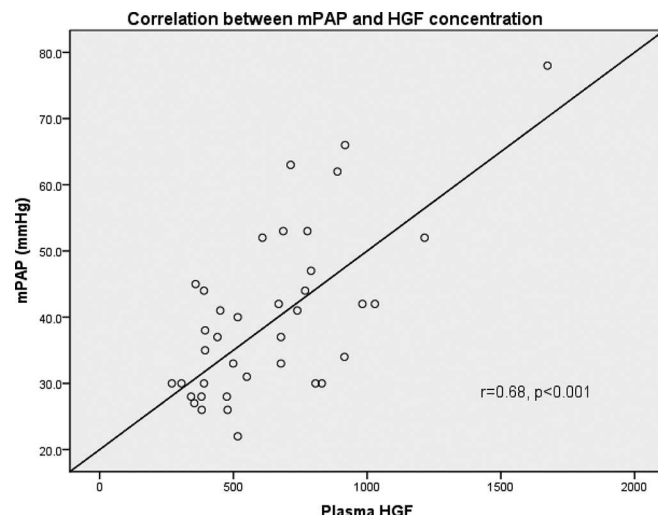following therapy with Bosentan (n = 6) or sildenafil (n = 1). Interestingly, improvement in CI at repeat RHC correlated positively with a further increase in HGF levels (r = 0.8, p = 0.03).

Abstract P157 Figure 1.

Conclusion Plasma HGF concentrations correlate with haemodynamic severity in CTD-PAH. Further work to determine whether high levels of HGF are involved in the pathogenesis of PAH or are instead elevated in a reparative response to underlying pulmonary vascular remodelling is required.

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P158   ENDOTHELIAL PROGENITOR CELLS FORM BIOLOGICAL EXCLUSION BARRIERS SIMILAR TO THAT OF MATURE ENDOTHELIAL CELLS- A THERAPEUTIC POTENTIAL IN SYSTEMIC SCLEROSIS?

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Abstract Vascular complications associated with systemic sclerosis (SSc) including pulmonary arterial hypertension (PAH-SSc), result from endothelial damage and loss of barrier function. Endothelial progenitor cells (EPCs) express endothelial (VEGFR2$^+$, CD31$^+$) and haematopoietic (CD133$^+$) markers. They home to sites of vascular injury and differentiate into endothelial cells restoring the endothelium. In SSc patients circulating levels of EPCs are reduced. This study aimed to develop a robust method to grow EPCs from peripheral blood mononuclear cells (PBMCs) and to compare cellular functions to mature endothelial cells.
Methods EPCs and human pulmonary artery endothelial cells (hPAECs) were seeded into transwell inserts and grown to confluence. Cells were incubated with TNFa (50ng/ml), and their capacity to form biological barriers assessed using FITC-albumin (5mg/ml). FITC-albumin 'leak' was quantified by fluorescent absorbance over time. We further assessed the responses of EPCs to TNFa stimulation by ELISA to quantify pro-inflammatory cytokine release.

Results EPCs form a biological exclusion barrier with similar capabilities as mature hPAECs. TNFa significantly enhanced the permeability of EPCs (P < 0.05) and hPAECs (P < 0.05) monolayers. There is no difference in EPC colony formation between HC and SSc EPCs.
Discussion We developed a robust method for isolating EPCs from PBMCs. We have demonstrated that EPCs can maintain an endothelial barrier consistent with that observed by mature hPAECs *in vitro*. We have established that EPCs respond to TNFa in a similar manner to mature PAECs. We have shown no significant difference in the capacity of PBMCs from SSc patients to form EPC colonies compared to HCs.

P159   PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION ARE HYPORESPONSIVE TO INFLAMMATORY STIMULI

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Background Inflammation is a key feature of pulmonary arterial hypertension (PAH); circulating levels of plasma cytokines and chemokines are raised, some of which (including interleukin (IL)-6 and CXCL8) are associated with increased mortality (1). We wished to determine whether it was possible to detect an altered inflammatory phenotype in circulating inflammatory cells from PAH patients compared to controls.
Methods Following ethical approval, patients with idiopathic PAH and age-matched non-smoking control subjects were selected. Peripheral blood mononuclear cells (PBMC) (10^5 cells) or citrated whole blood (WB) were plated into 96-well plates and treated with E. Coli LPS (0–1 g/ml) (Sigma) or recombinant TNF-a (0–10ng/ml) (R&D systems) for 24h at 37°C. Following centrifugation, plasma was aspirated and samples frozen at -20°c, and IL-6 and CXCL8 ELISAs later performed. Data are mean ± SEM.
Results All patients had idiopathic PAH (n = 12) (7 in NYHA class III-IV) with 6MWD 370 ± 18m, mPAP 72 ± 5mmHg). All patients were taking advanced PAH therapies but not conventional anti-inflammatory therapies. There was no age difference between patients and controls (n = 9) (36.5 ± 3.7 vs. 36.2 ± 2.7 years, NS).

At baseline, release of IL-6 and CXCL8 was higher from PBMC from PAH patients than controls (918.1 ± 280.5 vs. 195.2 ± 64.3 for IL-6 (p < 0.05) and 4203 ± 1354 vs. 1048 ± 288.4 p < 0.05 for CXCL8), although no difference was seen between groups from the whole blood assays. Following stimulation with LPS, however, release of both IL-6 and CXCL8 was lower from both WB and PBMC assays for PAH patients compared to controls. For example for LPS-induced IL-6 release from PBMC, both the maximal effect (E_{max}) was lower in PAH vs. controls (5148 ± 1320 vs. 9154 ± 2510 pg/ml, p < 0.05) and the log EC_{50} (half maximal effective concentration) was higher in PAH vs. controls (-2.70 ± 0.27 vs. -5.90 ± 1.16 g/ml, p < 0.01) (Figure 1). Similar results were seen following stimulation with TNF-a.
Conclusion The main findings of this study are that: (1) baseline CXCL8 and IL6 release was higher from PBMCs of patients with PAH compared to control donors; (2) PBMCs and WB from patients were hyporesponsive to LPS (and TNF-a),

suggesting an altered inflammatory phenotype, at least in these models, to these stimuli.

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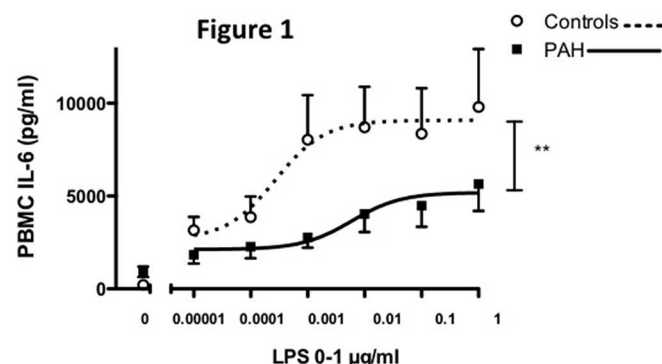
Abstract P159 Figure 1.

P160   **RETROSPECTIVE STUDY ANALYSING WHETHER OPTIMIZATION OF PULMONARY VASCULAR ENHANCEMENT INFLUENCES DIAGNOSTIC OUTCOME IN THE INTERPRETATION OF CT PULMONARY ANGIOGRAMS (CTPA)**

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Introduction and Objectives Pulmonary embolism (PE) is a common cardiovascular emergency and is the most common preventable cause of hospital deaths. Various factors can impact on the diagnostic accuracy of CTPA, which is now considered the 1st line investigation for PE diagnosis. Mis-timing of contrast medium administration or cardiac impairment can result in an indeterminate scan through hidden or mimicked emboli. This study focuses on the accuracy of CTPAs in excluding a diagnosis of PE.

Method This is a single centre study looking at all inpatient and outpatient CTPAs carried out over a 12-month period with matched CTPA and lower limb ultrasound Doppler events selected. Of these matched studies, all positive CTPAs for PE were excluded. Of the remainder, only those studies done within 6 months of the original CTPA were included in the study. CT pulmonary arterial (PA) opacification with intravenous contrast medium was objectively measured in Hounsfield units (HU) using an oval region of interest in the main pulmonary trunk. PA opacification was categorised as very poor (<100 HU), suboptimal (< 200 HU) and optimal (= 200HU) as measured at the PA trunk based on the estimation that a minimal opacification of 100 HU is required for identification of acute emboli and 200 HU is required for identification of chronic emboli.

Results From the 32 CTPAs included in the study, 28 had a negative initial CTPA with subsequent negative CT or US follow-up. 4 of these cases had an initial negative CTPA with a subsequent positive CTPA or lower limb US Doppler study. Of these latter cases only one initial CTPA was deemed poorly suboptimal (average 174HU) with optimal pulmonary arterial opacification in all other three initial CTPAs.

Conclusion This study demonstrates that despite a large number of initially negative CTPAs undergoing subsequent follow-up imaging due to presumed persistent or recurring patient symptoms, the majority (87.5%) of these negative scans were still negative and only 12.5% were positive for PE. No major pitfall has been identified in local CTPA acquisition technique that may have led to subsequent misses of potentially life-threatening PEs.

Clinical trials in asthma

P161   **A RANDOMISED CONTROLLED TRIAL OF SINGLE COMBINATION BUDESONIDE/FORMOTEROL INHALER AS MAINTENANCE AND RELIEVER THERAPY IN ASTHMA PATIENTS AT RISK OF SEVERE EXACERBATIONS**

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Introduction The Single budesonide/formoterol inhaler Maintenance And Reliever Therapy (SMART) regimen reduces severe asthma exacerbations, but it is uncertain whether it increases the risk of adverse effects due to high beta-agonist and corticosteroid doses with both short-term and cumulative exposure.

Objectives Our hypothesis was that treatment with the SMART regimen would reduce the risk of beta-agonist overuse but that when such episodes occurred, patients were less likely to seek medical review and, that any reduction in severe exacerbations would be at the cost of a higher systemic corticosteroid burden.

Methods This was a 24-week, open-label, parallel group trial in 303 asthma patients with a recent exacerbation, conducted at four primary healthcare practices and one secondary care hospital. Participants were randomised to 200/6 g budesonide/formoterol metered dose inhaler according to the SMART regimen (two actuations twice daily maintenance with one extra actuation as-needed) or a fixed-dose regimen (two actuations twice daily maintenance) with one to two actuations of 100 g salbutamol as-needed ('Standard'), with electronic monitoring of actual inhaler use. The primary outcome was the proportion of participants with at least one high beta-agonist use episode (>12 budesonide/formoterol actuations/day in SMART or >16 salbutamol actuations/day in Standard).

Results There was no significant difference between groups in the proportion of participants with at least one high use episode: SMART 84/151 (56%) vs Standard 68/152 (45%) participants; relative risk (95% CI) 1.24 (0.99–1.56), p = 0.058. There were fewer days of high use [mean (SD) 5.1 days (14.3) vs 8.9 days (20.9), relative rate (RR) (95% CI) 0.58 (0.39–0.88), p = 0.01] and days of high use without medical review [8.5 days (17.8) vs 18.3 days (24.8) per high use patient, RR 0.49 (0.31–0.75), p = 0.001] in the SMART group. The SMART regimen resulted in higher average daily inhaled corticosteroid exposure, but reduced oral corticosteroid exposure, with no difference in composite systemic corticosteroid exposure [ratio of means (95% CI) 1.03 (0.86–1.22), p = 0.76]. SMART participants had fewer severe asthma exacerbations [35 vs 66, RR 0.54 (0.36–0.82), p = 0.004].

Conclusions The SMART regimen has a favourable risk/benefit profile in patients at risk of severe asthma exacerbations.

P162   **EFFECT OF ADDING PROPRANOLOL OR INCREASED INHALED CORTICOSTEROID DOSE IN PERSISTENT ASTHMA**