479. New understanding of childhood lung disease through physiological measurement

P4598

***Plp1* mutation induces altered respiratory response to an airway challenge**

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Pelizaeus-Merzbacher disease (PMD) is a disease caused by mutations of the proteolipid protein1 (*PLP1*) gene that result in defective CNS myelination. Mice with an extra copy of *Plp1*, called *Plp1*dup, develop a syndrome that models the duplication form of PMD. Patients with all except the mildest forms have respiratory involvement.

Objective: We hypothesized that *Plp1*dup mice would lack protective airway responsiveness (AR) to an autonomic drug challenge. To address this, we investigated whether respiratory mechanics in these mice would be different at baseline (BL) or during methacholine (MCh) challenge.

Methods: Wild type (Wt) n=16, carrier (Car) n=8 & affected (Af) n=17 mice, 3 months (3m) and 6 months old (6m), were anesthetized, mechanically ventilated & challenged with 0.1-6 mg/ml of aerosolized MCh. We calculated resistance (R), dynamic/static compliance (Cdyn/Cstat), asynchrony (PhRTB); lung tissue biomarkers & histological analysis are ongoing.

Results: BL differences were found in R, between Wt6 vs.Car6 ($p=<0.05$) dependent on group ($p=<0.001$) & age ($p=<0.0001$), and in Cdyn, among Wt3 vs. Af3 ($p=<0.01$). MCh increased R as a function of dose in Wt6 & Car6, whereas Af6 mice lacked sensitivity to MCh ($p=<0.05$). No differences in body weight, gender (Wt females vs. Wt Males) and Cstat were found. Af6 mice had the highest PhRTB.

Conclusions: Wt mice compared with Af mice were lacking of AR to MCh at 6m, but not at 3m. These results indicate an age-associated lack of protective autonomic AR in the *Plp1*dup mouse model and suggest that respiratory autonomic disequilibrium may contribute to the respiratory involvement in PMD patients. The *Plp1*dup animal model may be used for testing therapeutic interventions.

P4599

Bronchiolar wall structure is altered in adult mice following neonatal hyperoxia

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Background: Very preterm infants often require supplemental oxygen, and can have an increased risk of poor lung function and asthma in later life. Follow-up studies suggest that factors associated with very preterm birth can cause long-term changes in the the small conducting airways.

Aim: To determine if bronchiolar wall structure is persistently affected by neonatal exposure to hyperoxic gas.

Methods: Neonatal mice (C57BL/6J) breathed 65% O_2 from birth until postnatal day 7 (P7), after which they lived in room air until P56 (n=26). Controls breathed room air from birth (n=27). Bronchiolar walls, lung parenchyma and bronchoalveolar fluid (BALF) were analysed at P56. In bronchioles, we measured epithelial thickness, proportions of proliferating epithelial cells, ciliated and Clara cells, the amount of collagen and airway smooth muscle (ASM), and the number of alveolar-bronchiolar attachments. In lung parenchyma, we measured percent tissue space and mean linear intercept (MLI).

Results: In bronchioles, adult mice exposed to neonatal hyperoxia had significantly thicker epithelium, more ASM and more collagen than controls ($p<0.05$). Compared to controls there were no significant differences in bronchiolar epithelial cell proliferation, Clara cells or ciliated cells. In lung parenchyma, MLI was increased and tissue fraction reduced (both $p<0.05$) in hyperoxia-exposed mice, but there was no effect on the number of alveolar-bronchiolar attachments. In BALF there were 60% more immune cells ($p<0.05$) after hyperoxia.

Conclusions: Exposing the developing lung to hyperoxic gas results in persistent airway remodelling and increased numbers of pulmonary immune cells suggesting on-going inflammation in adulthood.

P4600

Long term sensitisation of cough and expiration reflex in adult rabbits by 48 hour postnatal normobaric hyperoxia

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Rationale: Cough is a frequent report in follow up studies of late premature children at school age. On the other ahnd, the experimental evidence exists in adult animals that the short term effect of acute hyperoxia is to down regulate cough. The

Hypothesis that neonatal exposure to hyperoxia may interfere with incidence of cough at school age has not been tested previously.

The aim of the study was to determine whether postnatal administration of oxygen in pups alters the cough reflex in adult rabbits.

Methods: Rabbits were exposed within the first 8 days of life to 48h normobaric hyperoxia (FIO2 > 93%; n = 12) or ambient air (control; n = 9). At age 3 - 4 months the animal was anesthetized, tracheotomized and subjected to a series of discrete mechanical stimulations of the trachea lasting 50, 150, 300 and 600ms. Each stimulus was quadrupled so as to total 24 stimulations per animal. Cough and expiration reflex were identified from breathing flow and volume, respectively as a forced expiratory effort preceded (cough) or not (expiration reflex) by an increased inspiration.

Results: The incidence of either cough (66/288: 23%) or expiration reflex (124/288: 43%) in hyperoxic rabbits was significantly larger than its respective control (cough: 25/214: 12%; expiration reflex: 60/214: 28%; p = 0.0005).

Conclusion: The experimental evidence of long term sensitisation of airway defensive reflexes in rabbits breathing high oxygen soon after birth favours a role for neonatal hyperoxia in the pathogenesis of chronic cough at school age in late premature children.

P4601

Routine measurement of the LCI in CF with an ultrasonic device for multiple breath nitrogen washout

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During the last decade multiple breath washout technique (MBW) for calculating the Lung Clearance Index (LCI) has become very popular for assessing ventilation inhomogeneity (VI) as an early manifestation of Cystic Fibrosis (CF) lung disease. However, routine use has been difficult not only due to limited availability of licensed equipment. Inert tracer gases (e.g. SF_6, He) certified for medical purposes are not universally available. Switch to nitrogen washout (MBW_{N2}) using 100% oxygen may overcome this problem.
The aim of this cross sectional study was to assess whether LCI derived from MBW_{N2} discriminates as well as MBW_{SF6} between patients with CF and healthy controls.
19 controls (7-51years) and 11 unselected patients with CF (7-25 years) performed 2-3 single MBW_{N2} using the EasyOne Pro LAB™ (ndd Switzerland) with 100% oxygen.
Mean (SD) LCI was 6.5 (0.64) in controls and 9.3 (1.93) in CF with a mean difference (95% ci, p-value) of -2.83 (-4.14;-1.51, 0.001) between the groups. Within-test repeatability (CV%) was 5.3% in controls and 7.7% in CF.
Assessment of LCI using licensed equipment for MBW_{N2} was feasible and well tolerated in both, children and adults and patients and controls. LCI based on MBW_{N2} differed significantly between patients with CF and controls and results were comparable to published data obtained with different equipment and with using SF_6 as tracer gas. We conclude that MBW_{N2} reflects VI similar to MBW_{SF6} and may thus be used for clinical application of MBW in patients with CF.

P4602

Bench test of a mass spectrometer based multiple-breath washout system using a realistic lung model

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Background: Lung volume measurement accuracy of multiple-breath washout (MBW) systems should be validated under realistic conditions. Here we used a previously described lung model (ERS meeting 2011) incorporating BTPS conditions to validate a customized mass spectrometer (AMIS 2000; Innovision) MBW setup used in a series of clinical studies.
Methods: Functional residual capacity (FRC) measurement accuracy was assessed across a range of FRCs (430-4120 mL), tidal volumes (210-1010 mL), and respiratory rates (36-12 min^{-1}). A wash-in inert gas mixture containing 4% sulfur hexafluoride (SF_6) and 4% helium (He) was used for MBW. 63 MBWs were conducted over two test days. Measured gas and flow signals were processed in custom software (TestPoint). FRC was calculated as cumulative expired inert gas volume divided by the difference of MBW start minus end inert gas concentration.
Results: Mean (range) coefficient of variation of FRC was 0.6 (0.1-2.2)%. Mean difference between measured and nominal FRC was 46.7 mL (1.7%), upper and lower limits of agreement were 155.3 mL (5.1%) and -62.0 mL (-1.8%), respectively. Of 126 FRCs, 124 (98%) were within 5% of the nominal FRC, mean error (range) was 2.1 (0.1-6.0)%. Error was associated with respiratory rate ($R^2 = 0.23$). FRC was reproducible between tests, coefficient of repeatability was 73 mL (3.4%) and similar using either He or SF_6 for FRC calculation.
Conclusion: Using a lung model previously shown to be suitable for MBW system validation under physiological conditions, the custom mass spectrometer MBW system precisely and reproducibly measures FRCs ranging from preschool to adult lung volumes.

P4603

Validation of multiple breath washout technology in healthy children and children with CF

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Mass spectrometry based technology is considered the "gold standard" for measuring LC, but is not readily available. In this ongoing study we aim to validate a nitrogen washout system for use in CF children. In a cross-over design, healthy and clinically stable CF children performed MBW by mass spectrometry (AMIS 2000; Innovision A/S, Odense, Denmark) using 4% SF6 or by nitrogen washout (Exhalyzer D, Eco Medics AG, Switzerland). Results were independently scored by two operators; Bland-Altman plots were used to assess the agreement between the two systems. To date 24 healthy children (median age 11 years (range 3-17)) and 33 children with CF (median age 11 years (range 3-17) completed MBW measurements using both the mass spectrometry and N_2 washout. There was no systematic bias observed in LCI between the two methods. Overall there was good agreement healthy children (95% of all measurements agreed within –0.44; 0.83); however LCI_{SF6} was systematically 0.2 (95% CI 0.06; 0.33) units lower than LCI_{N2}. The mean difference between the two systems was greater in children with CF (0.4 (CI 0.29 to 0.55) and the limits of agreement were wider (-0.33; 1.17). Agreement between the two methods for moment ratios was better for the M1M0 (-0.43; 0.63) than M2M0 (-5.72; 8.60), and greater in healthy children compared with children with CF. Inter-observer agreement for nitrogen washout was high (-0.08; 0.08) for all outcomes. These data suggest that while there is no systematic bias between the two systems, LCI measured by nitrogen washout is higher compared to LCI measured by mass spectrometry. Inter-observer variability is low for nitrogen washout if analyzed by trained operators.
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P4604

Ways to shorten the lung clearance index measurement I – Are three measurements needed?

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Background: Inert gas multiple-breath washout (MBW) derived lung clearance index (LCI) is a sensitive lung function parameter in subjects with mild cystic fibrosis (CF) lung disease, but rarely measured in clinical routine due to lack of available equipment and lengthy protocols. Using an available nitrogen (N_2) MBW setup (Exhalyzer D, Eco Medics, Switzerland) we assessed shortened N_2MBW protocols for LCI.
Methods: We determined whether the LCI from the 1st (LCI_1) and the mean LCI of the 1st and 2nd valid N_2MBW (LCI_2), respectively, are comparable to the averaged information obtained from three N_2MBW (LCI_3). We analyzed data of 33 school-aged children with CF and 10 controls performing triplicate N_2MBW.
Results: LCI_1, LCI_2, and LCI_3 differed significantly between healthy and CF children. LCI_1 and LCI_2 were strongly associated with LCI_3 ($R^2 = 0.98$ for both), took less time, and were of similar diagnostic value. Comparing LCI_1 and LCI_2 with LCI_3, mean (range) test duration was 2.2 (0.5-5.2), 6.6 (3.6-12.7), and 11.1 (8.0-17.1) min, and upper limits of normal LCI (8.3, 8.3, and 8.4) all correctly classified 71% of children, respectively. Bland-Altman analysis of LCI_1 and LCI_2 showed good agreement with LCI_3: Mean difference was -1.1% and -0.7%, limits of agreement were 6.8 to -9.0% and 5.9 to -7.3%, respectively.
Conclusion: In the current study population, the 1st LCI or the mean of the 1st and 2nd LCI take less time and predict the mean LCI of three N_2MBW within physiological measurement variability. Using less N_2MBW measurements seems promising for time-saving LCI measurement in clinical routine.

P4605

Abnormalities in lung clearance index in CF infants diagnosed by newborn screening

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Newborn screening (NBS) in CF offers the potential to prevent lung damage before the onset of clinical symptoms. The lung clearance index (LCI), measured by multiple breath washout has shown promise as a marker of early lung disease in patients with CF.Therefore we aimed to determine whether LCI could be used to distinguish between subjects with CF and healthy subjects in the first 2 years of life. Healthy infants (3 months – 2 years) completed MBW testing as part of the infant pulmonary function protocol of the Canadian Healthy Infant Longitudinal Development Study (CHILD Study). Infants (3 months – 2 years) with diagnosed CF attending the Respiratory Medicine Clinic at the Hospital for Sick Children were invited to complete MBW testing. MBW was measured 51 healthy infants and 18 infants with CF diagnosed by NBS. Despite NBS, infants born with CF were smaller (Height-for-age z-score (-1.19 (-0.58; -1.81) and lighter (mean difference weight-for-age z-score (-0.40 (-0.98; 0.19) compared with healthy controls. The LCI was on average 0.4 units higher in CF (mean LCI 7.32 (SD 0.91)) compared to healthy infants (6.92 (SD 0.61)). Adjusting for the relationship between LCI and height in the first 2 years of life, LCI was 0.67 (95%CI -1.34; -0.01) z-scores higher in CF compared to healthy infants, although overlap was considerable (Figure). Therefore, despite newborn screening early abnormalities in LCI are present in CF patients LCI underscoring the need for new treatment approaches to address early lung disease.
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P4606

Assessment of ventilation inhomogeneity in patients with alpha-1-antitrypsin deficiency – A useful tool for monitoring early lung disease

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The risk of developing alpha-1-antitrypsin deficiency (α1-AT deficiency) related fatal emphysema during adulthood is high in patients with a PiZZ genotype. Currently, the FEV_1 is used for detecting and monitoring α1-AT deficiency related lung disease. However it is likely that the early manifestation starts in peripheral

airways that are not accessible by spirometry. Multiple Breath Washout (MBW) is a sensitive test for detecting ventilation inhomogeneity (VI) within the peripheral airways that can be quantified by calculating several indices such as the Lung Clearance Index (LCI).
We performed a preliminary analysis of an ongoing multi centre study assessing the clinical and prognostic value of measuring the LCI in patients with α1-AT deficiency.
19 controls (7-51years) and 20 unselected patients with α1-AT deficiency (12-72 years) performed 2-3 single MBW_{N2} using the EasyOne Pro LAB™ (ndd Switzerland) with 100% oxygen and subsequent spirometry.
Mean (SD) LCI was 6.5 (0.64) in controls and 9.0 (1.98) in patients with a mean difference (95% ci, p-value) of -2.57 (-3.54;-1.60, <0.001) between the groups. Within-test repeatability (CV%) was 5.3% in controls and 6.0% in patients. LCI correlated significantly with age in patients.
Assessment of LCI derived from MBW_{N2} was feasible, reproducible and well tolerated in both, patients and controls. LCI differed significantly between patients and controls. In patients, VI increased with age. We conclude from these preliminary data that the LCI reflects presence of VI in patients with α1-AT deficiency and may thus be useful for monitoring α1-AT deficiency related lung disease.

P4607
Lung clearance index and exercise capacity among children with bronchiectasis
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Background: In paediatric bronchiectasis, there has been limited experience on the relationship between disease severity, as assessed by exercise limitation and lung clearance index (LCI).
Aim: To compare LCI and exercise capacity among children with bronchiectasis.
Method: Fifteen stable children and adolescents with CF, 14 stable children with non-CF bronchiectasis and 15 healthy children and adolescents, participated in maximal incremental cardiopulmonary exercise testing using a cycle ergometer and they had an LCI assessment.
Results: The CF children's mean age was 13.7 years, mean FEV_1 74.4% predicted. The 14 non-CF bronchiectasis children's mean age was 13.8 years, mean FEV_1 75.1% predicted and the healthy children's mean age was 13.6 years, mean FEV1 94.7%. Among CF patients there was evidence of exercise limitation, with mean Peak Aerobic Capacity (V'Opeak) 62.2% predicted. Among the non-CF bronchiectasis patients there was evidence of exercise limitation, with mean V'Opeak 77.3% predicted. Mean V'Opeak did not differ significantly among children with CF and non-CF bronchiectasis (p: 0.06). LCI was significantly increased among CF patients (mean LCI 13.7), compared to healthy children (p<0.00001). LCI was also found significantly increased among patients with non-CF bronchiectasis (mean LCI 11.8), compared to healthy children (p<0.0001). LCI was not found significantly different among children with CF and non-CF bronchiectasis (p: 0.16).
Conclusions: Exercise testing and Multiple Breath Washout measurements can discriminate children with bronchiecatsis from healthy children. However, the burden of the disease is more prominent in children with CF compared with the non-CF bronchiectasis.

P4608
Sensitivity of lung clearance index and chest computed tomography in early lung disease among children with non-CF bronchiectasis
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Background: Lung disease starts before clinical symptoms become prominent, among CF children. Computed chest tomography (CT) is the reference method for identifying structural changes. It has been suggested that the Lung Clearance Index (LCI) is a sensitive marker allowing non-invasive monitoring of lung disease.
Aim: The aim of this prospective study was to investigate the diagnostic accuracy of the LCI in comparison to CT among children with non-CF bronchiecatsis, with early lung disease and normal FEV_1 (>80% predicted).
Method: MBW and low-dose HRCT were performed in 14 patients (6-21 years) with non-CF bronchiectasis and normal FEV_1 (>80% predicted.) HRCT scans, LCI and FEV_1 were recorded. A modified Bhalla score was used to assess HRCT scans. LCI was assessed with Multiple Breath Washout measurements.
Results: LCI was abnormal in 12/15 (80%) of children. 11/15 patients (73%) demonstrated both, increased LCI and structural changes on CT. 1/15 (6%) had normal results in both measurements. There was a significant linear correlation between CT-score and LCI in 12/15 (87%) of patients; whereas no such correlation was observed between CT-score and FEV_1. Sensitivity of the LCI to detect structural lung damage was 85%, whereas specificity of LCI was 50%.
Conclusions: Diagnostic accuracy of the LCI for detecting CF lung disease in patients with normal FEV_1 was good when compared to CT. Results indicate that structural changes are unlikely if a normal LCI is measured. The LCI may be a suitable surrogate marker for monitoring progression of lung disease among children with non-CF bronchiecatsis.

P4609
Lung clearance index in paediatric exercise induced asthma
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Introduction: Exercise induced bronchoconstriction is reported commonly by asthmatic children. We assessed whether lung clearance index (LCI) can detect it.
Methods: Following symptom questionnaire, spirometry, and Multiple Breath Washout (MBW, Innocor 0.2% SF_6), children performed a standard treadmill exercise challenge. One minute post exercise we measured MBW and spirometry. Measures repeated 10 mins post bronchodilatation. If baseline FEV_1 decreased > 20% baseline, testing only post salbutamol.
Results: 21 asthma patients and 21 healthy controls (13M:8F, mean 12 yrs in both groups). In asthma LCI 7.2(0.7) (mean (SD)) significantly > control 6.8 (0.4), p=0.02. In asthma, post exercise LCI increase by 4.8(10.2)% (Fig 1), with no change in FEV_1(-0.01(3.3)%). Post exercise LCI +1.4(5.5)% in healthy volunteers (ns). In those asthma with >20% fall FEV1 (n=3), post salbutamol LCI remained high, 8.0(0.3). Post exercise change in LCI higher in asthma who reported exercise intolerance; No symptoms 2.8(8.5)%; controlled symptoms 5.0(9.3)%; limited despite salbutamol 17.7(16.7)%. No trend for FEV_1; 0.3(3.9)%, vs -1.0(3.1)% vs -0.2(0.3)%.

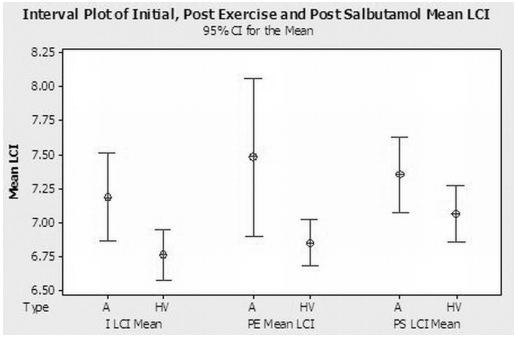

Figure 1. Initial, post exercise and post salbutamol LCI in asthmatic and healthy groups.

Conclusions: LCI tends to rise following exercise. However rise in LCI was non-significant, consistent with the discrepancy between symptoms and altered lung function in asthmatic children. Change in LCI is greatest in the symptomatic, with largest LCI change in those reporting poor treatment response (not demonstrated by FEV_1).

P4610
Hand-held tidal breathing nasal nitric oxide measurement as a targeted case-finding tool for primary ciliary dyskinesia
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Background: Nasal Nitric Oxide (nNO) measurement is a sensitive supplementary tool in diagnosis of Primary Ciliary Dyskinesia (PCD). Tidal Breathing (TB) nNO requires minimal cooperation, has potential as more widespread targeted case-finding tool for PCD in all age groups, and discriminative capacity between PCD and non-PCD has been previously established using stationary nNO analyzer (Marthin JK and Nielsen KG. Eur Respir J 2011; 37: 559-565).
Aim: Assess validity of hand-held TBnNO in a selected population.
Methods: TBnNO was measured in PCDs, cystic fibrosis (CF) patients and healthy subjects (HS) using both an electrochemical hand-held device, NIOX MINO® Nasal equipped with a nasal research application, and two chemiluminiscence stationary systems: NIOX® and ANALYZER CLD 88sp®. All systems allow passive nasal sampling at a flow rate of 5 ml/s during tidal breathing. 2 ml/s sampling is an additional option with NIOX MINO® Nasal. Data were analysed by ROCC and Bland-Altman plots.
Results: TBnNO values were compared in 41 subjects between 0.3 and 57 years: 15 PCDs, 13 CF patients, and 13 HS. MINO discriminated significantly between PCD and HS (P<0.001) and between CF and PCD (P<0.001).

	MINO5	MINO2	NIOX	ANALYZER CLD 88sp
Cut off, ppb (PCD vs HS)	142	363	202	175
Sensitivity, %	100	100	100	100
Specificity, %	100	92.3	100	100
CV% (all subjects)	10.5	19.4	13.8	13.7
LoA#, ppb (PCDs only)	–43.9 to 87.5	–120 to 89.5	–	–25.5 to 44.8

#Limits of Agreement: NIOX as reference method.

Conclusion: Hand-held TBnNO separated significantly between PCD and HS, and between PCD and CF, with cut off value and sensitivity/specificity comparable to those of stationary systems.

P4611

Upper and lower airway nitric oxide levels in primary ciliary dyskinesia

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Introduction: Patients with primary ciliary dyskinesia (PCD) have low nasal nitric oxide (nNO) and fractional exhaled NO (FeNO). The reasons are unclear but might be related to ciliary function. Analysis of nitric oxide (NO) from different regions of the airway and comparisons with other disease states may guide our understanding.
Aim: To compare differential bronchial (J_{NO}) and alveolar ($Calv_{NO}$) NO in patients with PCD, cystic fibrosis (CF), asthma and healthy subjects.
Methods: Exhaled NO at different flow rates (50, 100, 200 and 250 ml/s) and nNO were measured (NIOX flex®, Aerocrine, Sweden) in patients with PCD (n=12), asthma (n=18), CF (n=12) and healthy controls (n=17). J_{NO} and $Calv_{NO}$ were derived using a model of pulmonary NO exchange-dynamics.
Results: $FeNO_{50}$ and nNO were significantly lower in PCD than in healthy subjects, as was J_{NO}, 271 pl/s (228) vs. 965 pl/s (963) (p=0.004) (mean (SD)). However $Calv_{NO}$ was similar between the two, 1.6 ppb (0.5) vs. 2.4 ppb (1.4) (p=0.174). (Table 1 for CF and asthma data)
Conclusion: PCD patients have significantly lower J_{NO} but similar $Calv_{NO}$ to healthy controls. As there are no cilia in the alveolar region this might support the hypothesis that NO biosynthesis is coupled to ciliary function. Data collection continues.

P4612

Determinants of functional deficits assessed by spirometry and plethysmography in children with bronchial asthma

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Follow-up in asthmatic children is usually performed by spirometry, and hence without consideration of pulmonary hyperinflation. 'Effective' specific resistance(sR_{eff}) measures resistive changes throughout the whole breath cycle concomitantly to changes of resting end-expiratory level.[1]
Objectives: Defining the pattern of functional deficits and their changes over time in children with bronchial asthma.
Methods: Serial lung function measurements performed in 216 asthmatic children (age: 4.0 to 17.9 y) were analyzed retrospectively representing at least 3 annual tests, and providing functional residual capacity (FRC_{pleth}), sR_{eff}, volume-time and flow-volume indices (FEV_1, MEF_{50}, and $MMEF_{75-25}$). Data were expressed as SD-score computed by z-transformation using reference equations.
Results: Within the 1270 lung function tests spirometry failed to detect abnormal lung function in 24.1% of tests (plethysmography 1.7%). Bronchial obstruction (> 2SDS) was depicted by sR_{tot} in 93.8%, sR_{eff} in 74.6%, MEF_{50} in 73.8%, $MMEF_{75-25}$ in 62.1% and FEV_1 in 23.7% of tests. Moreover, pulmonary hyperinflation (FRC_{pleth} > 2 SDS) was present in 26.7%, mostly combined with obstruction (23.4%). Independent from age at entry pulmonary hyperinflation remained to a certain degree despite treatment (LABA and ICS).
Conclusions: Apart from spirometry, follow-up of asthmatic children should include plethysmographic measurements, because changes in static lung volumes influence airway dynamics, mimicking normal flow-volume curves. Moreover, patients with pulmonary hyperinflation are less responsive to standard treatment.
[1] Matthys H, Orth U. Respiration 1975: 32(2): 121-134.

P4613

Breath by breath specific airway resistance during panting in children

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Rationale: Panting is the optimal way to minimize the phase lag between the plethysmographic volume compression signal (Vplet) and airflow due to the thermal artifact when measuring specific airway resistance (sRaw). A drawback in children is that the end expiratory lung volume may be observed to increase during the measurement. It is not known to what extent sRaw is altered by the breathing strategy.
Hypothesis: The hypothesis was tested here that increased end expiratory lung volume during panting does not impact significantly on sRaw.
Objective: To test whether increased end expiratory lung level within the sRaw acquisition results in a systematic trend with time.
Methods: sRaw was measured in 10 children panting in a custom made pressure plethysmograph. Thirty measurements (1-5 per subject) that displayed a steady increase in end expiratory lung level throughout at least 4 breaths were reanalyzed breath by breath.
Results: Panting frequency (mean ± SD) was 3.1±0.5 Hz. sRaw was found to increase significantly throughout the acquisition (p = 0.006).

Significant breath by breath increase in sRaw (hPa.sec) during panting in 10 children

Breath 1	Breath 2	Breath 3	Breath4
7.0±1.5	7.3±1.8	7.8±1.6	8.1±1.7

mean ± SD.

Conclusion: The progressive increase in end-expiratory lung volume during panting in children is associated with parallel increase in sRaw. This may be explained by non linearities in the Vplet - airflow relationship while the end expiratory lung volume increases in relation with imbalance between inspiratory and expiratory efforts. The clinical relevance is not clear and desserves further evaluation.

P4614

Lung function and gender at twelve to thirteen years of age in children born very prematurely

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Lung function abnormalities are common in school aged children born extremely prematurely. Follow up of infants born at 23-28 weeks gestational age from the United Kingdom Oscillation Study (UKOS) showed males had a higher incidence of respiratory morbidity during infancy.
Aim: To test the hypothesis that lung function at 12-13 years of age in children born very prematurely would be worse in males than females.
Methods: Lung function was assessed in 65 children, the first to date assessed in follow-up of UKOS, which randomised babies to high frequency oscillation or conventional ventilation. Forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1:FVC, residual volume (RV), transfer factor for carbon monoxide (TLCO), functional residual capacity (FRC) and response to cold air challenge (CACh) were assessed. Results were abnormal if two standard deviations (SD) below expected, except RV results which were abnormal if >2 SDs.
Results: 29 females and 36 males have been assessed. A greater proportion of males compared to females were oxygen dependant at 28 days (89% versus 69%, p=0.063), had reduced FEV1 (22% versus 3.5%, p=0.036), higher RV (34% versus 4%, p=0.009) and higher FRCpleth (26% versus 0%, p=0.008), but there were no significant differences regarding the proportions with reduced FEV1:FVC (44% versus 31%, p=0.27 (FET p=0.31)) or reduced TLCO (29% versus 50%, p=0.18 (FET p=0.23)) or responding to a CACh (24% versus 26%, p=0.94).
Conclusion: Preliminary results suggest that amongst 12-13 year old children born at 23-28 weeks gestation, males have greater airways obstruction than females, but this is not explained by greater airway hyper-reactivity.

P4615

Lung function in infants and toddlers after the repair of congenital diaphragmatic hernia (CDH)

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There are only 4 studies on infant pulmonary function testing (IPFT) performed after repair of CDH. We used a wide spectrum of IPFT methods to test babies in whom CDH repair was performed.

Abstract P4611 – Table 1. Nitric oxide readings by respiratory disease

Reading	PCD	Healthy	Asthma	CF
nNO (ppb)*	23 (16-61)	856 (536-988) (p<0.001)	769 (560-1126) (p<0.001)	521 (457-609) (p=0.028)
$FeNO_{50}$ (ppb)**	9.2 (7.9)	21 (21) (p=0.019)	43 (41) (p<0.001)	15 (11) (p=0.161)
J_{NO} (pl/s)**	271 (228)	965 (963) (p=0.004)	2100 (1935) (p<0.001)	564 (492) (p=0.387)
$Calv_{NO}$ (ppb)**	1.6 (0.5)	2.4 (1.4) (p=0.174)	5.4 (3.5) (p<0.001)	2.3 (1.1) (p=0.195)

*Median (IQR), **Mean (SD), p values compared to PCD.

We tested 30 infants and toddlers after CDH repair (BW 3.10±0.54 kg [mean±SD]; body length 49.5±2.2 cm). In 4/30 Goretex patch was used (subgroup GORE). Age at testing was 1.32±0.54 (median 1.07) yrs; body weight 9.76±1.25kg (z-score -0,777), body length 78.8±6.7cm (z-score -0,024). The whole-body plethysmography (to measure FRCp and sReff), tidal breathing analysis (tPTEF%tE), baby resistance/compliance (specific Crs) and RTC method (V'maxFRC) were performed. Standard protocols1,2 and proper reference values3,4 were used.

FRCp equals 126.5±36.9% pred ($P<0.002$), sReff reached 109.9±58.9% pred (ns). A parameter of tPTEF%tE decreased (22.2±8.5%). Specific compliance of the respiratory system, rs (Crs/kg) was 14.1±2.3 ml/kPa/kg (76.1±20.1% pred) A value of V'maxFRC reached only 112±44ml/sec (z-score -2.387). Increased value of FRCp was found in GORE subgroup (165.7±51.9 vs. 120.4±31.2, $p<0.02$).

Our cohort had normal body length but mildly lowered body weight. Neither central airway (aw) obstruction nor restrictive pattern was found. Mild peripheral aw obstruction, mild (secondary) hyperinflation and mildly decreased specific Crs was found.

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References:

[1] Hammer J, Eber E (eds). Pediatric Pulmonary Function Testing, 2005, Basel, Karger.
[2] Gappa M et al. ERS/ATS Task Force. Eur Respir J. 2001 Jan;17(1):141-8.
[3] Hulskamp G et al. Am J Respir Crit Care Med 2003;168:1003-9.
[4] Koumbourlis AC et al. Pediatr Pulmonol. 2004 Apr;37(4):318-23.