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.

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**Mechanisms of airway injury in COPD**

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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% CS generated from IR5F 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,10⁻³M) 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 (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 (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-κB, 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 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.

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**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 H2D-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 H2D-2, and whether any clinically beneficial effects of adequate vitamin D exist in children with CF. Bronchoalveolar fluid (BALF) supernatant levels of LL37 and H2D-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 H2D-2 from <15.6 to >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 H2D-2. Contrary to our hypothesis neither LL37 nor H2D-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 (r²=0.01, p=0.4).

**Conclusions** Our results demonstrate that there is not a relationship between serum 25(OH)D₂ and BALF H2D-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.

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

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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).

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 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.