# Week 3: Neonatal Cardiopulmonary

## Neonatal Cardiac Physiology/Pathophysiology

**Friday, June 26  4:30-6:00 pm EDT**

**Moderators**  
Brian Scottoline  
Shahab Noori

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Note: Schedule subject to change based on presenter availability.
CONTROL ID: 3377831

TITLE: Increased MicroRNAs 125a and 34c contribute to impaired angiogenesis in a fetal lamb model of persistent pulmonary hypertension of the newborn (PPHN)

PRESENTER: Devashis Mukherjee

AUTHORS (LAST NAME, FIRST NAME): Mukherjee, Devashis1; RANA, UJALA1; Kriegel, Alison J.1;
Michalkiewicz, Teresa1; Konduri, G. G.1

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CURRENT CATEGORY: Neonatology

CURRENT SUBCATEGORY: Neonatal Cardiac Physiology/Pathophysiology

KEYWORDS: Persistent pulmonary hypertension of the newborn (PPHN), microRNA, angiogenesis.

SESSION TITLE: Neonatal Cardiac Physiology/Pathophysiology | Neonatal Cardiac Physiology/Pathophysiology

SESSION TYPE: Webinar|Platform

ABSTRACT BODY:

Background: MicroRNAs (miRs) are conserved, short, noncoding nucleotide strands. They bind to the mRNA 3’ untranslated region and degrade mRNA or repress translation, altering gene expression. In PPHN, pulmonary vascular pressures fail to decrease after birth. Decrease in angiogenesis contributes to PPHN; mechanisms remain unclear.

Objective: To identify differential expression of miRs in a ductal ligation lamb model of PPHN using next generation sequencing (NGS) and study the angiogenesis effects of altered miR expression in pulmonary artery endothelial cells (PAECs) in vitro.

Design/Methods: PPHN was induced by prenatal ductus arteriosus ligation. Small RNA libraries constructed from PAEC RNA from PPHN and control lambs was used for NGS to identify miRs differentially expressed in PAECs. TargetScan® was used to identify mRNA targets. MiRs having the strongest baseline PAEC expression, significant fold change in PPHN and predicted targets in angiogenesis signaling pathways were selected for study. PAECs were transfected with miR mimics and anti-miRs and harvested at 48 h for immunoblotting, PCR and angiogenesis assays (cell count, cell migration and tube formation). All tests were performed first in human umbilical vein endothelial cells (HUVECs) to standardize transfection protocol.

Results: We identified 470 miRs; 12 were significantly differentially expressed between control and PPHN (p <0.05, adjusted for multiple comparisons). MiR-125a-5p (miR125) and miR-34c-5p (miR34) were strongly expressed and up-regulated in PPHN (Table 1) and have targets in the angiogenesis pathway. Transfection of control fetal lamb PAECs with miR125 and miR34 mimics separately decreased capillary tube formation in Matrigel in vitro. Tube formation was impaired in PPHN PAECs, at baseline, as compared to control PAECs. Transfection of PPHN PAEC with anti-miR125 and anti-miR34 separately, improved the tube formation by these PAECs (Table 2). HUVECs showed similar results along with decreased cell count and migration (Table 3). Successful transfection was verified by PCR fold change in transfected HUVECs using human miR primers.

Conclusion(s): Both miR34 and miR125 are increased in PPHN lamb PAECs. They impair angiogenesis in control PAECs and their inhibition improves angiogenesis in PPHN cells. Finding their mRNA targets will help identify key angiogenesis regulators and develop novel therapeutic strategies.
Table 3: Angiogenesis assay results from transfection of HUVECs with control miRs and miR34 and miR125 mimics separately. All tests were performed at 48h after transfection.

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Table 3: Angiogenesis assay results from transfection of HUVECs with control miRs and miR34 and miR125 mimics separately. All tests were performed at 48h after transfection.

CONTROL ID: 3384052

**TITLE:** Extremely Preterm Neonates born 22-26 weeks who Positively Respond to Inhaled Nitric Oxide for Hypoxic Respiratory Failure have a Lower Risk of Death or Ventilator Dependence at 36 weeks

**PRESENTER:** Timothy J Boly

**AUTHORS (LAST NAME, FIRST NAME):** Boly, Timothy J.; Dagle, John M.; Klein, Jonathan M.; McNamara, Patrick J.; Giesinger, Regan E.

**AUTHORS/INSTITUTIONS:** J.M. Dagle, J.M. Klein, P.J. McNamara, R.E. Giesinger, Pediatrics, University of Iowa, Iowa City, Iowa, UNITED STATES; T.J. Boly, Stead Family Department of Pediatrics, University of Iowa, Iowa City, Iowa, UNITED STATES;

**CURRENT CATEGORY:** Neonatology

**CURRENT SUBCATEGORY:** Neonatal Cardiac Physiology/Pathophysiology

**KEYWORDS:** inhaled nitric oxide, periviable neonates, bronchopulmonary dysplasia.

**SESSION TITLE:** Neonatal Cardiac Physiology/Pathophysiology | Neonatal Cardiac Physiology/Pathophysiology

**SESSION TYPE:** Webinar|Platform

**ABSTRACT BODY:**

**Background:** Hypoxic respiratory failure (HRF) is common among neonates born ≤26 weeks gestational age (GA). The value of inhaled nitric oxide (iNO) at the limits of viability is highly controversial. We hypothesized that extreme preterms who respond to iNO have improved survival with less bronchopulmonary dysplasia (BPD).

**Objective:** To evaluate whether response to iNO among extreme preterms is associated with the composite of death or ventilator dependence at 36 weeks postmenstrual age (PMA).

**Design/Methods:** A retrospective cohort of neonates ≤26 weeks GA who received iNO for ≥12h for HRF was collected [2010-17]. HRF was defined as fraction of inspired oxygen (FiO₂ ≥ 0.5) or oxygenation index (OI ≥ 10). After 2h, iNO response was classified as **positive** if either FiO₂ or OI declined by 0.2 or 20% respectively, **negative** if either increased by the same margin, and **non-response** if neither occurred. Response was independently categorized by 3 neonatologists by and finalized by consensus. Primary outcome was a composite of death or severe BPD defined as ventilator dependence at 36 weeks PMA. Secondary outcomes included duration of post-iNO ventilation and neonatal morbidities. Univariate/ANCOVA analysis were performed. Logistic regression was used to evaluate the relationship between response, postnatal age and GA with outcome.
Results: Of 107 infants included, 67 were classified as responders, 27 as non-responders, and 13 as negative responders. Demographics and illness severity were similar across groups but responders received iNO at a younger postnatal age [Table 1, p<0.001]. On logistic regression, postnatal age [OR 1.1(1.0, 1.1), p=0.01] but not GA[OR 0.9(0.7, 1.3), p=ns] was associated with positive response.[Figure 1] Responders had a reduced risk of death or severe BPD (p=0.01) which was independent of GA [OR 4.7(1.4, 15.4), p=0.01]. Responders required fewer days of positive pressure ventilation following iNO [Table 2, p=0.01]. Negative responders (12%) had high rates of death, severe BPD and ROP requiring laser therapy

Conclusion(s): Among neonates 22-26+6 weeks GA with HRF, iNO is associated with improved oxygenation, particularly in the transitional period. Responders have lower risk of death or severe BPD and negative response is associated with poor prognosis. Further study into modulators of iNO response may identify patients likely to benefit.

Table 1: Baseline characteristics of responders, non-responders and negative responders ≤26+6 weeks gestation at birth who received iNO for at least 12 consecutive hours. PPROM = premature preterm rupture of membranes; mean ± standard deviation; median [interquartile range]; frequency (percent)

Figure 1: Proportion of neonates who were classified as responders, non-responders and negative responders by gestational age. A: All neonates who received inhaled nitric oxide at any time during hospitalization (n=107) and B: Those receiving inhaled nitric oxide in the transitional period [≤96 hours of age] (n=77).

Table 2: Neonatal Outcomes. BPD = bronchopulmonary dysplasia; iNO = inhaled nitric oxide; ROP = retinopathy of prematurity.

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Table 2: Neonatal Outcomes. BPD = bronchopulmonary dysplasia; iNO = inhaled nitric oxide; ROP = retinopathy of prematurity.
Background: Targeted neonatal echocardiography (TnECHO) performed by neonatologists as part of a hemodynamics consultation is increasingly being utilized in NICUs. Current guidelines mandate that first echos should be performed by pediatric cardiology due to perceived high risk of congenital heart disease (CHD) among newborns with hemodynamic instability. To minimize delays in obtaining physiological data, some institutions have adopted a policy whereby first echos, including imaging sufficient to rule out relevant CHD, may be performed by the neonatal hemodynamics team and reviewed by pediatric cardiology. [Figure 1] The safety of this practice has not been systematically evaluated.

Objective: To compare concordance between the anatomic impression from the hemodynamics team and review by Pediatric Cardiology.

Design/Methods: We retrospectively evaluated infants admitted to two large referral centers with established neonatal hemodynamics programs [The Hospital for Sick Children and The University of Iowa Stead Family Children’s Hospital] where comprehensive TnECHO was the modality of first postnatal Echo. The hemodynamic consultation note was compared to the Cardiology Echo reports for anatomical concordance. The pediatric cardiologist reviewing the images was blinded to the neonatologist’s impression. The protocol included comprehensive imaging including aortic arch, pulmonary veins and evaluation for shunts. The study was approved by the ethics board of both institutions. Any discordances were agreed upon by consensus.

Results: TnECHO was performed in 720 patients (January 2015-November 2019). [Figure 2]. 329 infants were included and anatomical concordance occurred in 96%. Table 1 depicts baseline characteristics and indication for TnECHO. There were 22 infants (6.6%) diagnosed with CHD, out of which only 1 (0.3%) had a ductal-dependent lesion (Coarctation of the aorta) which was identified by both teams. Additional imaging was performed by echocardiography technicians for 4 infants (1.2%) but neither a change in diagnosis nor intervention was required.

Conclusion(s): There is high diagnostic concordance, particularly for major CHD, between trained Neonatal Hemodynamics Specialists and Pediatric Cardiology. First Echos performed by subspecialist neonatologists may provide imaging of sufficient quality to evaluate a critically unwell neonate for CHD. This study highlights the importance of close collaboration between neonatologists with hemodynamics expertise and pediatric cardiology to provide timely and clinically effective neonatal cardiovascular care.

Figure 1: Hemodynamics Consultation Flowchart
Figure 2: 1) Concordance: no difference in anatomic impression; 2) Major discordance: ductal-dependent lesions or major congenital heart disease (i.e.: TAPVR, CoA, etc) and/or major mixing lesions, coronary artery anomalies, and arch abnormalities (i.e.: vascular ring setup), perimembranous or malalignment VSD; 3) Minor discordance: small muscular VSD, ASD, anomalous vessel (i.e. insignificant aorto-pulmonary collateral, coronal branch from the right coronary artery, tiny coronary fistula)

IMAGE CAPTION:
Figure 1: Hemodynamics Consultation Flowchart

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Background: Targeted neonatal echocardiography-based hemodynamic consultations (TNEc) typically involve neonatologists with additional expertise in functional echocardiography and hemodynamic physiology, in relation to the provision of neonatal critical care. While its use is expanding, its impact on clinical outcomes is not known.

Objective: To evaluate the clinical impact of TNEc among preterm neonates with acute critical illnesses (ACI).

Design/Methods: This retrospective study, conducted over an 8-year period in two tertiary perinatal centers in Toronto, included neonates with gestational age <37 weeks and ACIs, defined for this study as exposure to rescue treatment with inotropes or inhaled nitric oxide (iNO), for hypotension and acute refractory hypoxemia respectively. Exclusion criteria were: transfer in or out of the unit while receiving therapies, complete resolution of symptoms or death ≤ 4 hours from treatment onset (time-0), and congenital anomalies. Neonates who received TNEc ≤ 24 hours from time-0 (TNEc group) were compared to those managed without TNEc (non-TNEc group). Only the first ACI episode per patient was included. Death ≤7 days from time-0 was the primary outcome, while pre-discharge death, death or chronic lung disease (CLD), and new intraventricular hemorrhage ≥grade 3 (IVH) were secondary outcomes. Multivariable logistic regression analyses were performed to evaluate the impact of TNEc on outcomes adjusted for potential confounders identified in the univariate analyses. Presuming mortality of 40% from ACIs, 150 neonates in each group were needed to show a difference of 15% (80% power, alpha 0.05). Subgroup analysis included neonates receiving only inotropes, only iNO or both were also conducted.

Results: 258 and 170 patients formed the non-TNEc and TNEc groups respectively. Median (IQR) time to TNEc was 2.5 (0 – 9) hours after time-0. While all baseline variables were similar between groups, TNEc demonstrated higher ACI related illness severity (Tables 1 and 2). TNEc was associated with lower odds of death ≤ 7 days, pre-discharge death and death or CLD in the whole cohort (Table 3), and a trend towards lower death and IVH in those receiving only inotropes and only iNO respectively (Table 4).

Conclusion(s): TNEc performed early in the course of ACIs in preterm neonates is associated with improved survival from illness and till discharge, without an increase in major morbidities.

Table 1
Table 2

Table 3

Table 4

IMAGE CAPTION:
Table 1

Table 2

Table 3

Table 4

CONTROL ID: 3382566

TITLE: Marked Alterations in the Diversity and Gene Expression of Pulmonary Mesenchymal Cells After Birth Revealed at Single Cell Resolution

PRESENTER: David N. Cornfield

AUTHORS (LAST NAME, FIRST NAME): Alvira, Cristina M.; Zanini, Fabio; Che, Xibing; Domingo-Gonzalez, Racquel; Cornfield, David N.

AUTHORS/INSTITUTIONS: C.M. Alvira, X. Che, R. Domingo-Gonzalez, D.N. Cornfield, Pediatrics, Stanford University, Palo Alto, California, UNITED STATES; F. Zanini, University of New South Wales, Sydney, New South Wales, AUSTRALIA;

CURRENT CATEGORY: Neonatology

CURRENT SUBCATEGORY: Neonatal Cardiac Physiology/Pathophysiology

KEYWORDS: lung, genomics, BPD.

SESSION TITLE: Neonatal Cardiac Physiology/Pathophysiology | Neonatal Cardiac Physiology/Pathophysiology

SESSION TYPE: Webinar|Platform

ABSTRACT BODY:

Background: The lung undergoes marked structural and physiologic change in moving from fetal to air-breathing life
and during postnatal alveolarization. However, knowledge about lung mesenchymal cell (LMC) diversity and function during later development is limited due to lack of specific markers to identify and study key populations.

**Objective:** To determine if single cell RNA-sequencing can map lung mesenchymal cell (LMC) subpopulations and dynamic changes in gene expression during postnatal alveolarization.

**Design/Methods:** ~5600 LMC were isolated from female and male mice at 4 stages of lung development, early saccular (embryonic 18.5), late saccular (P1), early alveolar (P7), and late alveolar (P21). Single EPCAM-CD45-CD31- LMC were FACS sorted, cDNA libraries generated and sequenced on Illumina NovaSeq at a depth of $10^7$ reads per cell. Reads were mapped, counted with HTSeq and data analyzed using a Python-based script.

**Results:** Unsupervised clustering identified 15 transcriptionally distinct LMC clusters. At E18.5, two clusters predominated, one expressing high levels of *Tgfbi*, a matricellular protein highly expressed by myofibroblasts, and the other expressing high levels of *Tcf21*, which regulates epithelial to mesenchymal transition and normal lung branching. After birth, LMC diversity increased to include 13 more distinct clusters comprising airway and vascular smooth muscle cells (SMC), pericytes, myofibroblasts (early, late, proliferating), lipofibroblasts, fibroblasts, and multiple, distinct matrix fibroblasts. Developmental shifts in the myofibroblast transcriptome were observed, with P1 and P7 myofibroblasts present in clusters distinct from P21 cells. The early myofibroblast cluster expressed 18.2-fold more *Nrep*, a gene that promotes myofibroblast commitment and alveolarization, and 11.3-fold more *Mdk*, a HIF and glucocorticoid regulated cytokine that promotes angiogenesis. In contrast, in the late myofibroblast cluster, SMC specific genes (*Tagln*, *Acta2*) were downregulated, and anti-angiogenic (*Dcn*, *Serpinf1*, *Dpt*), and collagen synthesis (*Col1a1*, *Col3a1*, *Col14a1*) genes were up-regulated.

**Conclusion(s):** Single cell RNA-seq reveals distinct LMC subpopulations that dramatically increase in diversity after birth, peaking in the early alveolar stage (P7). Temporal specific diversity and unique molecular signatures of distinct LMC subpopulations underscores complexity and vulnerability of the neonatal lung. Loss of specific LMC at critical time points may compromise lung development.
Design/Methods: The New Zealand very low birth weight (VLBW) study is a population-based, longitudinal cohort study of all infants born <1500g in 1986. Of 323 VLBW who survived to adulthood, 228 (71%) had echocardiograms at 26-30 years together with 100 age and sex-matched controls born healthy at term. Right heart size and function were evaluated after indexing for body surface area (BSA) where appropriate: right atrial (RA) area and volume, right ventricular (RV) dimensions, fractional area change (FAC), and tissue Doppler peak velocity of tricuspid annular motion (S’), with tricuspid regurgitation (TR) peak velocity interrogated as an indicator of pulmonary artery pressure. Analysis was blinded to group allocation.

Results: Baseline characteristics were similar between participants who had echocardiography and non-participants (Table 1). RA area and volume were significantly smaller in VLBW. There were no differences in RV size. Although measures of right heart function (FAC, S’) were similar, TR peak velocity was higher in VLBW. VLBW with a history of BPD had lower mean FAC compared with those without BPD (40.1% (SD 5.9) versus 43.1% (6.5), p=0.025) but no difference in S’ (p=0.788) or TR velocity (p=0.606). Male but not female VLBW had lower mean FAC than controls (42.2% (6.4) vs 45.0% (5.8), p=0.044). Female but not male VLBW had lower mean S’ than controls (12.3 (1.6) vs 12.9 (1.8), p=0.025). When analysed by gender, differences in RA size persisted but there were no differences in RV size.

Conclusion(s): Young adults born VLBW had smaller RA but not RV. A history of BPD was associated with reduced global RV function. Increased TR velocity implies higher pulmonary artery pressures in those born VLBW. Evaluation over time to assess the impact of aging and assessment of RV volumes and pulmonary pressures and RV function under stress, will be important.

Table 1: Demographics, perinatal factors, and adult baseline cardiovascular risk factors for VLBW study adults who were and were not assessed at 26-30 years and of controls

Table 2: Echocardiographic measures of right heart size and function
Background: Initiating respiratory support prior to umbilical cord clamping (UCC), termed physiologically-based cord clamping (PBCC), has cardiovascular advantages over immediate UCC during delivery of moderately hypoxic newborns, but its efficacy during advanced cardiopulmonary resuscitation (CPR) is not known.

Objective: To determine whether providing advanced CPR, including respiratory support, chest compressions and adrenalin, is feasible prior to umbilical cord clamping, restores spontaneous circulation (ROSC) as quickly as the current standard of immediate UCC, and whether it provides any physiological benefit compared to CPR after UCC.

Design/Methods: Near term sheep fetuses (139 ± 2 (SD) days gestation) were instrumented to measure umbilical, carotid, and pulmonary arterial flows and pressures. Systemic and cerebral oxygenation were recorded using pulse oximetry and near infrared spectroscopy, respectively. Fetal asphyxia was induced until asystole ensued whereupon lambs underwent immediate UCC (ICC) and initiation of CPR before (n=10), or after UCC (PBCC; n=16). PBCC lambs were further randomised to cord clamping 1 min (PBCC \textsubscript{1}, n=8) or 10 min (PBCC\textsubscript{10}, n=8) after ROSC. Cardiovascular parameters were measured continuously and blood gas samples taken regularly.

Results: There were no differences in the duration of chest compressions to restore cardiac output between groups: mean (range) ICC: 231 s (119 – 467s), PBCC\textsubscript{1}: 208 s (101 – 409s) and PBCC\textsubscript{10}: 230 s (87 – 692s). There was no difference between any blood-gas variable between groups, however, cerebral oxygenation (as measured by NIRS) in ICC and PBCC\textsubscript{1} lambs was significantly higher than PBCC\textsubscript{10} for the first 6 minutes after ROSC (Fig 1). There was a significant overshoot in blood pressure for the first 3 minutes and carotid blood flow for the duration of the study in ICC and PBCC\textsubscript{1} groups which did not occur in PBCC\textsubscript{10} lambs (Fig 1).

Conclusion(s): Advanced CPR before umbilical cord clamping was possible, however no discernible benefit was found if UCC occurred 1 minute after ROSC. However, delaying UCC until 10 min after ROSC prevented an overshoot in cerebral oxygen delivery, blood pressure and cerebral blood flow which may reduce cerebrovascular injury resultant from hyperperfusion. The timing of UCC before or after advanced CPR is critical for improving cardiovascular stability and
Cerebral oxygenation (SctO2), pulmonary blood flow, mean blood pressure and carotid blood flow measured as a fetus (F), end of asystole (A) and upon return of spontaneous circulation (R) in lambs undergoing CPR after (ICC; red), or 1 min (light blue) or 10 min (dark blue) before umbilical cord clamping. * p<0.05.

**IMAGE CAPTION:**
Cerebral oxygenation (SctO2), pulmonary blood flow, mean blood pressure and carotid blood flow measured as a fetus (F), end of asystole (A) and upon return of spontaneous circulation (R) in lambs undergoing CPR after (ICC; red), or 1 min (light blue) or 10 min (dark blue) before umbilical cord clamping. * p<0.05.