65* Acute respiratory infections in young CF children

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Aims: Local defense is impaired in CF lung disease, which might imply a higher impact of acute respiratory infections (ARI). Acquisition of *P. aeruginosa* (PA) has been associated with a preceding viral respiratory infection in CF patients.We hypothesised that ARI causes more symptoms in CF children compared to healthy controls (HC) and is associated with an increased rate of PA acquisition.
Methods: In a 6 months period 20 PA free CF children aged 0 to 7 years and 19 unrelated age-matched HC were contacted 2×/week with a standard survey regarding symptoms of ARI. If any symptom was present physical examination, naso- and oropharyngeal swabs for viral PCR analysis and bacterial culture were performed.
Results: In CF we found 4.4 periods of ARI/child compared to 4.2 in HC, with no significant differences in upper respiratory tract symptoms. CF children showed significantly more cough with sputum (54 vs. 27×, $p < 0.01$), more dyspnea (18 vs. 6×, $p = 0.04$), higher respiratory rate (32.2 vs. 25.9/min, $p < 0.01$), lower oxygen saturation (96.2 vs 97.4%, $p = 0.02$) and more abnormal auscultation compared to HC (21 vs. 7×, $p = 0.02$). In a sample of 99 nasopharyngeal swabs we found a viral pathogen in 64% (36/56) in CF children, compared to 53% (23/43, $p = 0.42$) in HC. Higher than expected numbers of children had at least one PA positive culture, 6 of 20 CF patients (30%) compared to 7 of 19 HC (37%). Follow-up cultures were always negative for PA in HC, while in 4 of 6 (67%) CF children they remained positive for PA.
Conclusions: Young CF children have as many ARI as healthy children, but more lower respiratory tract symptoms.Viral associated ARI are frequently linked with PA acquisition in both CF and healthy children.While healthy children easily clear PA, this often results in a persistent infection in CF children.

66 Bacterial diversity in the Cystic Fibrosis lung: geographical differences in community composition and distribution

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Respiratory failure, driven by bacterial infection, leads to mortality in the majority of CF patients. Our understanding of these bacteria has been deepened by applying molecular profiling techniques such as T-RFLP to sputum samples. T-RFLP, which characterises the total bacterial community without prior growth, avoids biases associated with culture-based techniques. These studies have reported a significantly higher level of bacterial diversity than observed previously by using diagnostic culture-based analyses. The vast majority of species detected were not those regarded as traditional CF pathogens e.g. *Pseudomonas aeruginosa*. Here, we compare sputum sampled from CF patients attending either an American or a British CF clinic. This allowed the extent of similarity of lung bacterial communities from both patient sets to be determined. These data were used to test hypotheses relating to the impact of geographical separation on the bacterial communities present in the lungs of CF patients. In order to do so, sputa from patients at Southampton and UNC were subjected to T-RFLP profiling of the 16S rRNA gene. Initial findings suggest species diversity was slightly lower in US than UK samples (9.5 ± 1.5 and 13.3 ± 7.9 respectively). For both sample sets, *P. aeruginosa* was the dominant species (*c.*60% of cases), with non-"key CF pathogens" dominant in remaining samples. However, further investigation is vital to determine the generic significance of these findings. Studies such as these will deepen our understanding of the geographical component to CF lung infection and may provide new strategies that will improve the treatment of lung disease.

67 Use of anaerobic bacteriological culture for the detection of anaerobic bacteria in sputum from Cystic Fibrosis patients

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Pulmonary infection by *Pseudomonas aeruginosa* is the leading cause of morbidity and mortality in Cystic Fibrosis (CF) patients. The reduced oxygen concentration observed in the sputa of CF patients coupled with respiration of *P. aeruginosa* is believed to create anoxic zones within the CF lung. If the airway mucus of CF patients is anaerobic, there is the potential that these anoxic zones contain obligate anaerobes which may also contribute to the infection.
In this study, we used strict anaerobic bacteriological culture techniques to detect anaerobic bacteria in sputa samples from CF patients colonized with *P. aeruginosa*. Potential anaerobes were checked for oxygen sensitivity and identified by 16S rDNA PCR.
Anaerobes were detected in 80% of the 60 samples examined to date and the total viable count of the majority of anaerobes isolated from each individual patient was equal to or exceeded the total viable count of P. aeruginosa isolated from that same patient. Anaerobes isolated have belonged to the genus's Bifidobacterium, Prevotella, Veillonella, and Propionibacterium.
These results indicate that anaerobes are present in the lungs of CF patients in significant numbers and may, therefore, contribute to a polymicrobial infection in the lungs of CF patients.

68 The physiological state of *Pseudomonas aeruginosa* populations in lungs of Cystic Fibrosis (CF) patients

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In the research of chronic infections in CF patients, we focused on the growth characteristics of *P. aeruginosa* to understand better the persistence of the infection. *P. aeruginosa* isolates from 6 chronically infected CF patients grow 2–3 fold slower than environmental isolates in laboratory media. We indirectly measured growth rates of the bacteria in sputum samples (reflecting the in vivo conditions in the lung) using quantitative fluorescent in situ hybridization (FISH). Different clinical isolates were cultivated in various media resulting in a range of growth rates, and 16S rRNA targeted quantitative FISH was applied to measure the amount of ribosomal RNA per cell volume at each growth rate. Based upon these standard curves, estimates of the in situ growth rates of *P. aeruginosa* in CF sputum were derived from determinations of the rRNA content in a large number of single cells in individual sputum samples. The result showed that most of the cells were growing with rates of 0.2–0.5/h, some even faster. Only small stationary sub-populations seemed to be present in the sputum samples. This suggests a high turnover rate of bacterial biomass in the CF lung, most likely caused by antibiotic treatment, immune system attacks and/or removal of biomass by coughing. Consequently the bacterial population may be confronted with two opposing selection forces: One in favour of reduced growth rates, perhaps coupled to a high stress level in the CF lung, and another in favour of fast growth coupled to competition for nutrients. This scenario constitutes a new perspective on the adaptation of *P. aeruginosa* in chronically infected CF patients, which must be taken into consideration in relation to the design of antibiotic therapies.