Background EarlyCDT®-Lung Test detects autoantibodies to abnormal cell surface proteins from the early stages of lung cancer with a specificity of 93%. This may allow earlier tumour detection thus altering prognosis.

The primary research question is: Does using the EarlyCDT®-Lung Test to identify those at high risk of lung cancer, followed by x-ray and CT scanning in the test positive group, reduce the incidence of patients with late-stage lung cancer (III and IV) or unclassified presentation (U) at diagnosis, compared to standard practice? Recruitment was completed in June 2016 with 12,018 subjects randomised.

Methods A RCT in Scotland recruiting from the most socially disadvantaged quintiles. Adults aged 50 to 75 (ECOG 0–2) who were at high risk for lung cancer (>20 pack years or relevant family history) were eligible. The intervention was the EarlyCDT®-Lung Test, followed by chest x-ray and CT in those with a positive result. The comparator is standard clinical practice in the UK. The primary outcome is the difference, after 24 months, between the rates of patients with stage III, IV or unclassified lung cancer at diagnosis in test v no-test group. Secondary outcomes include: all-cause mortality; cancer specific mortality; a range of morbidity outcomes; cost-effectiveness and measures examining the psychological and behavioural consequences of screening.

Participants with a positive test result had an initial chest x-ray which was used to determine the urgency and the need for contrast in the initial screening CT. Those in whom the initial CT scan did not lead to a lung cancer diagnosis were offered annual chest CTs for 24 months. Participants who are found to have lung cancer will be followed-up to assess both time to diagnosis and stage of disease at diagnosis.

Results 575/6120 (9.8%) of the test group had a positive test. The primary research question is: Does using the EarlyCDT®-Lung Test, followed by chest x-ray and CT in those with a positive result. The comparator is standard clinical practice in the UK. The primary outcome is the difference, after 24 months, between the rates of patients with stage III, IV or unclassified lung cancer at diagnosis in test v no-test group. Secondary outcomes include: all-cause mortality; cancer specific mortality; a range of morbidity outcomes; cost-effectiveness and measures examining the psychological and behavioural consequences of screening.

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Conclusions DNA methylation profiles are significantly different between airway and parenchymal fibroblasts but only small modifications are associated with COPD. Future work will focus on validating a methylation based markers of parenchymal versus airway fibroblasts and assessing modification to these profiles in cells isolated from individuals with COPD.

Methods DNA was isolated from parenchymal and airway fibroblasts at passage 4, and bisulphite treated. Site specific, quantitative genome wide methylation was determined using the Illumina 450K Infinium Methylation BeadChip array. Linear modelling and DMRcate functions identified differentially methylated sites and regions respectively between airway and parenchymal fibroblasts isolated from individuals with normal lung function versus those with COPD.

Results 3980 CpG (methylation) sites significantly differed after Bonferroni correction between airway and parenchymal fibroblasts isolated from healthy individuals. These sites had a broad distribution of effect size, with 240 CpG sites displaying a difference in methylation of >50%. 78 of these sites validated in a second cohort of 7 sets of paired airway and parenchymal fibroblasts isolated from the same individual. There was genomic proximity to these sites and DMRcate was used to refine the individual CpG sites to 5 regions of interest associated with 5 genes; HLX, TWIST1, CREB5, SKAP2 and PRDM16. Differences in methylation were less pronounced when comparing cells isolated from healthy individuals to those with COPD. In airway fibroblasts 47 DMRcate regions were identified with a maximum difference in methylation of at least 20%. In parenchymal fibroblasts 3 DMRcate regions were identified with a maximum difference in methylation of at least 20%.

Conclusions DNA methylation profiles are significantly different between airway and parenchymal fibroblasts but only small modifications are associated with COPD. Future work will focus on validating a methylation based markers of parenchymal versus airway fibroblasts and associating our differential observations with gene/protein expression.

Respiratory Science

INVESTIGATING GENOME WIDE DNA METHYLATION IN AIRWAY AND PARENCHYMAL FIBROBLASTS FROM HEALTHY INDIVIDUALS AND INDIVIDUALS WITH COPD

Rationale Lung fibroblasts are implicated in respiratory disease pathology including chronic obstructive pulmonary disease (COPD). Phenotypic differences between fibroblasts isolated from the airway versus the parenchyma have been described but no studies have compared the cell types on a genome wide scale. DNA methylation is a reversible modification of the DNA structure with the ability to affect cell function via the alteration of gene expression. Here we compared genome wide DNA methylation profiles from airway and parenchymal fibroblasts and assessed modification to these profiles in cells isolated from individuals with COPD.

Methods DNA was isolated from parenchymal and airway fibroblasts at passage 4, and bisulphite treated. Site specific, quantitative genome wide methylation was determined using the Illumina 450K Infinium Methylation BeadChip array. Linear modelling and DMRcate functions identified differentially methylated sites and regions respectively between airway and parenchymal fibroblasts isolated from individuals with normal lung function versus those with COPD.

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HOW SPECIFIC ARE FLUOROREGENIC SUBSTRATES DESIGNED TO ANALYSE ACTIVE PROTEASE BIOMARKERS OF RESPIRATORY DISEASE?

Introduction Active proteases, such as neutrophil elastase (NE) and matrix metalloproteases (MMPs), have been established as inflammatory biomarkers in lung diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis. Therefore, biological samples collected during clinical investigations are often analysed using fluorogenic substrates to determine protease activity and identify correlations with clinical and/or demographic parameters. Due to the nature of these diseases, samples often contain numerous active proteases from both human and bacterial origins which collectively have significant substrate crossover. This study investigates the ability of fluorogenic substrates to distinguish between proteases in complex clinical samples and provide an indication of the predictive capability of this assay type.

Methods Expectorated sputum was randomly collected from patients with CF who were hospitalised for an acute exacerbation. Samples were processed within 30 minutes of collection, and the aqueous sol recovered, pooled, aliquoted and stored at −80°C until analysis. The capacity of sputum proteases to hydrolyse fluorogenic substrates with and without the presence of inhibitors specific for serine (multiple subclasses), metallo and matrix metalloproteases was assessed. A fluorogenic assay was used to determine the activity of each protease and specific inhibitors were used to determine which protease(s) were responsible for hydrolysing the fluorogenic substrates. The fluorogenic assay was based on a method described by Walsh et al. (2004) with modifications to increase the sensitivity of the assay. The fluorogenic substrates used were Boc-Val-Val-Leu-Arg-AMC for NE, Boc-Leu-Leu-Glu-Arg-AMC for MMP-1 and MMP-9, and Boc-Val-Val-Arg-AMC for MMP-2 and MMP-9. The fluorogenic assay was validated using sputum from normal healthy volunteers and patients with bronchiectasis.

Results The fluorogenic assay was able to distinguish between NE and MMPs in sputum samples from patients with CF. The fluorogenic assay was also able to distinguish between NE and MMPs in sputum samples from patients with bronchiectasis.

Conclusions The fluorogenic assay developed in this study is a reliable and sensitive method for the detection of active proteases in sputum samples from patients with CF and bronchiectasis. This method can be used to identify the specific proteases responsible for the hydrolysis of fluorogenic substrates, which can be used to identify the presence of specific inhibitors and to monitor the effectiveness of treatment.