# Cutting edge pulmonary hypertension

## S1* DOES PARADOXICAL EMBOLI OF PARTICULATE MATTER THROUGH PULMONARY ARTERIOVENOUS MALFORMATIONS PRECIPITATE MIGRAINES?

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**Introduction and objectives** Pulmonary arteriovenous malformations (PAVMs) are an example of a right-to-left shunt resulting in deoxygenated, unfiltered venous blood bypassing the pulmonary capillaries to re-enter the systemic arterial circulation. Patients with PAVMs are known to have an increased incidence of migraines, reducing following PAVM treatment by embolisation. This study aimed to examine if paradoxical embolism of particulate matter through PAVMs may precipitate migraines.

**Methods** A structured survey was designed for online completion by people with hereditary haemorrhagic telangiectasia (HHT), the most common cause of PAVMs. Question logic directed participants through a series of unbiased questions that asked about HHT features including presence of PAVMs; variables in relation to migraines; and whether participants had undergone imaging tests. Stratified by whether contrast had been given, participants reporting migraines were asked whether any difference in migraines had been noted following scans, by ticking that "migraines were no different really"; "seemed a bit better"; "seemed a bit worse"; "seemed to bring on a migraine"; "seemed to stop a migraine". Participants were recruited from 26/07/2013- 21/04/2015, yielding 702 consented responses. Data were downloaded to an Excel spreadsheet for participant stratifications, and statistical analyses using GraphPad Prism 6.0 and STATA 13.1 (Statacorp LP).

**Results** Overall, 557 participants had HHT, of whom 180 (32.3%) reported features consistent with migraines. HHT participants with migraines more commonly reported PAVMs than those without migraines (62.8% vs 38.5%, $p < 0.00001$). For computerised tomography (CT) scans, images "with injection of contrast" were associated with a higher proportion of participants reporting worsening migraines than "without injection of contrast" CT scans (11.7% vs 3.4%, $p = 0.0065$). This association strengthened following paired analysis of participants who had undergone both methods (13.6% vs 3.2%, $p = 0.0032$). In multiple regression analyses, there was no additional contribution from other participant demographics such as alcohol consumption or smoking habit. Analysis of magnetic resonance imaging (MRI), contrast echocardiography and ultrasound data is ongoing.

**Conclusion** This study strongly indicates that an association between injecting contrast media and the worsening of migraines, in participants with right-to-left shunts due to PAVMs, exists. Further research is required to establish the exact mechanism responsible for this phenomenon.

*S1- BTS Medical Student Award Highly Commended.

## S2 VASCULAR QUIESCENCE FACTOR BMP9 IS REGULATED BY INFLAMMATION AND NEUTROPHIL ACTIVATION

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**Introduction** Endothelial bone morphogenetic protein type II receptor (BMPR-II)-mediated signalling is essential for protecting vascular endothelium. Loss of BMPR-II predisposes human pulmonary artery endothelial cell (hPAEC) monolayers to apoptosis and increased permeability. *In vivo*, reduced BMPR-II function promotes endothelial permeability and the development of pulmonary arterial hypertension (PAH). Importantly, BMP9, the only confirmed active circulating BMP, signals preferentially via BMPR-II and induces BMPR-II expression to maintain endothelial integrity and homeostasis. It was recently shown that administration of recombinant BMP9 prevented LPS-induced lung vascular leakage *in vivo* and reversed established PAH in three rodent models.[1] However, it is not known how circulating BMP9 is regulated during LPS-induced inflammation and in PAH.

**Objective** To investigate whether BMP9 is regulated by inflammatory stimuli *in vivo* and *in vitro*.

**Results** Intraperitoneal LPS challenge in mice led to a significant increase in circulating neutrophil elastase levels with a reciprocal reduction in BMP9 levels (both measured by ELISA) within 24 h. Since this reduction in BMP9 might be due to reduced BMP9 synthesis in the liver or cleavage of BMP9 by neutrophil-derived proteases, we quantified BMP9 synthesis in the liver after LPS challenge, as well as changes in alpha-1 antitrypsin, the major elastase inhibitor in man. Synthesis of BMP9 fell sharply 3 h after LPS-challenge but recovered completely by 18 h. No increase in the synthesis or levels of circulating active alpha-1 antitrypsin was observed. Supernatants from purified human peripheral blood neutrophils activated *in vitro* degraded recombinant BMP9. Inhibition studies confirmed that the BMP9-cleavage activity released by activated neutrophils was largely attributable to neutrophil elastase.

**Conclusions and discussions** Synthesis of the endothelial protective factor BMP9 is actively regulated by inflammation, and BMP9 is subject to neutrophil elastase-mediated cleavage. Since inflammation has been shown to be a second hit in the pathogenesis of PAH, this study could provide a potential link between inflammation and reduced endothelial BMPR-II signalling.

### REFERENCE

1 Long L, Ormiston ML, Yang X, *et al*. Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. *Nat. Med*. 2015;**21**: 777–785

## S3 REDUCED BMPR2 EXPRESSION POTENTIATES A PULMONARY ARTERY SMOOTH MUSCLE CELL SPECIFIC IL-1ß RESPONSE

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**Introduction and objectives** Bone morphogenetic protein receptor type 2 (BMPR2) mutations are found in heritable and idiopathic pulmonary arterial hypertension however penetrance is incomplete implying necessity for a 'second hit'. IL-1ß and IL-6 are increased in PAH patients and animal models and are

thought to have a role in disease. We aimed to determine pulmonary specific interplay between BMPR2 and IL-1ß signalling through assessing IL-1ß responsiveness of pulmonary artery and aortic smooth muscle cells (Ao/PA SMC) and determine the effects of reduced BMPR2.

**Methods** Microarray analysis of PASMC and AoSMC mRNA was performed using microarray on mRNA isolated from cells cultured in SMGM-2 (Lonza) +/- functional BMPR2 (by use of siRNA) and stimulation with 10 ng/ml IL-1ß for 6 h. Subsequent bioinformatics was performed using R. Findings were validated using quantitative PCR and western blotting. Furthermore $R899X^{+/-}$ BMPR2 transgenic mice were fed western diet for six weeks and injected daily with IL-1ß then assessed for inflammatory activation and PAH phenotype (catheter/echo). mRNA and protein changes were measured by TaqMan PCR, western blotting and serum ELISA. Immuno-staining of paraffin embedded lung sections assessed pulmonary vascular remodelling.

**Results** Array data shows reduced inflammatory activation in response to IL-1ß in PASMC compared with AoSMCs, analysis of cells lacking functional BMPR2 identified an exaggerated inflammatory response to IL-1ß in PASMC lacking BMPR2 (siRNA). Significant up-regulation of IL-6, IL-1α and adhesion molecules (>2-fold) shown by array analysis was validated by qPCR. In the absence of BMPR2 a 1.5 fold increase in proliferation was observed in response to IL-1ß compared to PASMC with functional BMPR2. Mice treated with IL-1ß show higher white blood cell counts (1.7-fold), and protein levels of OPG and IL-6 (serum) matching *in vitro* data.

**Conclusion** IL-1ß induces a pulmonary specific transcriptome altered by suppression of BMPR2 signalling indicating cross-talk between the pathways. In the presence of BMPR2, PASMCs show limited response to IL-1ß however reducing BMPR2 exacerbated this response increasing the likelihood of a PAH phenotype in PASMCs. This highlights a mechanism that increased IL-1ß may provide "second hit" to reduced BMPR2 to stimulate development of PAH.

## S4 PULMONARY ARTERY PRESSURE AND EXERCISE TOLERANCE IN PATIENTS WITH PULMONARY ARTERIOVENOUS MALFORMATIONS

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**Introduction and objectives** Pulmonary arteriovenous malformations (PAVMs) provide direct communications between pulmonary arteries and pulmonary veins, bypassing the pulmonary capillary bed. Greater improvements in exercise capacity following embolization therapy have been reported by PAVM patients with raised pulmonary artery pressures (PAPs). The objective of this study was to test the hypothesis that lower PAPs are associated with greater exercise capacity in hypoxaemic PAVM patients.

**Methods** Patients attending our tertiary care institution were recruited at the time of PAVM assessment or embolization. Pulmonary artery pressure measurements were obtained by right-heart catheterisation immediately prior to contrast injection and embolization. For both patients and healthy controls, incremental cardiopulmonary exercise tests were performed seated on an adjustable cycle ergometer (MasterScreen CPX; Via Sprint).

**Results** A total of 19 patients and 26 controls were recruited for the study. Significantly lower arterial oxygen saturations (median 91% vs. 98%, $p < 0.0001$) and end-tidal $PCO_2$ levels were demonstrated in patients alongside significantly raised V[dot]e/V[dot]$CO_2$ slopes (32.2 vs. 24.1 L/min/L/min, $p = 0.0003$). The regression models that best described peak oxygen uptake (V[dot]$O_2$) as a percent of predicted values, were similar between PAVM patients and controls. Surprisingly, despite the differences in $SaO_2$ between PAVM and control subjects, the addition of $SaO_2$ to the final models did not improve the significance of the final model nor did it raise the adjusted-$r^2$ value. For the PAVM subgroup, the median mean PAP was 14.5 mmHg, (IQR 12.5–16.0; range 6–22). Univariate and multiple regression analyses demonstrated no significant relationships between systolic, diastolic or mean PAPs, and peak exercise parameters.

**Conclusions** This study has demonstrated in a population of PAVM patients with hypoxaemia and PAPs essentially within the normal range, that PAP does not have a major influence on peak exercise capacity.

## S5 IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION DEMONSTRATES A PERIPHERAL BLOOD SIGNATURE OF DYSREGULATED IMMUNITY

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**Introduction** There is increasing evidence of an association between Idiopathic Pulmonary Arterial Hypertension (IPAH) and immune dysregulation. Amongst other evidence, autoantibodies are detected in IPAH and there is a high prevalence of autoimmune conditions associated with group 1 pulmonary arterial hypertension.[1] Previous work has focussed on a role for T-regulatory cells in IPAH, however, we have noted a more profound general lymphopaenia clinically, which we set out to define and investigate.

**Objectives** To phenotype circulating leucocytes in patients with IPAH.

**Methods** Fresh peripheral blood leucocytes from patients with IPAH were compared to age and sex-matched healthy controls. A standardised flow cytometry panel for cell surface markers of leucocyte sub-populations was adapted from the Human Immunology Project.[2] Additionally Ig subclasses in serum were analysed by PEG enhanced immunoturbidometric assay and nephelometry. Peripheral lung tissue from explants in patients with IPAH was compared to control tissue from lung tumour resection (distant to the tumour margin). Tissue was immunostained for complement fragments C3d and C4d by streptABC peroxidase technique and visualised with 3,39-DAB hydrochloride and imaged by light microscopy.

**Results** Overall lymphocyte counts were significantly reduced in IPAH compared to controls ($p = 0.0187$). Despite this, IPAH patients demonstrated significantly increased populations of T follicular helper (Tfh) cells ($p = 0.0007$) and $PD1^+CD4^+$ T-cells ($p = 0.0028$), associated with T-cell exhaustion and control of Tfh mediated humoral immunity. There was evidence of altered B-cell differentiation with increased transitional cells ($p = 0.01$) and decreased non-switched memory cells ($p = 0.001$). Ig subclasses were not statistically different between IPAH and control