Role of Antenatal and Postnatal Infection in the Development of Chronic Lung Disease of Prematurity – On Line Supplement


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Reasons for admission to the neonatal unit

Any infant born at 34 weeks or less or 37 weeks and more were admitted into the study. Preterm infants were classified into the respiratory distress syndrome (RDS) group on the basis of developing respiratory distress within four hours of birth, needing additional oxygen and having a chest x-ray change compatible with RDS. However, any term infant admitted to the neonatal unit was entered into the study to ascertain the incidence of “background” colonisation by 16s rRNA genes or *Ureaplasma*. From the 61 term infants, 29 were admitted due to respiratory reasons due to RDS, pneumonia or transient tachypnoea of the newborn; 24 for surgery with the vast majority with gastroschisis (n=18, with rest due to miscellaneous conditions including duodenal atresia, diaphragmatic hernia, etc.) and the rest (n=8) were due to miscellaneous reasons including hypoxic ischemic encephalopathy, hypoglycaemia, etc. From those requiring respiratory management 3 were intubated and ventilated, 12 received continuous positive airway pressure (CPAP) and the remainder were self breathing needing oxygen. None were oxygen dependent beyond two weeks of age, i.e. none developed CLD.

In total 8 infants, who were all preterm of ≤ 30 weeks gestation, died from respiratory causes (n=4) including two with pulmonary haemorrhages; 3 from sepsis and/or necrotising enterocolitis and 1 from a cardiac tamponade.

Bronchoalveolar Lavage Procedure (BAL)
BAL was performed according to European Respiratory Society guidelines [19, 20]. Two aliquots of 1ml/kg of normal saline were instilled and suctioned immediately. Bronchoalveolar lavage was performed at the time of clinically indicated tracheal suctioning as previously described (1-4). With the baby lying supine with the head turned to the left, a FG 5 end-hole catheter was advanced through the end porthole of the endo-tracheal tube until resistance was felt. Two aliquots of 1 ml/kg (maximum 2 ml) of saline were instilled and immediately, after each aliquot, a suction pressure of 7-8 kPa (50 mmHg) was applied to the catheter and the returned bronchoalveolar lavage fluid collected in suction 'trap'. Additional oxygen was given to maintain an oxygen saturation, as measured by an oximeter, at 90 - 95%. BAL fluid (BALF) was collected daily for the first week of life and then twice a week up to 28 days or until extubation. Samples were centrifuged at 1000g for 10 minutes at 4°C within 30 minutes of collection. The cell pellet and aliquoted supernatant were stored at -80°C until further analyses.

**Measurement of Interleukin-6 and Interleukin-8**

IL-6 and IL-8 were measured in duplicate in BAL fluid samples (5), from 63 infants including 11 term infants, 21 from the RDS group, 24 who developed CLD and 7 (6 for IL-8) who died, by ELISA (IL-6 DuoSet ELISA kit, R&D Systems, UK; IL-8 OptiEIA ELISA kit, BD, UK) or by multiplex cytometric bead assay, CBA, (BD Europe, San Diego, CA, USA) (6) according to manufacturer’s instructions. Range of sensitivities was 9.375-600pg/ml for IL-6 and 125 - 200pg/ml for IL-8 for the ELISA and 1-2,500 pg/ml for IL-6 and IL-8 for the CBA. Those BALF samples with IL-6 or IL-8 over these ranges were diluted and re-measured in duplicate. Measurements below the limit of detection for the assay were assigned zero for the purposes of statistical evaluation.
References:


