| Studies investigating the microbiome and virome in IPF and their main results and microbial populations | | | |
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| **Study** | **Method** | **Disease** | **Main results related to IPF/Microbial population in IPF patients** |
| Richter et al (28) | Quantitative cultures of BAL | IPF<br>ANCA-related Cocciomatosis (Wegener granulomatosis) | BAL cultures positive in 8/22 IPF patients: *Haemophilus influenzae*<br>Haemophilus parainfluenzae<br>*Moraxella catarrhalis*<br>*Pseudomonas aeruginosa*<br>*Proteus mirabilis*<br>*Streptococcus pneumoniae* |
| Friaza et al (29) | 16S-rDNA PCR - denaturing gradient gel electrophoresis - bacterial band identification in BAL | IPF | Identified organisms normally present in the oropharynx<br>Bacterial sequences corresponding to the *Streptococcus*, *Neisseria* and *Actinobacterium* genera.<br>*Pneumocystis jirovecii* may impair bacterial colonization of the airways |
| Garzoni et al (30) | 16s-rRNA gene sequencing of BAL | IIP<br>Sarcoidosis<br>Normal controls<br>PCP | No significant differences between ILD and healthy controls.<br>*Prevotellaceae, Streptococcaceae* and *Acidaminococcaceae* |
| Han et al (31) | 454 pyrosequencing was used to assign OTUs in BAL | IPF | *Prevotella, Veillonella* and *Cronobacter spp*<br>*Streptococcus* OTU1345<br>*Staphylococcus* OTU1348 |
| Molyneaux et al (24) | 16S rRNA qPCR and pyrosequencing in BAL | IPF | Patients with IPF had double the burden of bacteria in BAL fluid<br>Baseline bacterial burden predicted the rate and risk of death and was associated independently with the rs35705950 polymorphism of the MUC5B mucin gene<br>*Veillonella*, *Neisseria*, *Streptococcus* and *Haemophilus* spp were more abundant in IPF than controls |
| Invernizzi et al (32) | Amplification of the V4 hypervariable region of the 16S rRNA gene in BAL | IPF vs HP | Distinct differences in the microbial profiles were evident in the lower airways of subjects with CHP and IPF<br><br>IPF *Firmicutes* phyla*, Actinomyces* and *Veillonella* genera<br>HP *Proteobacteria* phyla*, Actinomyces* and *Veillonella* genera |
| Takahashi et al (33) | Amplification of the V2–4-8 and V3–6, 7–9 hypervariable region of the 16S rRNA gene in BAL | IPF<br>Bleomycin-treated mice | The most prevalent lung phyla were *Firmicutes*, *Proteobacteria* and *Bacteroidetes*.<br>Decreased microbial diversity was associated with low FVC and early mortality.<br>Diversity and abundance of *Firmicutes*, *Streptococcaceae*, and *Veillonellaceae* were significantly associated with FVC, 6-min walk distance, and serum surfactant protein D<br>Bleomycin-induced lung fibrosis resulted in decrease of diversity and alteration of microbiota |
| Valenzi et al (34) | PCR amplification and sequencing of the 16S rRNA gene in parenchymal and airways tissue samples | IPF<br>CTD-ILD<br>CF<br>COPD<br>Normal Subjeccts | Airway-based samples had higher bacterial load compared to parenchymal<br>Bacterial load was lower in IPF compared to CF and controls |
| Calabrese et al (36) | Nucleic acids were extracted from fresh lung tissues using a modified RNAzol method RT-PCR, PCR or nested-PCR were used to detect principal respiratory viral genomes | IPF<br>Normal subjects | Higher frequency of virus positive cases was found in IPF<br>Only herpes virus genomes were detected<br>Viral cases showed higher mPAP, poorer performance in the six minute walking test and higher frequency of primary graft dysfunction |

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| Yin et al (43) | Surgical lung biopsies<br>viral RNA qPCR | IPF | 740 viruses were quantified<br>Key sequencing results were confirmed for specific viruses (EBV, HCV, herpesvirus saimiri and HERV-K) |
| Molyneaux et al (46) | DNA extraction from BAL<br>A hyper-variable region of the 16S ribosomal RNA gene (16S rRNA) was amplified, quantified and pyrosequenced | IPF | Increased burden 4-fold<br>Shift from stable disease OTUs<br>A significant increase was noticed for *Proteobacteria*, *Campylobacter spp*. and *Stenotrophomonas spp*., while a significant decrease was found in *Veillonella spp*. and *Campylobacter spp* |
| Weng et al (47) | Antiviral/bacterial IgM in Sputum cultures<br>RNA sequences of pathogens in nasopharyngeal swab of IPF patients were detected by PathChip | IPF | Thirty-eight different bacterial strains were detected in IPF patient sputum - 89% were gram-negative bacteria:<br>*Klebsiella pneumonia* 26%<br>*Mycobacteria tuberculosis* 21%<br>*Acinetobacter baumannii* 10%<br>Fifty-seven different viruses were detected in nasopharyngeal swabs of IPF patients. |
| O'Dwyer et al (49) | DNA extraction from mouse tissue and human BAL<br>Droplet digital PCR for the 16S rRNA gene | IPF<br>Bleomycin-treated mice | Lung Bacterial Burden Predicts Disease Progression in IPF<br>Bleomycin induces fibrosis of equal severity to both germ-free and conventional mice; however the germ-free mice were protected against mortality |
| Wootton et al (51) | Multiplex PCR, pan-viral microarray and high-throughput cDNA sequencing in BAL | IPF<br>AE-IPF<br>ALI | Viral nucleic acid was detected in cases of AE-IPF but not in stable disease<br>Evidence of viral infection with herpes simplex virus, Epstein-Barr virus and torque teno virus (TTV) in patients with acute exacerbation<br>TTV infection was significantly more common in patients with acute exacerbation |

*BAL Bronchoalveolar lavage; IPF Idiopathic Pulmonary Fibrosis; ANCA Antineutrophil Cytoplasmic Antibody; PCR Polymerase Chain Reaction; IIP Idiopathic Interstitial Pneumonia; PCP Pneumocystis Jirovecii Pneumonia; ILD Interstitial Lung Disease; OTU Operational Taxonomic Unit; qPCR Quantitative Polymerase Chain Reaction; CHP Chronic Hypersensitivity Pneumonitis; FVC Forced Vital Capacity; CTD-ILD Connective Tissue Disease – Interstitial Ling Disease; CF Cystic Fibrosis; COPD Chronic Obstructive Lung Disease; mPAP mean Pulmonary Artery Pressure; EBV Epstein-Barr Virus; HCV Hepatitis C Virus; HERV-K Human Endogenous Retrovirus type K;*