Methods Studies were performed on 125 infants <29 weeks gestation who required mechanical ventilation at 7–14 days and received 24 days of iNO at 20–2 ppm. A control group of 19 infants did not receive iNO.

Results In NO-treated infants there was a significant dose-dependent increase of both urinary NOx and cGMP per creatinine (maximal 3.1- and 2-fold, respectively, at 10–20 ppm iNO) compared to off iNO. The ratio of cGMP to NOx, which may reflect efficiency of NO signalling via guanylate cyclase, was lower (mean 38%) at all doses of iNO compared to off NO. NOx and cGMP concentrations at both 2 ppm and off iNO were inversely related to severity of lung disease (Respiratory Severity Score) during the first month, and the NOx levels were lower in infants who died or developed BPD at term. NOx was higher in Caucasian compared to other infants at all iNO doses.

Conclusions Urinary NOx and cGMP are biomarkers of endogenous NO production and lung uptake of iNO, and levels at some doses reflect the severity of lung disease in infants. These results support a role of the NO-cGMP pathway in lung development and repair from injury.

PS-206 MATERNAL/NEONATAL VITAMIN D DEFICIENCY MAY BE A RISK FACTOR FOR DEVELOPMENT OF BRONCHOPULMONARY DYSPLASIA IN PRETERM INFANTS

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Background and aims Vitamin D seems to play an important role in the pathogenesis of respiratory system diseases. The aim of this study was to evaluate the possible association between both maternal and neonatal 25-hydroxyvitamin-D (25-OHD) levels and the subsequent risk of bronchopulmonary dysplasia (BPD) development in preterm infants.

Methods Premature infants ≤ 32 gestational age and admitted to Neonatal Intensive Care Unit with a diagnosis of respiratory distress syndrome (RDS) between December 2012 and December 2013 were included to this prospective study. Blood for neonatal and maternal vitamin D levels were obtained from all infants and their mothers at the time of hospital admission. The maternal and neonatal demographic features, maternal vitamin D usage, maternal head cover status, birth season and neonatal morbidities and mortality were all recorded.

Results A total of 100 preterm infants were included and 31 of them developed BPD. The mean birthweight, gestational age, duration of ventilation and duration of oxygen supplementation were significantly higher in infants with BPD compared with those who did not develop BPD ($p < 0.05$). Both maternal (19 ± 2.2 vs 28.7 ± 7.6) and neonatal (7.1 ± 1.6 vs 14.8 ± 4.7) 25-OHD levels were significantly lower in infants with BPD (both $p > 0.05$). All of the infants with BPD had a 25-OHD level < 10 ng/ml that represented severe vitamin D deficiency ($p < 0.05$).

Conclusions This study suggested for the first time that maternal/neonatal vitamin D deficiency might be associated with increased risk of BPD in preterm infants.

PS-207 PLASMA PRO-ENDOTHELIN-1 AS MARKER OF BRONCHOPULMONARY DYSPLASIA

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Background Endothelin-1 (ET-1) is a potent pulmonary vasoconstrictor, involved in lung injury and remodelling. ET-1 can be estimated by measuring its stable by-product, C-terminal pro-ET-1 (CT-proET-1), in plasma.

Aims To investigate CT-proET-1 values in very preterm infants (gestational age < 32 weeks) from birth to postmenstrual age (PMA) of 36 weeks, and their relationship with lung injury and bronchopulmonary dysplasia (BPD).

Methods Prospective cross-sectional study of 391 CT-proET-1 measurements (fully automated immunofluorescent assay) from 267 very preterm infants. Measurements were performed at birth ($n = 72$ infants), on day of life (DOL) 2 ($n = 89$), on DOL 6 ($n = 49$), on DOL 28 ($n = 106$), and at PMA 36 ($n = 75$). Trial registration: ClinicalTrials.gov NCT01644981.

Results CT-proET-1 values were (median) 151 pmol/L (IQR 118–186) at birth, peaked on DOL 2 (319 pmol/L (235–382)), and declined thereafter to 214 pmol/L (148–293) on DOL 6, 184 pmol/L (149–233) on DOL 28, and 150 pmol/L (118–188) at PMA 36. Infants with BPD had higher CT-proET-1 values on DOL 2 ($p = 0.001$), DOL 6 ($p < 0.001$), and DOL 28 ($p = 0.007$), with no differences at birth and PMA 36 as compared to those without BPD. CT-proET-1 on DOL 6 was significantly correlated with days of mechanical ventilation, nasal CPAP, and oxygen requirement (Spearman's R_s 0.657, 0.713, 0.745, respectively, $p < 0.001$ each). Moderate correlations were found for the same parameters on DOL 2 and DOL 28 but not for birth and PMA 36.

Conclusions The levels and the pattern of CT-proET-1 increase during the first week of life might serve as an early marker of BPD.

PS-208 GENETIC PREDISPOSITION TO THE DEVELOPMENT OF BRONCHOPULMONARY DYSPLASIA IN INFANTS BORN PREMATURELY

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Background and aims Bronchopulmonary dysplasia (BPD) is an unfortunately common outcome following premature birth. Genetic factors influence BPD development but their role is part of a complex interaction with environmental factors. We postulated that alterations in the gene as well as imbalances in gene products may affect BPD development.

Methods The NIH human genome database was interrogated for previously identified gene polymorphisms that have been associated with neonatal respiratory conditions. Angiotensin converting enzyme (ACE) and surfactant proteins A-D gene candidates were selected based upon clinical plausibility for the study

population. DNA was extracted from whole blood and Guthrie card specimens. Surfactant single nucleotide polymorphism (SNP) genotyping was arranged using the Taqman technique. ACE gene primers were obtained for the previously known insertion/deletion polymorphism and PCR analysis was performed.

Results 236 infants born prematurely survived to 28 days post-natal age and contributed to genotype analysis. 106 infants did not develop BPD and 130 infants did develop BPD (54 mild, 29 moderate, 47 severe). Both gestational age and birth weight were significantly different between those infants who did and did not develop BPD and predicted BPD development with an area under the ROC of 0.88 and 0.82 respectively. We demonstrated using multifactorial statistical analysis that the inclusion of the ACE genotype to a predictive statistical model of BPD development improves the predictive potential of the model (area under ROC curve 0.88).

Conclusion The presence of the ACE DD genotype is associated with a higher likelihood of developing BPD.

PS-209 BCG VACCINATION CAN PREVENT HYPEROXIC LUNG INJURY?

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Introduction The aim of this study effects of BCG vaccine on the histopathologic and gene expression changes seen in hyperoxia induced neonatal rat lung injury.

Method Twenty-three rat pups were divided into four groups: air-exposed control group (n = 5), hyperoxia-exposed placebo group (n = 7), hyperoxia-exposed BCG-vaccinated group (n = 7), and air-exposed BCG-vaccinated group (n = 4). neonatal hyperoxic lung injury model was established according to the previous studies. Measurement of alveolar surface area, quantification of secondary crest formation, microvessel count, evaluation of alveolar septal fibrosis, and smooth muscle actin immunostaining were performed to assess hyperoxia-induced changes in lung morphology. The gene expression level was evaluated by RT-PCR.

Results The alveolar surface areas were significant different between the oxygen exposed placebo group and oxygen exposed BCG vaccinated group (alveolar surface area; $0.29 \pm 0.02 \mu\text{m}^2$ and $0.52 \pm 0.04 \mu\text{m}^2$ $p < 0.05$ respectively). Number of crests and microvessel count was found to be significantly increased in the oxygen exposed BCG vaccinated group compared with the animals in the oxygen exposed placebo group ($p < 0.05$). Exposure to hyperoxia resulted in a significant decrease in mean alveolar surface area and number of crests formed compared with air-exposed animals ($p < 0.05$). The degree of fibrosis was found to be significantly increased in the oxygen exposed placebo group compared with the animals in the oxygen exposed BCG vaccinated group (degree of fibrosis: 1.88 ± 0.33 and 0.91 ± 0.66 $p < 0.05$ respectively). Immunostaining for SMA demonstrated hyperoxia-exposed animals with BCG vaccine in a significantly decrease in smooth muscle content compared with hyperoxia-exposed placebo animals ($p < 0.05$). The expression of VEGF, FGF1, IL13, NF κ B1 and TNF α in the lungs of vaccinated animals was significantly higher than that of non-vaccinated animals ($p < 0.05$).

Conclusion Our results suggest that BCG vaccination can be a new protective strategy against neonatal hyperoxic lung injury.

These beneficial effects may be interpreted with its immunomodulatory effects on proinflammatory-antiinflammatory cytokine balance and expression of growth factors.

PS-210 ALVEOLAR CAPILLARY DYSPLASIA: A GENETICALLY DETERMINED DISRUPTION OF THE ALVEOLAR/MESENCHYMAL CROSS-TALK CAUSING NEONATAL HYPOXIC FAILURE

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Background/aims Alveolar capillary dysplasia (ACD) is characterised by pulmonary veins misalignment, capillary paucity and alveolar misdevelopment, and caused by FOXF1 mutations only in 40% of cases. Objectives were 1. to identify known and new gene defects and 2. to correlate them with molecular/cellular mechanisms.

Methods We recruited a cohort of 23 pathology-confirmed cases. When DNA was available, genome-wide copy number variation was analysed through Array Comparative Genomic Hybridization (aCGH). Mutations were tested by direct sequencing of FOXF1 and candidate genes identified by aCGH; Molecular pathways were analysed by multi-channel immunofluorescence microscopy of ACD cases compared to human fetal/neonatal lung tissue at various development stages.

Results 1. Genomic deletions or mutations were identified in 57% of tested cases. Besides FOXF1, two of the genes involved stand out as potential candidates: MEOX2 and TBX4.

2. ACD cases showed a markedly decreased expression of c-kit, a marker expressed in pulmonary small arteries and capillaries in fetal lung controls. In normal fetal lungs FOXF1 and TBX4 were prevalently expressed at the mesenchymal-epithelial border, and MEOX2 in pulmonary vascular smooth muscle cells (PVSMC). Their expression pattern and intensity were altered in all ACD cases, indicating that decreased FOXF-1 and/or its downstream transcription factor TBX4 disrupt lung micro vessel formation and homing to alveolar epithelium, and that a similar phenotype may derive from dysregulated PVSMC proliferation and angiogenesis related to MEOX2 insufficiency.

Conclusion Genetic defects affecting the FOXF1 pathway affect the mesenchymal, endothelial and epithelial cross-talk leading to lung developmental disruption, pulmonary hypertension and hypoxic respiratory failure.

PS-211 EARLY POSTNATAL SYSTEMIC LIPOPOLYSACCHARIDE INCREASES PRO-INFLAMMATORY CYTOKINES AND ANGIOGENIC GROWTH FACTORS IN THE LUNGS AND LEADS TO THE PHENOTYPE OF NEW BPD IN NEONATAL RATS

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