PCR and Next-Generation Sequencing Techniques to Identify Respiratory Pathogens in the Sputum of Children and Adults with Cystic Fibrosis and Non Cystic Fibrosis Bronchiectasis

Application deadline: Applications accepted all year round

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Summary

This is a very exciting project that employs the latest molecular microbiological technology to identify organisms in the lungs of individuals with cystic fibrosis and non cystic fibrosis bronchiectasis. The roles of these techniques in clinical practice is currently uncertain but have the potential to transform sputum microbiological analysis in such patients and to have an immediate impact on the management of lower respiratory tract infections. This is an emerging field and there is currently worldwide interest in this technology not least as a result of the human microbiome project.

Study aims: To perform modern PCR and employ next-generation sequencing techniques to detect and quantify bacteria in the sputum of children and adults with cystic fibrosis (CF) and non cystic fibrosis bronchiectasis.

Hypothesis: Compared to routine microbiology, modern molecular techniques are superior at detecting, quantifying and identifying bacteria in lower respiratory tract samples from CF and non CF bronchiectasis patients experiencing chest infections.

Reasoning behind the proposal: Prompt detection and treatment of lower respiratory tract infections is essential in the management of patients with CF and non-CF bronchiectasis. The true sensitivity of standard culture techniques to detect bacteria in lower respiratory tract secretions is not known and in a significant number of cases treatment may be delayed. Moreover, frequently no organisms are identified using routine microbiological techniques making it difficult to choose the correct antibiotic. Also, the contribution of anaerobic organisms in causing lung disease in such adults and children is unknown.

Methodology: We will obtain respiratory secretions by sputum induction with hypertonic saline, from patients with CF and non-CF bronchiectasis seen at the University Hospitals Leicester NHS Trust. Sputum is obtained when patients experience an acute exacerbation of cough and breathlessness and after treatment with antibiotics. Induced sputum samples will be divided into two portions, one for routine microbiology and the second portion for batched molecular microbiological analyses. DNA barcode ultra-high throughput pyrosequencing to profile microbiota, block PCR and real-time PCR assays targeting the principal lung pathogens and the conserved 16S rDNA bacterial gene will be used to analyse the samples. A sample-specific multi-locus sequencing typing approach will also be used to detect and/or quantify the presence of multiple strains of the same bacterial species.