Introduction CT pulmonary angiography (CTPA) is the recommended imaging modality for suspected pulmonary embolism (PE). Current NICE guidelines recommend using clinical prediction scoring systems to estimate the probability of PE and guide further investigation[1]. A low or intermediate probability score, coupled with a negative D-dimer, reliably excludes PE, thereby avoiding the need for CTPA.

Objectives We undertook a retrospective audit to examine adherence to NICE guidelines for diagnosis of suspected PE in patients admitted to a district general hospital, and identify patients who may have undergone unnecessary CTPA.

Methods We obtained a list of all CTPAs undertaken in our hospital between December 2012 and February 2013. D-dimer tests are poorly specific within hospitalised patients; therefore, we excluded post-surgical and obstetric patients, and pre-existing inpatients where primary admission was not for suspected PE. We also excluded outpatient CTPAs. We searched the records for contemporaneous PE probability scores and D-dimer results. For patients without a probability score result, we reviewed the clinical notes and calculated a probability score retrospectively using a local scoring system adapted from BTS guidelines.

Results There were 115 CTPAs during the study period – 36 were excluded and 4 patients' case notes were unavailable. 75 patients fulfilled the inclusion criteria (mean age 68.2 years), and PE was confirmed in 20%. 50 patients (66.7%) had a contemporaneous documented clinical probability score. There were 5 patients (6.7%) with a low/intermediate probability score and negative D-dimer, who underwent unnecessary CTPA (PE excluded in each case). There were 9 patients (12%) with retrospectively calculated low/intermediate clinical probability scores and no D-dimer result, who may have avoided CTPA had D-dimer been undertaken (CTPA excluded PE in each case).

Conclusions In our district general hospital, the underuse of clinical probability scoring and D-dimer testing in patients with suspected PE is contributing to unnecessary CTPAs. Introducing mandatory documentation of PE clinical probability score on CTPA request forms may reduce the number of unnecessary CTPAs.

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NEUTROPHIL AND REDOX DEPENDENT PROTEOLYSIS OF BONE MORPHOGENETIC PROTEIN 9: POTENTIAL ROLE IN THE PATHOGENESIS OF PULMONARY ARTERIAL HYPERTENSION

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Introduction A critical reduction of bone morphogenetic protein type II receptor (BMPRII) in the pulmonary circulation, either due to the genetic loss-of-function mutations, heightened inflammation or prolonged hypoxia, is one of the major causes behind pulmonary arterial hypertension (PAH), a fatal disease with poor prognosis. BMPRII is highly expressed in the vascular endothelium and undergoes rapid turnover. Bone morphogenetic protein 9 (BMP9), the only active circulating BMP, signals via endothelial BMPRII, inducing BMPRII expression and maintaining endothelial homeostasis. Although BMPRII function has been studied

extensively, factors that regulate BMP9 stability and activity remain unclear.

Objective To investigate how BMP9 activity and stability are regulated and whether this regulation plays a role in pulmonary arterial hypertension.

Results Two forms of BMP9 dimer could be co-purified, with (D-form) or without (M-form) intermolecular disulphide bond. M- and D-forms BMP9 are interchangeable with redox potential, but have different stability. While the M-form is more susceptible to redox-dependent cleavage and proteases present in serum, the D-form is a preferred substrate for neutrophil elastase. Freshly isolated human peripheral blood neutrophils, when activated by hypoxia or inflammatory stimuli, released elastase that cleaved BMP9 effectively.

Conclusions and Discussions This study demonstrates a novel proteolytic regulation of BMP9 under physiological and pathological conditions, suggesting neutrophil elastase could be a potential link between inflammation/hypoxia and BMPRII signalling, and the recognised benefits of elastase inhibition in rodent models of PAH may be due in part to reduced degradation of BMP9 and preservation of endothelial BMPRII signalling.

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HEPATOCYTE GROWTH FACTOR CONCENTRATION CORRELATES WITH HAEMODYNAMIC SEVERITY IN CONNECTIVE TISSUE DISEASE-ASSOCIATED PULMONARY ARTERIAL HYPERTENSION

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Introduction Hepatocyte growth factor (HGF) acts via the tyrosine kinase receptor, c-MET, on endothelial and epithelial cells. It has angiogenic, mitogenic, motogenic and anti-apoptotic effects. Administration of HGF has been shown to ameliorate pulmonary arterial hypertension (PAH) in the monocrotaline rat model.^{1,2} Little is known regarding circulating HGF levels in human disease.

Methods 47 incident, treatment naive patients with PAH in association with connective tissue disease (CTD-PAH) had blood sampling at or within 1 day of diagnostic right heart catheterisation. Plasma HGF concentrations were measured using Bio-Plex bead array. A proportion of patients also had NT-proBNP measured and underwent cardiac MRI.

Results Baseline characteristics were (mean, sd): Age 64(10)yrs, mean right atrial pressure (mRAP) 9.6(11.7)mmHg, mean pulmonary arterial pressure (mPAP) 40.6(13)mmHg, pulmonary arterial wedge pressure 10.5(4.5)mmHg, cardiac index (CI) 2.97 (0.7)L/min, pulmonary vascular resistance (PVR) 531(350)dyns. HGF levels correlated positively with mRAP (0.6, $r < 0.001$), mPAP ($r = 0.68$, $p < 0.001$: fig 1), PVR ($r = 0.51$, $p = 0.001$) and negatively with CI ($r = -0.43$, $p = 0.008$) and right ventricular ejection fraction measured by MRI ($r = -0.53$, $p = 0.034$). N-terminal pro B-type natriuretic peptide (NT-proBNP) measured in approximately 50% of patients correlated more strongly with CI ($r = -0.72$, $p < 0.001$) and PVR ($r = 0.61$, $p = 0.003$) but did not correlate with mPAP. A small proportion (7) of patients underwent repeat right heart catheterisation (RHC)

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 TNF α (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 TNF α stimulation by ELISA to quantify pro-inflammatory cytokine release.

Results EPCs form a biological exclusion barrier with similar capabilities as mature hPAECs. TNF α 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 TNF α 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- α (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 $\pm$ SEM.

Results All patients had idiopathic PAH ($n = 12$) (7 in NYHA class III-IV) with 6MWD 370 ± 18 m, mPAP 72 ± 5 mmHg. 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- α .

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- α),