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

doi:10.1136/thoraxjnl-2012-202678.089

¹RM Thursfield, ²A Bush, ¹EWFW Alton, ¹JC Davies. ¹National Heart and Lung Institute, Imperial College, London, UK; ²Department of paediatric respiratory medicine, Royal Brompton Hospital, London, UK

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

doi:10.1136/thoraxjnl-2012-202678.090

¹S Alam, ²S Janciauskiene, ¹R Mahadeva. ¹University of Cambridge, Cambridge, UK; ²Hannover Medical School, Hannover, Germany

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)

doi:10.1136/thoraxjnl-2012-202678.091

¹NJ Sinden, ²E Sapely, ²GM Walton, ¹RA Stockley. ¹ADAPT project, Queen Elizabeth Hospital, Birmingham, United Kingdom; ²University of Birmingham, Birmingham, United Kingdom

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.