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 xray 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 xray 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 xray 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 biannual 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 with 207 found to have lung nodules >8 mm, 16 cancers have been detected so far, 12 of which are at early stage. Eleven have abnormalities undergoing current investigation. At this stage of the trial we have no outcome data for the comparison group.

Conclusion The study will determine EarlyCDT-Lung test's clinical and cost effectiveness.

Respiratory Science

S133 INVESTIGATING GENOME WIDE DNA METHYLATION IN AIRWAY AND PARENCHYMAL FIBROBLASTS FROM HEALTHY INDIVIDUALS AND INDIVIDUALS WITH COPD

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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.

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.

S134 HOW SPECIFIC ARE FLUOROGENIC SUBSTRATES DESIGNED TO ANALYSE ACTIVE PROTEASE BIOMARKERS OF RESPIRATORY DISEASE?

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Introduction Active proteases, such as neutrophil elastase (NE) and matrix metalloproteinases (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

cysteine proteases was examined. Fluorogenic substrates analysed include those for various inflammatory proteases including elastase-like (MeOSuc-AAPV-AMC), MMPs (MCA-PLGL-Dpa-AR-NH2), trypsin-like (Z-GGR-AMC) and chymotrypsin-like (Suc-AAPF-AMC). Substrate hydrolysis by a relevant recombinant enzyme (± inhibitors) was analysed as a control.

Results Data analysis indicates that alternative enzymes actively hydrolyse substrates designed to be specific for one group of proteases. Inhibitors specific for metallo, trypsin-like, chymotrypsin-like and cysteine proteases all decreased elastase-like substrate turnover (~10–50%). A similar trend was seen for chymotrypsin-like substrate using metallo, trypsin-like and elastase-like protease inhibitors (~10–40%).

Conclusion This investigation has suggested that there is significant non-specific hydrolysis and cross-reactivity when fluorogenic substrates are utilised to measure active proteases in complex biological samples. Thus, using such fluorogenic substrates may produce elevated readings, impacting on the accuracy of results when such assays are used for clinical or research purposes.

S135 CIRCULATING METABOLITES IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION AND CHRONIC THROMBOEMBOLIC PULMONARY VASCULAR OCCLUSION

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Introduction Recent studies have demonstrated that metabolomic profiling can identify metabolites and pathways which may have importance in the pathobiology of pulmonary arterial hypertension. However, the plasma metabolome in chronic thromboembolic pulmonary hypertension (CTEPH) and chronic thromboembolic vascular occlusions without pulmonary hypertension (CTED) has not been well characterised.

Objective To profile circulating metabolites in CTEPH and CTED and assess metabolite gradients across the pulmonary circulation.

Methods In the patient group, multisite blood sampling was performed at the time of right heart catheterisation. Blood samples were collected from the superior vena cava, pulmonary artery and radial artery.

Venous blood samples from patients were compared to healthy controls to identify the metabolites present and to assess the difference between health and disease. Additionally, in the disease group, transpulmonary gradients were assessed by analysis of fold change in metabolite concentration between paired samples from the pulmonary artery and radial artery.

Untargeted, semi-quantitative metabolic profiling of plasma was performed using the Metabolon DiscoveryHD4™ platform (Metabolon, NC, USA), utilising 2 ultra-high performance liquid chromatography methods, coupled with tandem mass spectrometry. Kruskal-Wallis analysis was used to compare metabolites between disease and control, with false discovery rate correction for multiple testing.

Results The disease group included patients with a spectrum of chronic pulmonary vascular occlusions (Table 1). A total of 1375 metabolites were detected in 70 venous plasma samples analysed from 43 patients and 27 healthy controls. Amongst endogenous metabolites, 266 showed a significant difference between disease and control. In the disease group there were increases in acylcarnitine metabolites, long chain fatty acids, polyamines, glycogen metabolites and primary bile acid metabolites compared to healthy controls. There was a reduction in lysolipids, plasmalogens, aminosugars, branched chain amino acid metabolites, glutathione metabolites and a number of steroids (Table 1). Analysis of transpulmonary gradients revealed primarily a reduction in metabolite concentration across the pulmonary circulation. This included depletion of energy substrates, lysolipids, lysoplasmalogens and acylcholines.

Conclusions This pilot study of circulating metabolites in patients with CTEPH, CTED and healthy controls reveals differences between health and disease in several biological pathways. Measurement of the transpulmonary gradient of metabolites indicated predominant clearance of circulating metabolites associated with energy metabolism and cell turnover. These findings require confirmation in a larger population.

Abstract S135 Table 1 Study population and changes in metabolite groups in venous blood of patients compared to healthy controls

	Chronic pulmonary vascular occlusions (n = 43)	Controls (n = 27)
Group demographics		
Age (years)	58 (22–77)	44 (18–75)
Sex (% male)	64	60
Patient group		
Proximal CTEPH- treatment naive (n)	**11**	
Distal CTEPH (n)	**3**	
Proximal CTEPH- previous pulmonary endarterectomy- residual PH (n)	**11**	
Proximal CTEPH- previous pulmonary endarterectomy, no residual PH (n)	**10**	
Chronic thromboembolic vascular occlusions without PH (n)	**8**	
Changes in metabolites in disease		
Acylcarnitines	↑	
Long chain fatty acids	↑	
Polyamines	↑	
Glycogen metabolites	↑	
Primary bile acid metabolites	↑	
Lysolipids	↓	
Plasmalogens	↓	
Aminosugars	↓	
Branched chain amino acids	↓	
Glutathione metabolites	↓	
Steroids	↓	

S136 POTENTIAL THERAPEUTIC BENEFITS OF THE HUMAN AMNIOTIC EPITHELIUM CELL SECRETOME DURING EX-VIVO PERFUSION OF DONOR LUNGS

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Introduction Ex-vivo lung perfusion (EVLP) is used to assess and potentially recondition donor lungs that are not initially suitable for transplantation. In a recent UK study, EVLP was associated