonas aeruginosa continues, both in stability periods and in exacerbations. These findings from microbiome analysis have potential implications for recommendations in therapeutic guidelines.
One additional advantage of the identification of the composition of respiratory microbiome by culture-independent techniques in respiratory samples is the characterization of the functional features of the bacterial genome, through metagenomics. This approach has shown that in COPD exacerbations the functional activity of the background flora may change, in spite of the absence of changes in their composition, with increases in the abundance of genes involved in carbohydrate metabolism and carcinogenesis, and parallel decreases in genes related to cell growth and catabolism.
Thus, the introduction of microbiological identification techniques not based on culture has demonstrated that the diversity of bacterial flora is reduced with advanced COPD, with an overrepresentation of potentially pathogenic microorganisms. In exacerbations, 16S rRNA gene sequencing have identified causal pathogenic bacteria not recovered through cultures, and functional changes in bacterial flora with potential clinical significance.

The microbiome in respiratory medicine

Understanding the microbiome: How can we determine/analyse it? Technical issues

Vicente Pérez Brocal
Valencia, Spain.

The microbiome harboured in the human respiratory tract represents a complex ecosystem, consisting of more than 600 species, and subject to environmental fluctuations and changes. Determining the diversity of such an elusive community has been a permanent challenge for researchers until the advent of metagenomics contributed to circumvent it. However, these culture-independent approaches are not exempt from limitations, not only in the experimental steps, but also during the bioinformatic analyses arising from the huge amount of data generated. Those have to be suitably processed in order to extract proper conclusions, therefore avoiding erroneous interpretations of the results. Here, the relevance of choosing an appropriate database is set out. Moreover, after outlining the initial processing steps, the problems of chimeras and their removal are addressed. Finally, other shortcomings of the metagenomic approach are also tackled and suggestions to avoid them in order to reliably assign taxonomy are suggested. The main conclusion is that there are still technical issues associated with the biological limitations inherent to the data themselves and the databases that bioinformatic tools alone cannot easily discriminate, despite our efforts. Therefore, we must be cautious when it comes to drawing conclusions, since results represent usually a trade-off between their accuracy and the necessity to present them unambiguously.

"Sala La Lengua": Study of human mouth microbiome as a large citizen science project

Julia Ponomarenko
Barcelona, Spain.

Sala La Lengua is a citizen science project studying the human mouth microbiome. Bacterial profiles of over 1500 school children across Spain were obtained using 16S rRNA sequencing and analyzed in relation to their diet, mouth hygiene, geographic area and other environmental characteristics and lifestyle. In my talk I will discuss the results of the project and also bioinformatics challenges we have encountered undertaking the project.

SYMPOSIUM

The Dynamics of the Lung Microbiome during Health and Disease

Gary Huffnagle
Ann Arbor, USA.

The macro- and micro-anatomic features of the lungs are very much distinct from that of the gastrointestinal tract. The factors that regulate microbial residence, growth and metabolism in the lungs will, therefore, be very different from those in the GI tract. In the past decade, culture-independent techniques of microbial identification have revealed a previously unappreciated complexity to the microbial ecosystem of the lungs. Numerous studies have shown that the airways are not sterile and the composition of the lung microbiome is determined by the balance of three factors: (1) microbial immigration into the airways from the nose, mouth and air, (2) elimination of microbes from the airways, and (3) the relative reproduction rates of its community members, as determined by regional growth conditions. Any change in the microbiome - within an individual or across disease states - must be due to some perturbation in these factors. Thus, the microbiome of the lungs can significantly change during disease, which has potential significant implications for respiratory disease pathogenesis and therapeutic interventions.

Microbiome in COPD: Pitfalls and Progress

Sanjay Sethi
Buffalo, USA.

Microbiome studies of respiratory secretions have provided exciting new observations regarding the bacterial causation of exacerbations of COPD, and the impact of treatment of the exacerbation on the airway microbiome. Bacterial exacerbations likely represent abrupt major changes in the microbiome with resultant large increases in airway and systemic inflammation. Microbiome studies could lead to discovery of new pathogens that have been difficult to obtain with standard culture techniques. The Vicious Circle Hypothesis embodies the likely contribution of an altered microbiome to COPD progression, with an unhealthy microbiome driving the inflammatory process in stable COPD. While studies with conventional microbiology found potential pathogenic bacteria to be virtually absent in bronchoscopic samples in healthy controls, smoking and development of COPD was associated with 35–50% incidence of isolation of pathogenic bacteria. In contrast, recent microbiome studies have found no or minor differences from controls in smokers and COPD. These contradictory findings could be explained by the extreme sensitivity of the microbiome technique to upper airway contamination. Several challenges need to be tackled to fully utilize the benefits of microbiome research in COPD. Paramount is the issue of upper airway and environmental contamination of lower airway samples. Significant concentration thresholds in microbiome data need to be defined. The microbiome differs in sputum, bronchoalveolar lavage and lung parenchyma samples obtained from patients with COPD. Determination as to which of the various new microbes that will be identified in microbiome studies are pathogenic is still unclear, especially for unculturable pathogens. Though various obstacles need to be surmounted, ultimately lung microbiome studies will provide new insights in to how infection contributes to COPD.

The microbiome in respiratory medicine

Microbial dysbiosis in bronchiectasis and cystic fibrosis.

James Chalmers
Dundee, UK.

Bacterial infection is central to our understanding of the pathophysiology of bronchiectasis and cystic fibrosis. Traditional culture based microbiology techniques have revealed the importance of well characterised pathogens such as Haemophilus influenzae, Pseudomonas aeruginosa and Moraxella catarrhalis in bronchiectasis, and Staphylococcus aureus and Burkholderia cepacia in cystic fibrosis among many others. The management of these diseases and drug development efforts have been largely devoted to antimicrobial therapies targeting these pathogens. P. aeruginosa represents a special case, having been shown to impact prognosis in both diseases and being both frequently multidrug resistant and difficult to eradicate.

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SYMPOSIUM

Host-microbe interplay sets the lower airway microenvironment in lung transplantation

Eric Bernasconi
Lausanne, Switzerland.

The lower airways can be considered as an ecosystem, whose equilibrium post-transplantation conditions graft survival. Due to long-term use of immunosuppressive and antibiotic therapy, the transplanted lung offers particular microenvironmental conditions. Despite prophylactic and therapeutic regimens, strong disturbance in local conditions may be observed. In particular, inflammatory episodes are elicited, most often by lower airway infections and during the first months post-transplant. Later, the onset of bronchiolitis obliterans syndrome, a clinical manifestation of chronic lung allograft dysfunction (CLAD) associated with obstructive fibrotic airway remodeling, has also been linked to host-microbe interactions, through pathogen-driven inflammatory triggers and/or impaired host innate responses affecting bacterial clearance. Hence, a concept of growing interest suggests that host-microbe interactions in lower airways are involved in controlling resistance to disturbance, or supporting a return to equilibrium. Correspondingly, microbiota dysbiosis may reflect a substantial disturbance in local conditions. Our work allowed us to determine the microbiota composition based on 16S ribosomal RNA analysis in a total of 203 bronchoalveolar lavages obtained from 112 patients, up to 12 months post-lung transplantation. Host cellular gene expression profiles were characterized in parallel, by quantifying expression of a set of genes involved in prototypic macrophage functions. We found that the characteristics of the pulmonary microbiota align with distinct innate cell gene expression profiles. While a non-polarized activation was associated with bacterial communities consisting of a balance between pro-inflammatory (e.g. Staphylococcus and Pseudomonas) and low stimulatory (e.g. Prevotella and Streptococcus) bacteria, “inflammatory” and “remodeling” profiles were linked to bacterial dysbiosis. Mechanistic assays provided direct evidence that bacterial dysbiosis could lead to inflammatory or remodeling profiles in macrophages, while a balanced microbial community maintained homeostasis. We conclude that host-microbe interactions determine the lower airway microenvironment post-lung transplantation, and consequently could impact upon graft survival.

The microbiome in respiratory medicine

Lesson from the human gut microbiome

Chaysavanh Manichanh
Barcelona, Spain.

Functions of the gut microbiota affects many aspects of our systems physiology, ranging from processing and harvesting of nutrients from our diets, to shaping the features of our innate and adaptive immune system. Any factors that disturb this mutualism could result in diseases. Over the last decade, the limitations of culture-based methods have been overcome thanks to Next Generation Sequencing techniques, allowing us to understand the microbial gut community in greater depth through the study of microbial genes or full genomes, called metagenomics. To catalyse the field, the NIH and the European Commission launched, in 2008, the Human Microbiome and the MetaHIT Projects, respectively. These initiatives have allowed a deep characterization of the human gut microbiome in health and disease states. The human GI-tract harbours one of the most complex and abundant microbial ecosystems colonised by more than 100 trillion microorganisms, and the number of microbial genes is about 100 times higher than that of our own genes. Although stable across ages, the composition and functions of the microbiome may be influenced by a number of factors including genetics, mode of delivery, age, diet, geographic location and medical treatments. Alteration in the microbiome structure and function has been linked to inflammatory, functional and metabolic disorders such as IBD, IBS and obesity.

The gut microbiome in HIV infection

Roger Paredes
Barcelona, Spain.

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Outcomes

Intended publication of a position paper on the topic of "The Microbiome in Respiratory Medicine" in the European Respiratory Journal. http://erj.ersjournals.com/

On the BRN website, you will find all the necessary information related to the holding of the meeting. That includes videos of the talks, abstratcs of the presentations, speaker's CVs, images, scientific documents, press documentation and other related materials.
We invite you to visit it on http://brn.cat/microbiome2016/

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PULMONARY PERSPECTIVE
Personalized Respiratory Medicine: Exploring the Horizon, Addressing the Issues
Summary of a BRN-AJRCCM Workshop Held in Barcelona on June 12, 2014
Personalized Respiratory Medicine – 2014
05. Introduction to Network Medicine
Introduction to Network Medicine
Edwin K. Silverman, M.D., Ph.D.

Organizers

BARCELONA RESPIRATORY NETWORK
Collaborative research

Barcelona Respiratory Network (BRN) is a network of different institutions based in Barcelona that seeks to promote cutting-edge translational research in Respiratory Health. Founded in 2012 as a non-profit organization, its Board of Trustees is composed of researchers from prestigious university hospitals and research centres in the field of respiratory health, pharmaceutical & healthcare companies, and civil society organizations. Therefore, BRN is a clear example of private-public partnership. BRN's mission is to strengthen and streamline research and innovation in the field of respiratory health, in order to improve the quality of life and well-being of patients and the population in general. BRN encompasses the basic, preclinical, and clinical research performed in the field of respiratory health by 7 major tertiary hospitals (H. Clinic, H. Bellvitge, H. Sant Pau, H. Mar, H. Germans Trias i Pujol, H. Parc Taulí, H. Arnau de Vilanova), an epidemiological research centre (CREAL) and 7 companies (Aldo-Unión, Astra Zeneca, Boehringer Ingelheim, Esteve, Esteve-Teijin, Ferrer, Leti and Linde Medicinal). BRN members hold a high degree of expertise in COPD, Asthma, Pulmonary Hypertension, Mechanical Ventilation, Interstitial Lung Diseases, and Sleep Apnea, among other respiratory research topics. BRN has recently launched two initiatives such as:

BRN Reviews: an official journal of Barcelona Respiratory Network. An online, open access, quarterly journal that publishes cutting-edge, high quality, internationally authored reviews on timely topics in respiratory medicine, with an emphasis on their translational aspects. More information at: http://www.brnreviews.com/

BRN Seminars: thematic scientific meetings specifically created to foster scientific debate, to share the latest advances and ideas and to explore opportunities for new collaborative projects in the field of respiratory health. BRN SSeminars are open to all interested researchers, either clinical, basic or involved in industrial research. More information at: http://brn.cat/brn-seminars/
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The Centre for Genomic Regulation (CRG) is an innovative centre for basic research created in December 2000 by an initiative of the former Department of Universities, Research and Information Society (DURSI) of the Catalan Government. CRG is legally constituted as a non- profit foundation with the participation of the Catalan Government through the Economy and Business Department, the Health Department, the Pompeu Fabra University (UPF) and the Spanish Ministry of Economy & Competitiveness (MINECO). On this basis, CRG performs as a full member of the Barcelona Biomedical Research Park (PRBB), one of the scientific parks in the city, which is physically connected to the Hospital del Mar.

CRG believes in the fundamental value of scientific knowledge and that the medicine and biotechnology of the future depends on the ground-breaking science of today. As an essential platform to achieve that, CRG offers its scientists a cutting-edge environment that allows them to focus on top-level interdisciplinary research into the complexity of life.
Since 1st July 2015, CRG has integrated the National Centre for Genomic Analysis (CNAG-CRG). CNAG-CRG was created to carry out projects in DNA sequencing and analysis in collaboration with local researchers from Catalonia and Spain as well as with international researchers, to ensure Spanish competiveness in the strategic area of genomics.

Visit http://www.crg.eu/
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Biomedical Research Networking Center (CIBER) is a Public Research Consortium created in 2006 under the leadership of the Carlos III Health Institute (ISCIII, the main Public Research Entity responsible of funding, managing and carrying out biomedical research in Spain), to promote research excellence and build a critical mass of researchers in the field of Biomedicine and Health Sciences. Organized in 8 research areas including Bioengineering, Biomaterials and Nanomedicine (CIBERBBN), Mental Health (CIBERSAM), Hepatic Diseases (CIBEREHD), Diabetes and Metabolic Diseases (CIBERDEM), Rare Diseases (CIBERER), Respiratory Diseases (CIBERES), Public Health and Epidemiology (CIBERESP) and, Obesity and Metabolic Diseases (CIBEROBN), CIBER is the largest Research Network in Spain.

CIBER numbers with more than 830 dedicated employees and 4,000 associated researchers integrated in 399 leading-edge research groups. Thanks to its network structure, CIBER is capable of bringing together more than 92 different associated institutions including Hospitals, Research Centers, Technology Centers, Private Institutions and Universities selected to join CIBER consortium on the basis of their excellence.
CIBERES is the area of CIBER devoted to respiratory diseases, with 10 specific research lines regarding, Lung Cancer, Acute lung Injury (ALI), Asthma, Chronic Obstructive Pulmonary Disease (COPD), Pathogen-Host Interaction, Pneumonia, Pulmonary Fibrosis, Sleep Apnea, Tuberculosis and Pulmonary Hypertension. CIBERES brings together 33 leading-edge Spanish research groups in the area of respiratory diseases selected on the basis of scientific excellence.

Sociedad Española de **Neumología y Cirugía Torácica** SEPAR

The Spanish Society of Pneumology and Thoracic Surgery (SEPAR) is the scientific society bringing together more than 3,600 respiratory health professionals in Spain, including pulmonologists, thoracic surgeons, and other specialists, both national and international, who all share common interests. The SEPAR goal is to work on scientific projects that advance pulmonology and thoracic surgery and to perform respiratory health initiatives that positively impact society.

Main Partners

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Menarini is an international pharmaceutical group with over 125 years of history that is present in over 100 countries around the world. Grupo Menarini España is one of the strategic subsidiaries of the group, with a production of more than 60 million units of medicines per year and employs about 700 workers. Based in Badalona, with an area of 15,000 m2, it includes the production plant and one of the six R&D centres that Menarini International Group has in Europe. Menarini, present in Spain for 55 years, ranks among the top 20 pharmaceutical companies in the Spanish sector.

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Collaborators

AstraZeneca

AstraZeneca's mission is to make a meaningful difference to healthcare through great medicines. AstraZeneca's vision is to be a global biopharmaceutical business delivering great medicines to patients through innovative science and excellence in development and commercialization. Everything they do at AstraZeneca is driven by its commitment to improving the lives of patients. Whether that's working to reach more people with their medicines, or applying their science skills to developing the next generation of treatments, or collaborating with others in the fight against disease – everything centers on understanding and meeting the needs of people facing serious health challenges. They focus primarily on cancer, cardiovascular / metabolic disease and respiratory, inflammatory and autoimmune disease.

Chiesi

People and ideas for innovation in healthcare

Chiesi Farmaceutici is a multinational pharmaceutical company based in Parma (Italy) created in 1935 and R&D oriented, investing the 18% of its turnover in the last years. It has a strong presence in Europe and worldwide (United States, Brazil, Mexico, Pakistan, China, Russia).

LETI

Laboratorios LETI, SL Unipersonal (LETI) is a biopharmaceutical research company founded in Barcelona in 1919. Today the company is one of the first four World Laboratories in the field of allergen based immunotherapy. In addition immunology, preventive medicine and biological products are key pillars of the Company.
The headquarters is located in Barcelona and its Industrial Plant and Research Laboratory are located in Tres Cantos (Madrid), where individualized allergy vaccines are produced. The Company has subsidiaries in Germany, Portugal and the United States, and exclusive distributors in several countries of Europe, Latin America and Africa. LETI invests between 15-20% of its turnover in R&D. LETI collaborates with research centers, universities and hospitals in numerous countries.

NOVARTIS

Novartis is a world-leading healthcare company that operates in 140 countries, with global headquarters in Basel, Switzerland and with more than 135,000 associates. Novartis mission is to care and cure and for that his objective is to discover, develop and successfully market innovative products to prevent and cure diseases, to ease suffering, and to enhance quality of life.

Notes

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With the support of:

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