ePS06.1 *Stenotrophomonas maltophilia* is increased in patients with cystic fibrosis-related diabetes and displays enhanced growth under physiologically-relevant levels of glucose

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The CF lung facilitates the cohabitation of diverse microbial organisms. These communities diversify as a result of interactions with both the environment and each other. Cystic fibrosis-related diabetes (CFRD) is an increasingly recognised and complex issue in the management of CF patients. There has been little research into the effect that this change in the host has on the associated microbiome.

Objectives: The main objective of this study was to determine the bacterial community structure in patients with CFRD.

Methods and Results: Clinically significant dysglycemia was confirmed using continuous glucose monitoring showing interstitial glucose readings of 7.8 mmol/L for more than 4.5% of the time. Amplicon-sequencing using Illumina Miseq of the 16S rRNA gene was performed on DNA extracted from sputum samples taken during outpatient visits. Analysis was performed using uSearch/Qiime pipeline and R package. Sequencing of 19 diabetic and 22 non-diabetic patients revealed a diverse microbial population. In CFRD patients, a significantly higher level of *S. maltophilia* was detected and confirmed using qPCR. In vitro studies were performed using physiologically relevant levels of glucose (0–10 μM). *S. maltophilia* displays a significant increase in growth in glucose rich environments suggesting that this bacterium is adept at exploiting this niche.

The importance of glucose control in CFRD cannot be underestimated as it has potential to predispose the growth of resistant organisms. Increased understanding of these complex interactions between the microbiome and host may ultimately lead to altered CF patient management.

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ePS06.2 Immune cell localization in different compartments of human end-stage CF lungs

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Objectives: The localization of immune cells along airways, blood vessels or in parenchyma in CF lung disease has insufficiently been established. A comprehensive quantitative immunohistochemical analysis was performed on lung tissue from CF patients and healthy controls (HC).

Methods: Sections (8 μm) of lung biopsies of CF patients taken during transplantation ($n=20$) and control lung biopsies ($n=22$) were stained for Th-lymphocytes (CD4), mast cells (tryptase) and eosinophils (EG-2). Quantification was performed by counting all single cells normalized over total area (mm^2) of biopsy (Th cells), or in 10 high power fields averaged per compartment (airways, blood vessels and parenchyma) per subject (mast cells and eosinophils).

Results: $CD4^+$ T-lymphocyte counts were significantly increased in CF vs. HC ($p=0.001$) and were present as dispersed single cells (CF & HC), or organized in follicles mainly situated in parenchyma (predominantly CF). Both total & compartmental mast cell counts were significantly increased in CF vs. HC (all $p<0.001$), whereas eosinophil counts showed no difference. Mast cell and eosinophil counts were higher around airways than around blood vessels or in parenchyma. Female CF patients ($n=10$) had significantly higher Th-lymphocyte, mast cell and eosinophil counts than males (all $p<0.01$).

Conclusion: There is an increased presence of Th lymphocytes and mast cells in the end-stage CF lung, albeit in different compartments of the lung. Inflammation was more severe in female CF patients irrespective of FEV_1, colonization status or genotype, corroborating previous data suggesting a direct deleterious effect of female hormone on inflammation in the CF lung.

ePS06.3 Neutrophil elastase activity on the surface of sputum neutrophils is associated with severity of cystic fibrosis lung disease

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Objective: Previous studies identified free neutrophil elastase (NE) in bronchoalveolar lavage fluid and sputum of patients with cystic fibrosis (CF) as a key risk factor of early bronchiectasis and decline in lung function. However, the pathophysiologic relevance of membrane-bound NE in CF lung disease is not well understood. We therefore used a ratiometric Foerster resonance energy transfer reporter (NEmo-2) to determine NE activity on sputum neutrophils and correlated NE levels with lung function measurements.

Methods: Inflammatory cells isolated from spontaneous or induced sputum of CF patients ($n=37$) and healthy non-smokers (control, $n=9$) were incubated with NEmo-2. To control for NE-specific cleavage of NEmo-2, cells were preincubated with the NE inhibitor sivelestat (T0). NE activity was calculated as the ratio of donor and acceptor fluorescence measured by confocal microscopy and normalized to T0.

Results: Membrane-bound NE activity (CF: 1.57 ± 0.29 vs. control: 1.07 ± 0.13, $p<0.001$) and percentage of sputum neutrophils (CF: 93.82 ± 4.89 [%] vs. control: 42.12 ± 24.22 [%], $p<0.001$) were significantly increased in CF. However, NE activity did not correlate with differential neutrophil count in CF or control sputum (CF: $R=0.22$, $p=0.19$ and control: $R=0.11$, $p=0.78$). In CF, levels of NE activity inversely correlated with FEV_1% predicted ($R=-0.33$, $p<0.05$) and FEV_1/VC ($R=-0.44$, $p<0.01$)and directly correlated with residual volume ($R=0.58$, $p<0.01$) and intrathoracic gas volume ($R=0.53$, $p<0.01$).

Conclusion: We conclude that NE activity is elevated on the surface of neutrophils in CF airways. This may contribute to severity of lung disease and serve as valuable biomarker in CF.

ePS06.4 Upper airway infection and inflammation in CF and healthy controls during exacerbation and stable phases

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Objectives: Upper airway (UAW) infections are common in CF patients with nasal congestion, impaired climate function and reduced quality of life. Moreover, by postnasal drip, infections can descend to the lungs. Actually, there are no data comparing UAW infection and inflammation in CF and healthy controls in stable phases and during exacerbation.

Methods: We collected epithelial lining fluid from 49 CF patients and 38 healthy controls by nasal lavage in stable phases and during UAW infection. Microbiological (only CF cohort), cytological and immunological analyses (NE/IL-6/IL-8/IL-1β/MMP-9/TIMP-1) were performed.

Results: During UAW infection all measured inflammatory mediators increased in both, CF patients and the control cohort. Whereas levels of IL-6 and IL-8 were significantly higher in CF under stable conditions compared to healthy controls, the levels of the control cohort exceeded those of CF patients during infection. Age-stratified analyses showed higher releases of mediators during acute UAW infection in CF patients ⩽12 years, especially for MMP-9, IL-8 and TIMP-1. These findings were not present in controls.

Conclusion: In CF patients, chronic inflammation with higher values of inflammatory mediators compared to healthy controls was observed. However, the increase of inflammation during UAW infection is not higher in CF compared to healthy controls. In contrast, for all measured parameters except TIMP-1 a weaker increase was observed in CF, suggesting a deregulation of the immune system in CF with a decreased ability of mediator release. This hypothesis is also supported by the observation of higher releases of MMP-9, TIMP-1 and IL-8 in younger CF patients.