431. Issues in paediatric and neonatal intensive care

P4306
Downregulated BMPR2 signaling pathway in nitrofen-induced pulmonary hypoplasia

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Background: Despite remarkable progress in resuscitation and intensive care, morbidity and mortality in congenital diaphragmatic hernia (CDH) remain high due to severe pulmonary hypoplasia. However, pathogenesis associated with CDH is still not clearly understood. The bone morphogenetic protein receptor (BMPR) type 2 signaling pathway plays a crucial role in fetal lung development.

Hypothesis: We sought to determine whether BMPR2 signaling pathway is altered in the nitrofen-induced pulmonary hypoplasia associated to CDH.
Methods: Pregnant rats were exposed to either 100 mg nitrofen or olive oil on day 9 (D9) of gestation. At D17 and D19, embryos were delivered by cesarean and sacrificed to check if diaphragmatic hernia existed. Fetal lung, heart and liver tissue weights and body weight of each fetus were recorded. Lung tissue was harvested for pathobiological evaluation (by immunohistochemistry and RTQPCR).
Results: Lung, heart and liver weight-to-body weight ratios decreased by 20, 30 and 25% ($p<0.05$) at D17 and by 25, 15 and 25% ($p<0.05$) at D21. In the CDH group, at D21, the airway septa were thicker and the radial alveolar count was significantly lower compared to controls. In the lungs, gene expression of BMPR2 was decreased in the nitrofen group at D17 and D21, together with decreased gene expression of the DNA binding protein 1 (Id1), the major target of the BMP signaling pathway. At D17 (but not at D21), pulmonary gene expression of gremlin, a BMPR antagonist, was increased, while pulmonary gene expression of BMP4, a BMPR agonist, decreased.
Conclusions: In nitrofen-induced CDH, BMPR2 signaling pathway is downregulated in hypoplastic lungs at both early and late stages of lung development.

P4307
Rho-kinase inhibitor ameliorates bleomycin-induced chronic lung injury in neonatal rats
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Bleomycin (BLEO) induces a chronic neonatal lung injury (CNLI) in rats that is characterized by inflammation, arrest of lung development and pulmonary hypertension (PHT), similar to severe bronchopulmonary dysplasia. Increased Rho-kinase (ROCK) signaling contributes to experimental inflammatory lung injury in adult animals but its role during early life remains unknown.
Methods: Rat pups received BLEO (1 mg/kg/d i.p.) or saline vehicle from postnatal days 1-14 ± Y27632 (a ROCK inhibitor; 10 mg/kg/d i.p.). Inflammation was assessed by tissue counts of immunoreactive macrophages (CD68) and neutrophils (MPO). Chronic PHT was assessed by right ventricle/left ventricle+septum weight ratio and% medial wall thickness of pulmonary resistance arteries. Markers of lung growth, injury and alveolarization included weight, tissue fraction, mean linear intercept and secondary crest counts.
Results: Lungs of BLEO-exposed pups had up-regulated ROCK activity, as evidenced by increased phosphorylation of ROCK targets, MYPT-1 and LIMK1, which was completely inhibited by Y27632. Treatment with Y27632 completely prevented neutrophil influx to the BLEO-exposed lung while having no effect on increased macrophages. Y27632 completely prevented BLEO-induced PHT and partially improved septal thinning, but did not affect inhibited lung growth or alveolarization. Complete abrogation of BLEO-mediated neutrophil influx by treatment with SB265610 (a CXCR2 antagonist; 4 mg/kg/d) had no effect on parenchymal or vascular injury.
Conclusions: ROCK inhibition prevented chronic PHT and improved parenchymal injury (septal thinning) in BLEO-mediated CNLI, independent of changes in inflammatory cells. *Funded by the CIHR.*

P4308
Characterization of miRNAs circulating in sepsis patients' serum
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Background: MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate mRNA expression at the post-transcriptional level and thereby regulate fundamental biological processes. A number of methods, such as multiplex polymerase chain reaction microarrays, have been developed to profile the levels of known miRNAs. However, these methods cannot identify novel miRNAs or accurately determine their expressions over a range of concentrations. Deep sequencing methods provide a suitable platform for genome wide transcriptome analysis and can identify novel transcripts.
Methodology/Principal findings: We isolated total RNA from serum samples of 9 sepsis patients. We sequenced circulating miRNAs in small RNA libraries using Solexa sequencing. This revealed a total of 154 known mature and 25 mature-star sequences, and predicted 38 novel miRNAs candidates. The miRNA expression profiles of sepsis patients were different from those of healthy controls previously reported by Chen et al. The uniquely biased distributions of nucleotides may be related to the stability of circulating miRNAs.
Conclusion: Some of these novel candidate miRNAs may be specific to sepsis patients and could be used as biomarkers to evaluate sepsis prognosis by measuring their levels in blood. Follow-up studies on the functional roles of these novel miRNAs and identifying their targets should provide additional insights on the development and progression of sepsis.

P4309
Spontaneous respiratory activity during mechanical ventilation of term born infants
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Aim: Prematurely born infants frequently breathe while being mechanically ventilated and the pattern of the respiratory interaction influences outcome. The different interactions seen are the result of stimulation of respiratory reflexes (Head's and the Hering-Breuer reflexes). Respiratory reflexes may be weaker in term born compared to prematurely born infants and thus may not influence the outcome of the former. The aim, therefore, of this study was to characterise any spontaneous respiratory efforts of mechanically ventilated infants born at term.
Methods: To date, ten infants (median gestational age 38 weeks) have been studied at a median postnatal age of five days: five infants on intermittent positive pressure ventilation (IPPV) and five on synchronised intermittent mandatory ventilation (SIMV). Oesophageal, gastric and airway pressures, flow and volume were simultaneously recorded for at least 20 minutes, 100 consecutive breaths were analysed for each baby.
Results: All the infants breathed during mechanical ventilation. Four patterns of interaction were noted: synchrony, augmented inspiration, active expiration, prolongation of expiration:

Pattern of interaction (%)

	Synchrony	Augmented inspiration	Active expiration	Prolongation of expiration
IPPV	23.9	4.5	21.6	50
SIMV	21.4	2.5	74.9	1.2

Active expiration was significantly more common in the SIMV group ($p<0.005$), whereas prolongation of expiration was significantly more common in the IPPV group ($p<0.005$).
Conclusions: Respiratory reflexes are provoked in term born infants by mechanical inflations. SIMV does not prevent active expiration, this may relate to trigger delay.

P4310
The influence of angiotensin-converting enzyme (ACE) genotype on the development of severe perinatal asphyxia in the neonates
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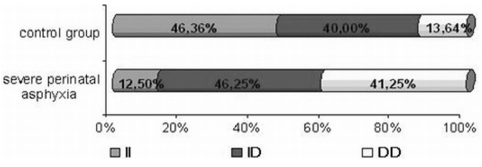
Background: The cardiovascular disturbances are the important pathways in the development of perinatal asphyxia. Study of genetic markers associated with the development of severe asphyxia in newborns is of great practical importance to develop preventive measures and child health in the future. The aim of this study was to evaluate the influence of the (I/D) gene polymorphism on the development of severe perinatal asphyxia.
Methods: We conducted a case-control study of 80 cases of severe perinatal asphyxia and 110 control group. For the genotyping was used polymerase chain reaction (PCR) with further restriction fragments length polymorphism analyses. The differences in comparative groups were assessed by the Pearson chi-square test analyses and Odds Ratio determination.
Results: The incidence of the homozygous DD alleles in the neonates with severe perinatal asphyxia was 33 (41,25%), of the heterozygous ID alleles 37 (46,25%), of the homozygous II alleles 10 (12,50%). The neonates of control group had following alleles: DD - 15 (13,64%), ID- 44 (40,00%), II -51 (46,36%).



Conclusion: DD gene polymorphism of the neonates is a risk factor for the development of perinatal asphyxia. We suggest using these genetic markers in prognosis of severe perinatal asphyxia in the neonates.

P4311
Determinants of lung function in school aged children prematurely born before 32 weeks of gestation
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Rationale: Persistent respiratory sequelae have been reported in children prema-

turely born. The aim of this work was to find the part of neonatal and childhood determinants of the lung function of school aged children born with a gestational GA< 32 weeks.
Methods: All the children born with a GA<32 wks, at the CHU de Bordeaux, between 1997 and 2000 were eligible for the study. Their respiratory outcome was evaluated by a respiratory questionnaire and lung function measurements: spirometry, static lung volumes, exercise bronchial responsiveness test, pulmonary diffusing capacity DLCO. Multivariable analysis was used to evaluate the neonatal and childhood determinants of their lung function at school age. The study was approved by the Hospital Ethical Review Committee.
Results: Of the 444 eligible children, 151 (Birth Weight= 1355±379g; GA = 30,1±1,7 wks) were included: Age= 8,6±0,8 yrs, Body Weight= 28,4±6,5 kg, Height= 132±8cm. Bronchopulmonary dysplasia BPD occurred in 36,4% of them. At school age 60% had respiratory symptoms. Lung function abnormalities were found in 53,5% of them: obstructive abnormalities with or without distension in 41%, restrictive or mixed abnormalities in 12.5%. Exercise induced bronchial responsiveness was positive in 41%. DLCO was reduced in 15,5%. Prior BPD was associated with restrictive or mixed abnormalities (OR:6,1, CI 95%:[1,1- 33,9], p=0,04]. Surfactant treatment was protective from lung abnormalities (p=0,03).
Conclusion: After premature birth, school aged children remain at risk of impaired lung function especially among those who did not received surfactant. Restrictive lung abnormalities are likely associated with prior BPD.

P4312
Infant spirometry at three months after birth in term and preterm infants
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Background: Ex-preterm infants have more airway symptoms in childhood, and forced expiratory flows have been shown to remain low or even diminishing during the first two years after birth in moderately preterm compared to term infants. Questions:Is airway function impaired both in very and moderately ex-preterm babies? Do viral infections affect preterms more severely during the first year?
Methods: 110 term and 150 preterms, born at 27+0 – 33+6 weeks gestation, were examined with infant spirometry during tidal breathing at term and with raised volume forced expiration in Jaegher Baby box at three months after birth. Nasopharyngeal viral swabs were taken during all URI and LRI during the first year. New spirometry will be performed at 18 months after birth, corrected for prematurity.
Results: Babies born at 27-31 weeks gestation had lower FVC, FEV 0,4 and FEV 0,5 compared to term infants of the same gender (p<0,02 for boys, 0,01 for girls). Vmax FRC was also lower than in term babies of same gender (p<0,01). Flows in babies born at 32-33 weeks did not differ from term infants.In term babies, girls had a longer Time to PEF, higher compliance and lower FRC than boys, but forced expiratory flows did not differ. Infections: During an intensive RSV season, 20% of both term and preterms <one year were diagnosed to have RSV airway infection. Significantly more preterm than term infants needed hospitalization (p<0,05). Mean gestational age of hospitalized preterms was 31,5 weeks.
Conclusions: Very preterm (GA <32 weeks)but not moderately preterm infants did have impaired expiratory flows compared to term infants. Infection rate was similar but need of hospitalization was higher in preterms. Follow-up will be performed.

P4313
Predicting the safety of air travel in ex-premature infants
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Background: Hypoxia is reported in preterm neonates during medical air transfer. Predicting in-flight hypoxia in preterm infants is difficult from available data.
Aims: Further investigate the response of preterm infants to flight, identify factors that may predict in-flight hypoxia and the need for in-flight oxygen.
Methods: A retrospective review of neonatal and in-flight data of all infants born <37 weeks gestation undertaking medical air transfer between 2005-2008. In-flight oxygen was commenced if oxygen saturation decreased to <85% for >2 minutes. The impact of post-menstrual age (PMA), birth weight, duration of ventilatory support/oxygen, demographics at time of flight and duration of flight on the need for in-flight oxygen was assessed by Mann-Whitney tests.
Results: 141 infants completed flights during the review period. The mean (range) PMA at birth and flight was 31.9 (24-36) weeks and 36.1 (33.1-47.6) w, respectively. In-flight, 43 required supplemental oxygen. PMA at birth and flight, birth weight, gender and duration and type of ventilatory support and oxygen did not differ between those infants that required in-flight O2. Paradoxically, infants requiring in-flight oxygen (n=43) were heavier at the time of flight (p=0.024) and undertook shorter flights (p=0.001).
Conclusion: A significant proportion of preterm infants require oxygen in the immediate post-natal period during air travel. In this analysis a requirement for oxygen could not be predicted by a range of neonatal outcomes. Neonatal units considering medical transfers of preterm infants should include options for in-flight oxygen to be available for these infants. Further research is required to understand the responses of young infants to air travel.

P4314
Outcome of congenital diaphragmatic hernia (CDH) in a non-ECMO unit
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Introduction: CDH management involves gentle ventilation, hypercarbia, iNO & delayed surgery. ECMO use is limited & associated with greater neurodevelopmental sequelae.
Aim: Study mortality and neurological morbidity of CDH patients at 12 months of age from a tertiary level non-ECMO unit.
Methods: Retrospective review of all CDH neonates presenting to the Children's Hospital at Westmead over 5 years [01/2005 to 12/2009]. Infants were assessed at 1 year using the Bayley Scales of Infant and Toddler Development, Version III. Visual reward orientation audiometry was performed.
Results: Of 37 babies [M=17] referred, 5 [13%] died perioperatively, 6 lost to follow-up & 2 missed developmental review. 30/32 [93%] of the survivors were seen at 1 year and 24/32 [75%] had a neurodevelopmental assessment. Mean GA 37.9 wks (SD ±1.7) & BW 2983gm (± 722.5). CDH diagnosed: 17 antenatal ultrasound [US] < 22 weeks of gestation, 6 later antenatal US and 14 [44%] postnatally. Median age at surgery was 7 days (range 0 to 55). 8 (21%) had an associated cardiac anomaly [4 had surgery]. Below average outcomes in 2 (8%) infants on cognitive skills & expressive language; 6 (23%) receptive language, 7 (27%) in gross motor skills & 2 (8%) deficient in fine motor skills. No sensorineural hearing deficits. Neither mortality nor abnormal neurodevelopmental outcome were significantly associated with prematurity, gender, time of diagnosis, pneumothorax, type of closure and oxygen at discharge.
Conclusion: Mortality rates in a tertiary level non-ECMO unit are comparable with ECMO centres and 1 year neuro-morbidity is lower. The outcome from conventional strategies is comparable in the treatment of most CDH patients where ECMO is not available.

P4315
Positive end-expiratory pressure affects the value of intra-abdominal pressure in acute respiratory distress syndrome in newborn with diaphragmatic hernia
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Introduction: To examine the effects of positive end-expiratory pressure (PEEP) on intraabdominal pressure (IAP) acute respiratory distress syndrome (ARDS) in newborn with diaphragmatic hernia.
Methods: Thirty sedated and mechanically ventilated patients with ARDS admitted to a twenty-bed surgical medical ICU were included. All patients were studied with sequentially increasing PEEP (0, 6 and 12 cm H2O) during a PEEP trial.
Results: Age was 5±1,7 days, weight was 1770, 4±302,0 g, SAPS II was 44±14 and PaO2/FIO2 was 192±53 mmHg. The IAP was 9,2±0,5 mmHg at PEEP 0 (zero end-expiratory pressure, ZEEP), 10,8±0,8 mmHg at PEEP 6 and 13,4±0, 6 mmHg at PEEP 12 (P < 0.05 vs PEEP). In the patients with intra-abdominal hypertension defined as IAP≥12 mmHg (n= 15), IAP significantly increased from 15±3 mmHg at ZEEP to 20±3 mmHg at PEEP 12 (P < 0.01). Whereas in the patients with IAP<12 mmHg (n= 15), IAP did not significantly change from ZEEP to PEEP 12 (8±2 vs 10±3 mmHg). In the 13 patients in whom cardiac output was measured, increase in PEEP from 0 to 12 cmH2O did not significantly change cardiac output, nor in the 8 out of 15 patients of the high-IAP group. The observed effects were similar in both ALI (n=17) and ARDS (n=13) patients.
Conclusions: PEEP is a contributing factor that impacts IAP values. It seems necessary to take into account the level of PEEP whilst interpreting IAP values in patients under mechanical ventilation.

P4316
The effect of body position on the arterial oxygen saturation of healthy premature neonates
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Background: Since the first time, when the prone position was introduced as a therapeutic maneuver in lung diseases, numerous studies in both adult and pediatric subjects have almost uniformly reported an improvement in PaO2 in the prone position compared to supine.
Aims and objective: This study was conducted to determine the effect of body position on the arterial oxygen saturation of healthy premature neonates in Vali-e-asr hospital, Zanjan, Iran.

Method: In this trial, totally 88 healthy premature neonates which were just feeding and being prepared to discharge, randomly selected. The neonates first randomly placed in prone or supine position, and 30 min later SpO2 was measured during 30 minutes. Then, the infants turned from prone to supine or from supine to prone, and a repeat set of measurement was made. The collected data was analyzed by utilizing SPSS 11.5 for windows package, using Paired Sample T Test.
Results: 60.2% (53 cases) of neonates were male and 39.8% (35 cases) were female. Their mean birth weight and gestational age were 2330.9 gram (range: 1080-3400) and 34.3 weeks (range: 26-36), respectively. Their mean postnatal age was 4.2 days (range: 1-28). Mean SpO2 of these neonates during 30 min in supine position was significantly higher than prone position (94.5±3.3 Vs 91.8±5; P< 0.001).
Conclusion: These finding suggest that prone position have not offer any advantage over the supine position in the improvement of arterial oxygenation of healthy premature neonates.

P4317
Association between severe bronchopulmonary dysplasia and serum HGF levels in premature infants during early postnatal life
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Background: Severe bronchopulmonary dysplasia (BPD) in very low birth weight infants often poses a therapeutic challenge. Therefore, it is important to make a prognosis. In this study, we investigated the relationship between the severity of BPD and serum HGF levels in infants less than 30 weeks of gestational age.
Methods: The subjects were 16 infants less than 30 weeks of gestational age born in our hospital. Cord blood was taken at delivery. Thereafter, blood was collected from the infants once a week. Serum HGF levels were measured by ELISA. Medical records were reviewed to collect information about the mothers and the clinical course of the infants.
Results: In 7 of the 16 infants, the serum HGF level exceeded 1 ng/dL during the clinical course. All those infants received more than 30% oxygen, nasal-CPAP, or mechanical ventilation at a corrected age of 36 weeks. Nine infants showed a serum HGF level of less than 0.6 ng/dL, and only 1 received 30% oxygen at a corrected age of 36 weeks. Infants exhibited high serum HGF levels within 2 weeks of birth. Serum HGF levels were not significantly correlated with the presence of chorioamnionitis (CAM).
Discussion: High serum HGF levels within 2 weeks of birth were associated with a significant increase in the risk of severe BPD. Serum HGF levels were not correlated with CAM, suggesting that fetal inflammation alone does not lead to the development of BPD. We speculate that some inflammation with or without CAM within 2 weeks of birth plays an important role in the development of severe BPD.
Conclusions: The periodic measurement of serum HGF levels may allow the early diagnosis of severe BPD.

P4318
Cardiorespiratory variables of preterm infants near term
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Introduction: Significant advances in neonatal care have improved survival in preterm infants. However chronic neonatal lung disease (CNLD) continues to be a significant problem. There is very limited data in literature describing the cardiorespiratory variables at 35-36 weeks of infants who are born preterm.
Aim: To collect sleep pattern and normative cardiorespiratory data in preterm infants born under 31 week's gestation at corrected gestation of 35-36 weeks. (We present the cardiorespiratory data).
Methods: Using an in-house polysomnography system prospective data on respiratory effort using effort bands, pulse oximetry, actigraphy and visual scoring of sleep using video camera were collected in a cohort of preterm infants under 31 weeks gestation over a 6-10 hour period continuously. We analyzed the heart rate, oxygen saturations from the data collected on the pulse oximetry channel. The respiratory rate was derived using the Labchart software. We are presenting heart rate, oxyhaemoglobin saturation and respiratory rate profile on 25 preterm infants.
Results: The mean heart rate with 5,95th centiles was 155 (129-181). The mean oxyhaemoglobin saturation with 5, 95th centiles was 94.7 (85.9-98.8). The respiratory rate had significant variability between awake and sleep with the range between 34-100/min. The average respiratory rate was in the 50s for this group of infants. Cumulative frequency curves constructed with the heart rate and oxyhaemoglobin data provides us with reference ranges for this specific group of preterm infants.
Conclusion: The description of reference ranges for cardiorespiratory variables would provide us with objective data in management of CNLD infants and ascertaining home oxygen requirement.

P4319
Association between C-reactive protein levels and outcome in acute lung injury in children
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Background: Acute lung injury (ALI) is a life threatening condition affecting both children and adults. High plasma C-reactive protein (CRP) levels are associated with favorable outcome in adults with ALI, suggesting a protective physiological effect of high CRP levels. The association between CRP levels and outcome has not been studied in ALI in children.
Aim: We hypothesized that increased plasma CRP levels are associated with favorable outcome in ALI in children in terms of 28-day mortality and ventilator free days (VFD).
Methods: We performed a historical cohort study in 98 mechanically ventilated children (0-18 years) with ALI. The CRP level within 48 hours of disease onset was tested for association with mortality and VFD. Clinical parameters and ventilator settings were evaluated for possible confounding.
Results: Fourteen patients (14%) died within 28 days. The median (Q1;Q3) CRP level in non-survivors was 126 mg/L (64;187) compared to 56 mg/L (20;105) in survivors (p=0.01). For every 10 mg/L rise in CRP level, the unadjusted odds for mortality increased 8.7% (95% CI 2.1%-15.8%). Cardiovascular organ failure (CVOF) at onset of ALI was the strongest predictor for mortality (OR 30.5, 95% CI 6.2-152.5). After adjustment for CVOF, for every 10 mg/L rise in CRP level, the odds for mortality increased 5.0% (95% CI -2.7%-12.6%). Increased CRP levels were associated with a decrease in VFD (ρ -0.26, p=0.01).
Conclusion: We conclude that increased plasma CRP levels are not associated with favorable outcome in ALI in children. Based on our findings and existing evidence that pathophysiology in ALI in adults and children differ, we suggest future research should take these differences into consideration.