COPD: from genetics to old age

S62 POLYCLONAL FREE LIGHT CHAINS: A POTENTIAL BIOMARKER FOR IMMUNE ACTIVATION IN ALPHA-1-ANTITRYPSIN DEFICIENCY (A1ATD) RELATED CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Introduction A1ATD is a hereditary condition associated with the premature onset of chronic obstructive pulmonary disease (COPD). In COPD, the immune response within the lung is known to involve cells of both the innate and adaptive immune systems. However, the antigenic stimulus to the adaptive immune response remains unknown. Bacterial colonisation of the lower airways is one potential factor. Alternatively this could reflect an autoimmune component. The production of antibodies by B-cells is a key element of the adaptive immune system. An excess of free light chains (FLCs) are produced as a by-product of antibody synthesis. The recent discovery of high polyclonal FLC levels in a number of autoimmune and inflammatory conditions has led to the assessment of their value as a biomarker of

adaptive immune activation. Our aim was to investigate the use of serum FLCs as a marker of immune activation, phenotypic variation and disease severity in COPD related to A1ATD.

Methods We measured FLC levels in 294 patients with A1ATD using the Freelite serum FLC assay. We then compared combined (? & ?) FLC levels (cFLC) in different subgroups defined by the presence of chronic bronchitis, CT findings, smoking status, subsequent mortality and colonisation status. In addition we determined any correlation with lung function parameters in a cross sectional analysis.

Results Significantly higher cFLC levels were found to be associated with, subsequent mortality ($p = 0.005$), the presence of chronic bronchitis ($p = 0.008$) and chronic colonisation of the lower respiratory tract ($p = 0.036$) (Table 1). FLC levels are known to increase with age and reducing renal function. A partial correlation controlling for these factors revealed no relationship between cFLC levels and the severity of lung function impairment.

Conclusions These results suggest that A1ATD patients with chronic bronchitis and chronically colonised airways have a greater adaptive immune response compared to those without. Whether this response is driven directly by microorganisms residing in the airways, or through an autoimmune phenomenon remains to be elucidated. Whether the use of FLCs in sub cohorts could have an impact on the clinical management of patients with COPD is yet to be determined.

Abstract S62 Table 1. Comparison of combined FLC (cFLC) concentrations in different sub groups (using Mann Whitney U test)

	Group 1	Group 2	P value
Mortality	Dead 11.2% median cFLC = 28.8 (IQR 16.2)	Alive 88.8% median cFLC = 25.2 (IQR 10.1)	0.005**
Emphysema	Yes 77.8% median cFLC = 25.2 (IQR 10.3)	No 22.2% median cFLC = 26.8 (IQR 11.2)	0.222
Bronchiectasis	Yes 31.9% median cFLC = 25.8 (IQR 10.4)	No 68.1% median cFLC = 26.4 (IQR 10.5)	0.446
Chronic bronchitis	Yes 33.7% median cFLC = 27.8 (IQR 14.3)	No 66.3% median cFLC = 25.0 (IQR 9.4)	0.008**
Current smokers	Yes 9.2% median cFLC = 25.4 (IQR 8.0)	No 90.8% median cFLC = 26.1 (IQR 10.5)	0.944
Culture positive (1 stable sputum PPM >10 ⁵ CFU/ml)	Yes n = 47 median cFLC = 29.9 (IQR 17.3)	No n = 24 median cFLC = 28.8 (IQR 15.3)	0.584
Chronically colonised ***	Yes n = 8 median cFLC = 38.9 (IQR 9.7)	No n = 13 median cFLC = 26.3 (IQR 17.1)	0.036*

* $p \leq 0.05$

** $p \leq 0.01$

PPM = potentially pathogenic organism, CFU = colony forming unit

***Only patients who had provided a minimum of 3 stable state sputum cultures included in analysis

S63 F ALPHA-1 ANTITRYPSIN POLYMERISES AND PROMOTES AN EXAGGERATED INFLAMMATORY RESPONSE

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Alpha-1 antitrypsin (AT) is the most important anti-elastase in the lung. Z-AT (342Glu>Lys) polymerises within the hepatocyte resulting in severe plasma deficiency and is the commonest genetic reason for the development of COPD. The F₁-anti-trypsin variant (223Arg>Cys) has been associated with mild plasma deficiency, and when found in association with the Z allele linked to emphysema and liver cirrhosis. We investigated the properties of F-AT in a cell-model.

Human F-AT cDNA was generated by site-directed mutagenesis and overexpressed into hepatocytes (HepG2 cells). Supernatants, lysates and inclusion bodies were assessed for total AT. F-AT cells were assessed for polymeric-AT, PERK, NF-kB, AP-1, TNF- α and IL-6 by ELISA, immunoblot or RT-PCR in comparison with normal (non-polymerising) M-AT and Z AT (polymerising control).

F-AT cells had no cell cytotoxicity or apoptosis upto 72h. At 24h (unless stated) F-AT secretion was slightly lower, but comparable to M-AT secretion (1479.3 ± 142 pg/ml vs. 1745.5 ± 102.3 pg/ml, $P = 0.413$) Secreted F-AT had significantly reduced elastase inhibitory capacity compared to M-AT (0.972 ± 0.069 (OD at 405nm) vs. 0.449 ± 0.085 , $P < 0.001$). F-AT formed insoluble aggregates of polymeric-AT (375 ± 32 pg/ml vs. undetectable), upregulated PERK mRNA (at 3h) and significantly increased NF- κ B (at 16h), AP-1, TNF- α (34.5 ± 5.3 pg/ml vs. 10.53 ± 3.2 , $P = 0.023$) and IL-6 (at 48h) (132.3 ± 20.8 pg/ml vs. 23.9 ± 16) ($P = 0.006$). All of which were inhibited by treatment with an inhibitor of polymerisation ($P < 0.001$ for all). In comparison to Z-AT, F-AT secretion was greater ($P < 0.001$) but had significantly reduced anti-elastase activity ($P = 0.032$) and

lower ER accumulation of polymeric-F-AT ($P < 0.001$). In the F-AT cell, PERK mRNA was upregulated at 3h compared to 0.5h in Z-AT. Although elevated compared to M-AT cells, F-AT cells had lower NF- κ B activity ($P < 0.001$), TNF- α production ($P = 0.046$) and IL-6 production ($P = 0.012$) compared to Z-AT.

In conclusion, F-AT secretion was comparable to M-AT. However, secreted F-AT was defective as an anti-elastase. F-AT was found to polymerise and aggregate in inclusion bodies. ER accumulation of F-AT activated the ER overload response; PERK-dependant-NF- κ B mediated inflammatory response, greater than M-AT but to a lesser degree than Z-AT. This data indicate that FZ phenotype may be at risk for liver and lung disease.

S64 CIRCULATING POLYMERS ARE FOUND IN ALPHA-1-ANTITRYPSIN DEFICIENCY AND ARE ASSOCIATED WITH LUNG DISEASE

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Introduction and Objectives The severe Z deficiency allele of alpha-1-antitrypsin (Glu³⁴²Lys) results in the formation of polymers that are retained within hepatocytes, leading to hepatitis, cirrhosis and hepatocellular carcinoma. The concomitant lack of circulating protein predisposes to early onset emphysema. Polymers are also found in the lung, skin and kidney and are known to be proinflammatory, but it is unknown whether those polymers are produced locally or are deposited from a circulating source. We wished to establish whether polymers are present in the plasma of individuals with alpha-1-antitrypsin deficiency, from where they originate and whether they are associated with any clinical phenotype.

Methods We used a novel anti-alpha-1-antitrypsin polymer monoclonal antibody (2C1) in an ELISA assay to evaluate whether polymers are present in a cohort of 513 individuals with ZZ alpha-1-antitrypsin deficiency. Serial samples from an individual with ZZ alpha-1-antitrypsin deficiency undergoing liver transplantation were used to investigate the source of circulating polymers. We then used a 2nd cohort of 293 individuals with mixed alpha-1-antitrypsin phenotypes to determine whether circulating polymers could be used as a screening test for the presence of a polymerogenic allele. Disease associations were sought using clinical data from the ZZ alpha-1-antitrypsin deficient cohort.

Results In the cohort of 513 individuals with PiZZ alpha-1-antitrypsin deficiency, we found 512 had quantifiable polymers present within serum, the 513th having previously had a liver transplant. Circulating polymer levels were higher in men and individuals with COPD and a there was a correlation with older age and lower lung function. The presence of circulating polymers was 100% sensitive and 89% specific in identifying 20 PiZZ alpha-1-antitrypsin homozygotes in a mix of 293 alpha-1-antitrypsin genotypes. Serial blood samples from a PiZZ

individual undergoing liver transplantation showed that circulating polymers originate from the liver, clearing with a half-life of approximately 30 hours and becoming undetectable 5 days after transplantation.

Conclusions We have shown that circulating polymers are present in PiZZ alpha-1-antitrypsin individuals and originate from the liver. Polymer levels are associated with COPD, suggesting they may play a role in disease pathogenesis.

Abstract S64 Figure 1. Z 1 antitrypsin polymers are present in the circulation and are cleared following liver transplantation. Mouse monoclonal antibodies that detect all conformers of α_1 -antitrypsin, or only polymeric (α_1 -antitrypsin (2C1), were used to quantify total (α_1 -antitrypsin and (α_1 -antitrypsin polymers respectively using sandwich ELISA. Time points marked * are below the lower limit of quantification (0.4 μ g/mL).

S65 LARGE AND SMALL AIRWAY EPITHELIAL CELL SENEESCENCE PRESENT IN COPD AND BRONCHIECTASIS?

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Introduction and Objectives Accelerated lung ageing has been implicated in the pathogenesis of COPD and therefore targeting cellular senescence may have therapeutic benefit. COPD is increasingly felt to have significant sub-phenotypes with large and small airway involvement. The airway epithelium likely endures the majority of potentially senescence-inducing insults. However, data on airway epithelial cell (AEC) senescence in COPD is limited and comparisons between large and small airways are lacking. Furthermore, the role of infection in COPD-associated senescence is unclear. To date, senescence in bronchiectasis has not been investigated as a model for infection-induced senescence. We sought to determine AEC expression of senescence-associated markers in COPD and bronchiectasis and to compare large and small airways.

Methods Lung explant tissue from our transplant programme from COPD (n = 19) and bronchiectasis (n = 14) with resection tissue from smokers without lung disease (control) (n = 11) was stained for senescence-associated markers by immunohistochemistry. Staining was quantified semi-quantitatively. Fluorescence *in situ* hybridisation (FISH) was used to investigate telomere length and possible co-localisation with DNA damage-associated proteins.