45 Development of a novel ProteaseTag™ immunoassay for the detection and measurement of cathepsin G in adult cystic fibrosis patients

C. Robb1,2, K.L. Moffitt1,2, T. Ferguson1,2, B. Walker1, J.S. Elborn3, D. Ribeiro2, S.L. Martin1,2, 1Queen’s University Belfast, School of Pharmacy, Belfast, United Kingdom; 2ProAxsis Ltd, Belfast, United Kingdom; 3Queen’s University Belfast, Centre for Infection and Immunity, Belfast, United Kingdom

Objectives: Cathepsin G (CatG) has been linked to chronic lung disease such as emphysema, COPD, CF and ALI. However, the highly autolytic nature of the protease and lack of a truly selective assay for measuring its activity in clinical samples has hindered the validation of CatG as a biomarker for these diseases. We report on the use of a novel assay approach with the potential to overcome these problems.

Methods: Expected sporadic sputum was randomly collected from CF patients (n = 15) hospitalised for acute exacerbation (8M/7F; age 27±6.49 years). Sol was recovered and analysed using our ProteaseTag™ Active Cathepsin G Immunoassay and the data compared with that obtained using a fluorogenic substrate assay for the protease. Statistical analysis was carried out to observe correlations between CatG activity and other clinical and demographic parameters: CRP, white cell and neutrophil count, BMI, FEV1 and age.

Results: Active CatG could be detected and quantified by the ProteaseTag™ Immunoassay in all of the patient samples (mean activity 1.2±0.9 ng/ml), but only in 33% of samples using the fluorogenic substrate assay (mean activity 1.6±3.5 ng/ml). Furthermore, correlations were found between CatG as measured by Immunoassay with patient age (r = 0.7; p = 0.009) and white cell count (r = 0.5; p = 0.04). No such correlations were evident using the fluorogenic substrate assay.

Conclusion: We report, for the first time, on the measurement of active CatG, using our patented ProteaseTag™ Active Cathepsin G Immunoassay, in Sputum Sol obtained from CF patients. This assay has advantages over substrate-based kinetic activity assays in terms of specificity and sensitivity.

46 Inflammatory markers in epithelial lining fluid from CF patients with ABPA obtained non-invasively by nasal lavage

C. Arnold1, T. Fleischmann1, J. Hentschel1, T. Lehmann1, J. Riedel1, K. Boer2, K. Hanner2, O. Kurzai2, J.G. Mainz1, Jena University Hospital, Cystic Fibrosis Centre, Jena, Germany; 2University Hospital of Leipzig, Institute for Medical Genetics, Leipzig, Germany; 3Jena University Hospital, Institute of Medical Statistics, Jena, Germany; 4Jena University Hospital, Medical Microbiology, Jena, Germany; 5Jena University Hospital, Institute for Clinical Chemistry, Jena, Germany; 6Friedrich Schiller University Jena, Septomics Research Centre, Jena, Germany; 7University of Jena, Department of Dermatology, Jena, Germany

Introduction: In CF, airway colonization with Aspergillus fumigatus (Asp.f.) is common. The prevalence of Asp.f. sensitization is reported to be 35% and 9% develop allergic bronchopulmonary aspergillosis (ABPA). Diagnosis of ABPA remains challenging due to an overlap of signs and symptoms with common CF manifestations. This is the first study to longitudinally assess inflammatory markers in epithelial lining fluid non-invasively sampled by nasal lavage (NL) in CF patients with ABPA.

Methods: We examined 78 NL samples from 15 patients with the diagnosis of ABPA (2010–2015; median age: 18±8; female: 8). Selection criteria were total serum immunoglobulin (IgE) >500 IU/mL, elevated specific Asp.f. IgG and IgE as well as specific IgE against recombinant Asp.f allergens (rAsp.f) 4 and 6. Interleukins (IL)-1β, IL-5, IL-6, IL-13, neutrophil elastase (NE) and thymus- and activation-regulated chemokine (TARC) in NL were assessed by multi-analyte profiling and ELISA.

Results: Twelve patients had the clinical diagnosis ABPA according to Nelson’s criteria. Another three patients exhibited elevated values for IgE or IgG-Asp. Concentrations of IL-1β in NL were directly related to serum IgE (r = 0.63; p = 0.006). IL-5, IL-6, IL-13, NE and TARC did not show an association to serological ABPA markers, attributed to low sensitivity of tests and instability of some mediators during prolonged storage at −80°C.

Conclusion: Upper airway sampling allows non-invasive detection of biomarkers that may contribute to diagnosis of ABPA. Following studies should optimize test kits, identify further markers in NL and compare their diagnostic value with established markers for diagnosis of ABPA.

47 Nasal and exhaled nitric oxide in cystic fibrosis (CF) in relation with lung function and blood leukocytes

C. Krantz1, K. Alving1, A. Malinovschi2, 1Uppsala University, Department of Women and Childrëens Health, Uppsala, Sweden; 2Uppsala University, Department of Medical Sciences, Clinical Physiology, Uppsala, Sweden

Objectives: To examine if patients with CF have lower levels of nNO and FeNO than controls and to see if NO levels correlate with lung function and blood leukocyte count.

Methods: 38 patients (19 adults) with CF were recruited. They performed measurements of nNO, FeNO and spirometry and venous blood samples were drawn. A total of 38 healthy controls and 38 asthma patients, matched for gender and age, from the MIDAS-study were included as reference groups.

Results: Levels of nNO in patients with CF (319 [193–447] ppb) were less than half found in healthy controls (797 [664–984] ppb) and asthma patients (780 [619–961] ppb), the difference remained in adults and children when analysed separately. FeNO levels were lower in CF patients (72 [4.7–11–2] ppb) than in healthy controls (114 [8.3–14.6] ppb) and asthma patients (147 [8.7–24.7] ppb). This difference remained in adults, but not in children. No correlation was seen between nNO and blood leukocytes, FENO or lung function in patients with CF. Patients with CF showed a positive correlation between FeNO and FEV1 (r = 0.51; p = 0.001), which was consistent in both adults and children. No correlation between FENO and lung function was seen in the control groups. There was no correlation between FeNO and eosinophil count, but a negative correlation between FeNO and neutrophil count (r = 0.37; p = 0.03), in patients with CF but not in the reference groups.

Conclusion: Patients with CF have lower levels of nNO and, in adults, lower FeNO than healthy controls and asthma patients. In patients with CF, FENO correlates positively with FEV1 and negatively with blood neutrophil count.

48 The humoral immune response against Pseudomonas aeruginosa correlates with decreased lung function in a cohort of Brazilian CF patients

R.M. Mauch1, C.L. Rossi1, T.B. Aiello1, M.T. Nolasco da Silva2, C.C.S. Gomez2, A.F. Ribeiro2, J.D. Ribeiro2, C.E. Levy1, 1University of Campinas, Clinical Pathology (Faculty of Medical Sciences), Campinas, Brazil; 2University of Campinas, Pediatrics (Faculty of Medical Sciences), Campinas, Brazil

Objectives: To evaluate the association of IgG antibody levels to Pseudomonas aeruginosa (Pa) and lung function markers.

Methods: This was a cross-sectional analysis, including 51 patients [median age 16.6 years (8.1–30.0)], addressing the period of 2012 to 2014. A total of 99 lung function tests were made by spirometry, and the variables of lung function were FVC, FEV1, and FEF25–75. Variable values were expressed as percentages of the predicted values for age, according with current guidelines. The anti-Pa IgG measurement was made by ELISA, using the polivalent antigen St-Ag:1-17, with a cut-off value of 15.3 U/mL. The median IgG levels in the period of one year before each spirometry were used to evaluate the associations, which were made by Pearson Correlation Coefficient (r). A result was considered statistically significant when p < 0.05.

Results: The median values for lung function markers were: FVC = 79% (28.1–132), FEV1 = 67% (18–117), FEF25–75 = 50% (06–132). The median anti-Pa IgG level was 10.81 U/mL (1.48–497.1). We found inverse correlations between anti-Pa IgG levels and FEV1 (r = −0.25; p = 0.01), FVC (r = −0.22; p = 0.32), and FEF25–75 (r = −0.27; p = 0.008).

Conclusion: Despite the limitations of a cross-sectional study, our findings show a significant association between the deterioration of FVC and FEV1 and lower anti-Pa IgG levels in CF patients.