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A CLINICAL EVALUATION OF QUANTITATIVE, CULTURE-INDEPENDENT METHODS FOR THE IDENTIFICATION OF BACTERIA IN CYSTIC FIBROSIS SPUTUM AND BRONCHO-ALVEOLAR LAVAGE FLUIDS

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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 were 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*, *Haemophilus 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 10⁷ 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.

S81

LUNG BIOPSY FOR CHRONIC PULMONARY DISEASE IN CHILDREN

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Introduction and Objectives Lung biopsy is frequently used in the management of children with chronic pulmonary disease to obtain a histological diagnosis. Previous studies have reported a high diagnostic yield and a low complication rate following lung biopsy

in children. We set out to further evaluate the role of lung biopsy in this group of clinically challenging patients by reviewing our experience of this procedure.

Methods A retrospective case-note review was carried out of all patients under 18 yrs who underwent a lung biopsy from 1998 to 2011. Data collected included age, mode of biopsy, histological findings, pre and post biopsy diagnosis, pre and post biopsy treatment, and complications of the procedure.

Results Thirty three children (12 boys) underwent lung biopsy in the period studied. Their ages ranged from three months to 16 years (median 5 years 5 months). Five biopsies were obtained using a thoracoscopic approach, the remainder via thoracotomy. Following the procedure, 17 patients required ventilation on the intensive care unit for a median of two days. Eight children (24%) experienced a direct complication of the procedure. Seven required an intercostal drain for a pneumothorax, one a tension pneumothorax presenting six days post-operatively. The operative mortality was 12% (4/33). Three children (9%) died within 28 days of surgery, two due to progressive respiratory failure and one following pneumonia. One patient died three months post-operatively due to a persistent air leak from the biopsy site. Twenty-six (79%) biopsies provided a sample adequate for definitive histological diagnosis. In 16 (48%) children the working diagnosis and treatment was changed following lung biopsy.

Conclusion Lung biopsy retains a place in the management of children with chronic pulmonary disease when there is diagnostic doubt. However it carries a significant morbidity and mortality which must be borne in mind when considering the need for histological diagnosis.

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 airway infection and inflammation. UK nationwide screening began in 2007.

Aims It is usual clinical practise at our centre to perform fibreoptic 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 13 (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 $p<0.01$).

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.

Abstract S82 Table 1

Table 1: BALF cell counts $\times 10^3$. Figures given are medians (range)

	NBS CF micro +ve	NBS CF micro -ve	Established CF micro +ve	Established CF micro - ve	Healthy controls
n (absolute cell count)	10	22	33	15	6
Absolute cell count $\times 10^3$	418 (155.0- 2060)	378 (75-1877)	1495 (140-48990)	555 (143- 14780)	143 (70-270)
n (neutrophil Differential)	13	24	22	15	6
Neutrophil differential (%)	17.2 (2.0-73.0)	8.4 (0.3-57.3)	53.2 (15.7-92.0)	19.3 (1.7-87.7)	1.4 (0.3-2.7)

S83 LEVELS OF ANTIMICROBIAL PEPTIDES IN THE AIRWAY OF CHILDREN WITH CYSTIC FIBROSIS ARE NOT RELATED TO SERUM VITAMIN D CONCENTRATION

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Introduction There is convincing evidence of the clinical health benefits of adequate vitamin D in many respiratory diseases but the evidence in cystic fibrosis (CF) is unclear. There are increasing data on the role of vitamin D as an immunomodulatory agent and it is thought, from *in-vitro* data, that this may be via induction of the antimicrobial peptides, LL37 and H β D-2. We hypothesised that antimicrobial peptide levels would be increased in children with adequate vitamin D and this could account for reported improvements in lung function via improved airway defence.

Aims and methods The main aim was to establish if a relationship exists between vitamin D and antimicrobial peptides, LL37 and H β D-2, and whether any clinically beneficial effects of adequate vitamin D exist in children with CF. Bronchoalveolar fluid (BALF) supernatant levels of LL37 and H β D-2 were by measured by ELISA and serum 25(OH)D₂ by mass spectrometry coupled with high-performance liquid chromatography.

Results Samples were collected from 120 children with CF (58% female); median age (range) 6.9 (0.1–17.6) years. One third of patients were vitamin D insufficient (<50 nmol/L). Median 25(OH)D₂ was 57 nmol/L (range 7–191 nmol/L). LL37 ranged from 0.1–21.9 ng/mL with median value of 0.49 ng/mL and H β D-2 from <15.6 – >1000 pg/mL, median 150 pg/mL.

LL37 was significantly correlated with serum neutrophils ($r=0.4$, $P<0.0001$), BALF total cell count ($r=0.7$, $p<0.0001$), and BALF neutrophil differential ($r=0.5$, $p<0.0001$). These relationships were not seen with H β D-2. Contrary to our hypothesis neither LL37 nor H β D-2 correlated with vitamin D and no differences were seen between vitamin D 'adequate' and 'insufficient' patients. There was no association seen between vitamin D and FEV₁ ($r^2=0.01$, $p=0.4$).

Conclusions Our results demonstrate that there is not a relationship between serum 25(OH)D₂ and BALF H β D-2 or LL37. If

vitamin D is involved in the induction of such defence peptides *in-vivo*, the impact of this on protein levels may be limited in the degradative environment of the inflamed airway. In addition, we found no clinical or physiological effects of vitamin D deficiency. If any beneficial effect of vitamin D on respiratory health does exist in CF, it is small and not mediated via the antimicrobial pathway.

Mechanisms of airway injury in COPD

S84 CIGARETTE SMOKE PROMOTES EXAGGERATED INFLAMMATORY RESPONSE IN THE PRESENCE OF Z-AT

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Severe deficiency of the major anti-elastase α_1 -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 1R3F 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- κ B and AP-1 by ELISA, immunoblot or RT-PCR. N-acetylcysteine (NAC, 10^{-5} 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 ± 20.71 pg/ml vs. 37.1 ± 2.7), IL-6 (421.4 ± 20.8 pg/ml vs. 159.3 ± 12.1), IL-8 (8763 ± 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- κ B ($p<0.001$) and AP-1 ($p=0.001$) at 0.5h. In CSE-Z-AT treatment with NAC significantly inhibited TNF- α , IL-6, IL-8, MCP-1, NF- κ B, AP-1 and Ox-pZ-AT formation ($p<0.001$ for all). These findings were confirmed on NHBE cells.

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.

S85 NEUTROPHIL CELL MEMBRANE EXPRESSION OF PROTEINASE 3 AND ITS RELATIONSHIP TO ALPHA-1-ANTITRYPSIN DEFICIENCY (A1ATD)

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Introduction Serine proteinases such as proteinase 3 (PR3) and neutrophil elastase (NE) on the surface of activated neutrophils retain their activity, which may be central to the tissue damage in emphysema. However, little is known about this expression in A1ATD when it may be more critical.