and Meniotrix®), with repeat samples collected two months later. We also recorded blood and sputum eosinophil counts, radiological findings such as bronchiectasis and bronchial wall thickening, total IgE, smoking status, exacerbations in the last year and ITU admissions.

Results 101 patients were followed up (69 asthma, 32 fungal) 67 female, mean (SD) age 53 (15) years, FEV1 69 (21.9)% predicted, ICS dose 1818 (1244) µg, and BMI 29.9 (8.9) kg/m^2. Specific antibody levels and responses to vaccination are presented in Figure 1. Immune deficiency at baseline and post vaccination did not correlate with lung function, radiological findings such as bronchial wall thickening or exacerbation frequency.

Conclusion Specific antibody deficiency is commonly seen in patients with asthma and fungal disease. Vaccination can provide protection and should be considered in this patient group. We need further analysis with a larger cohort of patients to study the association between antibody deficiency, lung function, radiological changes and disease progression.

P244 HAEMOGLOBIN MEDIATED PROLIFERATION AND IL-6 RELEASE IN HUMAN PULMONARY ARTERY ENDOTHELIAL CELLS: A ROLE FOR CD163 AND IMPLICATIONS FOR PULMONARY VASCULAR REMODELLING

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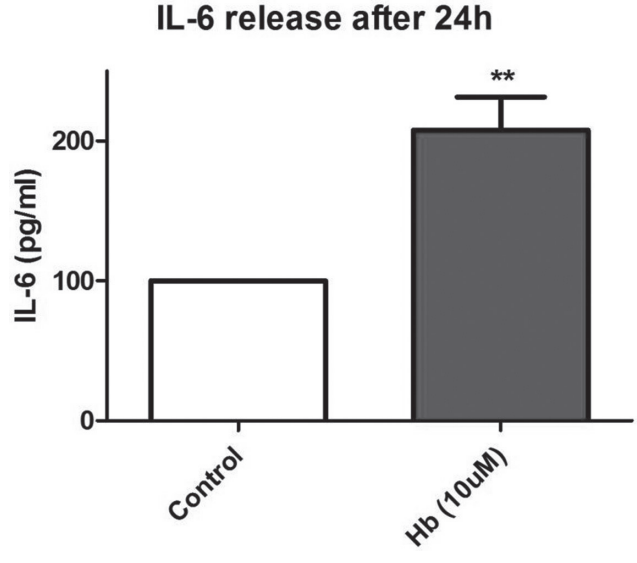
Introduction Pulmonary arterial hypertension (PAH) is characterised by vascular remodelling of pulmonary arterioles. Disrupted iron homeostasis as well as subclinical haemolysis are implicated in PAH, although exact mechanisms remain unknown. IL-6, a proinflamatory cytokine and regulator of iron homeostasis is elevated in PAH patients and also been implicated in pulmonary vascular remodelling in murine models.

Objectives In this study we explored the influences of free haemoglobin (Hb) on proliferative responses and secondary mediator, IL-6 release in human pulmonary artery endothelial cells (hPAECs).



Abstract P244 Figure 1 IL-6 relaese after 24h

Methods Cells were challenged with Hb (10 uM) and/or IL-6 (1–10 ng/mL). Transcriptional regulation was analysed by RT-PCR, protein expression by immunocytochemistry, secretion by ELISA and proliferation by BrdU incorporation.

Results Novel findings demonstrate that Hb and IL-6 individually and in combination increased proliferation of hPAECs (by 32%, 47% and 63% respectively; $p < 0.05$). CD163, a Hb scavenger receptor, was basally expressed as mRNA and protein (cell surface) on hPAECs and further modulated by Hb or IL-6 exposure. Hb treatment also caused increased transcription (30%; $p < 0.05$) and release of IL-6 (107%; $p < 0.01$) from hPAECs.

Conclusion This is the first report of Hb-mediated proliferation, CD163 expression and IL-6 release in hPAECs with potential implications for autocrine and paracrine signalling in pulmonary vasculature. Hb uptake may be facilitated via CD163. These studies may provide novel insights regarding mechanisms for haemoglobin driven proliferative and second messenger responses of relevance to PAH.

P245 WHOLE BLOOD LEVELS OF MICRORNA-34A PREDICT SURVIVAL AND REGULATE GENES ASSOCIATED WITH PULMONARY ARTERIAL HYPERTENSION

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Introduction Despite advanced therapies for pulmonary arterial hypertension (PAH), the hyperproliferative pulmonary vasculopathy persists. Circulatory microRNAs (miR) offer considerable promise as both a prognostic biomarker, and to identify molecular mechanisms underlying PAH. Previous study from our lab identified whole blood miR-34a as downregulated in patients with PAH.

Objectives To validate changes in whole blood miR-34a levels in patients with PAH and relate them to disease severity and survival, and determine the phenotypic effect on pulmonary artery smooth muscle cells (PASMC).

Methods Whole blood RNA was isolated from 27 treatment-naive patients with PAH, 12 age-matched healthy volunteers (HV) and experimental models of PAH (Monocrotaline-MCT, Sugen5416/hypoxia-SuHx and controls, n = 5/group). Whole blood miR-34a-5p and −3p levels were measured by qPCR. The phenotypic effect of miR-34a-5p and −3p levels was assessed on PASMC *in-vitro*. Differences between groups were determined by Student's t-test or ANOVA-Tukey.

Results Whole blood miR-34a-5p was reduced in patients with PAH ($p < 0.0001$) and experimental models of PAH (MCT $p < 0.05$, SuHx $p < 0.001$). Receiver operating characteristic curve identified that miR-34a-5p levels discriminates patients with PAH from HV (AUC = 0.86, $p = 0.001$). MiR-34a-5p levels were significantly lower in patients with severe PAH, as defined by a cardiac index of <2 vs >2.5 l/min/m^2 ($p < 0.05$) and NT-proBNP > 300 vs <300 ng/l ($p < 0.001$) and predict survival at 5 years. MiR-34a-5p levels were negatively correlated with pulmonary vascular resistance ($r = -0.4$, $p < 0.05$) and pulmonary arterial wedge pressure ($r = -0.4$, $p < 0.05$). Preliminary data showed that whole blood miR-34a-3p was reduced in patients with PAH ($p = 0.0267$) and experimental models of PAH (MCT $p < 0.01$, SuHx $p < 0.01$); and delineates patients with PAH from HV (AUC = 0.925, $P = 0.01$). Transfection of PDGF-stimulated PASMC with miR-34a-5p or −3p inhibitor

promote PASMC proliferation ($p < 0.001$). In contrast, miR-34a-5p and −3p mimic suppress PASMC proliferation ($p < 0.05$ and $p < 0.001$ respectively). Additionally, transfection with miR-34a-3p increases caspase-3/7 activities in PASMC ($p < 0.0001$).

Conclusions Reduced miR-34a-5p levels associate with increased disease severity and poor prognosis in PAH. MiR-34a-5p and −3p levels regulate PASMC proliferative-phenotype in response to PDGF. This research identifies miR-34a-5p and −3p as potential biomarkers, subsequent network analysis may identify novel disease mechanisms. Further experiments in preclinical models are currently underway.

P246 THE IN VITRO EFFECT OF COMMONLY USED VASODILATORS ON HUMAN PULMONARY ARTERY

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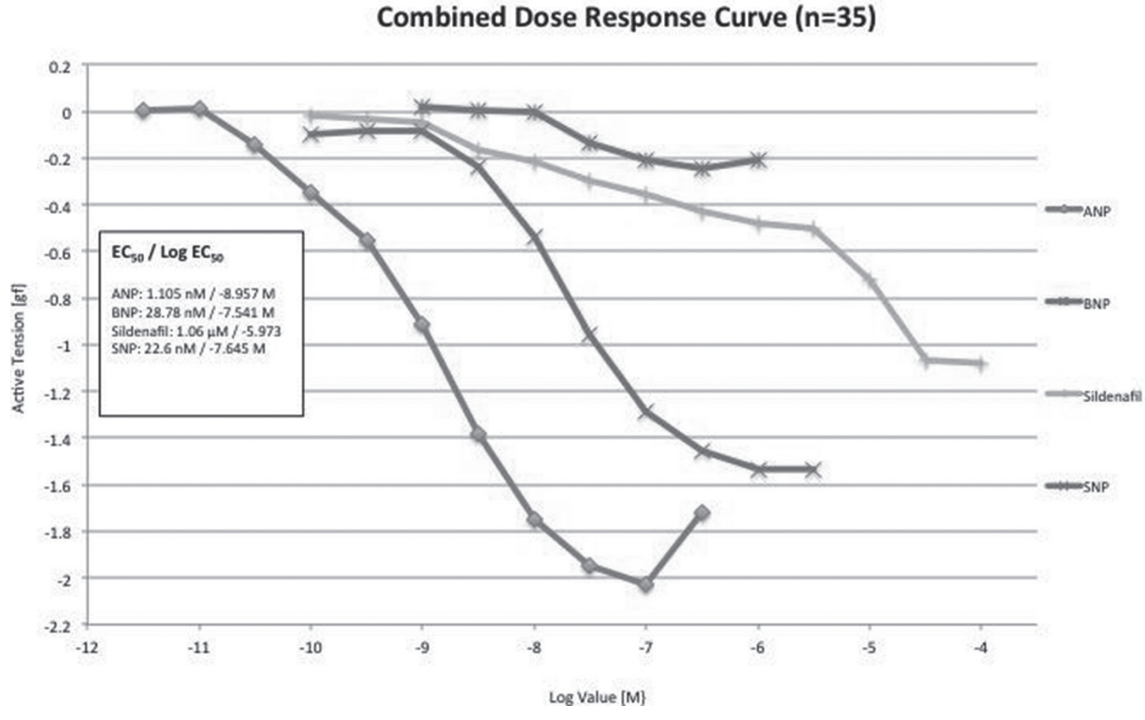
Introduction Acute pulmonary hypertension following cardiac surgery can have a significant effect on post-operative morbidity and mortality. The phosphodiesterase inhibitor sildenafil and the nitric oxide donor Sodium-Nitroprusside (SNP) are commonly used to treat pulmonary hypertension. The aim of this study was to characterise the pharmacological effects of clinically used vasodilators on the human pulmonary vasculature in comparison to the endogenous pulmonary vasodilators Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP).

Methods Research ethics committee approval was obtained for the use of human tissue for this study. Patients undergoing lung resection were consented for their resected lung tissue to be included in the study. Patients under the age of 18 and who cannot give informed consent were excluded from the study and twelve patients were enrolled in this study.

Pulmonary arteries were dissected from disease free areas of lung resection and 35 PA rings of internal diameter 2–4 mm and 2 mm long were prepared. PA rings were mounted in a multiwire myograph system containing Krebs-Henseleit solution (aerated with 21% O_2: 5% CO_2 at 37°C) for measuring changes in isometric tension. A basal tension of 1.61 g was applied and the rings left to equilibrate for 60 min. After equilibration rings were pre-constricted to 11.21 μM PGF2α (EC_{80}) then concentration response curves were constructed to Sildenafil, SNP, ANP and BNP by cumulative addition to the myograph chambers. The Integrity of the endothelium was confirmed with 1 μM Acetylcholine and smooth muscle viability was confirmed by exposure to potassium chloride.

Results ANP was the most potent and effective vasodilator whereas BNP had little effect. SNP was marginally less potent and effective than ANP and the maximum effect of sildenafil was about 50% that of ANP. The EC_{50} for ANP, BNP, Sildenafil and SNP were 1.105 nM, 28.78 nM, 1.06 uM and 22.6 nM respectively.

Conclusion This study demonstrated the differential effect of commonly used agonists on pulmonary vascular reactivity and this is the first comparison of these agents in human pulmonary arterial tissue. These effects may need to be considered in the clinical setting.



Abstract P246 Figure 1 Combined concentration response curse to ANP (n = 8), BNP (n = 7), Sildenafil (n = 12) and SNP (n = 8), n = number of PA rings used. The EC_{50} of ANP, BNP, Sildenafil and SNP were 1.1 nM, 28.78 nM and 22.6 nM respectively