Introduction and objectives: The detection of bacteria contributing to respiratory exacerbations in cystic fibrosis patients is routinely performed using standardised microbiological culture techniques. These methods often fail to identify a significant pathogen in symptomatic patients and have been found to underestimate the burden of fastidious organisms such as *Streptococcus pneumoniae* in CF sputum. There is also increasing evidence linking respiratory exacerbations to infection with organisms that are not identified by routine microbiology.

The primary objective of this cross-sectional study was to test the agreement between standard microbiology and molecular (culture-independent) techniques in detecting five common pathogens in sputum of children with CF.

Methods: Culture-independent microbiology was performed on samples obtained from children experiencing respiratory exacerbations over a 12 month period from April 2010 to March 2011. Sputum samples were either produced spontaneously or obtained either by sputum induction with hyper tonic saline or bronchoalveolar lavage. Aliquots of sputum we refrozen either neat or mixed 1:1 with 0.1% dithiothreitol, prior to batch extraction of total nucleic acid. Total bacterial load was determined using broad-range 16S rDNA quantitative real-time PCR. Specific real-time qPCR assays were used to quantify the numbers of *Staphylococcus aureus*, *Hemophilus influenzae*, *S pneumoniae*, *Pseudomonas aeruginosa* and *Moraxella catarrhalis*. Routine microbiology was performed by adhering to the laboratory standards published by the UK CF Trust.

Results: Fifty five samples (44 sputum and 11 bronchoalveolar lavage fluid) from 33 children were available for molecular analysis. In 40, the total bacterial load was calculated to be 10^7 or greater per ml of fluid. The qPCRs detected clinically relevant pathogens at significant levels (>1% of total) more frequently than standard microbiology.

Conclusion: We found that standard microbiology alone revealed fewer of the clinically relevant respiratory pathogens studied compared to molecular techniques. PCR based analysis also offers the potential to identify pathogens more rapidly and to detect organisms that are difficult to detect by routine culture. This may have an impact on the choice of antibiotic for the treatment of an exacerbation. Compared to standard microbiology, however, these assays are expensive and not readily available for routine diagnostics.

S82 AIRWAY INFLAMMATION IS PRESENT BY 4 MONTHS IN CF INFANTS DIAGNOSED ON NEWBORN SCREENING

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Introduction: Cystic fibrosis (CF) newborn screening (NBS) allows early introduction of treatment, often before any symptoms arise with the aim of reducing aireway infection and inflammation. UK nationwide screening began in 2007. Aims: It is usual clinical practise at our centre to perform fibroptic bronchoscopy (FOB) at the age of 3 months for all children with CF. The aim of this study was to establish the presence of infection and degree of inflammation in airways in the NBS cohort. Bronchoalveolar lavage fluid (BALF) was cultured and examined for cellular inflammation. Results: Infants diagnosed by NBS undergoing routine FOB who had either BALF absolute cell count or differential cell count assessed were included in the study. 44 infants (48% female), median age (range) 15 (7–28) weeks met these criteria. The majority of these infants were symptom free however 15 (29%) had bacterial isolates from their BALF. Comparable data are also available for 71 children with established CF (median age 9.5 years; range 1.9–16.7 years) of whom 46 (65%) were BALF culture positive and for 6 healthy controls (median age 12.3 years, range 10.5–15.4 years).

Cellular inflammation was present in the airways in infants diagnosed by NBS, both in those who were culture positive and negative in their BALF. Absolute BALF cell count and neutrophil differential were significantly higher in both NBS and established CF patients compared with healthy controls (p<0.02). Median absolute cell counts and neutrophil differentials can be seen in table 1. For both NBS and established CF, in those who had bacteria isolated from their BALF, an increase in neutrophil differential was seen compared with those culture negative at the time of FOB although....
this did not reach significance in the NBS group. (NBS CF p = 0.05, established CF p < 0.001).

Conclusion Our results demonstrate that inflammation is already present by 4 months of age in asymptomatic infants diagnosed through NBS, although at a lower level than seen in established CF. The results underscore the importance of early surveillance and lend support to the evolving focus on this age group for interventional trials.

Conclusions

Mechanisms of airway injury in COPD

Severe deficiency of the major anti elastase α1-antitrypsin (AT) due to the Z (Glu342Lys) variant is the commonest genetic reason for the development of COPD. Cigarette smoke (CS) accelerates decline in lung function in Z-AT homozygotes. We investigated whether Z-AT is associated with an exaggerated inflammatory response compared to normal AT (M-AT).

Lung epithelial (A549 and NHBE) cells transfected with human M-AT or Z-AT (M-AT/Z-AT cells) were exposed to 12.5% CSE generated from IR3F cigarettes. Supernatants, lysates and inclusion bodies were assessed for total AT to confirm a successful cell-model system. Supernatant was assessed for TNF-α, IL-6, IL-8 and MCP-1, oxidised pZ-AT (Ox-pZ-AT), NF-κB and AP-1 by ELISA, immunoblot or RT-PCR. N-acetylcysteine (NAC, 10⁻³ M) was used to probe the effect of oxidants.

At 24h CSE in Z-AT (CSE-Z-AT) compared to CSE-M-AT (unless stated significantly induced TNF-α (212±20.7 pg/ml vs. 37.1±2.7), IL-6 (421.4±20.8 pg/ml vs. 159.3±12.1), IL-8 (5763±497 pg/ml vs. 2593±450) and MCP-1 (23564±1852 pg/ml vs. 5329±706), p<0.001 for all. CSE-Z-AT had significantly induced mRNA for TNF-α, IL-6, IL-8 and MCP-1 at 0.5h (p<0.001 for all). Development of Ox-pZ-AT were exclusively detected in CSE-Z-AT inclusion (3246±433 ng/ml vs. undetectable, p<0.001). CSE-Z-AT had significantly activated NF-κB and AP-1 by ELISA, immunoblot or RT-PCR. N-acetylcysteine (NAC, 10⁻³ M) was used to probe the effect of oxidants.

In conclusion, following CS exposure Z-AT cells had significantly elevated inflammatory mediators compared to M-AT cells, which was inhibited by NAC. We propose that during CS exposed lung inflammation Z-AT monomer undergoes oxidation to form oxidised polymers thereby further reducing the level of protective monomeric AT, which predisposes to increased lung inflammation.