capacity. The ISWT is one field exercise test which is often employed in the assessment of pulmonary and cardiac rehabilitation (PR, CR) patients and also to prescribe a walking speed. In PR and CR programmes the ISWT is employed to calculate walking speed set at a desired training threshold. The aim of the study was to establish reference values for the ISWT and an equation for its prediction in a healthy population. This will allow comparison between patients from PR and CR programmes and healthy age-matched controls.

Methods Subjects were aged between 40 and 90 years, had normal spirometry defined as FEV₁ % pred >80% and/or a FEV₁/FVC >70% and had no known co-morbidities affecting mobility. The best distance from two ISWT was recorded along with body mass index (BMI) and leg length. Quadriceps maximal voluntary contraction (QMVC: Kg) was measured using a strain gauge (Kern). Physical activity was assessed using the DUKE physical activity questionnaire and an activity monitor (SenseWear PRO² Armband). The number of steps and energy expenditure achieved over 2 days was recorded. Subjects also completed the Hospital Anxiety and Depression Scale.

Results 114 patients completed the study [mean (SD) age 60.48 (10.99) years, FEV₁ 108.82% (15.13) predicted, 37 male]. Mean ISWT distance was 690 m (152.68). There were no significant differences in walking distance between males and females ($p>0.05$). ISWT distance showed significant correlations with age, BMI, FEV₁, QMVC, DUKE physical activity score and height ($p<0.01$). Stepwise multiple regression analysis showed that age, BMI, FEV₁, QMVC and DUKE physical activity score were independent contributors to the ISWT distance achieved by healthy subjects, explaining 50.4% of the variance. (Abstract S120 figure 1).

Conclusions Variance in the ISWT can be measured using a composite score, comprising of: age, BMI, FEV₁, QMVC and DUKE physical activity score. These findings would allow clinicians to express results of the ISWT as a percentage of the predicted values making results more meaningful for patients with chronic conditions.

Mast cells, smooth muscle and inflammation in asthma

S121 MEDIATOR PROFILING OF SEVERE ASTHMA PHENOTYPES

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¹D Desai, ¹C J Newby, ¹P Haldar, ¹S Shah, ¹S Gupta, ¹M Bafadhel, ¹A Singapuri, ¹S Siddiqui, ¹J Woods, ²A Herath, ²I K Anderson, ¹P Bradding, ¹R H Green, ¹A J Wardlaw, ¹I D Pavord, ²R D May, ¹C E Brightling. ¹Institute for Lung Health, Department of Infection, Immunity & Inflammation, University of Leicester, Leicester, UK; ²Medimmune Ltd, Milstein Building, Granta Park, Cambridge, UK

Background Severe asthma is a heterogeneous disease. Defining its phenotypic heterogeneity is likely to shed light upon its immunopathogenesis and direct therapy. We sought to determine the relationship between phenotypes of severe asthma and sputum mediator profiles.

Methods Subjects were recruited from a Difficult Asthma Clinic at a single centre (n=164) and assessments of lung function, atopic status, asthma control and sputum induction were undertaken. Sputum was obtained and supernatants were analysed for 23 mediators using the Meso-Scale Discovery platform. We performed k-means cluster analysis to determine clinical clusters using the baseline characteristics and sputum differential counts. The pattern of mediator expression was determined by factor analysis to identify biological factors. The biological factors were related to the clinical clusters and subjects stratified by asthma control, exacerbation frequency, treatment and sputum cell counts. The repeatability of the individual clinical characteristics and biological mediators was

assessed in paired samples in 106 subjects and in three samples in 66 subjects.

Results We identified four clinical clusters and five biological factors. The biological factors were differentially expressed in subjects stratified by sputum cell counts, asthma control and exacerbation frequency, but were not significantly different across the clinical clusters. The within subject repeatability of mediators was moderate; biological factors were consistent and tracked with sputum cell counts for the repeated visits.

Conclusions Sputum mediator profiling of severe asthma revealed repeatable biological factors that were strongly associated with cellular profiles and inform our understanding of asthma phenotypes.

Abstract S121 Figure 1 Relationship between clinical clusters and biological factors. The clinical clusters are plotted in two dimensions with airway inflammation on the x axis and asthma control (ACQ) on the y axis. The size of the ellipsoid represents the number of subjects within each clinical cluster. The distribution of the five biological factors for each cluster is shown as the mean (SEM) factor scores.

S122 SPUTUM CYTOKINE PROFILES IN ASTHMA AND THE IMPACT OF SMOKING-A FACTOR ANALYSIS

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¹M Spears, ²C Dewet, ¹C McSharry, ¹R Chaudhuri, ¹I Donnelly, ¹L Jolly, ¹E Cameron, ¹N C Thomson. ¹Institute of Infection, Immunity & Inflammation, University of Glasgow, Glasgow, UK; ²NHS Education for Scotland, Glasgow, UK

Introduction Cigarette smokers with asthma have a distinct clinical phenotype from non-smokers with asthma. This may reflect altered airway inflammation although how cigarette smoking directs this is unclear. We employed exploratory factor analysis to examine the impact of smoking on airway inflammation.

Abstract S122 Table 1 Factor loadings

Rotated component matrix			
	Factor		
	1	2	3
IFN- γ	0.991		
IL-4	0.986		
IL-5	0.986		
IL-6		0.864	
CXCL9		0.964	
CXCL10		0.908	
CCL2			0.813
CCL3			0.889
CCL4			0.920

Methods 22 smokers (sm), 10 ex-smokers (ex-sm) and 21 never smokers (ns) with asthma performed spirometry, induced sputum and completed asthma control questionnaires pre and post an oral corticosteroid trial. Sputum fluid cytokines were quantified using a 25-plex bead system (Invitrogen, Paisley). Factor analysis was performed (SPSS V.17) using principal component analysis and Varimax rotation. Factors were identified according to; visual inspection of the scree plot, eigenvalues >1.1 , minimum of three cytokines loading >0.4 . Sequential removal of cytokines was performed in stages according to; moderate to strong (>0.4) loadings, followed by requirement for 'strong' loading (>0.6) to only one factor then removal of cytokines that reduced the reliability of the data set. In a final step cytokines with the lowest loadings were removed if a factor had >3 .

Results The subjects were well matched except for higher asthma control questionnaire scores and inhaled corticosteroid dose in sm. Sm failed to demonstrate a lung function response to oral corticosteroids in contrast to nsm. No sputum cell differential differences were evident between smokers and non-smokers with asthma. A number of pre-steroid sputum cytokines were elevated in sm compared to nsm. The greatest difference present for interleukin 6 (sm 34.4 pg/ml (IQR 14.1, 72.4), nsm 8.1 pg/ml (4.4, 11.1), $p<0.001$). Factor analysis of the pre-steroid cytokines demonstrated that three factors explained 90% of the variance in the data. Sequential processing revealed three cytokines per factor (Abstract S122 table 1).

Discussion Sputum cytokine profiling of subjects with asthma with differing smoking histories reveals distinct groupings when examined by exploratory factor analysis providing insight into airway inflammation in asthma and the impact of smoking. Larger cohorts of patients with asthma should be examined to confirm these preliminary findings.

S123 THE EFFECT OF RHINOVIRUS INFECTION ON COUGH RECEPTORS ON HUMAN SENSORY NERVE AND HUMAN PRIMARY BRONCHIAL EPITHELIAL CELLS

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H Abdullah, S L Cosby, L Heaney, L McGarvey. *Centre for Infection and Immunity, Queen's University Belfast, Belfast, UK*

Human rhinovirus (HRV), a member of the *picornaviridae* family is a single stranded RNA virus. Rhinovirus infection in non-asthmatics rarely causes serious problems. However in asthmatic subjects HRV contributes to more than 60% of asthma exacerbations where the cough reflex is hyper-reactive and provokes severe coughing and wheezing.

Rational Members of a novel transient receptor potential (TRP) channels, the TRP vanilloid 1 (TRPV1), ankyrin like protein with transmembrane like domain 1 (TRPA1) and TRP melastatin 8 (TRPM8) have been shown to be involved in physiological and pathological aspects of cough. Subsequently understanding the interaction between HRV and 'cough receptors' is crucial as it may indicate potential therapeutic targets and strategies to block these interactions. In this study we investigated the effect of HRV infection on the cough reflex by determining the expression of receptors implicated in the cough process. We hypothesised that HRV may directly and/or indirectly interact with these receptors on sensory nerves and epithelial cells in the airways to provoke cough reflex.

Methods Human primary bronchial epithelial cells (PBEC) were obtained following informed consent and the human neuroblastoma (IMR-32) cell line was used to represent the fundamental cell type

which controls the reflex to cough. The IMR-32 cells undergo differentiation (dIMR-32) to acquire characteristics of peripheral nerve cells with positive neuronal markers. The expression of 'cough receptors' at the protein level in both cell types were detected by fluorescent staining using confocal microscopy and flow cytometry analysis. Receptors mRNA levels were measured by quantitative real-time PCR at different time points post-infection with HRV or treatment with UV-inactivated virus or supernatant.

Results Both cell types dIMR-32 and PBEC were susceptible to HRV infection and showed positive staining for TRPA1, TRPV1 and TRPM8. Up-regulation of the 'cough receptors' mRNA occurred at low multiplicity of infection moreover, higher level of 'cough receptors' expression was detected in PBEC isolated from subjects with lung disease compared to healthy volunteers.

Conclusions These results suggest that virus may both induce cough and interfere with cough related airway clearance depending on the level of infection at different time points.

S124 RAGE IS EXPRESSED BY HUMAN AIRWAY SMOOTH MUSCLE CELLS AND EXPRESSION IS INCREASED IN ASTHMA

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L Di Candia, E Gomez, J Challiss, C E Brightling, R Saunders. *University of Leicester, Leicester, UK*

Background Airway smooth muscle (ASM) dysfunction contributes to the airway hyper-responsiveness and airflow obstruction observed in asthma. The receptor for advanced glycosylation end products (RAGE) is a pattern recognition receptor activated by endogenous danger signals, including high mobility group box 1 (HMGB1). RAGE and HMGB1 are implicated in many pathophysiological states, such as diabetes and Alzheimer's disease. RAGE is abundantly expressed in healthy adult lung, where it might serve a homeostatic function, for example, in alveolar type I epithelial cells; however, RAGE expression/function in other airway cell-types is poorly characterised. We hypothesised that changes in RAGE and HMGB1 expression may contribute to ASM dysfunction in asthma. Therefore, RAGE/HMGB1 expression was investigated in human primary ASM cells.

Methods ASM was microdissected from bronchial biopsies and large airway specimens obtained at lung resection surgery. *Ex-vivo* ASM cells were characterised for α -smooth muscle actin and used between passages 2–5. Cells were serum deprived in ITS medium for 72-h prior to experimentation. RAGE and HMGB1 mRNA expression was measured using RT-PCR and qRT-PCR, and protein expression by western blotting, immunofluorescence and flow cytometry.

Results ASM cells were shown to express the full-length and three soluble RAGE transcripts by RT-PCR ($n=5$), and an HMGB1 transcript by qRT-PCR ($n=6$). By flow cytometry membrane-localised RAGE expression was shown to be significantly increased in ASM cells isolated from asthmatics ($18.0 \pm 5.4\%$ of the ASM population expressed RAGE, $n=6$) vs non-asthmatics ($8.6 \pm 6.5\%$, $n=7$; $*p<0.05$), and a trend towards decreased HMGB1 expression in asthmatics ($18.8 \pm 10.5\%$, $n=6$) vs non-asthmatics ($35.7 \pm 19.1\%$, $n=6$) was observed; however, these values were not significantly different. RAGE and HMGB1 expression were confirmed by immunofluorescence and western blotting.

Conclusion Human ASM cells express RAGE and its ligand HMGB1 at the mRNA and protein levels. Membrane-localised RAGE protein expression is significantly increased and there is a trend towards a decrease in HMGB1 protein expression in ASM isolated from asthmatic vs non-asthmatic subjects. The contribution of these changes to ASM dysfunction in asthma requires further investigation.