138 Urinary inflammatory markers in the assessment of clinical status in adults with CF

E.F. Nash[1], G. Parekh[2], P. Davis[2], J.L. Whitehouse[1], M. Stevens[3]. [1]*West Midlands Adult CF Centre, Birmingham Heartlands Hospital, Birmingham, United Kingdom;* [2]*Mologic Ltd, Sharnbrook, United Kingdom;* [3]*Department of Endocrinology, Diabetes and Medicine, University of Birmingham, Birmingham, United Kingdom*

Introduction: Sputum and blood inflammatory markers, including MMP-9, TNF-alpha and IL-8, are elevated in CF and these same biomarkers increase during pulmonary exacerbations. In this prospective study, we examine whether urinary inflammatory biomarkers are detectable and reflect clinical status in CF adults.
Methods: Subjects were recruited at our regional centre, data included: demographics, spirometry and clinical status ('stable' or 'exacerbation'). Subjects were chronically infected with *P. aeruginosa*. Pulmonary exacerbations were defined according to the Fuchs criteria. Urinary MMP-9, TIMP-1, TIMP-2, neutrophil elastase, alpha-1-antitrypsin and neutrophil gelatinase-associated lipocalin (NGAL) were measured.
Results: 14 subjects (8 male) had provided 24 urine samples at the time of abstract submission, FEV1% predicted (mean±SD) 49±14.2%. A1AT was significantly higher in 'stable' compared to 'exacerbation' samples [535.0 (210.1–653.1) ng/ml vs. 86.0 (32.5–185.3) ng/ml, $p < 0.006$]. TIMP-2/A1AT ratio [0.0095 (0.0055–0.019) vs. 0.046 (0.022–0.115), $p = 0.013$], TIMP-1/A1AT ratio [0.002 (0.002–0.003) vs. 0.01 (0.002–0.04), $p = 0.03$] and NGAL/A1AT ratio [0.129 (0.01–0.3) vs. 0.535 (0.23–1.07), $p = 0.04$] were significantly lower in 'stable' compared to 'exacerbation' samples. All other inflammatory markers tested were detected, but no significant differences were seen in this preliminary analysis.
Conclusions: We demonstrate for the first time that relevant inflammatory biomarkers are present in the urine of CF adults. The assessment of urinary inflammatory markers may prove to be a useful non-invasive method to diagnose pulmonary exacerbations, as well as to assess treatment response.

139 Gamma delta IL-6, IL-12 correlate with FEV1 in children with cystic fibrosis

M. Singh[1], N. Anil[1], A. Rajwanshi[2], H. Vohra[3]. [1]*PGIMER, Pediatrics, Chandigarh, India;* [2]*PGIMER, Cytology, Chandigarh, India;* [3]*PGIMER, Experimental Medicine and Biotechnology, Chandigarh, India*

Objectives: To determine the expression of gamma delta IL-6 and IL-12 in children with CF and controls. Furthermore to evaluate the relationship between gamma delta IL-6 and IL-12 and lung function.
Material and Methods: Twenty children with CF (Age 6–14 yrs), in a stable clinical condition were enrolled from the Outpatient Clinic of Department of Pediatrics, PGIMER, Chandigarh, India. Ten healthy controls were also enrolled. Sputum induction and processing were carried out according to standard guidelines. Cell acquisition and analysis following antibody staining with anti human gamma delta (PE labeled), IL-6, IL-12 (FITC labeled) was carried out flowcytometrically on FACS Calibur, using FACS Calibur™ software. Percentage positive cytokines were reported as mean±SD. Spirometry was performed according to standard ATS/ERS guidelines.
Results: We observed a high percentage of gamma delta IL-6 in children with CF (17.9±2.8) as compared to controls (8.2±2.1) $p < 0.05$, gamma delta IL-12 in children with CF (21.5±3.4) as compared to controls (10.4±2.5) $p < 0.05$. Gamma delta IL-6 correlated negatively FEV_1 ($r = -0.582$, $P < 0.05$). Furthermore gamma delta IL-12 correlated negatively FEV_1 ($r = -0.742$, $P < 0.05$).
Conclusion: Gamma delta T cells contribute to the dysregulated inflammatory lung response in CF airways through increased production of IL-6 and IL-12. Dysregulated gamma delta IL-6, IL-12 are associated with the clinical status in children with CF. Increased expression of gamma delta IL-6 might contribute to the enhanced IL-17 production in CF airways resulting in excessive neutrophilic mediated lung tissue damage.

140 Association between host immunological and pro-inflammatory mediators with survival in cystic fibrosis patients chronically colonised with *Pseudomonas aeruginosa* (PA)

K. Moffitt[1], S.L. Martin[2], L. Wei[1], A. Jones[3], A.K. Webb[3], M. Tunney[1], M. Ennis[4], J.S. Elborn[1,4]. [1]*Queen's University Belfast, CF and Airways Microbiology Research Group, Belfast, United Kingdom;* [2]*Queen's University Belfast, Biomolecular Sciences Group, Belfast, United Kingdom;* [3]*Manchester Adult Cystic Fibrosis Centre, Manchester, United Kingdom;* [4]*Queen's University Belfast, Centre for Infection and Immunity, Belfast, United Kingdom*

Introduction and Aims: Chronic PA pulmonary infection is associated with an increased host inflammatory response involving the release and activation of damaging inflammatory mediators. Quantification of these mediators may give an indication of lung damage and changes in clinical status and mortality outcome. The aim of this study was to determine whether serum IgG and IgA titre against PA in clinically stable, chronically colonised adult CF patients ($n = 40$) correlates with mortality and host inflammatory mediators.
Methods: Serum was assayed by ELISA for IgG and IgA against PA antigens. Sputum HNE, Cathepsin S and Cathepsin B were measured by spectrophotometric and fluorogenic assays with sputum IL-8 and TNFa, plasma IL-6 and urine TNFr1 measured by ELISA. Correlations were calculated between inflammatory mediators and antibody titre and results compared with a 10-year survival outcome.
Results: A significant positive correlation was observed between IgG and IL-6 ($p = 0.0005$), TNFa ($p = 0.0068$), TNFr1 ($p = 0.037$), Cat S ($p = 0.0106$) and Cat B ($p = 0.0006$). No correlation was found between IgG and IL-8 or HNE ($p = 0.6229$, 0.3921 respectively). In addition, IL-6 levels positively correlated with mortality ($p = 0.037$). No correlations were found between IgA and any of the inflammatory mediators.
Conclusion: Increases in IgG titre and IL-6 levels were shown to be associated with 10 year mortality. Pro-inflammatory mediators correlated with IgG response, and suggest that IgG to PA is an immunological marker of chronic infection and survival.

141 TIM-3 is required for neutrophil mediated Gram-negative bacterial killing: an effect abrogated within the cystic fibrosis lung

I. Vega-Carrascal[1], E.P. Reeves[1], N.G. McElvaney[1]. [1]*Royal College of Surgeons in Ireland, Dublin, Ireland*

The TIM family of membrane receptors have emerged as potential therapeutic targets to correct abnormal immune function in chronic inflammatory conditions. TIM-3 serves as a functional receptor in bronchial epithelial cells and through its natural ligand galectin-9 can modulate the inflammatory response. The aim of this study was to investigate whether TIM-3 was expressed in neutrophils and what function it may have in the healthy cell, which would be necessarily defective in the adult cystic fibrosis (CF) lung due to inactivation by neutrophil derived proteases. TIM-3 membrane expression in circulating neutrophils was examined using RT-PCR, western blotting and FACs analysis. Neutrophil degranulation was assessed by western blotting and superoxide (O_2^-) production by cytochrome C reduction assays.
Via signalling through TIM-3, galectin-9 induced neutrophil degranulation and O_2^- production. We have demonstrated that TIM-3 expressed on neutrophils plays a direct role in bacterial killing. Opsonisation of *Pseudomonas aeruginosa* with galectin-9 (50 nM) enhanced neutrophil-mediated bacterial killing by 25%, an effect abrogated by blockade of neutrophil TIM-3 receptors. This mechanism was gram-negative bacteria specific and mediated via galectin-9/lipopolysaccharide binding.
In conclusion, our data suggest a novel role for TIM-3/galectin-9 in neutrophil function with important consequences in neutrophil mediated killing of *Pseudomonas*. However, in the CF lung, membrane TIM-3 and its ligand galectin-9 are proteolytically degraded, potentially contributing to the defective bacterial clearance observed within the CF lung despite the high neutrophilic presence.