181* Development of a novel tool for the rapid detection of neutrophil elastase as a marker of inflammation within the clinic

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Neutrophil elastase (NE) is a biomarker of infection and inflammation which has been shown to correlate with the severity of several respiratory diseases, including cystic fibrosis (CF). Utilising our unique Protease-Tag molecules, we have developed a novel methodology for the capture and detection of active proteases in complex samples such as CF sol. The aim of this work was to provide initial clinical validation of the NE-Tag assay in CF.

Sputum (n = 45) was recovered from CF patients hospitalised for acute exacerbation. Sol was recovered and analysed for NE activity using the NE-Tag ELISA and two fluorogenic substrate-based assays [1. Suc-AAPV-AMC (Sigma) and 2. Innozyme™ Immunocapture assay (Calbiochem)]. NE activity between assays and with a range of clinical parameters was correlated.

A highly significant correlation was shown between assays. NE activity (NE-Tag) further correlated appropriately with clinical parameters: inversely with FEV_1 ($p = 0.036$) and positively with CRP ($p = 0.035$), neutrophils and total white cell counts ($p = 0.000$). The Innozyme™ assay showed similar correlations with the clinical parameters (with the exception of CRP). No correlations with any of the clinical parameters were observed when NE was measured using the standard fluorogenic substrate.

NE as a primary efficacy endpoint in clinical trials or as a marker of inflammation within the clinic has been hampered by the lack of a robust and simple to use assay. The NE-Tag assay has advantages over the InnoZyme™ assay in terms of time, ease of use and no dependency on a kinetic readout; it has also the capability of being transferred to a lateral flow device for routine monitoring.

182 Exhaled breath condensate (EBC) oxidative stress in children with cystic fibrosis

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Introduction: Cystic fibrosis is a frequent genetic disease with pulmonary inflammation, enhanced by repeated infections. This early inflammatory process leads to bronchial alterations and pulmonary functional defects.

The aim of the study was to compare oxidative stress markers [Malondialdehyde (MDA)] in exhaled breath condensate in children with cystic fibrosis vs. normal controls.

Patients and Methods: In this prospective case-control study, 15 children with cystic fibrosis were matched with 30 controls regarding age and sex. In both groups exhaled breath condensate, pulmonary function tests, urine and blood samplings were performed on day 1 AM. MDA levels were determined by Gas Chromatography. Repeat exhaled breath condensate measurements were performed on day one PM and 3 months later in CF patients.

Results: Median [range] age of patients was 9.6 [6.2–12.1] yrs, the sex ratio was M:F = 1:0.66. The MDA determinations were reproducible in both groups with a coefficient of variation of 6.3% (2 assays per sample). Median EBC MDA level on day 1 AM was 1.40 [0.55–7.53] in CF patients versus 0.80 [0.00–4.65] in controls ($p < 0.05$). In CF patients day 1 PM MDA levels were 0.95 [0.26–2.68] (NS vs. day 1 AM). MDA levels 3 months later showed a further increase in oxidative stress in CF patients: 1.62 [0.07–31.26] ($p < 0.05$ vs day 1).

Conclusion: Pulmonary oxidative stress markers in exhaled breath condensate are increased in children with CF compared to controls, with good reproducibility.

183 The relationship between resting cytokine levels and standard clinical measurements of disease activity in stable adult cystic fibrosis patients

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Background: Previous studies report patients with cystic fibrosis (PWCF) have persistently elevated circulating pro-inflammatory serum cytokine concentrations. In other chronic diseases, these cytokines have been associated not only with physiological and metabolic effects but also psychological ones. This study analyses the serum circulating cytokines in PWCF and their clinical relationships, including the 9 domains of health assessed in the Cystic Fibrosis Questionnaire-Revised (CFQR).

Methods: Circulating pro-inflammatory cytokines concentrations (IL-6, IL-8, TNF-α (cachexin) and IL-1β) were measured in the supernatant of whole blood samples obtained from stable adult PWCF >5 weeks free from an infective pulmonary exacerbation, and a healthy control population. Cytokine concentrations were correlated against CRP, CFQR, serum [1,25 OH D3], FEV_1% predicted, azithromycin use and neutrophil count using a Bonferroni correction.

Results: 68 PWCF and 57 controls were recruited. The pro-inflammatory cytokines were not significantly higher in PWCF. There were no significant correlations between [cytokine] and the 9 domains of health, azithromycin use, serum [1,25 OH D3] or neutrophil count. As expected physiologically, IL-6 correlated significantly with CRP ($r = 0.69$, $p < 0.001$), TNF-α correlated with reduced BMI ($r = 0.274$, $p = 0.02$) and the CFQR had multiple significant correlations with FEV_1% predicted, BMI and age in PWCF ($p < 0.005$).

Conclusion: In our cohort of stable adult PWCF there was no association between baseline cytokine concentrations and psychological well being. The lack of a significant elevation in resting cytokine levels in PWCF may contribute to this.

184* Basophil CD203c as a potential clinically relevant biomarker in cystic fibrosis and allergic bronchopulmonary aspergillosis

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Introduction: The opportunistic fungus *Aspergillus fumigatus* (*Af*) colonizes a significant fraction of cystic fibrosis (CF) patients, with some further progressing towards allergic bronchopulmonary aspergillosis (ABPA), a type I hypersensitivity response. ABPA significantly impacts short- and long-term prognoses in CF, yet may be avoided by early diagnosis and treatment. However, the diagnosis of CF-ABPA is clinically challenging, due in large part to the absence of an objective biological test. Since blood basophils play a major role in allergic responses, we hypothesized that discrete changes on their surface activation pattern could discriminate CF patients with ABPA from those without.

Method: We used a direct flow cytometry assay to measure surface CD203c levels on blood basophils, at baseline and upon rapid *in vitro* activation with *Af*-specific allergen, in 3 groups of subjects: (A) CF patients with chronic *Af* infection but without ABPA (N = 4); (B) CF-ABPA patients (N = 7); and (C) healthy controls (N = 7).

Results: In the CF-ABPA group, but not other groups, basophil CD203c levels increased specifically upon *Af* allergen stimulation ($P < 0.004$). Basophil CD203c levels upon *Af* allergen stimulation provided excellent predictive value for discriminating CF-ABPA from CF patients with chronic *Af* infection but without ABPA (area under the receiver operating characteristics curve = 0.95, $P < 0.009$).

Conclusion: These results support the notion that basophils play a role in the pathogenesis of CF-ABPA and can be used to diagnose this condition with a simple, clinically-relevant, blood assay.