Sociedad Española
de **Neumología**
y **Cirugía Torácica**
SEPAR
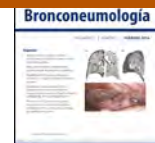
ARCHIVOS DE
Bronconeumología

www.archbronconeumol.org

Bronconeumología

Original Article

Core Microbiota in Central Lung Cancer With Streptococcal Enrichment as a Possible Diagnostic Marker

Salvador Bello [a,*], José J. Vengoechea [a], Manuel Ponce-Alonso [b], Ana L. Figueredo [a], Elisa Mincholé [a], Antonio Rezusta [c], Paula Gambó [d], Juan Manuel Pastor [e], Javier Galeano [e], Rosa del Campo [b,f]

[a] Department of Pulmonary Medicine, Miguel Servet University Hospital, CIBERES, Instituto de Investigación Sanitaria (ISS) Aragón, Zaragoza, Spain
[b] Department of Microbiology, Ramón y Cajal Health Investigation Institute (IRYCIS), Ramón y Cajal University Hospital, Madrid, Spain
[c] Department of Microbiology, Miguel Servet University Hospital, Instituto de Investigación Sanitaria (ISS) Aragón, Zaragoza, Spain
[d] Department of Pathology, Miguel Servet University Hospital, Instituto de Investigación Sanitaria (ISS) Aragón, Zaragoza, Spain
[e] Complex Systems Group, Universidad Politécnica de Madrid, Madrid, Spain
[f] University Alfonso X El Sabio, Villanueva de la Cañada, Madrid, Spain

A R T I C L E   I N F O

A B S T R A C T

Background: Dysbiosis in lung cancer has been underexplored. The aim of this study was to define the bacterial and fungal microbiota of the bronchi in central lung cancer and to compare it with that of the oral and intestinal compartments.
Methods: Twenty-five patients with central lung cancer and sixteen controls without antimicrobial intake during the previous month were recruited. Bacterial and fungal distribution was determined by massive sequencing of bronchial biopsies and saliva and faecal samples. Complex computational analysis was performed to define the core lung microbiota.
Results: Affected and contralateral bronchi of patients have almost identical microbiota dominated by *Streptococcus,* whereas *Pseudomonas* was the dominant genera in controls. Oral and pulmonary ecosystems were significantly more similar in patients, probably due to microaspirations. Streptococcal abundance in the bronchi differentiated patients from controls according to a ROC curve analysis (90.9% sensitivity, 83.3% specificity, AUC = 0.897). The saliva of patients characteristically showed a greater abundance of *Streptococcus, Rothia, Gemella* and *Lactobacillus*. The mycobiome of controls (*Candida*) was significantly different from that of patients (*Malassezia*). Cancer patients' bronchial mycobiome was similar to their saliva, but different from their contralateral bronchi.
Conclusions: The central lung cancer microbiome shows high levels of *Streptococcus*, and differs significantly in its composition from that of control subjects. Changes are not restricted to tumour tissue, and seem to be the consequence of microaspirations from the oral cavity. These findings could be useful in the screening and even diagnosis of this disease.

Núcleo de microbiota en el cáncer de pulmón central con enriquecimiento estreptocócico como posible marcador de diagnóstico

R E S U M E N

Antecedentes: La disbiosis en cáncer pulmonar no ha sido suficientemente estudiada. Los objetivos de este estudio fueron definir la microbiota bacteriana y fúngica de bronquios con cáncer central de pulmón, y compararla con la del compartimento intestinal en heces y saliva.

Métodos: Se reclutaron 25 pacientes con cáncer central de pulmón y 16 controles sin exposición antibiótica durante el mes anterior. Se determinó la composición de bacterias y hongos en biopsias de bronquio, saliva y heces. Se realizó un análisis computacional para definir el núcleo de microbiota del pulmón.

Resultados: Los bronquios afectados y contralaterales de pacientes presentaron una microbiota similar dominada por *Streptococcus*, mientras que *Pseudomonas* destacó en los controles. Los ecosistemas orales y pulmonares fueron significativamente más parecidos en pacientes, probablemente debido a microaspiraciones. La abundancia bronquial de estreptococos permitió diferenciar a los pacientes de los controles mediante una curva ROC (90,9% de sensibilidad, 83,3% de especificidad, AUC = 0,897). La saliva de los pacientes presentó mayor abundancia de *Streptococcus, Rothia, Gemella* y *Lactobacillus*. El micobioma de los controles *(Candida)* fue significativamente diferente al de los pacientes *(Malassezia),* con los bronquios afectados por el cáncer similares a su saliva, pero diferentes de sus bronquios contralaterales.

Conclusiones: En el cáncer de pulmón central hay enriquecimiento de *Streptococcus*, y su composición es significativamente diferente de sujetos control. Las alteraciones no se limitan al tejido tumoral, y parecen ser consecuencia de microaspiraciones desde la cavidad oral. Estos hallazgos podrían ser útiles para la detección e incluso el diagnóstico de esta patología.

Background

Culture-independent techniques have revealed the composition of a stable microbiota within the distal airways; and although normality criteria have not yet been established, atypical compositions linked to certain respiratory diseases have been detected.[1] Dysbiosis is typically detected surrounding tumours tissues, and this trait has been poorly explored in the respiratory airway tissues,[2,3] mostly using surgical samples.[4–8] An understanding of the microbiota is necessary to decipher its possible causal role in cancer, and also to elucidate the prognosis and response to immunomodulatory therapies, because microorganisms and/or their metabolites shape the local microenvironment, influence the immune response, and impact the final battle against cancer.[9] Finally, the presence and/or abundance of specific bacteria could be used as a biomarker, as occurs with *Streptococcus gallolyticus* sbsp. *gallolyticus* in the colorectal cancer.[10]

The aims of the present study were to define the bacterial and fungal microbiota of central lung cancer, in relation to the corresponding contralateral bronchus and compared with controls without cancer history, also defining the core of those microbiotas; and to correlate the pulmonary microbiota in lung cancer with the salivary and faecal compartments.

Methods

Patients and samples

Twenty-five patients (24 men, mean age of 68 years) diagnosed with central lung cancer, directly visible and biopsied by bronchoscopy, were recruited (Table 1). The exclusion criteria included the intake of antibiotics, pre or probiotics, and systemic corticoids during the previous 4 weeks; acute infection; radio or chemotherapy in the last year, and immunodeficiency. Tumours were histologically classified into non-small cell lung cancer ($n = 18$, including 10 squamous, 4 adenocarcinoma, and 4 undifferentiated); and small cell lung cancer ($n = 7$). Each patient contributed with 4 samples: (i) saliva collected from a rinse with sterile distilled water, prior to the bronchoscopic procedure, (ii) biopsies of affected, and (iii) contralateral bronchi, and finally (iv) a faecal sample (provided by 18 out of the 25 patients). The nasal route, or oral if not possible, with local instillation of lidocaine were used for bronchoscopies. Prior to the tumour sampling, biopsies for microbiota determination were obtained from contralateral non-affected tissues.

Simultaneously, 16 controls without history of cancer were included, and each contributed the following: (i) oral microbiota and (ii) a single biopsy of their healthy bronchi. Controls without respiratory symptoms (except 2 with chronic cough) underwent bronchoscopy for non-cancer related indications (benign tracheal stenosis 9, fake haemoptysis 3, chronic cough 2, control of a previous endobronquial hamartoma resection 1, and dyspnoea 1), and all of them had normal spirometries. All samples were immediately frozen at −80 °C after collection.

Microbiota composition

Total DNA was obtained by the QiaAmp kit (Qiagen) from the biopsies, from the pellet of saliva after centrifugation, and from 200 μl aliquots of a solution of 0.5 gr of faeces in 5 ml of water. Bacterial composition was determined by PCR amplification of the 16S rDNA V3-V4 region using published primers,[11] whereas the mycobiome was only analyzed in bronchial and saliva of the 16 controls and in a subset of 6 patients by amplification of the ITS-1 region.[12] PCR products were submitted to massive sequencing (2× 300 bp) on a MiSeq (Illumina, San Diego, CA, USA) platform, at FISABIO (Valencia, Spain). Raw sequence data were deposited in GenBank (BioProjects PRJNA586753 and PRJNA586768. QIIME2 software suite (2019.1 distribution)[13] and LEfSE[14] were used for analysis, and adequate negative sequencing controls were added in each process and run. A computational analysis has developed to define the microbiota core that was present in at least 95% of the individuals. This analysis is available at https://github.com/JJ-Lab/Cancer_Lung_Microbiota website.

Results

Sample filtering

Three samples from affected bronchi (patients 23, 24, and 25) and four samples from control bronchi (controls 2, 3, 7, and 16) were excluded of the analysis since they did not reach minimal sequencing depth requirements (>1000 reads/sample).

Alpha diversity

Alpha diversity indexes, as Chao1 and Shannon, express the mean diversity in microbial species in a single community, with the highest values corresponding to the greater number of species (richness). Faeces and saliva had similar alpha diversity values, while bronchi were significantly more diverse according to

Table 1

Demographic and clinical data of patients with lung cancer and healthy controls, differentiating between the entire control population ($n = 16$) and those included in the analysis of bacteria and fungi ($n = 12$).

	Cases (n = 25)	Controls (n = 16)	p-Value	Controls (n = 12)	p-Value
Age (years mean ± SD)	67.7 (12.8)	51 (14.4)	**.0003**	52.1 (14.9)	**.002**
Male (%)	24 (96%)	8 (50%)	**.002**	6 (50%)	**.003**
Active smoker (%)	14 (56%)	3 (19%)	**.02**	1 (8.3%)	**.01**
Exsmoker (%)	11 (44%)	4 (25%)	.3	4 (33.3%)	.7
Never smoked (%)	0 (0%)	9 (56%)	**<.001**	7 (58.3%)	**<.001**
Familiar cancer (%)	8 (32%)	11 (69%)	**.04**	8 (66.6%)	.07
Other cancer (%)	2 (8%)	3 (19%)	.3	3 (25%)	.3
Hypertension (%)	13 (52%)	3 (19%)	.05	3 (25%)	.1
Diabetes (%)	9 (36%)	0 (0%)	**.006**	0 (0%)	**.01**
Dyslipemia (%)	16 (64%)	5 (31%)	.08	5 (41.6%)	.1
Annual visit to dentist	3 (12%)	10 (63%)	**.001**	8 (66.6%)	**.001**
Lack of teeth	21 (84%)	12 (75%)	.6	9 (75%)	.6
COPD (%)	12 (48%)	0 (0%)	**.001**	0 (0%)	**.003**
Mild (FEV₁ > 80%)	3 (25%)	–		–	
Moderate (FEV₁ 50–79%)	8 (67%)	–		–	
Severe (FEV₁ < 50%)	1 (8%)	–		–	
Asthma (%)	0	0		0	
Interstitial lung disease	0	0		0	
Respiratory function test					
Normal (%)	10 (40%)	16 (100%)	**<.001**	12 (100%)	**<.001**
Obstructive (%)	7 (28%)	0 (0%)	**.03**	0 (0%)	.07
Mixed (%)	5 (20%)	0 (0%)	.1	0 (0%)	.1
FEV mean ± SD	73.4 (16.6)	97.6 (11.4)	**<.001**	96.6 (12.0)	**<.001**
FCV mean ± SD	89.3 (14.3)	103.7 (10.7)	**.001**	101.9 (10.3)	**.01**
FEV/FCV mean ± SD	73.2 (12.9)	78.7 (5.3)	.1	79.8 (8.4)	.2

Statistically significant values that can differentiate control cases are highlighted in bold.

Faith's PD index (which specifically assessed phylogenetic diversity) (Fig. 1). The diversity of saliva was comparable in patients and controls.

Microbiota composition

Beta diversity analysis compares the microbiota composition between different samples, and use to be referred to *phyla* (Fig. 2) and genera levels (Fig. 3). Patients' saliva presented a higher density of *Firmicutes* and *Actinobacteria* to the clear detriment of *Proteobacteria*, and this pattern was reproduced in the affected and contralateral bronchi. Faecal samples had a remarkably high proportion of *Firmicutes* (>75%) (Fig. 2).

Saliva

Up to 213 genera were detected in saliva samples, with 13 comprising the majoritarian in both controls and patients: *Streptococcus* (19% and 23%, respectively), *Prevotella* (15–13%), *Rothia* (4–7%), *Veillonella* (7–8%), *Neisseria* (6–4%), *Porphyromonas* (6–4%), *Haemophilus* (6–2%), *Gemella* (3–5%), *Fusobacterium* (5–3%), *Alloprevotella* (4–1%), *Actinomyces* (2–2%), *Granulicatella* (1–2%), and *Leptotrichia* (1–2%). The remaining genera represented less than 1% of the total microbiota abundance.

Saliva microbiota core from patients and controls were similar, and although significant differences on the bacterial proportions were detected (Figs. 3 and 4, and supplementary material), that differences are linked to relative abundance of common taxa and no to presence or absence of specific taxa. *Streptococcus, Rothia, Gemella* and *Lactobacillus* abundances are capable of distinguishing the saliva of patients from controls.

Bronchi

More than 450 bacterial genera were identified with different distribution among patients and controls, while the microbiota of the cancer-affected bronchus was almost identical to its contralateral counterpart (Figs. 3 and 4, and Supplementary material).

Particularities depending on the histological type of cancer were not detected.

Lung cancer-related genera were *Streptococcus* (19% in affected bronchus and 24% in contralateral), *Prevotella* (9–9%), *Blautia* (5–4%), *Veillonella* (4–5%), *Rothia* (3–4%), *Neisseria* (2–3%), *Gemella* (2–2%), and *Porphyromonas* (2–2%). Health bronchi from control group was dominated by *Pseudomonas* (21%), followed by *Streptococcus* (8%), *Actinobacter* (6%), *Veillonella* (4%), *Prevotella* (3%), *Delftia* (3%), *Janthinobacterium* (3%), *Escherichia-Shigella* (2%), *Haemophilus* (2%), and *Neisseria* (2%).

Streptococcal abundance characterizes the microbiota of patients

Streptococcus is a genus within the *Firmicutes* phylum that has a complex taxonomy. Our results showed that bronchial streptococcal abundance systematically distinguished patients from controls Up to 95 different amplicon sequence variants (ASV) assigned to *Streptococcus* were identified among all samples, and their particular distribution allowed us to hypothesize an exchange between oral and bronchial ecosystems (Supplementary material) Faeces were the most remote niche, with saliva and bronchi also distant. It is important to note that >95% of the ASV detected in the patients' bronchi were also present in their saliva, while on the contrary only 36% from saliva were also in the lung, indicating that saliva is the main source of pulmonary *Streptococcus*. An ROC curve representing *Streptococcus* bronchial abundance distinguished patients from controls, particularly when a relative abundance > 14.6% predicted lung cancer with 90.9% sensitivity and 83.3% specificity (AUC = 0.897) (Fig. 5). Unfortunately, this result was not reproduced in saliva.

The comparison of the whole microbiota in samples within each individual revealed that saliva and bronchi of patients with lung cancer were significantly the closest ecosystems ($p < 0.001$) (Fig. 5).

Mycobiome composition

Fungal characterization through ITS1 sequencing was determined in the 16 controls (saliva and bronchus) and a subset of
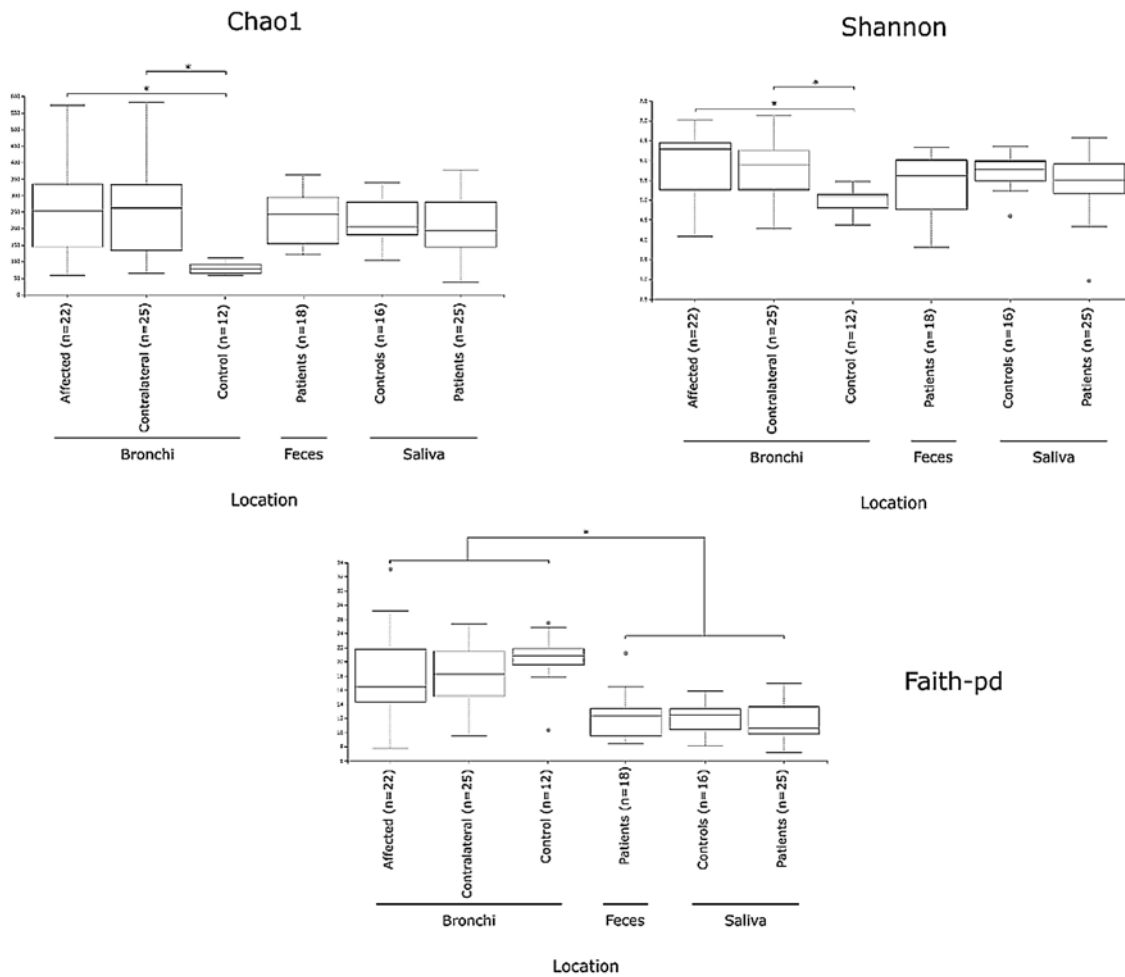
Fig. 1. Analysis of alpha diversity by sample location using Chao1, Shannon, and Faith's PD indexes. Significant differences ($p < 0.05$) between groups are highlighted by asterisks.
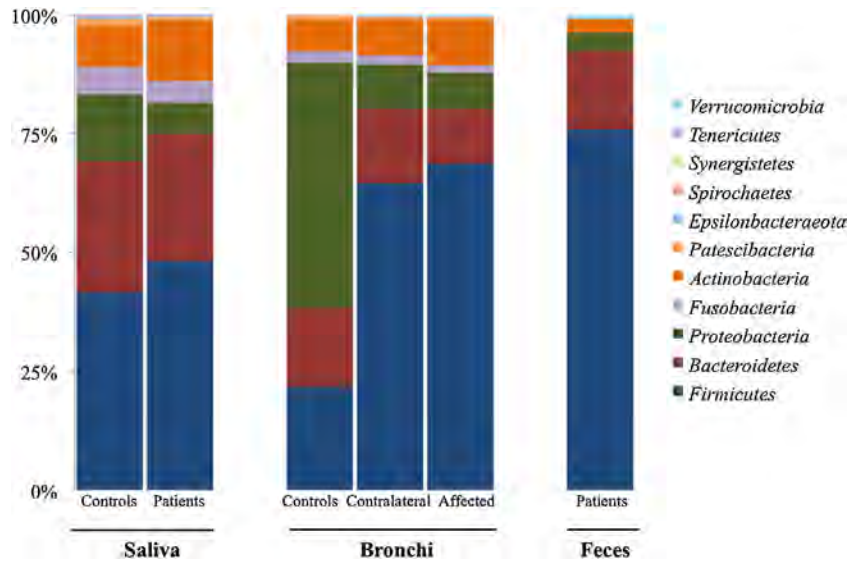
Fig. 2. Bacterial *phyla* distribution in each sample showing a high abundance of *Firmicutes* in lung cancer patients, combined with lower proportions of *Proteobacteria*.
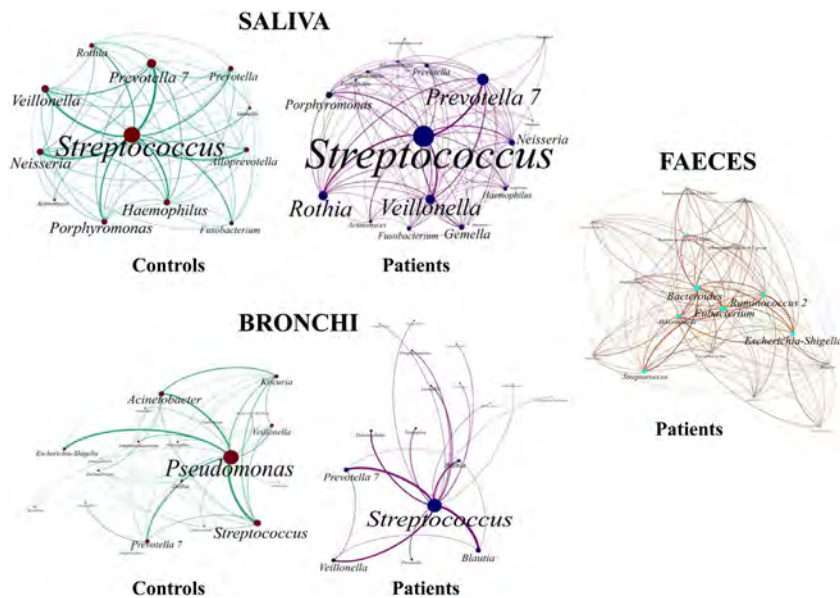
Fig. 3. Microbiota core of the most abundant genera accounting for 90% of total. The abundance of each genus is represented by the circle size and the numbers of connections were determined by their frequency.

6 patients (saliva, affected and contralateral bronchi). Regarding to alpha diversity, differences in Chao1 index were not observed, whereas Shannon was significantly higher in affected bronchi from patients compared to controls ($p < 0.03$) in accordance with the results obtained for bacteria (Supplementary material).

Affected bronchi and saliva from patients have similar fungal composition, but different from both their contralateral bronchi ($p < 0.006$), and the bronchi ($p < 0.001$) and saliva ($p < 0.007$) from controls. This trend supports the aforementioned bacterial findings, which also suggest an interconnection between both anatomical ecosystems in lung cancer patients. A differential abundance analysis between affected bronchi from patients and from controls showed an enrichment of *Malassezia* in patients, whereas controls had a higher abundance of *Candida* (Fig. 6).

Discussion

The role of microbiota in carcinogenic processes has not yet been elucidated and will probably be different for each tumour and localization. Recent data clearly indicate that the microbiota contributes to the prognosis of cancer and determines the response to treatments, particularly responses to the new immunomodulation therapies.[9,15] Criteria for the normal composition of lung microbiota have not yet been established, but the available data indicate that their composition in cancer patients differs considerably from that of healthy individuals.[2–8] Sampling in the respiratory system requires invasive methods, and here we have characterized the respiratory microbiota surrounding central lung cancer by direct sampling of the tumour tissue by bronchoscopy. Our results reinforce the previously known particularities of the lung microbiota, which considerably differ from oral and stool microbial communities.[16] Our results also allowed us to rule out significant contamination with the upper microbiota during the bronchoscopy, as other authors had previously suggested.[17–19] Moreover, we considered strict inclusion criteria to avoid the possible bias of antibiotic or corticosteroid therapy, and the bacterial exchange between the anatomically separated niches such as saliva and faeces. Finally, the mycobiome composition of central lung cancer was studied.

Lung microbiota has been described based on health status or a lung cancer diagnosis in sputum,[20] bronchoalveolar lavage,[5] protected specimen brushing,[6] cytological brushing,[21] and surgical tissue.[7,8,22] Our major contribution is the depiction of the microbiome surrounding central cancer via direct sampling, but our results are not necessarily applicable to the microbiota of the distal airway. Curiously, higher diversity indexes have been detected in bronchi than in faecal or oral compartments, contrarily to the biomass decreases from upper to lower tract described in healthy people.[19]

We found significantly higher alpha diversity values in cancer than in the control group, whereas other authors have published analogous[8] and opposing results.[5,6,22] Moreover, advanced cancer stages[16] and reduced recurrence-free survival and disease-free survival[22] have been associated with higher values of alpha diversity. In that sense, 80% of our patients were at tumour stage III or IV, and their mean survival was only of 198 days in the follow up. Furthermore, other factors such as environmental exposure, residence in high-population density areas,[4,16] and pack-years of tobacco smoking, can increase the biodiversity of the lung microbiota, whereas chronic bronchitis reduces it.[16] All our patients had been smokers, while most of the controls (56%) had not (Table 1).

In terms of beta diversity, cancer affected and contralateral bronchi had very similar compositions, probably reflecting the environmental influence which is not restricted to the cancer area.[2,6,21,22] Although the limited size of our sample prevents us from reaching solid conclusions, no differences were detected in the composition of the bronchial microbiota as a function of the histological variants of the cancer. The abundance of *Firmicutes* to the clear detriment of *Proteobacteria* was the most noticeable result in our patients, and this result was consistent in all samples. *Proteobacteria* dominance in health lung microbiota, especially *Pseudomonas*, has been also previously corroborated.[5,6,23] Higher concentrations of *Streptococcus, Blautia, Akkermansia,* and *Rothia* were observed in patients, but *Streptococcus* abundance was consistently the major marker linked to lung cancer. This fact has been previously reported in saliva,[5] sputum,[20,24] bronchoalveolar lavage,[5] lung tissue,[7] and protected specimen brushing.[6,21] The exhaustive phylogenetic analysis of ASVs allows us to suggest that the streptococcal variants present in lung tissue are similar to those found in saliva or faeces. New studies including *Streptococcus* cultures and molecular characterization of the species are needed to decipher whether oral lineages are different from those found in
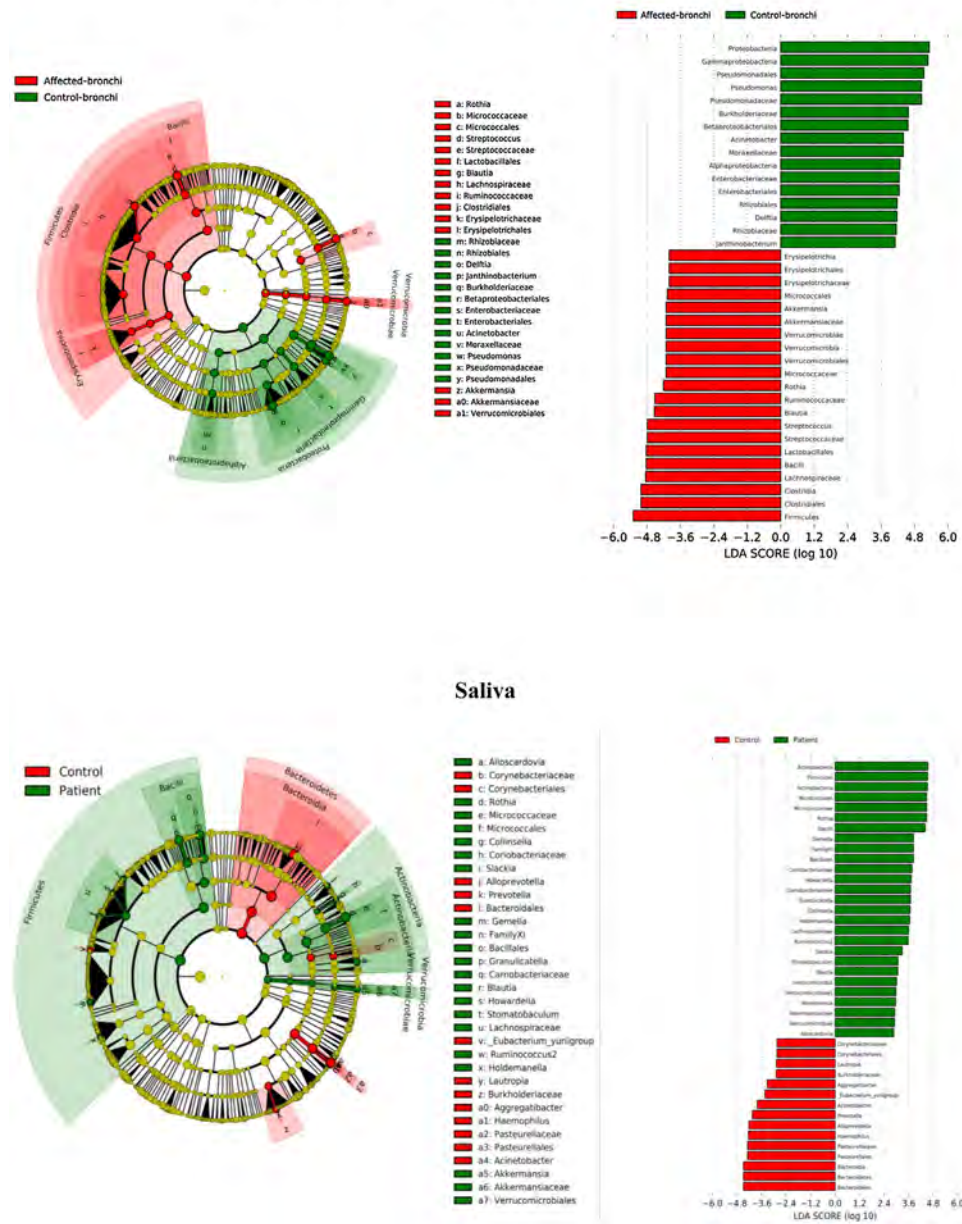
Fig. 4. Streptococcal abundance (A); ROC curves of the relative *Streptococcus* abundance to discriminate patients from controls (B), and distance between saliva and lung microbiota in controls and in patients (C).

the lungs or faeces and thus establish whether there are any markers truly associated with lung cancer, as occurs with *Streptococcus gallolyticus* subsp. *gallolyticus* and colorectal carcinoma.[10,25]

There is increasing evidence of a link between *Streptococcus* and lung cancer. Recently, Tsay et al.[21] detected *Streptococcus* and *Veillonella* enrichment in the lower airways with ERK and PI3K pathways upregulation – an early event that contributes to cell proliferation, survival and tissue invasion – combining microbiome and transcriptomic signatures. The major question that a remains to be answered is whether the abundance of streptococci is a cause or consequence of the tumour process.[26] *Streptococcus* is a natural inhabitant of the oral cavity, which is connected to the lower respiratory tract by the larynx and trachea, and the oral/lung bacterial exchange could occur via microaspirations.[6,21,23]

Microaspiration events are common, but their frequency is significantly increased in chronic inflammatory airway diseases,[23]

inducing Th17 lymphocytes, as well as expression of inflammatory cytokines (as IL-1α, IL-1β and IL-17). IL-23/IL-17 axis alteration is well known in the pathogenesis of both autoimmune diseases and tumours, and *Streptococcus mitis* facilitate the cancer development and expansion by induction of IL-1β, IL-6, IL-10 and IL-23 transcription, Th17 activation, and an increased immune checkpoint PD-L1 expression.[27] Lung resident γδ T cells with protective roles or pro-tumorigenic functions in cancer have been recently discovered, and local lung microbiota, including *Streptococcus*, can provoke inflammation and tumour cell proliferation via lung resident γδ T cells activation.[28] Our results demonstrated a global streptococcal enrichment in patients with cancer that affected more than just the respiratory tract, supporting the idea that microorganisms can orchestrate the balance between tumour-promoted inflammation and anti-tumour immunity depending on the specific microenvironment.
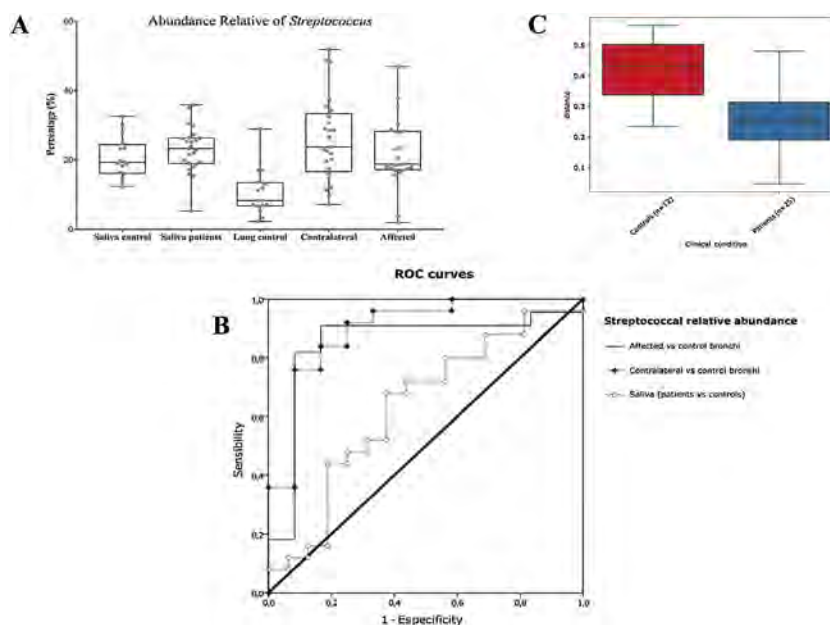
Fig. 5. Statistical differences between the fungal composition of patients and controls bronchi.
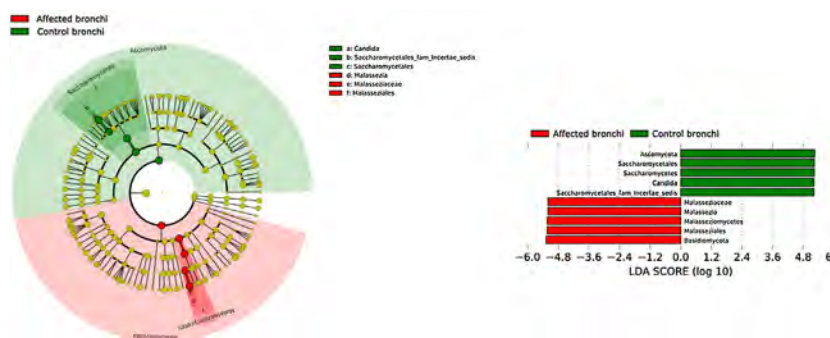

Fig. 6. Statistical differences (LEfSE) of the bacterial microbiota between patients and controls in bronchi and saliva. Only taxa with an LDA score > 4 are shown.

Streptococcal relative abundance in bronchial biopsies was a good predictor of lung cancer, but unfortunately was not reproducible in saliva. ROC curves suggested the contralateral bronchi as the best sample (90.9% sensitivity and 83.3% specificity; AUC = 0.897). Other authors found similar results in protected specimen brushing samples (87.5% of sensitivity and 55.6% specificity, AUC = 0.693).[6] The proportional abundance of *Streptococcus* should be validated in the early stages of lung cancer with subsequent follow-up to corroborate their potential use as biomarker. Along these lines, the intestinal enrichment of *Streptococcus* should be exhaustively explored to identify lung cancer markers in faeces.

The intestinal and the respiratory ecosystems harbour a diverse and abundant microbiota, but some particularities distinguish both ecosystems. Food intake favours a higher rate of microbial reproduction increasing the total mass that is significantly reduced after defecation. On the contrary, nutritional sources for bacteria in the airway are limited to mucus and cellular debris, while clearance is carried out by the ciliary system and the immune system, particularly by macrophages. Lung microbiota core, which is composed by those taxa that are present in >95% of individuals, had not yet been defined. We have implemented new computational strategies to define the lung microbiota core, which had not previously been defined. Our main results are the elevated alpha-diversity of the bronchial microbiota in comparison with saliva or faeces, and the dominance of *Pseudomonas* in controls. Presence of this

particular genus is linked to cystic fibrosis, being the major pathogen that decreases the respiratory functionality within a pathogenic colonization in those patients. However, the lack of respiratory symptoms reduces the potential pathogenic role of *Pseudomonas* in healthy individuals, although more studies are needed in that line.

The mycobiome results were consistent with those obtained for bacteria. The fungal community was slightly richer and more diverse in patients than in controls, although the contralateral bronchus was more similar to controls than to the affected counterpart. The mycobiome of saliva and the affected bronchus from patients matched perfectly, but differed in controls, again suggesting that in patients with cancer the bronchial microbiota is the result of a continuous exchange with that of the oral niche. In terms of taxonomy, affected bronchi from patients had an enrichment of the *Basidiomycota phylum* with higher populations of *Malassezia* genus, whereas the enriched taxon in healthy individuals was the *Ascomycota phylum* and the genera *Candida* and *Saccharomyces,* as previously described.[29] Although the public databases are increasing exponentially, it is important to note that a major limitation to describing the mycobiome is the lack of available taxonomic records. As far as we know, this is the first description of the lung mycobiome.

The main limitation of our work is that we cannot estimate the contribution effect of lung cancer factors on the bronchial

microbiota, mainly tobacco (all patients had been smokers but only the half of the controls were), and COPD (12/25 patients). However, it has not been established yet if tobacco has a significant influence on lung microbiota composition, and some important studies have shown contradictory results.[16,30,31] Whereas severe COPD has been linked with significant alterations in the lung microbiota composition,[32,33] mild and moderate COPD (92% of our COPD patients) has been associated with *Streptococcus* enrichment.[33] This finding might explain the association of mild/moderate COPD and lung cancer,[34] although further studies are needed to confirm this association.

Additional limitations are the small number of patients, all of them from the same hospital, and the lack of other -omic analyses based on genetic expression. On the other hand, our strengths include performing the first study of microbiota combined with mycobiome of bronchial tissue obtained directly from tumour and contralateral bronchi (not adjacent to a resected tumour), as well as performing analysis of the connected ecosystems including saliva and faeces.

In summary, patients with central lung cancer have a significantly different bronchial microbiota from controls, not restricted to tumour-involved tissue, and probably conditioned by continuous microaspiration events from the oral cavity, more than by the carcinogenic process. Lung cancer was associated with a considerable enrichment of *Streptococcus* and we propose that this feature could be used for the screening, diagnosis of this pathology. Lung mycobiota differ considerably in control individuals, and there are dissimilarities in patients between the affected and the contralateral bronchi. An innovative bioinformatics strategy used in this study has allowed us to define healthy individuals' the bronchial core microbiota, which is dominated by a non-pathogenic colonization of *Pseudomonas*.

Ethics approval

The Ethics Committee "CEIC Aragón" approved this project in 2016 with the reference 15/2016, and all participants signed the informed consent after receiving all the information from the clinical researchers.

Authors' contributions

SB conceived the study. JJV, ALF, EM, and AR contributed to the patients' selection and sample collection. JMP, JG carried out the computational analysis for core microbiota definition. MPA and RdC determined the microbiota composition. AR, PG, JMP, JG, and SB participated in the analysis and interpretation of data. SB, RdC, and MPA wrote and reviewed the manuscript, and finally all authors read and approved the final version.

Funding

Conflict of interest

RdC is the recipient of a Vertex grant IIS-2017-106179 for cystic fibrosis research. The remaining authors declare that they have no competing interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.arbres.2020.05.034.

References

1. Pulvirenti G, Parisi GF, Giallongo A, Papale M, Manti S, Savasta S, et al. Lower airway microbiota. Front Pediatr. 2019;7:393, http://dx.doi.org/10.3389/fped.2019.00393.
2. García-Pachón E, Padilla-Navas I. The lower airway microbiome and lung cancer. Arch Bronconeumol. 2020, http://dx.doi.org/10.1016/j.arbres.2020.01.020. S0300-2896(20)30052-1.
3. Garrido-Martín EM, Paz-Ares L. Lung cancer and microbiome. Arch Bronconeumol. 2020;56:3–4, http://dx.doi.org/10.1016/j.arbres.2019.04.012.
4. Mao Q, Jiang F, Yin R, Wang J, Xia W, Dong G, et al. Interplay between the lung microbiome and lung cancer. Cancer Lett. 2018;415:40–8, http://dx.doi.org/10.1016/j.canlet.2017.11.036.
5. Wang K, Huang Y, Zhang Z, Liao J, Ding Y, Fang X, et al. Preliminary study of microbiota diversity in saliva and bronchoalveolar lavage fluid from patients with primary bronchogenic carcinoma. Med Sci Monit. 2019;25:2819–34, http://dx.doi.org/10.12659/MSM.915332.
6. Liu HX, Tao LL, Zhang J, Zhu YG, Zheng Y, Liu D, et al. Difference of lower airway microbiome in bilateral protected specimen brush between lung cancer patients with unilateral lobar masses and control subjects. Int J Cancer. 2018;142:769–78, http://dx.doi.org/10.1002/ijc.31098.
7. Liu Y, O'Brien JL, Ajami NJ, Scheurer ME, Amirian ES, Armstrong G, et al. Lung tissue microbial profile in lung cancer is distinct from emphysema. Am J Cancer Res. 2018;8:1775–87.
8. Greathouse KL, White JR, Vargas AJ, Bliskovsky VV, Beck JA, von Muhlinen N, et al. Interaction between the microbiome and TP53 in human lung cancer. Genome Biol. 2018;19:12, http://dx.doi.org/10.1186/s13059-018-1501-6.
9. Ma W, Mao Q, Xia W, Dong G, Yu C, Jiang F. Gut microbiota shapes the efficiency of cancer therapy. Front Microbiol. 2019;10:1050, http://dx.doi.org/10.3389/fmicb.2019.01050.
10. Pasquereau-Kotula E, Martins M, Aymeric L, Dramsi S. Significance of *Streptococcus gallolyticus* subsp. gallolyticus association with colorectal cancer. Front Microbiol. 2018;9:614, http://dx.doi.org/10.3389/fmicb.2018.00614.
11. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. Bioinformatics. 2013;29:1072–5, http://dx.doi.org/10.1093/bioinformatics/btt086.
12. Toju H, Tanabe AS, Yamamoto S, Sato H, High-coverage ITS. primers for the DNA-based identification of Ascomycetes and Basidiomycetes in environmental samples. PLoS One. 2012;7:e40863, http://dx.doi.org/10.1371/journal.pone.0040863.
13. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7, http://dx.doi.org/10.1038/s41587-019-0209-9.
14. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60, http://dx.doi.org/10.1186/gb-2011-12-6-r60.
15. Roy S, Trinchieri G. Microbiota: a key orchestrator of cancer therapy. Nat Rev Cancer. 2017;17:271–85, http://dx.doi.org/10.1038/nrc.2017.13.
16. Yu G, Gail MH, Consonni D, Carugno M, Humphrys M, Pesatori AC, et al. Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. Genome Biol. 2016;17:163, http://dx.doi.org/10.1186/s13059-016-1021-1.
17. Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. The microbiome and the respiratory tract. Annu Rev Physiol. 2016;78:481–504, http://dx.doi.org/10.1146/annurev-physiol-021115-105238.
18. Moffatt MF, Cookson WO. The lung microbiome in health and disease. Clin Med (Lond). 2017;17:525–52, http://dx.doi.org/10.7861/clinmedicine.17-6-525.
19. Charlson ES, Bittinger K, Haas AR, Fitzgerald AS, Frank I, Yadav A, et al. Topographical continuity of bacterial populations in the healthy human respiratory tract. Am J Respir Crit Care Med. 2011;184:957–63, http://dx.doi.org/10.1164/rccm.201104-0655OC.
20. Cameron SJS, Lewis KE, Huws SA, Hegarty MJ, Lewis PD, Pachebat JA, et al. A pilot study using metagenomic sequencing of the sputum microbiome suggests potential bacterial biomarkers for lung cancer. PLOS ONE. 2017;12:e0177062, http://dx.doi.org/10.1371/journal.pone.0177062.

21. Tsay JJ, Wu BG, Badri MH, Clemente JC, Shen N, Meyn P, et al. Airway microbiota is associated with up-regulation of the PI3K pathway in lung cancer. Am J Respir Crit Care Med. 2018;198:1188–98, http://dx.doi.org/10.1164/rccm.201710-2118OC.

22. Peters BA, Hayes RB, Goparaju C, Reid C, Pass HI, Ahn J. The microbiome in lung cancer tissue and recurrence-free survival. Cancer Epidemiol Biomarkers Prev. 2019;28:731–40, http://dx.doi.org/10.1158/1055-9965.EPI-18-0966.

23. Segal LN, Clemente JC, Tsay JC, Koralov SB, Keller BC, Wu BG, et al. Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. Nat Microbiol. 2016;1:16031, http://dx.doi.org/10.1038/nmicrobiol.2016.31.

24. Hosgood HD, 3rd, Sapkota AR, Rothman N, Rohan T, Hu W, Xu J, et al. The potential role of lung microbiota in lung cancer attributed to household coal burning exposures. Environ Mol Mutagen. 2014;55:643–51, http://dx.doi.org/10.1002/em.21878.

25. Jans C, Boleij A. The road to infection: host–microbe interactions defining the pathogenicity of *Streptococcus bovis/Streptococcus equinus* complex members. Front Microbiol. 2018;9:603, http://dx.doi.org/10.3389/fmicb.2018.00603.

26. Engen SA, Schreurs O, Petersen F, Blix IJS, Baekkevold ES, Schenck K. The regulatory role of the oral commensal *Streptococcus mitis* on human monocytes. Scand J Immunol. 2018;87:80–7, http://dx.doi.org/10.1111/sji.12636.

27. Cheng M, Hu S. Lung-resident γδ T cells and their roles in lung diseases. Immunology. 2017;151:375–84, http://dx.doi.org/10.1111/imm.12764.

28. Jin C, Lagoudas GK, Zhao C, Bullman S, Bhutkar A, Hu B, et al. Commensal microbiota promote lung cancer development via γδ T cells. Cell. 2019;176, http://dx.doi.org/10.1016/j.cell.2018.12.040, 998–1013.e16.

29. Krause R, Moissl-Eichinger C, Halwachs B, Gorkiewicz G, Berg G, Valentin T, et al. Mycobiome in the lower respiratory tract – a clinical perspective. Front Microbiol. 2017;7:2169, http://dx.doi.org/10.3389/fmicb.2016.02169.

30. Morris A, Beck JM, Schloss PD, Campbell TB, Crothers K, Curtis JL, et al. Comparison of the respiratory microbiome in healthy non-smokers and smokers. Am J Respir Crit Care Med. 2013;187:1067–75, http://dx.doi.org/10.1164/rccm.201210-1913OC.

31. Panzer AR, Lynch SV, Langelier C, Christie JD, McCauley K, Nelson M, et al. Lung microbiota is related to smoking status and to development of acute respiratory distress syndrome in critically ill trauma patients. Am J Respir Crit Care Med. 2018;197:621–31, http://dx.doi.org/10.1164/rccm.201702-0441OC.

32. Budden KF, Shukla SD, Rehman SF, Bowerman KL, Keely S, Hugenholtz P, et al. Functional effects of the microbiota in chronic respiratory disease. Lancet Respir Med. 2019;7:907–20, http://dx.doi.org/10.1016/S2213-2600(18)30510-1.

33. Pragman AA, Lyu T, Baller JA, Gould TJ, Kelly RF, Reilly CS, et al. The lung tissue microbiota of mild and moderate chronic obstructive pulmonary disease. Microbiome. 2018;6:7, http://dx.doi.org/10.1186/s40168-017-0381-4.

34. de Torres JP, Marin JM, Casanova C, Cote C, Carrizo S, Cordoba-Lanus E, Baz-Dávila R, et al. Lung cancer in patients with COPD: incidence and predicting factors. Am J Respir Crit Care Med. 2011;184:913–9, http://dx.doi.org/10.1164/rccm.201103-0430OC.