

## Abstracts

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### The C-Terminal, Endoplasmic Reticulum-Luminal, Domain of Prosurfactant Protein-C Is a Chaperone That Prevents Surfactant Protein-C Misfolding

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**Background:** Newly synthesized prosurfactant protein-C (proSP-C) is a 197-residue integral membrane protein with a type II orientation in the endoplasmic reticulum (ER) membrane. Part of the cytosolic region and the transmembrane segment of proSP-C generate SP-C, an  $\alpha$ -helical 4.2 kDa acylpeptide. Mutations in the ER-luminal, C-terminal part of proSP-C (CTC) are associated with interstitial lung disease (ILD), intracellular accumulation of cytotoxic protein aggregates and lack of detectable mature SP-C. **Objective:** CTC binds to an unfolded poly-Val segment corresponding to the transmembrane domain of SP-C, but not to  $\alpha$ -helical SP-C. This, together with the phenotype associated with mutations in the proSP-C Brichos domain, led us to investigate the hypothesis that CTC promotes helix stability during biosynthesis of proSP-C. **Results:** Human embryonic kidney cells that express the ILD mutant proSP-CL188Q show (i) Congo red positive inclusions indicative of amyloid formation, and accumulate large proSP-C aggregates, (ii) transfection of CTC into these cells results in a CTC/proSP-CL188Q complex and prevents proSP-CL188Q aggregation, (iii) replacement of the SP-C poly-valine segment with a poly-leucine segment reduces proSP-CL188Q aggregation, and (iv) CTC is oligomeric, exposes hydrophobic surfaces, and binds to phospholipid membranes. **Conclusions:** The proSP-C, C-terminal, ER-luminal region has chaperone-like properties that prevent the transmembrane part of proSP-C from aggregating before it has attained helical conformation.

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### Variable Phenotype and Surfactant Protein Expression in Infants with ATP-Binding Cassette Transporter A3 Mutations

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**Background:** Mutations in the ATP-binding cassette transporter A3 (ABCA3) gene are increasingly recognized as a cause of acute and/or progressive respiratory failure in neonates and infants. However, the clinical spectrum of the disease and hence its actual incidence are poorly defined. **Objectives:** (1) To determine the incidence of mutations in the surfactant-related genes, Surfactant Protein-B (SP-B), SP-C and ABCA3 in neonates/infants with progressive lung disease of unknown origin; (2) to correlate these mutations with protein expression pattern, histology and ultrastructure. **Methods:** Ten neonates/infants with idiopathic lung disease underwent lung biopsy and SP-B/SP-C/ABCA3 sequencing. Optical (OM) and electronic (EM) microscopy, immuno-histochemistry confocal microscopy, and pulmonary gene expression studies (RT-PCR) were performed. Three neonates who died of a recognized cause of respiratory failure and 4 'healthy' infants with lung lobectomy for cystic adenomatous malformation were used as controls. **Results:** Five neonates presented with unexplained respiratory distress syndrome (URDS) and died within 6 months of age; 5 had infantile interstitial lung disease (ILD), 2 of which with severe pulmonary hypertension. In all 9 infants tested with lung biopsy, EM showed typical alterations of lamellar bodies that none of the controls had, whereas OM showed a non-specific picture of desquamative interstitial pneumonitis. Moreover, one infant with pulmonary hypertension also showed histologic features typical of alveolar capillary dysplasia. Bi-allelic ABCA3 mutations were found in 5 neonates, whereas 3 of the 5 infants with ILD carried a single ABCA3 mutation. The ABCA3 protein, normally co-expressed with SP-B and SP-C in type II cell lamellar bodies, showed 4 patterns in affected infants: normal, reduced,

absent and diffuse/mislocated. One case with monoallelic ABCA3 mutation showed abnormal processing of SP-B, pooled in alveolar lumen. **Conclusions:** Newborns with URDS and infants with ILD and idiopathic pulmonary hypertension should be investigated for SP-B, SP-C and ABCA3 mutations, the latter appearing the most frequent in our series, although unexplained cases suggest the involvement of other genes. These mutations affect lamellar body formation and surfactant homeostasis through various molecular mechanisms. Larger, multicenter studies are needed to better define the incidence and spectrum of these newly-defined entities.

### 3

#### Effect of Cholesterol on Biophysical and Biophysiological Properties of Surfactant Protein-C33 Surfactant

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**Background:** Cholesterol is the major neutral lipid in pulmonary surfactant, but its role for surfactant activity is uncertain. **Objective:** To evaluate the effect of cholesterol on Surfactant Protein-C33 (SP-C33) surfactant in vitro and in vivo. **Methods:** Cholesterol (2–10%, w/w) was mixed with 2% SP-C33 in dipalmitoylphosphatidylcholine:palmitoyloleoylphosphatidylglycerol 68:31 (w/w) and suspended in saline. Surface tension in the synthetic preparations (10 mg/ml) was recorded using a captive bubble surfactometer before and after rotation in capped glass tubes at 20 rpm at 37°C for different periods in order to obtain a maximal change of the surface area. In the in vivo experiments the animals were tracheotomised at birth, treated with surfactant (80 mg/ml, 2.5 ml/kg) and ventilated for 30 min with a standardized sequence of insufflation pressures without positive end-expiratory pressure. Curosurf<sup>®</sup> and non-treated animals were used as positive and negative controls, respectively. Tidal volumes were recorded during the experiment and lung gas volumes at the end of the experiment. **Results:** Synthetic surfactant mixtures containing 2–10% cholesterol needed less compression to obtain a surface tension of 5 mN/m than the preparation without cholesterol. After rotation for 1–7 days, SP-C33 surfactant without cholesterol did not reach a surface tension of 5 mN/m at 50% compression while preparations containing 5–10% cholesterol only needed 20–30% compression to reach a surface tension of 5 mN/m. Treatment of animals with SP-C33 surfactant containing 5% cholesterol gave significantly lower tidal volumes compared to those treated with only SP-C33 surfactant or Curosurf<sup>®</sup>. Animals treated with SP-C33 surfactant with or without 5% cholesterol had similar lung gas volumes, but these volumes were significantly lower than those obtained after treatment with Curosurf<sup>®</sup>. **Conclusions:** The presence of cholesterol in SP-C33 surfactant improved in vitro surface activity but had a negative effect on tidal volumes in experiments on premature newborn rabbits. The

results indicate that synthetic surfactant preparations should be prepared without addition of cholesterol.

**Ethical approval:** The experiments were approved by the local ethical committee for animal research, Stockholms Norra Djurförsöksetiska Nämnd.

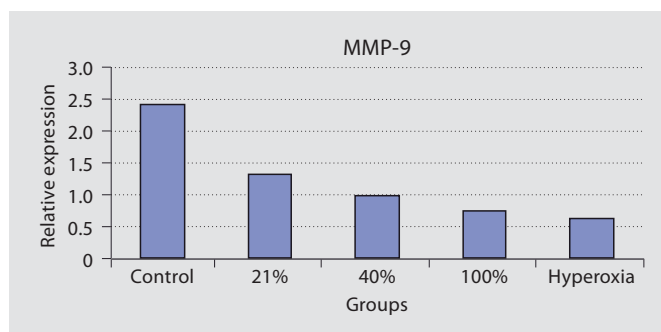
### 4

#### Hyperoxia or Newborn Resuscitation with 100% Oxygen Alters Gene Expression in Lungs and Liver: A Study of Gene Regulation in Newborn Piglets

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**Background:** Asphyxia and subsequent re-oxygenation cause a burst of oxygen-free radicals. Oxidative stress induced by re-oxygenation may be responsible for short- and long-term pathological consequences. **Objective:** To study gene regulation after hypoxia and re-oxygenation with 21, 40 or 100% oxygen. We also examined changes in gene expression if 100% oxygen was given without preceding hypoxia. **Methods:** Newborn piglets (12–36 h) underwent hypoxia until a base excess of –20 mmol/l or a mean arterial blood pressure <15 mmHg were obtained, followed by resuscitation for 30 min by ventilation with 21, 40 or 100% oxygen (n = 10, n = 12, n = 10) and thereafter the animals were observed for 9 h. The hyperoxia-group received 100% oxygen for 30 min without preceding hypoxia. RNA was isolated from snap-frozen lung and liver tissue and gene expression levels for betaglycan (TGFB3), vascular endothelial growth factor A (VEGF-A), VEGFR and VEGFR2 together with the matrix metalloproteinases, MMP-2 and MMP-9 were determined by RT-PCR. **Results:** VEGFR2 and TGFB3 in liver and MMP-9 in lung were significantly less expressed in the hyperoxia-group compared to with the controls (p = 0.016, p = 0.05, p = 0.038). MMP-9 expression in liver was lower in the 100% group compared to the 21% group (p = 0.039). **Conclusions:** Hypoxia and subsequent resuscitation for 30 min with high percentages of oxygen reduce MMP-9 expression, possibly due to an up-regulation of the endogenous inhibitors (TIMPs). Just ventilating newborn piglets with 100% oxygen for 30 min leads to a significant down-regulation of VEGFR2 and TGFB3 measured after 9 h. This may have a negative impact on vasculogenesis.



## A Randomised Comparison of Wide versus Narrow Saturation Monitor Alarm Limits for Controlling Oxygen Therapy in Preterm Infants

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**Background:** Saturation monitoring is used widely to guide oxygen therapy. The optimal target ranges are unknown. There is a general aim to minimise hyperoxia, hypoxia and variability. Chosen alarm limits may influence stability because alarm soundings prompt alterations to oxygen therapy. **Aim:** To determine whether the width of the alarm limits influences the stability of oxygenation in oxygen-dependent preterm infants. **Methods:** Infants born at <29 weeks' gestation and receiving supplemental oxygen were studied between days 3 and 14. Each infant was studied for 2 consecutive 3-hour periods allocated in random order. During one period the alarm limits were set at 80–94% and during the other at 86–94%. Saturation values were downloaded to a personal computer every second. For each period the percentage of time spent with saturation >94%, <86%, <80% and saturation variability (standard deviation) were calculated. Differences within babies between the two periods were analysed by Wilcoxon test. **Results:** Data are median (interquartile range). **Conclusions:** When wider saturation alarm limits were used, babies spent less time with high saturations but no more time with low saturations. They also showed less variable saturation. These results will facilitate improved oxygen saturation targeting.

	Wide (80–94%)	Narrow (86–94%)	Median difference
Mean SpO <sub>2</sub> , %	89.3 (88.1, 90.5)	89.0 (88.6, 91.8)	0 (–1.9, 1.7)
%time SpO <sub>2</sub> >94%	8.8 (5.3, 20.4)	12.9 (6.3, 31.0)	3.8 (–0.9, 10.2)*
%time SpO <sub>2</sub> <86%	16.0 (5.8, 24.7)	14.4 (9.1, 24.3)	0 (–8.4, 6.9)
%time SpO <sub>2</sub> <80%	3.8 (0.5, 7.9)	4.1 (2.0, 11.8)	0.5 (–1.0, 6.2)
SpO <sub>2</sub> variability	5.0 (3.3, 7.3)	6.2 (4.2, 10.7)	0.7 (–0.4, 3.8)*

\* p < 0.05.

## Contribution of Nurse Oxygen Adjustment Behaviours to Stability of Oxygenation in Preterm Infants

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**Background/Objective:** Hyperoxia and increased variability of oxygenation have been linked to adverse outcomes in preterm infants. We aimed to explore the contribution of nurse oxygen adjustment behaviours to the stability of oxygenation in preterm infants, after controlling for the intrinsic instability of the infants. **Methods:** We studied the oxygen adjustment behaviours of 24

trained neonatal nurses while caring for 13 ventilator-dependent infants during 133 12-hour shifts. We determined the average time per shift that each individual infant spent with saturation (SPO<sub>2</sub>) >94% whilst receiving supplemental oxygen, the variability (standard deviation) of SPO<sub>2</sub> and the time spent with SPO<sub>2</sub> <86%. After determining average values for each infant we then compared the oxygen adjustment behaviours of the nurses, who for ≥50% of their shifts had an infant in their care that was more unstable by these measures than the average of all the recorded shifts for that infant, with the behaviours of the remaining nurses. Behaviours compared were number of increases in FiO<sub>2</sub> per shift, mean size of increase in FiO<sub>2</sub>, mean FiO<sub>2</sub> variability, mean FiO<sub>2</sub> administered and mean SPO<sub>2</sub> maintained. Differences between groups of nurses were compared by independent samples T-test. **Results:** FiO<sub>2</sub> was increased a mean (SD) 24 (11) times per 12-hour shift overall. The mean (SD) size of the individual increases was 9.3 (3.2)%. Nurses whose babies spent more time hyperoxic than average (24 vs. 14%) made larger increases in FiO<sub>2</sub> (9.9 vs. 7.6%, p = 0.02) but not more frequent increases. Nurses whose babies showed greater than average variability in SPO<sub>2</sub> increased the FiO<sub>2</sub> more frequently (28 vs. 21 times per shift, p = 0.03) but not in larger steps. Nurses whose babies spent most time with SPO<sub>2</sub> <86% (16 vs. 10%) also made more frequent (29 vs. 20 times per shift, p = 0.003) but not larger increases in FiO<sub>2</sub>. **Conclusion:** After controlling for the intrinsic instability of the infant we found that larger and more frequent changes in FiO<sub>2</sub> contribute to instability of oxygenation. These should be modifiable behaviours.

Ethical approval: The study was approved by the local ethics advisory committee.

## Comparison of Curosurf (Porcine Lung Surfactant Extract) and Survanta (Bovine Lung Extract) in Treatment of Respiratory Distress Syndrome

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**Background:** Treatment with surfactant reduces morbidity and mortality in preterm infants with respiratory distress syndrome (RDS). There is limited information concerning the relative benefits of different preparations. **Objective:** We changed from beractant to poractant alfa as treatment of RDS in August 2006. We hypothesized that this change would reduce oxygen use and need for mechanical ventilation, and improve outcome in preterm infants. **Methods:** Retrospective data were obtained on all 298 inborn infants with gestational age (GA) <37 weeks and birth weight (BW) <3,000 grams, born at a large perinatal center between October 2005 and April 2007 with RDS requiring artificial ventilation, excluding those with anomalies, persistent pulmonary hypertension, or death within <72 h. We examined oxygen and ventilation requirements, doses of surfactant, mortality and major complications or morbidities. 153 infants were born between October 2005 and July 2006 and treated with beractant 100 mg/kg (Survanta<sup>®</sup>, Ross Laboratories, Columbia, Ohio, USA).

145 were born from August 2006 to April 2007 and were treated with poractant alfa (Curosurf®, Chiesi Pharmaceuticals, Parma, Italy) 200 mg/kg. Infants who remained dependent on artificial ventilation with a  $\text{FiO}_2 \geq 0.30$  received up to two additional doses (100 mg/kg) for each surfactant. **Results:** The groups were similar in GA and BW. The poractant-treated group received fewer doses (84.8 vs. 44.4% 1 dose, 15.2 vs. 43.8% 2 doses, 0 vs. 11.8% 3 doses,  $p = 0.000$ ), and required lower  $\text{FiO}_2$  and mean airway pressures (MAP) for 48 h after surfactant ( $p < 0.005$ ). They were also extubated sooner – mean (SD) duration of ventilation – 89.6 (249.8) vs. 160.8 (340.0) h ( $p = 0.006$ ) and required less total duration of ventilation – 127.2 (282.3) vs. 212.3 (340.0) h ( $p = 0.021$ ), oxygen device use – 495.1 (756.5) vs. 703.5 (946.3) ( $p = 0.009$ ), and less high frequency ventilation (13.8 vs. 22.2%,  $p = 0.059$ ). The rates of pulmonary interstitial emphysema (PIE) (0.7 vs. 5.2%,  $p = 0.037$ ), intraventricular hemorrhage (IVH) (2.1 vs. 7.8%,  $p = 0.032$ ), and persistent ductus arteriosus (PDA) (32.4 vs. 45.8%,  $p = 0.018$ ) were lower for the poractant-treated infants, but mortality, necrotizing enterocolitis, sepsis, chronic lung disease and severe retinopathy of prematurity were similar between the groups. Similar results were seen for infants born  $< 32$  weeks. **Conclusions:** Treatment with poractant alfa compared with beractant resulted in fewer doses and a reduction in supplemental oxygen and MAP, and a shorter time to extubation and reduced ventilator and oxygen device use. It was also associated with a decreased incidence of PIE, PDA and IVH.

Ethical approval was obtained for this study from the Brigham and Women's Hospital Institutional Review Board.

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### Changing Patterns of Early Respiratory Management in Extreme Prematurity

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**Background:** Respiratory care in extremely preterm infants has significantly changed in the past two decades. New European guidelines advocate targeted surfactant prophylaxis and less invasive respiratory support in this population. We wanted to evaluate the changing practice in respiratory care of preterm infants in Belfast to determine if we were complying with evidence-based practice. **Objective:** To describe and compare the early respiratory care of preterm babies admitted to the regional neonatal in-

Year	1993	2003	2006
Number	87	156	113
Gestational age, week	27 (25–28)	27 (25–28)	28 (25–29)
Mortality, %	34	21	17
Received surfactant, %	38	90	84
Timing of first dose, min	180 (121–297)	9 (5–15)	11 (7–39)
Ventilator, days	4 (0–9)	6 (1–21)	2.5 (0–7)
CPAP, days	2 (0–9)	12 (4–20)	10 (3–29)
Oxygen $> 36$ weeks PCA, %	13	20	24
Post-natal steroids, %	21	1	4

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tensive care unit in 1993, 2003 and 2006. **Methods:** This was a retrospective study using data from NICORE (Northern Ireland Neonatal Database). Data were collected on all babies born  $\leq 30$  weeks' gestation in 2006 on use of surfactant, timing of first dose, duration of respiratory support, postnatal steroid use and respiratory outcome. Bronchopulmonary dysplasia (BPD) was defined as oxygen requirement  $> 36$  weeks post-conceptual age (PCA). Data were compared with previous findings from similar babies in 1993 and 2003. **Results:** Results are shown as % and median (interquartile range). **Conclusions:** Extremely preterm babies are receiving more targeted and selective respiratory care in terms of surfactant therapy and mechanical ventilation. In the latest era mortality has been lower, but BPD continues to rise despite increased use of postnatal steroids.

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### Potential Risk Factors for the Development of Necrotizing Enterocolitis: A Retrospective Case-Control Analysis

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**Background:** During recent years we observed an increased incidence of necrotizing enterocolitis (NEC) among low birth weight (LBW  $< 2,500$  grams) infants cared for at our institution. **Objective:** To evaluate possible risk factors responsible for the development of NEC. **Methods:** In a retrospective evaluation during an 8-year period, LBW infants who had developed NEC grade 2 or 3 were each compared to 2 matched controls of similar birth weight and gestational age with respect to factors possibly inducing NEC: lowest  $\text{pCO}_2$  values and cumulative dosage of catecholamines (possible effects on mesenteric perfusion), time to complete meconium emptying (possible intestinal impaction leading to inflammation), and lowest rectal temperature (possible reactive down-regulation of mesenteric perfusion). To avoid bias by other factors, infants with NEC were also compared to controls with respect to prenatal steroids, small for gestational age (SGA) status, CRIB score, type of milk, highest and lowest hematocrit, occurrence of persistent ductus arteriosus (PDA), treatment with indomethacin and duration of CPAP. **Results:** 23 LBW infants were identified with NEC. Mean age at diagnosis was 16 days. 46 matched controls were not significantly different from the NEC patients with respect to gestational age, birth weight, gender, SGA, prenatal steroids, CRIB scores, highest and lowest hematocrit, incidence of PDA, indomethacin treatment, and days on CPAP until day 16. NEC infants were significantly less often fed with their mothers' milk (7/23 vs. 30/46;  $p = 0.01$ ). As possible risk factors, lowest  $\text{pCO}_2$  was lower in NEC patients (mean, SD 28.0, 6.2 mm Hg vs. 31.0, 7.7 mm Hg;  $p < 0.05$ ). Cumulative catecholamine dosages were higher (mean, SD 11, 20 mg/kg body weight until NEC diagnosis vs. 6, 16 until day 16,  $p < 0.05$ ). Time to complete passage of meconium was not significantly different (NEC, mean, SD: 6.7, 3.6 days vs. 5.6, 3.9 days,  $p = 0.2$ ), neither was lowest rectal temperature (NEC, mean, SD: 36.1, 0.9°C vs. 36.0,

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0.9°C). **Conclusions:** We showed, as others have, that feeding mothers' milk has a protective effect against NEC in LBW infants. We also found associations between occurrence of NEC and factors possibly affecting mesenteric perfusion. Therefore, it may be crucial for the prevention of NEC to avoid, amongst other factors, hypocapnia.

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### Metabolic Precursors for Biosynthesis of Surfactant Disaturated-Phosphatidylcholine in Preterm Infants with Respiratory Distress Syndrome

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**Background:** To date it is not known which are the major metabolic precursors for surfactant synthesis in humans and only scanty data exist on animals. Recent studies with stable isotopes in humans show that plasma free fatty acids (FFA), plasma glucose (GLUC) and body water (WAT) are all suitable metabolic precursors for disaturated phosphatidylcholine (DSPC) synthesis. **Objective:** To determine which is the preferential metabolic substrate for surfactant DSPC synthesis in preterm newborns with respiratory distress syndrome (RDS). **Methods:** We performed 46 DSPC synthesis studies in 23 preterm infants who required exogenous surfactant (Curosurf<sup>®</sup>) and prolonged mechanical ventilation for persistent respiratory failure [birth weight (BW) mean (SD) = 1167 (451) g, gestational age (GA) = 28.5 (2.0) weeks]. Infants were all admitted to the Neonatal Intensive Care Unit, Department of Pediatrics, University of Padua, Italy. The study was approved by the local Ethics Committee. Patients were given (1) intravenous (IV) U13C-glucose (U13C-GLUC), (2) IV albumin-labelled U13C-palmitate (U13C-PA), (3) IV albumin-labelled [16,16,16]-2H-palmitate (2H3-PA), and (4) IV deuterated water (D<sub>2</sub>O) in various combinations as metabolic precursors for DSPC synthesis (Bunt JE, AJRCCM 1998; Cavicchioli P, AJRCCM 2001; Cogo P, *Pediatr Res* 1999). Eight infants received a simultaneous infusion of GLUC and PA, 8 infants GLUC and D<sub>2</sub>O and 7 PA and D<sub>2</sub>O. Infants were not being fed orally and were on fat-free parenteral nutrition at 5 ± 0.5 mg/kg/min of glucose intake. DSPC was extracted from sequential tracheal aspirates and isolated by thin layer chromatography. Isotopic enrichment of DSPC and of the plasma precursors was measured by mass-spectrometry. DSPC secretion time (ST), fractional synthesis rate (FSR), peak time (PT) and half-life (HL) were measured for each metabolic precursor. **Results:** Measurable isotopic enrichment was detected from tracheal aspirates of all study infants. DSPC FSR from plasma GLUC was 17 ± 11% per day (8 studies), from FFA-PA it was 21 ± 16% per day (15 studies) and from body WAT it was 15 ± 6% (15 studies) (p = 0.36). Mean ± SD ST of all study infants was 24 ± 14 h, PT 100 ± 34 h and HL, available in 10 of the 23 infants was 125 ± 56 h. Paired data analysis in 8 infants who received the simultaneous tracing of GLUC and D<sub>2</sub>O gave a mean DSPC FSR

difference of -0.1 ± 3% (p = 0.91). FSRs from GLUC and D<sub>2</sub>O were highly correlated (R<sup>2</sup> = 0.93, p = 0.001). Fifteen infants received PA and D<sub>2</sub>O or GLUC simultaneously. Paired data analysis of FSRs showed a mean difference of +4.5 ± 11.8% per day (PA vs. GLUC) and +4.6 ± 16.3% per day (PA vs. D<sub>2</sub>O) (n.s.). There was only a tendency towards higher DSPC FSR from PA, not from GLUC or D<sub>2</sub>O (p = 0.21 by paired t test). **Conclusions:** Infants with RDS treated with exogenous surfactant and on fat-free parenteral nutrition showed variable contribution of GLUC and FFA to DSPC synthesis. Mean FSR from D<sub>2</sub>O or GLUC was 15–17% per day and the 2 methods were highly correlated. Further studies are in progress to study if different parenteral nutrition regimens affect DSPC synthesis from different plasma precursors.

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### Does Nasal Intermittent Positive Pressure Ventilation in Neonatal Intensive Care Prevent Bronchopulmonary Dysplasia?

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**Background:** The developing lung is a delicate structure easily injured by therapies employed to sustain ex-utero life. Bronchopulmonary dysplasia (BPD) was first described as a consequence of hyaline membrane disease among infants requiring mechanical ventilation. The incidence of BPD varies amongst centres. Approximately 40% of extremely low birth weight (ELBW) infants require reintubation within 7 days when extubated to nasal continuous positive airway pressure (nCPAP). In contrast to nCPAP, nasal intermittent positive pressure ventilation (NIPPV) augments airway pressure during inspiration. More than half (7 out of 11) large perinatal centres in Canada use NIPPV. **Objectives:** To present the current understanding and evidence for the use of NIPPV in neonatal intensive care units. Does NIPPV prevent or ameliorate BPD? **Methods:** A review of the current literature on the evidence for the use of NIPPV. Is synchronised NIPPV more effective than non-synchronised NIPPV? What are the long-term safety data? Does NIPPV prevent or ameliorate BPD? **Results:** There is evidence that NIPPV improves short-term physiological outcomes such as tidal and minute volumes. No randomised controlled trials (RCTs) have evaluated NIPPV as a primary mode of respiratory support. The evidence for NIPPV reducing apnoea is inconclusive. NIPPV is known to be effective at reducing the rate of reintubation in the short term (<72 h); meta-analysis of 3 RCTs suggests a number needed to treat of 3. Meta-analysis of 2 RCTs reports the effect of NIPPV on rates of BPD (RR 0.73; 95% CI 0.49–1.07). There is no clinical evidence that synchronised NIPPV is superior to non-synchronised NIPPV. There are no long-term safety data. **Conclusions:** There is evidence that NIPPV reduces the frequency of reintubation. No RCT has been powered to detect a clinically significant reduction in BPD or other long term outcomes. The NIPPV trial (ISRCTN15233270), a 1000-patient trial funded in Canada is now actively recruiting centres and patients with a view to answering this important question.

### Surfactant Dysfunction in Preterm Infants with Lung Disease

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**Background:** Infants born preterm are often deficient in pulmonary surfactant and benefit from surfactant replacement at birth. In addition, infants who require ventilatory support after 1-week experience episodes of dysfunctional endogenous surfactant due to a developmental lag and/or acquired deficiency in production of Surfactant Protein-B (SP-B) and SP-C. In some studies, exposure of animals or cultured cells to nitric oxide (NO) adversely affects surfactant content and/or function. **Objective:** To examine surfactant composition and function as well as outcome in a subpopulation of infants enrolled in the NO Chronic Lung Disease (CLD) Trial of inhaled NO to prevent bronchopulmonary dysplasia (BPD, *N Engl J Med* 2006). **Methods:** Large aggregate surfactant was prepared from serial tracheal aspirate samples of 100 preterm infants (mean 25 weeks' gestation) enrolled in the NO CLD Trial. Surfactant function was assessed by pulsating bubble surfactometry and SP-B was measured by immunoassay. The studies were approved by the Institutional Review Board and consent was obtained. **Results:** Phospholipid recovery in large aggregate surfactant was not different for placebo and iNO-treated infants (mean  $\pm$  SEM):  $180 \pm 9$  versus  $178 \pm 8$   $\mu$ g/mg tracheal aspirate protein, respectively. iNO therapy was associated with improved minimum surface tension (ST<sub>min</sub>) at 3–5 days ( $p = 0.008$ ) after starting NO but not at 9–12 days or thereafter. The content of SP-B in surfactant tended to decrease with time in placebo infants and was maintained in iNO-treated infants ( $p = 0.13$  at 3–5 days). Over the 3 weeks after initiating therapy, both placebo and iNO-treated infants experienced surfactant dysfunction (ST<sub>min</sub>  $>5$  mN/m) at similar rates (50 and 44% infant-weeks, respectively). Normal surfactant function in iNO-treated infants during the 3 weeks was associated with significantly improved outcome at 36 weeks (survival without BPD = 57.1%) compared to infants with abnormal surfactant status (16.7%,  $p = 0.05$ ). This association was not observed for outcome among placebo infants (30 vs. 29%). **Conclusions:** iNO therapy does not reduce surfactant content and transiently improves function, supporting the safety of this therapy. Some iNO-treated infants experience episodes of surfactant dysfunction, which are associated with worse pulmonary outcome. Combined therapy with iNO and late treatments with surfactant may improve outcome for preterm infants at risk for BPD.

### Preterm Infants Developing Bronchopulmonary Dysplasia Have Decreased Growth Factor Concentrations in Bronchoalveolar Lavage Fluid

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**Background:** Growth factors are important for normal lung development, an interplay between alveolarisation and vascularisation. New bronchopulmonary dysplasia (BPD) is characterised by an arrest in lung development, the mechanisms for which are largely unknown. **Objective:** We hypothesised that an early imbalance of growth factors within the lung contributes to development of BPD and is present soon after birth. **Methods:** Bronchoalveolar lavage fluid (BALF) was collected from ventilated preterm infants (32 weeks' gestation) at postnatal days 0, 1, 3 and 7. Growth factors involved in lung development [Vascular Endothelial Growth Factor (VEGF), Keratinocyte Growth Factor (KGF), and both active and latent Transforming Growth Factor-beta (TGF-beta)] were quantified by ELISA. Concentrations were compared between infants developing BPD at 36 weeks' corrected age and infants surviving without BPD. **Results:** 117 BALF specimens were obtained from 62 infants. Seven babies died before 36 weeks, 17 developed BPD. BALF concentrations of all growth factors increased over time, with lower initial values but a more pronounced subsequent increase in BPD patients. BALF from infants with BPD contained significantly less VEGF on day 0 (median 3 vs. 24 pg/ml;  $p < 0.01$ ) and 3 (median 43 vs. 119 pg/ml;  $p < 0.05$ ) and this increased to 203 pg/ml on day 7 in BPD. In addition, BPD infants were less likely to have detectable amounts of active TGF-beta on day 0 and 1. Also, lower concentrations of latent TGF-beta were found on day 0 in infants with BPD. Moreover, infants with BPD were more likely to have KGF detectable in BALF on day 7. **Conclusions:** Decreased concentrations of growth factors involved in lung development were found very early in BALF from babies who later developed BPD. Conversely, most of these factors were increased later. Early imbalance in pulmonary growth factors may contribute to developmental arrest of the lung in BPD. Interventions aimed at restoring this imbalance may help to prevent BPD.

### Transforming Growth Factor- $\beta$ , 'Bad' or 'Good'?

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**Background:** Bronchopulmonary dysplasia (BPD) is thought to result from inhibition of alveolization by mediators of injury. One mediator of injury is Transforming Growth Factor-Beta

(TGF- $\beta$ ) which is a multifunctional signaling molecule. Signaling is initiated by the binding of TGF- $\beta$  to its receptors T $\beta$ RI and T $\beta$ RII which activate Smads and lead to altered expression of target genes such as surfactant protein-B, *SpB*. Importantly, the level of bioactive TGF- $\beta$  in the bronchoalveolar lavage fluid of preterm infants correlates with severity of BPD. Therefore, reduction or inactivation of TGF- $\beta$  and its signaling cascade may present a potentially viable preventive strategy for BPD. **Objective:** To investigate the mechanisms involved in the role of TGF- $\beta$  in normal lung development and injury. **Methods:** We used a *Cre-LoxP* system of conditional, cell type-specific gene deletion approach to inactivate either *T $\beta$ RI* or *T $\beta$ RII* genes in the murine lung. The *Cre* gene was directed by a knockin approach into the *Nkx2.1* locus whose expression coincides with onset of lung morphogenesis. **Results:** Lung epithelial-specific deletion of *T $\beta$ RI* gene inhibited progenitor/stem cell lineages including those of airway Clara cells. Gene expression analysis showed abnormal expression of *Hes 1* and *Mesh1* in *T $\beta$ RI* (-/-) lung epithelium, two pathways with important roles in progenitor/stem cell homeostasis. Interestingly, epithelial-specific deletion of *T $\beta$ RII* gene resulted in precocious maturation of the lung with enhanced alveolization and protection against lung injury ('super mice'). **Conclusions:** The results demonstrate that the role of TGF- $\beta$  in the lung is both physiological ('good') and pathophysiological ('bad') dependent on context. These data have implications for considering potential gene or other preventive/therapeutic strategies to abrogate TGF- $\beta$  in the lungs of preterm neonates at risk for BPD.

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### Imbalance between Angiopoietin-1 and Endostatin in Tracheal Aspirate Fluid of Very Low Birth Weight Infants with Bronchopulmonary Dysplasia

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**Background:** Vascularization and alveolarization are closely related in normal lung development and profoundly disturbed in bronchopulmonary dysplasia (BPD). Systemic fetal inflammation, reflected by funisitis, is a known risk factor for this pulmonary disease. Angiopoietin-1 (ANG1) is a mediator of normal vascular development and maturation. Endostatin (END) is a potent inhibitor of angiogenesis. **Objective:** To evaluate the association between funisitis and concentrations of ANG1 and END in airways of very low birth weight (VLBW) infants. **Methods:** Tracheal aspirate fluid was obtained three times a day on days of life 1, 3, 5, 7, 10 and 15 from 42 ventilated VLBW infants [gestational age: 27.4  $\pm$  1.8 weeks, birth weight: 1,017  $\pm$  229 g (mean  $\pm$  SD)]. Specimens were centrifuged, supernatants of one day were pooled and ANG1 and END were measured by ELISA. The secretory

component for IgA was used as a reference protein. Placental tissue, membranes and umbilical cords were examined microscopically to distinguish three groups: choriomnionitis (n = 9), funisitis (n = 17) and controls without inflammation (n = 16). BPD was defined as need for supplemental oxygen on day 28. **Results:** The ratio ANG1/END steadily increased (p < 0.05 for day 1 and 3 vs. days 5–10) in the study cohort. Funisitis was associated with significantly lower concentrations of ANG1 and END but was not associated with any changes in the ratio. However, the ratio was decreased on days 1 and 3 in VLBW infants who developed BPD or died before day 28 (p < 0.01). **Conclusions:** During early postnatal life the balance of mediators in airways of VLBW infants is shifted towards angiogenesis. A systemic prenatal inflammation equally interferes with pro- and anti-angiogenetic mediators. Additional postnatal risk factors might contribute to the relative predominance of anti-angiogenetic mediators in those VLBW infants who develop BPD or die.

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### Safety and Cost Effectiveness of Inhaled Nitric Oxide in Preterm Infants at High Risk of Bronchopulmonary Dysplasia

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**Background:** Inhaled nitric oxide (iNO) significantly improved survival without bronchopulmonary dysplasia (BPD) from 37–44% (p = 0.03) in the NO chronic lung disease (CLD) multicenter randomized trial (NEJM 2006 & 2007) and decreased length of ventilatory support and hospitalization, and improved pulmonary outcomes at one year of age. On post hoc analysis the effect was primarily in the infants entered in the trial between 7 and 14 days of age (27 vs. 49%, p = 0.005). **Objective:** To examine the safety and cost-effectiveness of iNO therapy administered to prevent BPD in preterm infants <1,250 g and <32 weeks' gestation requiring ventilatory support between 7 and 21 days of age. **Methods:** Between May 2000 and April 2005, 582 infants were randomized to iNO or placebo in 21 US hospitals. 539 survived to discharge, 4 died later and 496/535 survivors (93%) were followed. Safety was determined by clinical and laboratory findings during the initial course and neurodevelopmental follow-up at 24 months corrected age. Neurodevelopmental impairment (NDI) was defined as one or more of: Bayley MDI or PDI <70, cerebral palsy, bilateral blindness or deafness. Cost-effectiveness was determined from previously reported hospital per diem costs stratified by intensity of ventilatory support, iNO costs (current US market prices) and physician fees (Center for Medicare and Medicaid Services). **Results:** There was no difference in incidence of the comorbidities of prematurity, laboratory indicators of inflamma-

tory or oxidant stress or incidence of NDI in control vs. treated infants (46.9 vs. 43.8%). iNO transiently improved endogenous surfactant function. For infants started on iNO between 7 and 14 days the point estimate for cost of care was USD 5,882 less than controls with a 71% probability that iNO cost less and improved outcome. **Conclusions:** We conclude that iNO therapy improves survival without BPD and is safe in both the short and long term. Further, we conclude that the therapy appears to lower cost while improving outcomes.

**Ethical approval:** The study was approved by the Institutional Review Boards at all the hospitals and informed consent was obtained from the parents.

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### Long-Term Follow-Up of Adult Survivors of Bronchopulmonary Dysplasia

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**Background:** Bronchopulmonary dysplasia (BPD) is the commonest form of chronic lung disease in infancy and the second most common after asthma in children. Despite advances in neonatal intensive care, it remains one of the major complications in mechanically ventilated preterm babies with a mortality of up to 40% in severe cases. The long-term outcome of survivors of BPD in adulthood has not yet been determined. Because many of these survivors are now adults, health care professionals are likely to see more cases of this chronic lung disease. Studies designed to elucidate the long-term outcome of BPD have been recommended as a research priority (AJRCCM 2001;163:1723 and NEJM 2007;357:1946). **Objective:** To trace and follow-up for pulmonary and quality of life outcomes 200 adults who were diagnosed as having BPD in the neonatal period. **Methods:** We will trace 200 adults (16–30 years old) who as infants had BPD and compare their pulmonary and quality of life outcomes with 2 control groups: one group of former preterm infants without BPD matched for gestational age, gender and date of birth (n = 200), and another group of age-matched adults who were born at full term (n = 200). We plan to perform lung function (spirometry and reversibility, flow-volume loops, lung volumes and diffusion capacity) measurement, and assess quality of life and intelligence in addition collecting data on smoking status, personal or family history of asthma or atopy, marital history, educational history, occupational history and health care utilization in the preceding 12 months. Cardiopulmonary exercise testing and bronchial hyper-reactivity testing will be performed on a randomly selected subgroup of participants from each group. **Results:** Previous studies have shown reduced pulmonary function with lung over-inflation in young adult survivors at a mean age of 18 years (NEJM 1990;323:1793). In more recent cohort studies of adult survivors of BPD (Acta Paediatr 2004;93:1294 and AJRCCM 2006;173:890) maximal FEV<sub>1</sub> values are less than 80% of predicted values and

up to 30% of these individuals are smokers. Consequently much interest has focused on the susceptibility of survivors of BPD to develop chronic lung disease in adulthood, in particular chronic obstructive pulmonary disease (Thorax 2001;56:317). **Conclusions:** Studies of long-term survivors of BPD in adulthood are urgently needed. While previous studies have focused mainly on respiratory health, the broader outcomes including neurocognitive effects, health care utilization and social functioning of BPD survivors have been almost entirely neglected. It is likely that BPD constitutes a significant burden far beyond the neonatal period. The rate of lung function decline in these individuals is unknown and the impact of additional environmental lung insults, in particular cigarette smoking, is unclear. BPD can no longer be considered a disease of infants and young children and follow-up studies are needed if we are to better understand the consequences for adult health.

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### C1-Inhibitor in Meconium-Induced Complement Activation and Cytokine Formation

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**Background:** Meconium aspiration syndrome has a complex and not well-defined pathophysiology. Meconium is a potent activator of the complement system both in vitro and in vivo associated with an inflammatory response. Meconium-induced cytokine formation is differentially mediated by complement and CD14. Other constituents of meconium than its low lipopolysaccharide (LPS) content seem responsible for the inflammatory response. C1-inhibitor (C1-INH) regulates complement and contact system activation by protease inhibition and interacts with endotoxins and selectins. The effects of C1-INH in sepsis and inflammatory disease are not only a result of complement and contact system activation since reactive center-cleaved, inactive C1-INH also is beneficial. C1-INH may inhibit alternative pathway by binding C3b and may also bind LPS, none of which involves the reactive binding site of C1-INH. **Objective:** The aim of the study was to investigate the effect of C1-INH on meconium-induced cytokine formation and complement activation in human cord blood. **Methods:** Cord whole blood, anticoagulated with lepirudin, was collected from 6 different donors and distributed into tubes containing phosphate-buffered saline (PBS), PBS with C1-INH or PBS with albumin in equivalent concentrations. The samples were preincubated for 5 min before either PBS or meconium (1 mg/ml) were added. Samples for complement and cytokine analyses were incubated at 37°C for 30 min and 4 h, respectively. Complement activation was measured by an ELISA for quantification of the terminal complement complex. A Bio-Plex Array was used to measure 27 different biomarkers (cytokines, chemokines and growth factors). **Results:** C1-INH dose-dependently reduced meconium-induced complement activation by more than

70%. Of the 27 mediators quantified, meconium induced the formation of 14. C1-INH dose-dependently reduced all 14 mediators in the range of 53–82%. **Conclusions:** C1-INH is an efficient inhibitor of meconium-induced complement activation and cytokine formation probably via several different mechanisms. C1-INH in supraphysiological concentrations may be a rational approach for reducing meconium-induced inflammation.

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### Effect of Intra-Amniotic Endotoxin-Induced Chorioamnionitis on T-Lymphocytes in Pulmonary Inflammation

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**Background:** Bronchopulmonary dysplasia is associated with chronic pulmonary inflammation which may be initiated by chorioamnionitis antenatally. Inflammation is controlled in part by regulatory T (Treg)-lymphocytes. The effects of fetal inflammation by chorioamnionitis on T-lymphocyte development and function are not well understood. **Methods:** We quantified plasma cortisol concentration, circulating lymphocytes in blood and lung, apoptotic death rate and transcription factors such as nuclear factor- $\kappa$ B and FoxP3, the latter inducing the development of Tregs, after induction of chorioamnionitis by a single injection of endotoxin (*E. coli*) intra-amniotically given 5 h, 1, 2 and 5 days before delivery at 124 days gestation (term 147 days). Thirty-six pregnant ewes were randomly assigned to the chorioamnionitis group or controls (saline injection). Lambs were delivered by caesarean section. FoxP3-positive cells, activated caspase-3-positive cells and NF- $\kappa$ B-positive cells were evaluated by immunohistochemical analyses. Plasma cortisol concentration was measured by radioimmunoassay. **Results:** Intra-amniotic endotoxin induced NF- $\kappa$ B signalling in fetal lung at all time points. The plasma cortisol concentration was not changed after endotoxin-induced chorioamnionitis compared with controls. Lymphocyte counts increased in both blood and lung within 1 day and remained increased at later time points. The number of FoxP3-positive lymphocytes in the lung was reduced on day 1 to 50%, on day 3 to 58% and on day 5 to 65% in comparison to controls (100%;  $p < 0.05$ ). The percentage of apoptotic cells that were positive for activated caspase-3 was not changed. **Conclusions:** Exposure to antenatal inflammation activated proinflammatory gene transcription, without affecting plasma cortisol concentration. The number of FoxP3-positive lymphocytes was dramatically decreased, which may be a part of the chronic inflammation in the airways after chorioamnionitis and preterm birth.

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### Use of the Capillary Surfactometer for Evaluation of Surfactant Preparations – A Methodological Study

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**Background:** Surfactant inactivation plays an important role in the pathophysiology of different lung diseases. In vitro techniques may afford valuable information on surfactant quality. **Objective:** The aim of the study was to evaluate if the capillary surfactometer, which mimics the terminal airways, is a useful tool to assess the resistance of pulmonary surfactant in the presence of inhibitor. **Methods:** Surfactant (Curosurf<sup>®</sup>, Chiesi Farmaceutici, Parma, Italy) was suspended in saline in concentrations of 0.25, 1 and 2 mg/ml. Human meconium was added at concentrations of 1 and 10 mg/ml and dextran (MW, 69 kDa; Sigma Chemicals) at concentrations of 10–50 mg/ml. The samples in volumes of 0.5  $\mu$ l were evaluated by capillary surfactometer CS-2005 (Calmia Medical, Toronto, Canada). Initial opening pressure and duration of capillary patency (in % of the observation period 120 s) were measured. **Results:** Initial pressure as well as the ability to keep the patency of the capillary were comparable at all three tested concentrations of surfactant (0.25, 1 and 2 mg/ml) without any significant differences ( $p > 0.05$ ). At higher surfactant:meconium ratio (1:4) the capillary was open for longer [mean (SD)] than at the lower surfactant:meconium ratio (1:10) [46.1 (14.6) vs. 13.3 (3.0)%;  $p < 0.05$ ]. The addition of dextran at a concentration of 10 mg/ml to surfactant (0.25 mg/ml) and meconium (1 mg/ml) did not significantly influence the initial pressure but it significantly increased the patency of the capillary in comparison to a sample without dextran [86.7 (17.2) vs. 46.1 (14.6)%;  $p < 0.01$ ]. With increasing concentration of dextran (up to 50 mg/ml) the capillary patency was gradually reduced probably due to the increasing viscosity of the sample. **Conclusions:** The data suggest the potential of the capillary surfactometer for testing the quality of surfactant preparations and their biophysical properties in the presence of inhibitor. The duration of capillary patency is a more sensitive measurement than the initial pressure needed to open the capillary.

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### Perinatal Risk Factors for Bronchopulmonary Dysplasia among Preterm Infants Less than 32 Weeks' Gestation

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**Background:** Irrespective of surfactant replacement therapy and modern respiratory treatment of respiratory distress syndrome (RDS) the incidence of bronchopulmonary dysplasia (BPD) has not changed recently. **Objective:** To determine perinatal risk factors for the development of BPD. **Methods:** In 5 years (2002–2006) 725 infants without lethal malformation ( $n = 9$ ) were born with gestation <32 weeks; 88.3% survived (7, 24, 58, 80%, with gestation 22, 23, 24 and 25 weeks, respectively; after 26 weeks survival rate was greater than 92%). 332 (46%) developed RDS, and 76 (10.5%) BPD; among infants with RDS the incidence of BPD was 18% (61 infants). To look for perinatal risk factors for BPD, a control group was selected matched by gestational age and gender, and relative risk (RR) was used to describe differences between

groups. Data were obtained from medical records. A backward stepwise logistic regression model was used to determine independent predictors of BPD. **Results:** The mean (SD) gestational age and birth weight of infants with BPD were 26.6 (1.9) weeks and 909 (275) g, respectively. Univariate analysis revealed statistically important differences between infants with BPD and controls only for prolonged rupture of membranes (>24 h) (RR 2.8; 95% CI 1.04–7.7;  $p = 0.04$ ) and resuscitation after birth (bag and mask ventilation or intubation) (RR 2.3; 1.2–4.4;  $p = 0.02$ ). There were no differences in incidence of hypertensive diseases, vaginal bleeding, intrauterine growth restriction in pregnancy, clinical chorioamnionitis, abdominal delivery, low 1-min Apgar score ( $\leq 3$ ), patent ductus arteriosus, RDS, severe infection (sepsis, pneumonia) and respiratory therapy, either with mechanical ventilation or continuous positive airway pressure. Multivariate analysis to take account of potential confounding variables confirmed prolonged rupture of membranes (RR 3.2, 1.1–9.2;  $p = 0.03$ ) and resuscitation after birth (RR 2.1, 1.1–4.2;  $p = 0.03$ ) as independent predictors of BPD. However, only 12% of the risk of BPD could be explained by both risk factors. **Conclusions:** In a group of preterm infants <32 weeks of gestation, prolonged rupture of membranes and bag and mask ventilation or intubation were found to be independent risk factors for development of BPD.