European Respiratory Society
Annual Congress 2013

Abstract Number: 2704

Publication Number: P663

Abstract Group: 3.3. Mechanisms of Lung Injury and Repair

Keyword 1: Biomarkers **Keyword 2:** Inflammation **Keyword 3:** Children

Title: Alpha-amylase as a marker of direct aspiration in children with neurodisability

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Body: Background: Pulmonary aspiration is thought to play a key role in the aetiology of recurrent respiratory problems in children with severe neurodisability (ND). At present there are no tests to differentiate between direct salivary (‘from above’) and indirect reflux (‘from below’) aspiration. The role of direct aspiration in the pathogenesis of aspiration lung disease is undefined. Aims and objectives: The aims of this study were to investigate alpha amylase (αA) as a biomarker of direct aspiration and its role in airway inflammation. Methods: αA was measured in BAL samples from children with ND when well (n=16) and during PICU admission (n=18), compared to healthy controls (n=10). In vitro studies were undertaken to investigate the effects of αA on airway inflammation, using an airway epithelial cell model (BEAS-2B). Results: αA activity was significantly increased in patients with ND when well compared to healthy controls (100[46-597] vs. 27[9-71] U/L, p=0.01). Furthermore αA activity was found to positively correlate with BAL neutrophils (r=+0.81, p<0.001), IL-8 (r=+0.77, p<0.001) and TGF-β (r=+0.66, p=0.005). We have gone on to show in vitro that αA induces a dose-dependent inflammatory (IL-8 & IL-6) response in BEAS-2B cells. Conclusions: A clinical biomarker to identify and quantify direct and indirect aspiration would be useful in the clinical assessment of patients with ND presenting with respiratory symptoms, and in guiding subsequent, potentially invasive management. This study suggests that αA may be a useful biomarker of chronic direct aspiration, and that it may have a role in respiratory disease pathogenesis in this group of children.