Conclusions Resolution of an empyema collection and cavity occurs immediately after surgery, and continues in the postoperative period. Similar follow-up results were achieved by debridement alone without decortication in patients presenting with empyema despite the presence of an underlying trapped lung.

S138 RECOVERY OF CALCINEURIN INHIBITOR-RELATED NEPHROTOXICITY WITH SIROLIMUS AFTER LUNG TRANSPLANTATION

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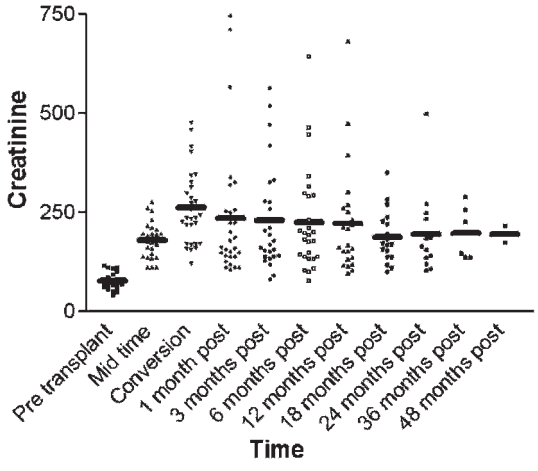
doi:10.1136/thx.2009.127100h

Introduction and Objectives Calcineurin inhibitor (CNI)-based immunosuppression regimens have contributed to the success of lung transplants by reducing early immunological injury and acute rejection rates. However, CNI-induced renal injury is a significant problem, with 16.5% of all non-renal transplant recipients having chronic renal failure at 36 months. Withdrawal of the CNI is a potential mechanism to prevent further damage. Sirolimus is a macrolide immunosuppressant with little impact on renal function. It inhibits T lymphocyte activation and proliferation, and antibody production. We report the medium term outcomes following institution of a ''renal-sparing'' protocol involving withdrawal of the CNI and replacement with sirolimus.

Methods Retrospective data were obtained on 29 lung transplant recipients with CNI nephrotoxicity who were converted to sirolimus between 1990 and 2008. Creatinine levels were followed up at regular intervals or until sirolimus was discontinued. Infective and rejection episodes per year were observed.

Results CNIs were withdrawn in 27 patients (93%), tapered in 1 patient and continued in 1 patient at a low dose due to an ABO mismatch transplant. Steroid cover with prednisolone was given in 23 patients until therapeutic sirolimus levels were obtained (5–10 ng/ml). All patients continued or were commenced on mycophenolate. Mean serum creatinine at conversion was 260 ± 94 μmol/l compared with 77 ± 20 μmol/l pretransplant. A switch to sirolimus showed a decrease in serum creatinine (fig 1) at 12 months ($n = 24$ $p<0.05$ Cr -31 μmol/l) and 24 months ($n = 14$ $p<0.05$ Cr -65 μmol/l). Our longest period of follow-up was at 48 months ($n = 2$ $p<0.05$ Cr -65 μmol/l) where the benefit of sirolimus was still maintained. The creatinine of nine patients remained unchanged and thus sirolimus was discontinued due to the need for renal transplantation/haemodialysis. Rates of infection and rejection were the same preconversion and postconversion to sirolimus (<1 episode/year). However, there were twice as many fungal infections postconversion to sirolimus. Treatment was well tolerated.



Abstract S138 Figure 1.

Conclusions Conversion to a sirolimus-based immunosuppression regimen can allow for stabilisation of renal function in the mid and long term, as well as some renal recovery in lung transplant patients with CNI nephrotoxicity.

S139 AN AUDIT OF SURVEILLANCE BRONCHOSCOPY IN LUNG TRANSPLANT RECIPIENTS

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doi:10.1136/thx.2009.127100i

Introduction Acute allograft rejection (AR) is the most important risk for obliterative bronchiolitis (OB) in lung transplant recipients (LTRs). Transbronchial lung biopsy (TBBx) is the gold standard for distinguishing AR from infections. Opinion is divided between surveillance bronchoscopy (SB) and clinically indicated bronchoscopy (CIB) as the procedure is not without risk.

Method We audited our current practice for SB performed in the year 2008. According to the unit guideline, SB and TBBx are performed at week 3, 6 and 12 after transplantation. The audit was designed to look at the adequacy of TBBx samples, microbiology results and complications.

Results A total of 28 transplants were performed in 2008 which included 25 (89%) bilateral lung transplants, 2 (7%) heart–lung transplant and 1 (3%) single lung transplant. 74 bronchoscopies and TBBx were performed besides CIB, depending on the patient's clinical conditions. Results of only SB were reviewed. Reportable adequacy of TBBx samples was obtained in 65 (88%) patients. Adequacy was low in the first biopsy, 20/27 (74%), as compared with the second, 26/27(96%), and third, 19/20 (95%). There were 7 (10%) A1 rejections and 9 (13%) A2 rejections. Bacteriology was positive in 29/74 (39%) samples, mycology in 4/74 (7%) and virology positive in 1/74 (1%). *Pseudomonas* was the most common isolate, 16/74 (22%), and *Aspergillus* was isolated on 4/74 (5%) occasions. One of 74 samples was positive for Epstein–Barr virus and metapneumovirus. Two of 74 (3%) patients had pneumothorax. No pneumothorax required chest drain. There was no major bleeding requiring blood transfusion or intubations and there was no mortality as a result of SB.

Conclusion SB has a high yield of diagnosing asymptomatic AR and infections in LTRs. The risk of serious complications is low. Identification and treatment of asymptomatic rejection may prove beneficial in preventing OB and we believe the low risk of TBBx with high yield makes this a beneficial approach.

Managing the airway defect in cystic fibrosis

S140 TOWARDS GENE THERAPY FOR CYSTIC FIBROSIS USING A LENTIVIRUS PSEUDOTYPED WITH SENDAI VIRUS ENVELOPES

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doi:10.1136/thx.2009.127100j

Gene therapy for cystic fibrosis (CF) is making encouraging progress into clinical trials. However, further improvements in transduction efficiency are desired. To develop a novel gene transfer vector that is improved and truly effective for CF gene therapy, a simian immunodeficiency virus (SIV) was pseudotyped with envelope proteins from Sendai virus (SeV), which is known to transduce unconditioned airway epithelial cells efficiently from the apical side. This novel vector was evaluated in vivo and in vitro directed

towards CF gene therapy. Here we show that (1) we can produce relevant titres of an SIV vector pseudotyped with SeV envelope proteins for in vivo use; (2) this vector can transduce the respiratory epithelium of the murine nose in vivo at levels that may be relevant for clinical benefit in CF; (3) this can be achieved in a single formulation, and without the need for preconditioning; (4) expression can last for 15 months; (5) readministration is feasible; (6) the vector can transduce human air–liquid interface cultures; and (7) functional CFTR (cystic fibrosis transmembrane conductance regulator) chloride channels can be generated in vitro. Our data suggest that this lentiviral vector may provide a step change in airway transduction efficiency relevant to a clinical programme of gene therapy for CF.

Funding: This work was in part funded by the CF Trust.

KM and UG contributed equally to this work.

S141 EVALUATION OF SAFETY AND GENE EXPRESSION WITH A SINGLE DOSE OF PGM169/GL67A ADMINISTERED TO THE NOSE AND LUNG OF INDIVIDUALS WITH CYSTIC FIBROSIS: THE UK CF GENE THERAPY CONSORTIUM "PILOT STUDY"

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doi:10.1136/thx.2009.127100k

The UK CF Gene Therapy Consortium is working towards a multidose gene therapy study, using the best currently available non-viral gene delivery complex, and whose end point will be to detect clinical benefit rather than proof-of-principle. Based on extensive preclinical testing our selected product is pGM169, a CpG-free human CFTR (cystic fibrosis transmembrane conductance regulator) plasmid with a CpG-free cytomegalovirus (CMV) enhancer and human elongation factor 1α (hCEFI) promoter complexed with GL67A (GL67, DOPE and DMP-PEG5000). We are currently undertaking a single dose study because of a requirement to confirm safety of this "first-in-man" product; however, study design has also been tailored to assess gene expression in vivo in cystic fibrosis (CF) lungs.

A single nebulised dose of 20 ml (53 mg of pGM169 and 286 mg of GL67A) is delivered by an Aeroeclipse II breath-actuated device; a nasal dose (10% of the nebulised volume) is administered on the same occasion using a standard nasal spray device. The latter allows assessment of gene expression without the sampling issues inherent in lower airway assessment, as well as anchoring to our previous clinical trials. Safety measures include physical examination, lung physiology (spirometry, pulse oximetry, lung clearance index), systemic and sputum inflammatory markers, renal and hepatic function and chest CT. We are also measuring antinuclear and antidouble-stranded DNA antibodies, and specific CFTR-related T cell responses. Measurements are made at intervals prior to dosing and during a 28-day follow-up period. Gene expression is assessed by (1) quantitative Taqman RT-PCR for transgene mRNA on nasal and bronchial brushings; (2) anti-CFTR immunohistochemistry; and (3) nasal and lower airway potential difference measurements. Given intersubject variability, paired measurements on individuals will be obtained; bronchoscopies are being performed prior to dosing and at two time points postdosing. Nasal potential difference is measured on serial visits.

To date, 3 patients in an initial non-bronchoscopic cohort have received a half dose of 10 ml and 3 have received the full 20 ml dose. The Data Safety Monitoring Board has granted permission to proceed further with 20 ml dosing, and available data will be presented on safety, tolerability and gene expression.

Funding: UK Cystic Fibrosis Trust.

S142 CERAMIDE IS INCREASED AND ASSOCIATED WITH NEUTROPHILIC INFLAMMATION IN THE LOWER AIRWAY EPITHELIUM OF PEOPLE WITH CYSTIC FIBROSIS

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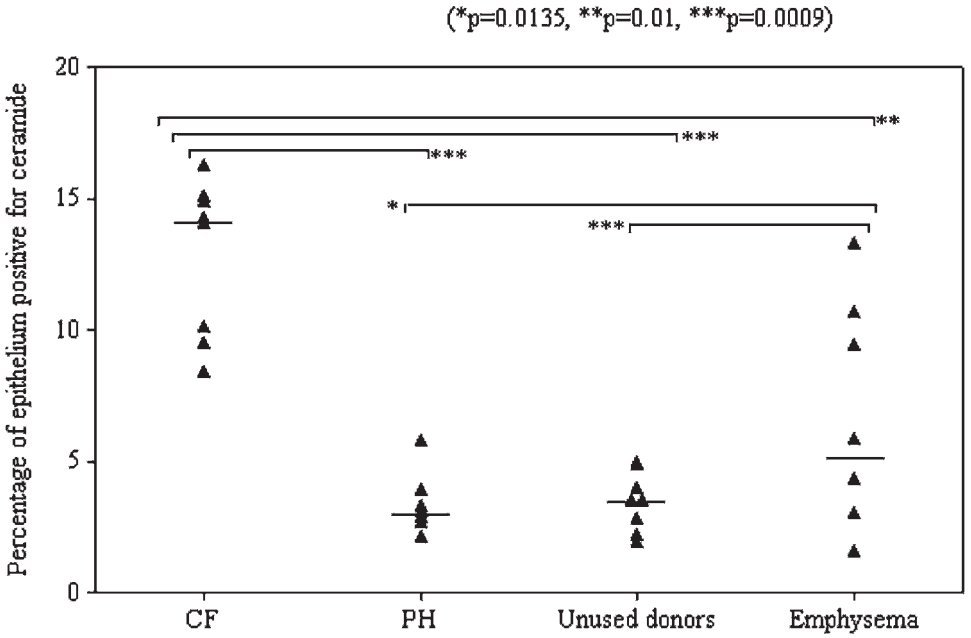
doi:10.1136/thx.2009.127100l

Introduction and Objectives Cystic fibrosis (CF) results from alterations in the CF transmembrane conductance regulator



Abstract S142 Figure 1 Ceramide staining. *p = 0.0135, **p = 0.01, ***p = 0.009. CF, cystic fibrosis, PH, pulmonary hypertension.

(CFTR) gene, but the exact pathogenesis of lung disease remains poorly understood. Ceramide is an essential constituent of plasma membranes and regulates many cellular responses. It has recently been shown that CFTR-deficient mice accumulate ceramide in airway epithelial cells, resulting in inflammation and susceptibility to *Pseudomonas aeruginosa*—two key features of CF lung disease.[1] Ceramide accumulation was also demonstrated in nasal epithelial cells from people with CF and qualitatively in a small number of sections of lung.[1] The objective of this work was to evaluate ceramide levels quantitatively in the lower airway of people with CF compared with pulmonary hypertension (PH), emphysema and unused donors, and to investigate relationships between epithelial ceramide levels and markers of neutrophilic inflammation and *P aeruginosa* infection.

Methods Immunohistochemistry was performed on airways from explanted lungs (8 CF, emphysema and PH, respectively) and 8 donor lungs using ceramide, neutrophil elastase (NE) and myeloperoxidase (MPO) antibodies. Ceramide staining was evaluated in the lower airway epithelium and expressed as percentage area. The number of cells staining positive in the airway for NE and MPO/mm basement membrane was also evaluated.

Results Staining for ceramide was significantly increased in the lower airway epithelium of people with CF (median 14.11%) compared with PH (3.03%, $p = 0.0009$), unused lung donors (3.44%, $p = 0.0009$) and emphysema (5.06%, $p = 0.01$) (fig 1). Ceramide staining was increased in emphysematous lungs compared with PH ($p = 0.0135$) and unused donors ($p = 0.0009$). The number of NE- and MPO-positive cells in the airway was positively correlated with the percentage of epithelium staining for ceramide ($p = 0.001$). Ceramide staining was significantly increased in lungs colonised with *P aeruginosa* (10.1%) compared with those not colonised (3.14% $p = 0.0106$).

Conclusions Ceramide accumulates in the lower airway epithelium of people with CF, and to a lesser extent in emphysema, and is positively correlated with markers of neutrophilic inflammation and *P aeruginosa* infection. These data support the hypothesis that ceramide plays a role in the pathogenesis of CF lung disease and may represent a target for pharmacotherapy.

1. **Teichgraber,** *et al*. *Nature Medicine* 2008;**14**:382–391.

S143 INCREASED INCIDENCE OF CYSTIC FIBROSIS-RELATED DIABETES IN PATIENTS INFECTED WITH TRANSMISSIBLE *PSEUDOMONAS AERUGINOSA* STRAINS

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doi:10.1136/thx.2009.127100m

Background Cystic fibrosis-related diabetes (CFRD) is associated with a sixfold increase in mortality. Although its pathophysiology is not well understood, repeated episodes of stress caused by recurrent respiratory infections may exacerbate its development. Furthermore, worsening clinical parameters in CF have also been attributed to chronic infection with *Pseudomonas aeruginosa* (Psa), but the relationship between infection with transmissible Psa strains (the most prevalent of which is the Liverpool epidemic strain (LES)) and the development of CFRD has not previously been explored. To investigate this further, we compared the incidence of CFRD in two cohorts of our clinic patients, one infected with unique Psa strains and the other with LES.

Methods All 204 patients chronically infected with Psa (139 with LES) were studied. The diagnosis of CFRD was based on World Health Organization criteria. We gathered information on clinical indices of disease severity, including hospital admissions as an index of exacerbation frequency. We carried out a raw univariate analysis on both matched and unmatched cohorts to identify any link between LES status and CFRD. Fisher two-tailed test was used to calculate statistical significance.

Results Using a raw univariate analysis of the entire population, the prevalence of CFRD was greater with LES infection and these patients were older, less well nourished, had worse pulmonary function and required more hospital admissions. However, in two matched cohorts (see table 1) there was still an increased prevalence of CFRD in patients infected with LES, who also had a significantly greater number of respiratory exacerbations.

Conclusions Patients infected with LES have an increased incidence of CFRD, perhaps due to the increased stress this places on their glucose/insulin axis as a result of repeated exacerbations. This study reinforces the need to prevent cross-infection of CF patients with transmissible Psa strains.

Abstract S143 Table 1

Matched cohorts	LES negative (n = 40)	LES positive (n = 40)	p Value
CFRD (%)	15.0	35.0	0.04
Number of hospital admissions	4.4 (4.9)	11.8 (10.3)	<0.01

CFRD, cystic fibrosis-related diabetes; LES Liverpool epidemic strain.

S144 BRONCHOALVEOLAR LAVAGE IN CHILDREN WITH CYSTIC FIBROSIS: WHICH LOBE IS BEST?

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doi:10.1136/thx.2009.127100n

Introduction Bronchoalveolar lavage (BAL) samples are increasingly being used to diagnose lower airway infection in infants and young children with cystic fibrosis (CF). Guidance from the European Respiratory Society (ERS) in 2001 recommended taking a BAL from the most affected lobe or, in diffuse disease, from the right middle lobe (RML). In 2007 an ERS-sponsored workshop recommended that in children with CF two BAL samples should be taken: a triple aliquot from the RML and a single aliquot from the lingula or the most affected lobe. At our unit we have traditionally taken single aliquot BAL samples from multiple lobes. We assessed if we would have missed positive cultures if we had only taken BAL samples from a single lobe (RML) or from two lobes (RML and lingula).

Methods A retrospective case note review was undertaken. It included all paediatric patients with CF who had undergone flexible bronchoscopy between May 2007 and May 2009.

Results We identified 39 bronchoscopies performed on 31 children with CF. BAL samples were sent from all six lobes in 38 bronchoscopies and from four lobes in one. Positive BAL cultures were obtained from 31 bronchoscopies. Had we only used the RML we would have missed 26 positive cultures (14 organisms) in 11 patients. The organisms were: 4 *Haemophilus influenzae*, 3 *Stenotrophomonas maltophilia*, 2 *Pseudomonas aeruginosa*, 2 *Sphingomonas paucimobilis* and one each of *Moraxella catarrhalis*, *Aspergillus fumigatus* and *Staphlococcus aureus*. Had we used the RML and lingula we would have missed 12 positive cultures (8 organisms) in 7 patients. The organisms were: 3 *S maltophilia* 2 *H influenzae*, 2 *S paucimobilis* and one *A fumigatus*.

Conclusions This is the first study that has BAL results from multiple lobes in children with CF. These data confirm that the bacterial distribution in CF is different between the two lungs and also between lobes of the same lung. A single lobe BAL is

insufficient in assessing patients with CF for lower airway infection. Even when BALs are taken from the RML and lingual, a significant number of infections are missed.

Is it asthma or not?

S145 HIGH CONCENTRATION OXYGEN CAUSES CARBON DIOXIDE RETENTION IN SEVERE ASTHMA: A RANDOMISED CONTROLLED TRIAL

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doi:10.1136/thx.2009.127100o

Introduction and Objectives The use of high concentration oxygen in acute exacerbations of chronic obstructive pulmonary disease (COPD) is well known to result in an increase in $PaCO_2$ in some patients. High concentration oxygen is often used routinely in acute severe asthma in the belief that it is safe and indicated in most patients; however, there is some evidence to suggest that this causes an increase in $PaCO_2$. In this randomised controlled trial we compared the effects of high flow vs titrated oxygen therapy on $PaCO_2$ levels in acute severe asthma.

Methods 80 patients with severe exacerbations of asthma (forced expiratory volume in 1 s (FEV_1) $\leqslant$50% predicted) presenting to the Emergency Department of Wellington Hospital, New Zealand were recruited. Participants were randomised to receive either high flow oxygen (8 l/min via a medium concentration mask) or titrated oxygen (via nasal prongs or a medium concentration mask) adjusted to achieve oxygen saturations of 93–95% for 1 h along with routine asthma treatment. Transcutaneous carbon dioxide measurements ($PtCO_2$) were made at 0 and 60 min. The primary outcome variable was the proportion of patients with a rise in $PtCO_2$ $\geqslant$4 mm Hg at 60 min. The secondary outcome variables were: the proportion of patients with a rise in $PtCO_2$ $\geqslant$4 mm Hg and a $PtCO_2$ $\geqslant$40 mm Hg at 60 min, the proportion of patients with a rise in $PtCO_2$ $\geqslant$8 mm Hg and the mean rise in CO_2.

Results Three subjects withdrew from the high flow group leaving 36 for analysis in the high flow group and 41 in the titrated group. The mean (SD) FEV_1 % predicted was 33.4% (10.5) in the high flow group and 35.4% (9.7) in the titrated group ($p = 0.35$). Results for the primary and secondary outcome measures are presented in table 1.

Conclusion These results show that uncontrolled high concentration oxygen therapy results in an increase in $PtCO_2$ when administered to patients with severe exacerbations of asthma. We propose that in severe asthma oxygen should only be used if hypoxaemia is present, and delivery should be titrated to achieve oxygen saturations within the normal range.

S146 AIRWAYS DYSFUNCTION AND EOSINOPHILIC INFLAMMATION IN ELITE ATHLETES WITH SYMPTOMS SUGGESTING EXERCISE-INDUCED ASTHMA

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doi:10.1136/thx.2009.127100p

Introduction and Objectives Symptoms suggesting exercise-induced asthma are common in athletes, particularly those participating in endurance sports. Increased use of asthma medications at elite level has led governing bodies to introduce legislation that requires proof of airways dysfunction prior to use of medication. The current gold standard is a drop in forced expiratory volume in 1 s (FEV_1) of 10% following the eucapnic voluntary hyperventilation (EVH) test. However, there remains controversy as to what the optimum criteria for a positive test should be and whether this test captures all domains of asthma. In order to explore the relationship between airway dysfunction and potentially steroid-responsive disease, we compared the response to EVH with markers of airway inflammation in a group of 30 international athletes who reported symptoms suggesting exercise-induced asthma.

Methods Inhaled steroids and long-acting β-agonists were withheld for at least 2 weeks prior to assessment, and short-acting β-agonists, caffeine and exercise for >8 h. Exhaled nitric oxide (FE_{NO}) was assessed using the NIOX Mino prior to spirometry and EVH challenge. Sputum induction was done after recovery and following pretreatment with inhaled salbutamol.

Results There was a significant correlation between the percentage fall in FEV_1 after EVH challenge and the sputum eosinophil count ($r = 0.46$, $p = 0.01$). A fall of 10% was a sensitive (100%) but not specific (45.5%) indicator of eosinophilic airway disease (defined as a sputum eosinophil count of >3%); a drop of 24% was a more valid marker (sensitivity 88%, specificity 91%). There was close correlation between FE_{NO} and the sputum eosinophil count ($r = 0.901$, $p<0.0001$), suggesting that FE_{NO} might be a simpler and more valid marker of eosinophilic, corticosteroid-responsive airway inflammation in elite athletes.

Conclusions We conclude that the current criteria for a positive EVH test identify significant numbers of athletes who do not have corticosteroid-responsive airway pathology. Either much greater falls in FEV_1 or alternative means are required to identify this dimension of the disease.

S147 HYPERTONIC SALINE CHALLENGE IN THE DIAGNOSIS OF VOCAL CORD DYSFUNCTION IN PATIENTS WITH ASTHMA ATTENDING A SECONDARY CARE CLINIC

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doi:10.1136/thx.2009.127100q

Introduction Symptoms of vocal cord dysfunction (VCD) are caused predominantly by vocal cord adduction during inspiration. It is

Abstract S145 Table 1

	High flow O_2	Titrated O_2	Relative risk	p Value
Subjects with a rise in $PtCO_2$ $\geqslant$4 mm Hg	15/36 (41.7%)	6/41 (14.6%)	2.8 (CI 1.2 to 6.6)	0.008
Subjects with a rise in $PtCO_2$ $\geqslant$4 mm Hg and $PtCO_2$ $\geqslant$40 mm Hg at 60 min	7/36 (19.4%)	1/41 (2.4%)	8.0 (CI 1.0 to 61.7)	0.022
Subjects with a rise in $PtCO_2$ $\geqslant$8 mm Hg	5/36 (13.9%)	3/41 (7.3%)	1.9 (CI 0.5 to 7.4)	0.35
	High flow O_2	Titrated O_2	$PtCO_2$ difference	p Value
Mean rise in $PtCO_2$	2.6 mm Hg (SD 4.2)	0.5 mm Hg (SD 4.4)	2.0 mm Hg (95% CI 0.08 to 4.0)	0.042

$PtCO_2$, transcutaneous CO_2.