Neonatal outcomes of extremely premature infants comparing patent ductus arteriosus management approaches

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Table of contents:

1 Abstract

Background: Extremely preterm infants (born at ≤29 weeks of estimated gestational age [EGA]), are at increased risk of mortality and morbidities. Although some evidence suggests that persistence of patent ductus arteriosus (PDA), common in premature newborns, contributes to adverse outcomes. Limited data on the benefits of PDA closure has led the American Academy of Pediatrics to recommend against nonsteroidal anti-inflammatory drugs (NSAIDs) use in preterm newborns for the purpose of PDA closure. Few studies to date have evaluated the outcome of this conservative approach. We hypothesize that a conservative approach policy is not associated with worsening of the outcome of death or bronchopulmonary dysplasia (BPD - defined as use of oxygen or respiratory support at 36 weeks corrected age) in babies born at ≤29 weeks, and most specifically in the most vulnerable ones born at <26 weeks.

Methods: We used the "difference in differences" (DID) method, a quasi-experimental design, to evaluate the impact of PDA non-intervention management policy on adverse outcomes. The analysis included all newborns ≤29 weeks born between 2011 and 2017 at two comparable sites, excluding those who had a congenital anomaly or had been transferred to either site after the first 24 hours after birth. **Site 1 (control)** continued using NSAIDs and/or ligation throughout the study period, while **Site 2 (exposed)** changed clinical practice in September 2013, abandoning the use of NSAIDs or ligation for PDA. Epoch 1 was defined as birth years 2011 to 2013, while Epoch 2 was defined as birth years 2014 to 2017. We assessed changes in death or BPD after the policy change at site 2, controlling for secular trends (site 1), among all newborns and in the sub-group born <26 weeks. We verified parallel trends assumption visually and performed several sensitivity analyses.

Results: The analysis comprised 1249 infants, including 341 born <26 weeks. Baseline characteristics of newborns were similar across sites in terms of EGA, severity of illness at birth, exposure to antenatal and post-natal steroids and sex. Distribution of EGA at birth between groups in each epoch were similar. Adherence to policy indicated that use of NSAIDs and ligation declined progressively to 0% at Site 2, while it remained stable at Site 1. After taking into account time-invariant differences between sites and secular trends, there was no change in death or BPD after policy change (5%-point, 95%CI: -7 to 17%) in the entire cohort of newborns. However, frequency of the primary outcome of death or BPD increased by 30% among infants born at <26 weeks [95% confidence interval (CI): 11 to 49%]. A model with a one-year lagged effect revealed a stronger association after policy change (35%, 95%CI: 17 to 53%). SNAP-2 score (*placebo*) did not change in either site following the change in policy, suggesting that our results were not due to changes in the severity of the patients in this time period.

Conclusion: Adherence to a strict policy of non-intervention towards the PDA in ≤29 weeks EGA population was not associated with an overall increase in death or BPD. However, the policy was associated with a significant rise in death or BPD in infants born at <26 weeks EGA. Future studies should specifically address if closure of PDA in this high-risk population is associated with improved outcomes.

2 Abrégé: Issues néonatales des nouveau-nés extrêmement prématurés en comparant deux approches médicales du canal artériel

Contexte: Les nouveau-nés extrêmement prématurés (né à 29 semaines d'âge gestationnel [AG] et moins) sont exposés à un risque accru de mortalité et de morbidités. La persistance du canal artériel (CA) a été associée à des issues défavorables. Les études portant sur la fermeture du CA n'ont pas été associées avec une amélioration du devenir. Ainsi, l'American Academy of Pediatrics a recommandé contre l'utilisation des anti-inflammatoires non stéroïdiens (AINS) chez les prématurés dans le but d'accélérer la fermeture du CA. À ce jour, peu d'études ont évalué cette approche conservatrice de tolérance du CA. Nous formulons l'hypothèse qu'une politique conservatrice n'est pas associée à une augmentation du décès ou une aggravation de la dysplasie broncho-pulmonaire (DBP – définie comme nécessitant un support respiratoire ou en oxygène à 36 semaines d'âge corrigé) chez les 29 semaines et moins, et spécifiquement chez les plus vulnérables nés à <26 semaines.

Méthodes: Nous avons utilisé la méthode de la double différence, un modèle quasi-expérimental, afin d'évaluer l'impact de la politique de non-intervention du CA sur nos variables dépendantes primaires (mortalité ou DBP). L'analyse a porté sur tous les nouveau-nés âgés de 29 semaines ou moins, sans anomalie congénitale, nés en 2011-2017 sur 2 sites comparables (ou ayant été transférés au cours des premières 24 heures de vie). Le site 1 (groupe témoin) a continué l'utilisation des AINS et/ou la ligature du CA tout au long de la période de l'étude, tandis que le site 2 (groupe exposé) a adopté une politique en septembre 2013 d'abandon de l'utilisation des AINS ou de la ligature. L'époque 1 a été définie comme étant les années de naissance 2011 à 2013, tandis que l'époque 2 comme les années de naissance 2014 à 2017. Nous avons évalué l'impact après le changement de politique au site 2, en contrôlant pour les tendances séculaires (site 1) dans l'ensemble de la cohorte et chez les <26 semaines. Nous avons vérifié visuellement pour s'assurer de la tendance parallèle de nos variables dépendantes primaires avant l'adoption de la politique et effectué plusieurs analyses de sensibilité.

Résultats: L'analyse a porté sur 1249 nouveau-nés (341 <26 semaines). Les caractéristiques des nouveau-nés étaient similaires d'un site à l'autre en termes de sévérité de la maladie à la naissance, de sexe, d'exposition aux stéroïdes anténataux et postnataux. La distribution des AG à la naissance entre les groupes de chaque époque était similaire. L'analyse de l'adhésion à la politique adoptée a démontré que l'utilisation des AINS et de la ligature a diminué progressivement jusqu'à 0% pour le site 2, alors que l'utilisation de stratégies de fermetures du CA est restée stable pour le site 1. Après avoir pris en compte les différences invariantes dans le temps entre les sites et les tendances séculaires, aucun changement de décès ou de DBP n'a été démontré après l'adoption de la politique (5% d'augmentation de la mortalité ou DBP, intervalle de confiance [IC] de 95%: -7 à 17%) pour les 29 semaines ou moins. Le nombre de décès ou de DBP a augmenté chez les nourrissons nés à <26 semaines (30%, IC 95%: 11 à 49%). Un modèle avec un effet décalé d'un an a révélé une association plus forte après l'adoption de la politique (35%, IC 95%: 17 à 53%). Le score SNAP-2 (placebo) n'a pas changé pour les 2 sites après l'adoption de la politique, ce qui suggère que nos résultats ne sont pas dus à des changements dans la sévérité de la maladie des patients au cours de cette période.

Conclusion: L'adhésion à une politique de non-intervention vis-à-vis du CA dans la population des 29 semaines et moins n'est pas associée à une augmentation du nombre de décès ou de DBP. Cependant, cette politique est associée à une augmentation du nombre de décès ou de DBP chez les moins de 26 semaines. De futures études devront déterminer si la fermeture du CA dans cette population à haut risque est associée à une amélioration des issues.

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Disclaimer: All the figures were extracted from ultrasound or echocardiography images from the picture archiving and communication system (Figures 4-6) by Gabriel Altit, or created using Adobe Illustrator Creative Cloud 2015 (version 2.0, 2015) by Gabriel Altit (Figures 1-3, and 7). The Figures 8 to 16 were created using Microsoft Excel (version 16.29, 2019)

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5 Contributions of Authors, co-Authors and collaborators

Gabriel Altit, MD, MSc(c): Gabriel Altit had the idea and designed the study, with input from collaborators and supervisors, and drafted the study protocol. He obtained ethics board (REB) approval after submission to the MUHC-RI (and CHU Sainte-Justine, through Dr. Anie Lapointe). He obtained local databases from the MUHC (Montreal Children's Hospital) and CHU Sainte-Justine. He cleaned the data and carried out the statistical analysis, using R and RStudio. He produced the figures and the tables. He drafted the abstract presented (oral presentation) at the 6th Annual Neonatal CardioPulmonary Biology Forum (Chicago, IL) and at the Child Health and Human Development Program Research Day of the MUHC-RI (Montreal, QC), as well as the thesis. Additionally, he presented the results of the study to the two neonatal units involved in the study and incorporated the feedback received in this document.

Sahar Saeed, MSc, PhD: Dr Saeed, (currently post-doctoral trainee at University of Washington – Saint-Louis, Missouri; graduate of McGill University PhD) advised GA on methods, data analysis, and interpretation of the results. She reviewed the written documentation and provided support in the construction of figures and tables.

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Marc Beltempo, MD, MSc: Dr Beltempo, Neonatologist at the Montreal Children's Hospital and Associate Director of the Canadian Neonatal Network (CNN), provided support to the project by reviewing the initial protocol prior to REB submission and facilitated access to the local CNN database at the Montreal Children's Hospital.

Anie Lapointe, MD, MSc: Dr Lapointe, Neonatologist at CHU Sainte-Justine and Assistant Professor in the Department of Pediatrics at *Université de Montréal,* reviewed the research protocol, submitted to the REB at CHU Sainte-Justine, and provided the local data, for which she is responsible. She reviewed the results of the analysis and provided feedback on the conclusions in light of the results of this study.

Gilles Paradis, MD, MSc: Dr Paradis, Chair of the Department of Epidemiology, Biostatistics, and Occupational Health at the Faculty of Medicine of McGill University, reviewed and commented on early drafts of the design and objectives of the protocol. He has provided support and guidance throughout the elaboration of the thesis.

Olga Basso, PhD: Dr Basso, Associate Professor in the Department of Obstetrics and Gynecology and the Department of Epidemiology, Biostatistics and Occupational Health at the Faculty of Medicine of McGill University is GA's supervisor. She supervised the elaboration of the research question and the study design, as well as the data analysis. She has reviewed the protocol, provided advice on data management and analysis. She has contributed to the interpretation of results and has provided critical input to this Thesis document.

6 Abbreviations

Bronchopulmonary dysplasia (BPD)

Canadian Neonatal Network (CNN)

Carbon dioxide (CO_2)

Cerebral palsy (CP)

Corrected age (CA)

Estimated gestational age (EGA)

Difference-in-Differences (DID)

Gestational age (GA)

Gross Motor Function Classification System (GMFCS)

Hemodynamically significant patent ductus arteriosus (hsPDA)

Intra-ventricular hemorrhage (IVH)

Montreal Children's Hospital (MCH)

Necrotizing enterocolitis (NEC)

Non-steroidal anti-inflammatory drugs (NSAIDs)

Patent ductus arteriosus (PDA)

Post-menstrual age (PMA)

Pulmonary vascular resistance (PVR)

Retinopathy or prematurity (ROP)

Neonatal Intensive Care Unit (NICU)

Non-steroidal anti-inflammatory medications (NSAIDs)

Randomized Control Trial (RCT)

Systemic vascular resistance (SVR)

7 Introduction and Objectives of the study

After birth, the rise in partial pressure of oxygen and loss of prostaglandins secreted by the placenta leads to the closure of the ductus arteriosus, a fetal vascular structure connecting the systemic and pulmonary vascular compartments. Preterm infants have an immaturity of these closure mechanisms, leading to a persistence of the ductus (patent ductus arteriosus - PDA). With shifting resistances between the two circulations, there is a post-natal reversal of flow at the ductal level, leading to a blood flow steal from the systemic circulation to the pulmonary circulation.

The PDA has been associated to numerous complications of prematurity, including bronchopulmonary dysplasia (BPD) and death (1, 2). In the hope to avoid these complications, numerous studies have shown that non-steroidal anti-inflammatory medications (NSAIDs), acting on prostaglandins inhibition, increase ductal closure (2, 3). However, these medications have numerous side effects (3), and surgical ligation is associated with adverse complications (1). Although trials assessing treatment effect have shown that medical and surgical approaches are successful at achieving PDA closure, none have been associated with improved outcomes (1, 2). While the benefits of therapy are unproven, there is still widespread use of NSAIDS or ligation in the extremely premature population. Although few studies have documented the safety of a "conservative" approach of non-intervention and its impact on BPD and mortality among premature newborns (3), the American Academy of Pediatrics (AAP) recently recommended against the use of treatments for PDA during the first two weeks of life of extremely premature newborns (1).

Our objective is to evaluate if premature newborns ≤29 weeks of gestational age exposed to a conservative approach to PDA management have similar outcomes to premature newborns exposed to a more traditional approach (NSAIDs and/or ligation). Furthermore, we were a priori interested in the outcomes of those born at <26 weeks, as they are the most at-risk for mortality and morbidity related to prematurity due to their vulnerability and immaturity. Our hypothesis is that the strict conservative approach is not associated with worst outcomes (death and/or BPD) in extremely premature newborns.

8 Literature review – Background and Rationale

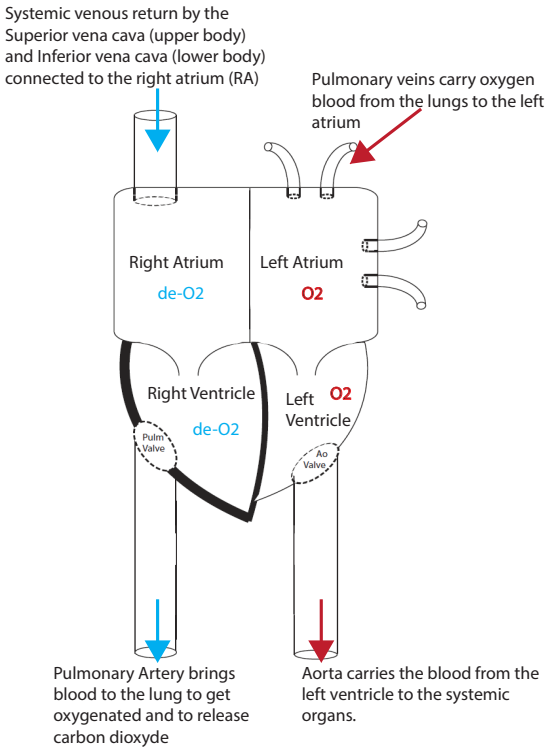
8.1 The normal heart: physiology and anatomy

The normal heart anatomy is composed of four chambers and four valves, as well as two outflow tracts (**Figure 1**). Oxygen in the blood is extracted by the systemic organs (brain, kidneys, gastro-intestinal tract, skin, etc.) and is used for generation of energy by aerobic metabolism, using energy substrates, such as: sugars and lipids. This process results in the production of carbon dioxide (CO_2), which must be eliminated by the lungs. Blood travels to the organs via a network of arteries, which in turns branch out in small capillaries. Oxygen is extracted at the level of the capillaries and CO_2 diffuses inside the blood. Capillaries collect into veins, which then brings blood to the heart via the superior vena cava (upper body) and the inferior vena cava (lower body).

The de-oxygenated blood reaches the right atrium, which is connected to the right ventricle. Ventricles are the muscular pumping chambers of the heart. The tricuspid valve, an atrio-ventricular valve, separates the right atrium from the right ventricle. The function of atrio-ventricular valves is to avoid backflow of blood into atriums during ventricular contraction. The blood is then transmitted from the right ventricle to the pulmonary artery, passing through the pulmonary valve, which opens during ventricular systole (contraction) and closes during ventricular diastole (relaxation), to avoid backflow into the ventricle.

Blood reaches the right and left lung via the right and left pulmonary arteries. The lungs allow for oxygenation of blood and for ventilation (the process of CO_2 clearance). Oxygenated blood reaches the left atrium via the pulmonary veins. During left ventricular diastole, the mitral valve opens and the left atrium empties into the corresponding ventricle. Following that, the left ventricle contracts, the mitral valve closes and the aortic valve opens, and the oxygenated blood is ejected into the systemic circulation. Due to the big vascular network covered by the systemic circulation, compared to the pulmonary circulation, the blood pressure generated in the systemic compartment is typically higher than in the pulmonary compartment.

Figure 1: Normal heart and circulation



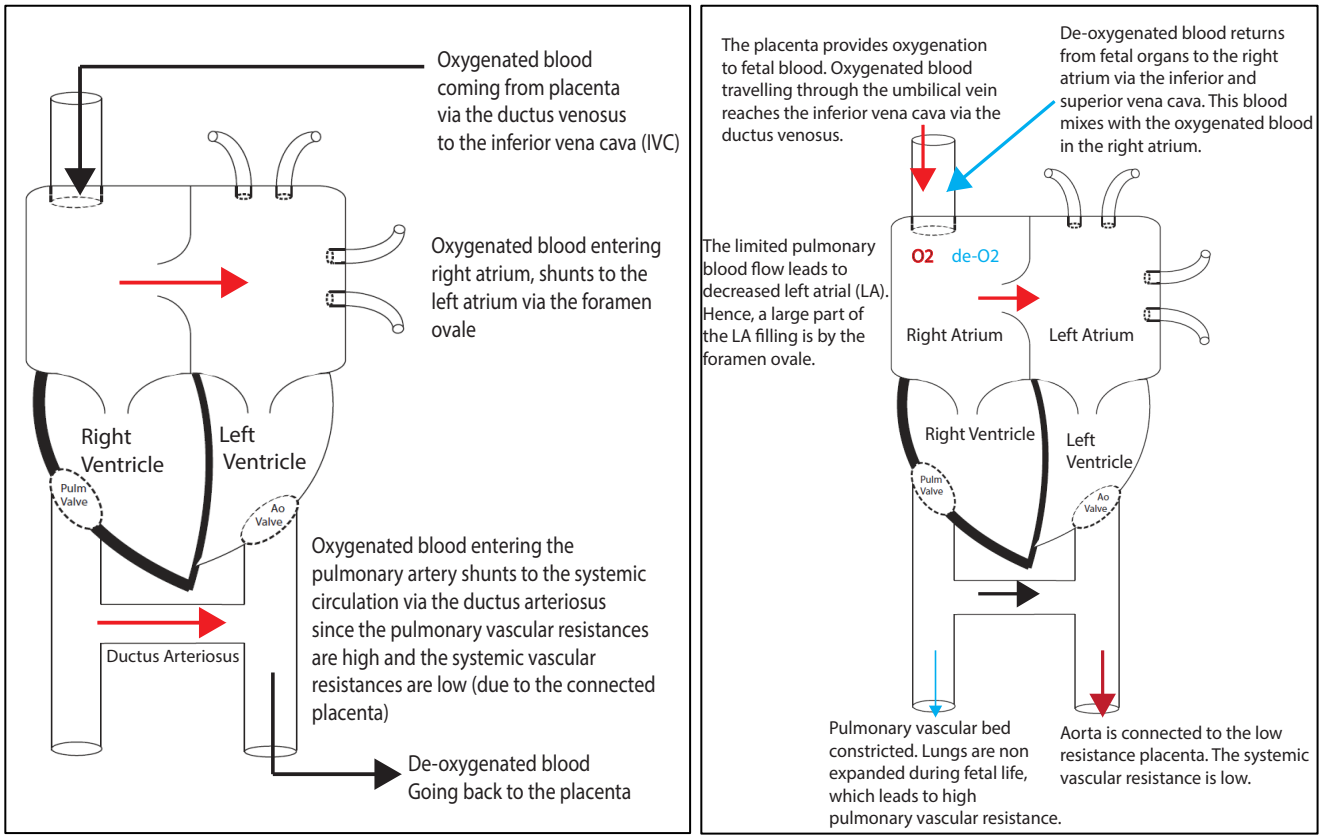
Legend of Figure 1: Schematic representation of normal cardiac anatomy and circulation to provide adequate oxygenation to systemic organs. Deoxygenated (de-O2) blood is pumped by the right ventricle to the lungs and oxygenated (O2) blood is pumped by the left ventricle to the body.

8.2 Fetal circulation

Throughout fetal life, oxygen and CO_2 exchange occurs at the level of the placenta (**Figure 2**). Oxygenated blood reaches the right atrium via the umbilical vein, which is connected to the inferior vena cava via the ductus venosus. The oxygenated blood enters the right atrium and goes towards the left atrium, which is under-filled. Indeed, in the context of lung compression, pulmonary vascular resistance (PVR) is high. Hence, only limited blood flow reaches the fetal lungs and, in turns, limited blood flow reaches the left atrium via the pulmonary veins. A fetal atrial opening, the "foramen ovale", allows for shunting of blood from the right to the left atrium. Part of the right atrium content reaches the right ventricle and is ejected into the pulmonary artery. Because of the high PVR and the relatively lower systemic vascular resistance (SVR), oxygenated blood in the pulmonary artery is largely shunted into the

aorta (systemic vessel), bypassing the lungs, via an anatomical bridge called the "**ductus arteriosus**". SVR is low during fetal life due to the connection of the fetal aorta to the placenta, which is a low resistance system. Hence, the shunt via the ductus is described as being "right to left", or "pulmonary to systemic". The ductus arteriosus remains open due to presence of prostaglandins (secreted by the placenta and the ductus itself) and the low arterial content in oxygen found in fetal blood.

Figure 2: Fetal circulation



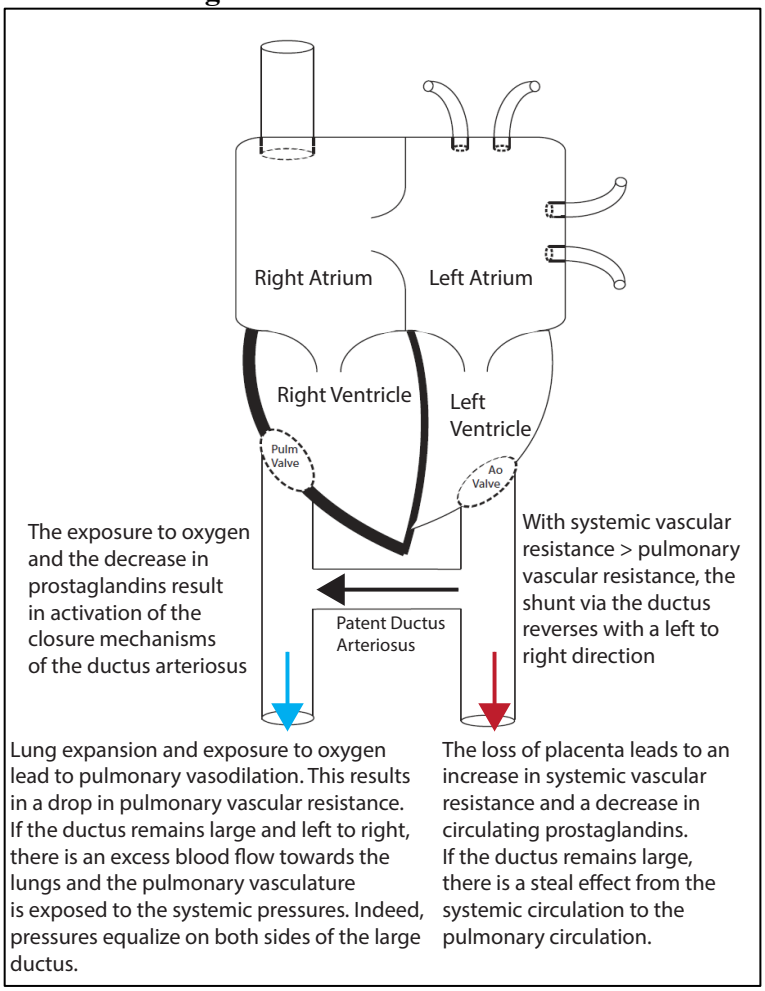
Legend of Figure 2: Schematic representation of normal fetal circulation. Blood is oxygenated in the placenta and is brought to the fetus via the umbilical vein. The oxygenated blood enters the right atrium, and partly enters the systemic circulation by the foramen ovale at the atrial level. Blood entering the right ventricle gets pumped in the pulmonary artery. The pulmonary vascular resistances are high because the lungs are compressed and the vessels in the lungs are constricted. The systemic vascular resistances are low because the fetus is connected to the low resistance placenta. Hence, a lot of blood flow entering the pulmonary artery is diverted to the aorta via the ductus arteriosus. The limited amount of blood flow travelling through the lungs leads to decreased filling of the left atrium by the pulmonary venous return, promoting the right to left shunt via the foramen ovale.

8.3 Transition to extra-uterine life

After birth, the lungs expand, leading to both mechanical stretching of the intra-pulmonary vasculature and an increased exposure to ambient oxygen. These events trigger a cascade of intra-cellular mechanisms, which cause a rapid drop in the PVR by arterial vasodilation (4, 5). Also, the clamping of the umbilical cord leads to the loss of the low-resistance placental vascular network and to a marked increase in the SVR. During the transition to extra-uterine life, the increase in partial oxygen tension and the loss of placental prostaglandins lead to vasoconstriction of the ductus, by contraction of the smooth muscles surrounding the vessel wall, with complete obliteration in the first few days of life (6). Indeed, vascular wall of the ductus arteriosus responds to increasing oxygen exposure by constricting (6). Following that, there is progressive fibrosis of the ductus arteriosus, which becomes the "ligamentum arteriosum" (6). Also, the increase in pulmonary blood flow results in an increase in the filling of the left atrium by increased pulmonary venous return.

The progressive decrease of resistance in the pulmonary vascular network exposes the right ventricle to decreasing afterload after birth, which triggers re-modelling and thinning of the right ventricular muscular wall. The post-natal rise in SVR leads to the muscularization of the left ventricle. The relative increase in left atrial pressure, compared with right atrial pressure, promotes shunting of blood from the left to the right atrium via the foramen ovale, which has a flap closing in the left to right direction. Overall, transition to extra-uterine life is marked by the progressive decrease in the PVR and increase in the SVR (**Figure 3**). With shifting resistances between the two circulations, there is a reversal of flow at the ductal level, leading to a physiological steal from the systemic circulation to the pulmonary circulation (left [systemic] to right [pulmonary] shunt), which occurs before the expected final closure.

Figure 3: Post-natal transition



Legend of Figure 3: Schematic representation of the post-natal transition. Transition to extra-uterine life is marked by a sudden increase in systemic vascular resistances (due to the clamping of the cord and the loss of the low resistance placenta) and by the progressive drop of pulmonary vascular resistances.

8.4 The persistence of patency of the ductus arteriosus

The persistence of the ductus arteriosus after transition to extra-uterine life depends strongly on gestational age at birth, as closure mechanisms are still immature in preterm infants. The ductus itself produces prostaglandins, a function that is lost at later gestational ages (7). Spontaneous closure before hospital discharge is expected in 85% of premature newborns with a birth weight <1500 grams (8). However, PDA persists for a longer time in infants born at the extreme of prematurity (8-10).

Rolland *et al.* described a French cohort of 103 infants born at 24 to 27 weeks estimated gestational age (EGA) between June 2009 and July 2010 (10). Of these, 12 (10%) died within the first 72 hours of life and were excluded. Spontaneous closure within the first 72 hours of life was achieved in 8 out of the

91 remaining patients (9%), all of whom were born at later gestational age (27 weeks ± 5 days, vs. $26^{2/7}$ weeks ± 7 days among those with no closure by 72 hours). Patency could not be determined for 13 patients, since no echocardiography was carried out beyond the first week of life. PDA was present beyond 72 hours of life in 70 infants, of whom 73% achieved spontaneous closure (and 1 required ligation). Date of closure could not be determined for 18 infants due to death or discharge (n=11 and 7, respectively). Only 28 infants were <26 weeks (27%), of whom 25 (89%) had a PDA, compared with 45 of 63 (71%) of those born at 26–27 weeks. Rate of spontaneous closure was 60% (15/25) in the younger group, compared with 80% (36/45) in the 26-27 weeks newborns.

Semberova *et al.,* also described a cohort from two European level-3 neonatal units (Dublin-Prague) (11). They included 368 very low birth weight newborns, 297 of whom had no congenital malformations and survived to discharge. Of these, 280 (94%) received a conservative PDA management and 237 (85%) achieved spontaneous closure. Median time to ductal closure was 71, 13, 8, and 6 days in <26+0, 26+0 to 27+6, 28+0 to 29+6, and ≥30 weeks, respectively. In addition, 70% of the 48 newborns born before <26 weeks achieved closure at follow-up.

Sung *et al*. followed 195 newborns born between January 2011 and June 2014 with EGA 23 to 28 weeks to evaluate spontaneous closure of the PDA. Out of the 54 newborns in the <25 weeks category, only 6 achieved early closure (within one week of life) and 93% (n=50) were considered "hemodynamically significant" by the authors. The <25 weeks was exposed to the longest time of patency (compared with the 25-28 weeks) (9).

In at-term newborns, persistence of the ductus is related to inherent abnormalities at the ductal vessel structure and/or to the mechanisms related to its closure. Hence, PDA in term infants has been associated with certain underlying genetic conditions or to congenital infections such as rubella (6, 12). Being born at higher altitude is also associated with an increased risk of PDA persistence, potentially secondary to chronic hypobaric hypoxia exposure (13). The incidence of PDA in term infants is estimated at 800 babies per million births (14), but increases to 1 per 500 births with echocardiographic

detection (15). This represents 5 to 7% of all congenital heart diseases in children at term (12). Some studies have shown familial associations, with 3% recurrence in a first-degree relative (16). Long-term persistence may lead to pulmonary vascular remodeling, ultimately leading to severe pulmonary hypertension (Eisenmenger syndrome) (17) .

8.5 Physiological impact of PDA

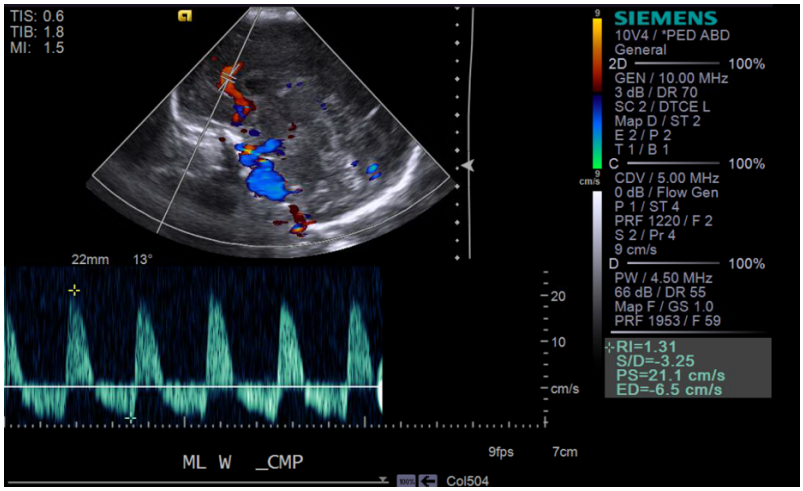
The Krichenko's classification describes several variants of the PDA anatomy (18). In the majority of cases, the aortic side is larger in diameter than the pulmonary side, giving the PDA a funnel-like appearance. The pressures on both sides of the PDA result in a trans-ductal flow that has a shunting direction. The anatomical characteristics and resistance on either side of the ductus result in a pressure and volume lesion whose properties characterize its hemodynamic effects. If the ductus arteriosus remains patent, the blood flow follows the path of least resistance: usually from the high resistance systemic compartment to the low resistance pulmonary compartment.

Signs and symptoms related to the PDA is thought to depend on the diameter, the direction, the duration of its persistence, the severity of the systolic and diastolic steal phenomenon and the compensatory mechanisms of the cardiac myocardium and of the other organs (especially in the context of immaturity related to prematurity) (19, 20). In the majority of newborns, the progressive PVR decrease following birth leads to an initial bidirectional trans-ductal flow, due to similar pressures on both sides of the PDA. With the drop in PVR, the left to right shunt via the PDA towards the pulmonary circulation can hypothetically lead to clinical symptoms of pulmonary edema, while the steal from the systemic circulation is thought to trigger systemic hypo-perfusion, decreased tolerance to feeding and decreased urine output (21). When large and unrestrictive, the PDA exposes the pulmonary vasculature to excess pulmonary blood flow, as well as to the systemic vascular pressure (during both systole and diastole). Vessels exposed to excessive flow react by vasoconstriction, which leads to remodelling and abnormal growth (22). This is of particular significance in newborns with immature lungs, abnormal growth and

impaired repair mechanisms, who may be exposed to an open ductus during a prolonged period of time. Also, pulmonary edema is thought to contribute to abnormal pulmonary compliance, rendering oxygenation and ventilation of the immature lung more challenging.

Finally, upon the drop of the PVR, the excessive blood flow and pressure in the immature lung vasculature of premature newborns may lead to pulmonary hemorrhage, which can be catastrophic and cause premature death (23). Signs and symptoms often attributed to a large left-to-right PDA include: presence of a heart murmur, hyperdynamic precordium, bounding peripheral pulses, and increased work of breathing leading potentially to increased respiratory support requirements (7, 24). In the context of a PDA, there is a compensatory increase in left ventricular cardiac output. However, despite this increase, studies have shown that blood flow can be impaired, affecting first the skin, the bones and the muscles, and eventually, the kidneys and the gastro-intestinal tract (25). The cerebral blood flow is maintained in premature babies, even in the presence of a PDA (26). However, studies have shown that in the context of a large PDA, there are disturbances in cerebral hemodynamics and growth (27), with decreased cerebral saturations by near infrared-spectroscopy (28, 29) (30). Furthermore, the Doppler profile (**Figure 4**) of the middle cerebral artery has been found to be altered in the context of a PDA (31).

Figure 4: Doppler of the anterior cerebral artery in a premature newborn with a left to right patent ductus arteriosus



Legend of Figure 4: Depicted here is an example of Pulse-Wave Doppler of the Anterior Cerebral Artery of a premature newborn with a left to right patent ductus arteriosus. During systole (contraction of the heart), the blood flow is anterograde and with a positive velocity (here 21.2 cm/second). During diastole (relaxation of the heart), there is steal effect by the open ductus arteriosus from the systemic circulation to the pulmonary circulation, which has experienced a drop in pulmonary vascular resistances. Hence, the blood flow has a retrograde direction with a negative velocity (peak at -6.5 cm/second). This steal effect from the immature brain is thought to be contributive to blood flow instability in the premature brain, potentially contributing to intra-ventricular hemorrhage. Image obtained by G. Altit via PACS.

8.6 Prematurity

The most vulnerable premature newborns, in terms of mortality and long-term morbidities, are those born at ≤29 weeks of EGA, considered extremely preterm (32) (33). A significant proportion of preterm infants are in this category (e.g., 11.1 % of all preterm births in Canada) (33, 34). Of those, the less than 26 weeks are at the highest risk of death, long-term neurodevelopmental impairment and prolonged hospitalisation (35, 36). The most recent reports of the Canadian Neonatal Network (CNN), which comprises members from 17 universities and 30 hospitals (out of 32 Neonatal Intensive Care Units (NICU) in Canada), reports that, between 2015 and 2017, 4910 newborns were born extremely prematurely (1637 cases per year) (37). Furthermore, the CNN reported 3830 live births below 26 weeks between 2010 and 2015 (638 cases per year) (35). To put this figure in context, 1445 Canadian children (0 to 18 year-old – 289 cases per year) were diagnosed with leukemia, the most frequent childhood cancer, between 2009 and 2013, and 4715 children aged 0 to 14 years were diagnosed with any cancer (all types - 943 cases/year). (38). Worldwide, prematurity is the leading cause of childhood mortality from birth to age five with 1 million (<37 weeks) reported in 2015 (39), and the cost for neonatal hospitalization of an extreme premature newborns is estimated at more than $200,000 per birth, not taking into account parental loss of productivity and post-discharge costs (37).

8.7 PDA and neonatal morbidities

PDA has been associated with numerous complications of extreme prematurity: lung injury due to over-circulation, necrotizing enterocolitis (NEC) due to intestinal hypo-perfusion and infection, as well

as intra-ventricular (cerebral) hemorrhage (IVH) due to blood flow and pressure instability. In observational studies, PDA has been associated with bronchopulmonary dysplasia (BPD), neuro-developmental impairment, cerebral palsy (CP) and death (1). This is consistent with the reports by the CNN, which described a rate of BPD of 62 to 70% in those with prematurity 23 to 26 weeks of gestational age at birth from 2011 to 2017 (**Table 1**).

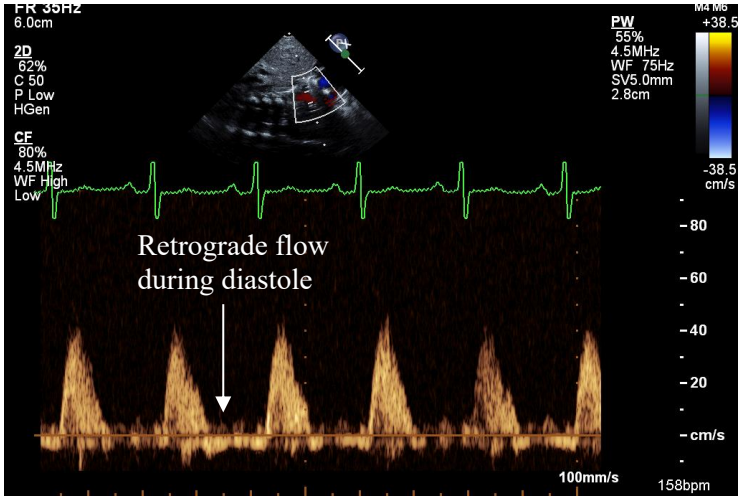
Table 1: Rate of BPD in the Canadian Neonatal Network

Year	Rate (Network)	No. of Cases (Network)	Total Infants
2011	61.87%	417	674
2012	52.64%	389	739
2013	57.77%	409	708
2014	57.31%	443	773
2015	58.32%	431	739
2016	67.75%	481	710
2017	70.16%	515	734

Legend of Table 1: Rate of BPD in the CNN for gestational age (in weeks): 23 to 26. Admission Date from: January 1st, 2011 to December 31st, 2017. Rate is defined as Number of infants with oxygen dependency or respiratory support at 36 weeks post-menstrual age / Number of infants who are alive at 36 weeks. The 2nd column represents the absolute number of newborns with a diagnosis of BPD. The 3rd column represents the denominator of all premature newborns 23 to 26 weeks during that corresponding year. Table provided by Dr. Marc Beltempo and the Canadian Neonatal Network.

Bronchopulmonary dysplasia is characterized by alveolar paucity, pulmonary fibrosis and abnormal pulmonary vasculature (40-43). BPD is associated with increased mortality (44) and poor long-term respiratory and neuro-developmental outcomes (45, 46). BPD is associated with important health care costs (home oxygen, ventilation, respiratory care, prolonged hospitalization in intensive care setting) (47). Thus, strategies to reduce its incidence may have large impact on survival, long-term outcomes and costs.

Figure 5: Doppler of the descending abdominal aorta by Echocardiography in a premature newborn with a left to right patent ductus arteriosus.



Legend of Figure 5: Pulse-Wave (PW) Doppler by echocardiography of the subcostal view of the descending abdominal aorta in a premature newborn with a left to right shunt across the PDA. During systole, there is anterograde (positive velocity) blood flow. During diastole, there is a steal effect from the systemic circulation with retrograde (negative velocity) blood flow.

Large PDA can lead to retrograde or decreased intestinal blood flow (48), which, in observational studies (49), has been associated with a higher risk of NEC. In the presence of a large PDA, the retrograde diastolic flow in the abdominal aorta appears to resolve after its closure (30) (**Figure 5**).

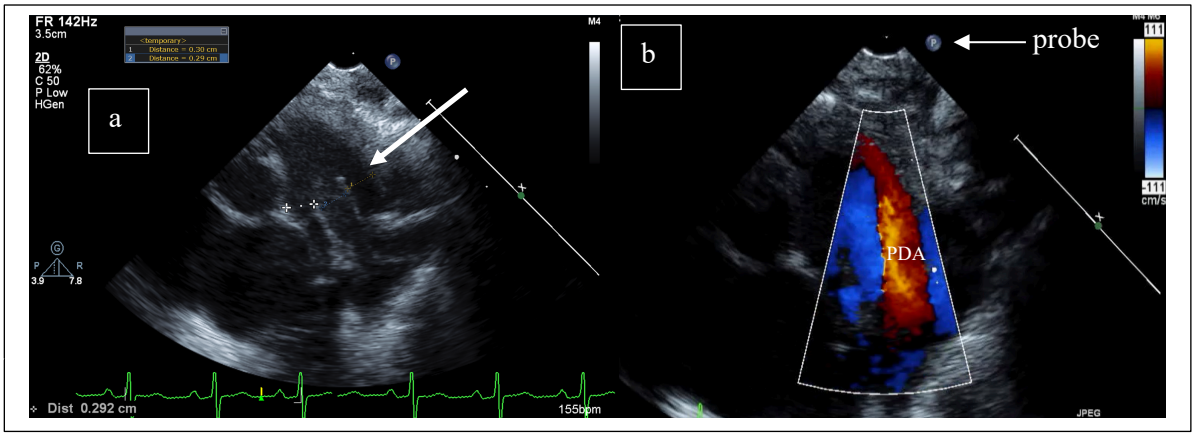
One small randomized controlled trial (RCT) on management of the PDA in <1000 grams newborns has demonstrated a decreased NEC rate in a group of 40 newborns (n=3 NEC; 8%) exposed to early prophylactic PDA ligation compared to 40 newborns (n=13 NEC; 30%) who were not exposed to early ligation (50). No other outcomes (death, BPD, retinopathy of prematurity or IVH) were different between groups. This was a small study with a high rate of NEC in the control group (baseline rate of 10% in the CNN database for Canada in <1000 grams) (37). No other RCT has shown that PDA closure leads to prevention of NEC. A causal association between PDA and respiratory morbidities of prematurity, severe cerebral hemorrhage or death before discharge remains unproven. Many studies have considered an open PDA as a binary risk factor (patent or not), regardless of its hemodynamic significance, which renders their conclusion difficult to generalize to those with large PDA, possibly associated with important hemodynamic consequences. The ductus arteriosus may be open to various degrees and its

physiological impact may be different in the context of various degree of patency (19, 20, 51). Premature newborns are at higher risk for sepsis or NEC, due to their immaturity and fragility. These factors are also associated to the patency of the ductus in observational studies (49, 52), and are, on their own, associated to BPD, adverse neurological outcomes and increased mortality; potentially confounding (or mediating) the association between PDA and BPD-Mortality.

8.8 Evaluation

Echocardiography remains the tool of choice for the diagnosis and characterization of the PDA anatomy (**Figure 6**). Coupled with clinical hemodynamic evaluation, echocardiography informs on the volume and direction of the trans-ductal shunt, while eliminating ductal-dependent pathologies. The diameter of the PDA is measured at the end of the systole in black and white (2D image – refer to **Figure 6**), at its narrowest part. In cases of an excessive and prolonged left to right shunt, the chest x-ray may demonstrate an enlarged pericardial silhouette with an increased cardio-thoracic index, associated with pulmonary vascular congestion. Renal and cerebral near-infrared spectroscopy monitoring has recently been described as a modality for estimating end-organ perfusion (28). Cerebral and renal saturations are decreased in the context of a large PDA in preterm newborns (28). Cardiac catheterization allows for evaluation of the geometry, measurement of the pressures on both sides of the PDA and for its closure by prosthesis (53).

Figure 6: Echocardiographic measurement of a Patent Ductus Arteriosus:



Legend of Figure 6: a) 2D-Echocardiography with measurement of the patent ductus arteriosus in an extremely preterm newborns (0.30 cm), which is larger than the left pulmonary artery (0.29 cm) and connecting the main pulmonary artery to the aorta. b) Color flow by 2D-Echocardiography showing a left to right direction of the blood flow via the ductus (red means that blood flow is moving towards the ultrasound probe, which is indicated at the top of the image). Hence, the blood flow is going from the aorta towards the main pulmonary artery.

8.9 Treatment of the PDA

In an attempt to avoid complications of prematurity linked to the PDA, numerous observational studies have shown that non-steroidal anti-inflammatory medications (NSAIDs), acting on prostaglandins inhibition, accelerates ductal closure. Indeed, the inhibition of prostaglandin synthesis using nonselective inhibitors of the cyclooxygenase 1 and 2 (such as indomethacin and ibuprofen) is the most commonly described approach for first-line treatment to promote PDA closure in premature newborns. The rate of non-closure for the two molecules is similar (about 35% after a 3-day course) (54).

However, these medications have several side effects, such as multi-organ vasoconstriction, increased bleeding time, renal hypo-perfusion and fluid overload (2). Indomethacin is associated with a decrease in blood flow in the superior mesenteric artery, thus compromising the ability of the intestinal wall to regulate its consumption of oxygen. It has also been associated with decreased mesenteric (55), renal (56) and cerebral blood flow (57), necrotizing enterocolitis, intestinal perforation (58) and increased bleeding time due to its effect on platelet aggregation. Age at the beginning of treatment with NSAIDs affects its effectiveness to achieve a full closure (or reduction in the caliber of the ductus). The rate of closure is inversely proportional to postnatal age (59) and decreases significantly after 2 weeks of life (60). Studies have found no differences between indomethacin and ibuprofen for the prevention of surgical ligation (61).

a) Prophylactic medical approach

The Trial of Indomethacin Prophylaxis in Preterm Infants (TIPP) was a multi-centric randomized placebo-controlled trial on the use of prophylactic indomethacin in extreme premature newborn (62-

65). The prophylactic use of indomethacin administered within the first 12 hours of life, regardless of a diagnosis of PDA, decreased the incidence of early cerebral bleeding (62-65). The main concern associated with this approach is that many infants are exposed to a medication with various potential side effects, despite the fact that the PDA closes spontaneously in a large proportion of newborns (1, 2, 9-11, 66-68). This approach has not been associated with improvements in survival without neurosensory impairment at 18 months of corrected age (65). After the publication of the results of the TIPP trial, most units abandoned the use of prophylactic indomethacin due to the lack of convincing long-term benefits (69). However, prophylactic indomethacin is still used and has been recently described in a retrospective observational study on outborn premature newborns (a newborn is considered *outborn* when being admitted to the neonatal unit after a birth outside of the walls of the institution, such as in the context of a birth: at home, in a level-2 community hospital, or in another tertiary care facility transferring the care of the patient) (70). Although the study did not show any effect on intraventricular hemorrhage, selection bias and confounding by indication may have compromised its validity (71).

b) Early medical treatment

A recent meta-analysis by Mitra *et al.* described RCTs carried in the neonatal population comparing various NSAIDs regimen between each other, or to placebo/no treatment (72). The studies included in the meta-analysis evaluated various regimens in terms of dosages and timing of administration after birth. The studies that had a placebo or "no treatment" arm were, for the vast majority, done before the years 2000 and had heterogenous reported outcomes. Indeed, most were trials done in the 1980s on small cohorts evaluating pharmacological aspects of the drugs (ibuprofen, indomethacin), with sparse description of early and long-term neonatal outcomes. Most trials were done on newborns of older gestational age at birth (28 to 32 weeks of EGA) and had a high open-label use of NSAIDs in the placebo/no treatment groups. In their analysis, Mitra *et al.* included 597

infants and did not find any significant difference between treatment and "placebo/no treatment" for the outcomes of mortality, BPD, NEC or IVH. Finally, some of the included trials had NSAIDs use implemented within the placebo arm of the study protocol (73).

More recent studies have shown an association between decrease in pulmonary hemorrhage and early closure of PDA by medical intervention (23, 74). The latest RCT (Kluckow *et al.*) assessing the effect of selective NSAIDs treatment versus placebo was published in 2014. It described a population of premature newborns more representative of the current cohorts that clinicians encounter in terms of underlying perinatal characteristics and perinatal management approaches (23). Researchers recruited 92 infants <29 weeks screened for the presence of a PDA at 12 hours of life by echocardiography. Neonates were eligible to enter the study given a pre-specified minimum size of the PDA by echocardiography. Participants received indomethacin (n=44) or placebo (n=48). While those exposed to NSAIDs showed a marked decrease in pulmonary hemorrhages at 72 hours of life (2 vs. 21%), the study had insufficient power to detect an effect on death or BPD (23). Infants in the placebo group had an unexpectedly high incidence of pulmonary hemorrhages at 72 hours of life (21%), precluding generalizability of this study to most North American centers (75). For the outcome of "all pulmonary hemorrhage" (throughout the entire hospitalization), the difference was not statistically significant. However, nearly half of controls (40%) were exposed to open-label use of indomethacin after 72 hours of life.

c) Later medical treatment

There is no evidence that differences in timing of therapy with NSAIDs affect mortality or BPD (76). However, early administration of NSAIDs has been associated with increased efficacy on PDA closure compared to later administration (77, 78).

d) Use of acetaminophen (paracetamol)

Recent reports suggest that acetaminophen may play a role in the closure of the ductus arteriosus in newborns with contraindications to NSAIDs (contraindications include: unconjugated

hyperbilirubinemia, active bleeding, thrombocytopenia, renal failure, concomitant use of steroids, NEC, intestinal perforation, persistent pulmonary hypertension of the newborn or electrolyte imbalances) (79-81). Both oral and intravenous forms are associated with similar efficacy to ibuprofen in achieving PDA closure in premature newborns (72, 79, 80, 82-91). In addition, acetaminophen may also play a role in late PDA closure in patients who are candidates for surgical ligation, after failure of NSAIDs (91). No studies have shown improvement in neonatal mortality or morbidities using acetaminophen to achieve PDA closure.

e) Mechanical closure approach

Ligation has been associated with numerous adverse complications, such as pneumothorax, chylothorax, reactive lung inflammation due to manipulation during surgery, scoliosis (92), infections and vocal cord injury. These are found in up to 67% of extremely low birth weight infants (<1000 grams) undergoing this procedure (1, 93). Ligation may be associated with sudden hemodynamic changes that result in an immediate increase in afterload and a decrease in left ventricular preload (filling). These effects, coupled with the immaturity of the myocardium and stress responses, can lead to a decrease in left ventricular cardiac output (called “post-ligation syndrome”). Up to 50% of premature newborns have a significant decrease in diastolic and systolic left ventricular function following PDA ligation (94). Signs of low cardiac output typically appear within six to twelve hours post-operatively and manifest as: low systemic blood pressure requiring inotropic support and increased respiratory support (95). A recent retrospective study reported that ligation was not associated with death and/or neurodevelopmental impairment after adjusting for confounders (96). With the advent of new technologies and improvements in cardiac catheterization techniques, closure of the PDA is now possible in premature infants (97, 98) with beneficial effects in the short and long term, compared with surgical ligation. Further studies are needed to identify the necessary expertise/training, benefits and potential side effects of this procedure.

The decision to close the PDA is often based on clinicians' preferences, when faced with what are believed to be its clinical consequences. Confounding by indication is likely to affect observational studies of this topic. Despite the physiological changes attributed to the PDA, use of NSAIDs has not been shown to result in a decrease in the incidence of NEC, spontaneous bowel perforation, retinopathy of prematurity (ROP), chronic lung disease or later neurodevelopmental impairment (99). In addition, when combined with postnatal corticosteroids, indomethacin has been associated with an increased risk of spontaneous bowel perforation and/or NEC (100, 101). There is also evidence linking cerebral hypo-perfusion and NSAIDs use, potentially leading to some degree of neurological injury (1, 2). With respect to pulmonary disease, some studies have shown adverse effects of treatment (102-104), while others have not (2, 105). Ibuprofen has also been associated with severe pulmonary hypertension when administered early after birth (106, 107). Overall, despite the lack of evidence of benefits, NSAIDs continue to be widely used in the extremely premature population, despite the possibility that it may lead to cerebral hypo-perfusion and neurological injury. Surgical closure is often used as a "rescue" strategy (1, 2).

Although trials have shown that medical and surgical approaches are successful at achieving closure of the PDA, they have not demonstrated that such closure leads to better outcomes (survival, less BPD or neuro-developmental sequelae) (1, 2).

8.10 Conservative approach

Given the lack of supporting evidence for the treatment of PDA, several experts have advocated for a more conservative approach (1, 2, 66-68). A recent meta-analysis of 68 studies by Mitra *et al.* (2018), including a total of 4802 infants (no stratification by gestational age status) and evaluating 14 various indomethacin, ibuprofen, or acetaminophen regimens, also reported no evidence supporting treatment of the PDA in preterm newborns (72). Furthermore, Benitz & Bhombal published in 2017 the results of a

meta-analysis of 51 trials addressing the use of NSAIDs for PDA management in premature newborns, and did not find any improved outcomes of mortality (n=4298), BPD (n=3747), death or BPD (n=3698), NEC (n=4189) or IVH (n=3988) (2). The included trials mostly compared different regimens of NSAIDs (dose, route of administration, length of courses) to each other, with some trials with a placebo arm (included in the Mitra *et al.* analysis) (72). The authors (Benitz & Bhombal) attempted to describe the lower gestational age group and extracted the data for babies born <29 weeks, since a lot of the studies included newborns up to 32 weeks of EGA. They did not find any improvement of outcomes in this subgroup but did not provide the specifics of the most vulnerable population composed of the <26 weeks. Indeed, few studies included a large number of 22 to $25^{6/7}$ weeks babies, and, as such, conclusions from these analyses should not be generalized to that particular subgroup. Furthermore, stemming from the recommendation to avoid medical treatment with NSAIDs, other strategies ("conservative management") are often introduced by clinicians to attenuate the clinical effects attributed to the PDA. Hence, this "conservative management" consists of various interventions to decrease the PDA's hemodynamic consequences. These interventions include: fluid restriction, increase in respiratory support, use of diuretics and post-natal steroids.

a) **Fluid restriction:** A decrease in the total amount of fluid administered to premature infants with a PDA has long been the treatment adopted by many experts (108, 109) in the hope to decrease blood volume and, consequently, pulmonary vascular edema. A recent report described decreased superior vena cava (SVC) flow, a surrogate for brain perfusion (110), in very premature infants with fluid restriction at 100-120 mL/kg/day beyond 10 days of life despite maintenance of blood pressure (111).

b) **Increase in positive end-expiratory pressure (PEEP):** The use of a higher PEEP (8 cmH_2O vs. 5 cmH_20) seems to decrease the magnitude of the left-to-right shunt via the PDA, without deleteriously affecting the flow of the SVC (112, 113). However, at present, there are no safety data regarding the potential impact of this approach on venous return and on pulmonary

parenchymal damage. In addition, there is no study evaluating the benefits of this approach on the clinical effects attributed to the PDA.

c) **Furosemide – diuretics:** Similar to the management of a significant ventricular septal defect or to congestive heart failure, furosemide (a loop diuretic) is sometimes used in the hope of decreasing pulmonary vascular congestion (114). However, this medication stimulates renal production of prostaglandins E_2 (115), which promotes persistence of a non-restrictive ductus (116). Furthermore, there is no evidence of benefit or safety for this practice. Furosemide is oto-toxic and can lead to electrolyte imbalances.

d) **Post-natal steroids:** Post-natal steroids have been associated with decreased mechanical ventilation support and pulmonary inflammation in the premature newborn (117, 118). While steroids may help in improving respiratory management of the very preterm newborn, their use has been associated with adverse neurodevelopmental outcomes (119). In addition, steroid-associated brain injury has been detected on neuro-imaging in adolescence (120). Studies have shown that dexamethasone may be associated with accelerated PDA constriction (121, 122). With a more conservative approach towards PDA treatment, management of extremely preterm newborns may shift towards the use of more post-natal steroids, if the association of PDA with pulmonary vascular congestion and worsening of pulmonary compliance were shown to be causal. Although management of the PDA using NSAIDs and mechanical closure may not be associated with clear benefits, the potential secondary increase in post-natal steroids use in an attempt to improve pulmonary function would also be worrisome.

8.11 The confusion

The need for closure of the PDA during the neonatal period is uncertain (123, 124). In the past decade, several studies have described spontaneous closure of the ductus in the majority of premature newborns (8-10). The absence of evidence for the improvement of long-term outcomes following

medical or surgical treatment of PDA has fueled the current uncertainty surrounding treatment (1, 2, 66, 67, 88, 125-127). In a recent population-based cohort study, PDA treatment has been associated to greater risk of abnormal neurodevelopmental outcomes at 2 to 3 years of age, especially in the premature newborns less than 25 weeks, supporting non-intervention (128). However, this conclusion, based largely on observational studies, may be due to confounding by indication. In light of the lack of proven efficacy of NSAIDs in preventing the complications of the PDA (1), in 2016, the AAP, recommended against the use of NSAIDs in extremely premature newborns during the first two weeks of life (1). However, few studies have documented the safety and outcomes of a conservative approach of "watchful waiting" in extremely premature babies. The few available reports are biased by the "rescue" use of NSAIDs or ligation in the babies managed conservatively (129, 130). Hence, the goal of the current study was to fill knowledge gaps regarding the outcomes of a recent extremely premature cohort of newborns exposed to a strict conservative management policy, specifically those born at <26 weeks.

9 Thesis Methodology and Research findings:

9.1 Methodology

9.1.1 Research Question

What is the effect of a conservative PDA management policy targeting premature newborns on the outcome of death and/or BPD compared with premature newborns managed with selective NSAIDS and/or ligation?

9.1.2 Objectives

To determine the impact of a conservative approach to PDA management, compared with a more traditional approach (NSAIDs and/or ligation), on death or BPD in premature newborn born at 29 weeks or less of EGA and in newborns born at less than 26 weeks EGA, as they are the most at-risk for mortality and morbidities related to prematurity due to their vulnerability and immaturity.

9.1.3 Hypothesis

Our hypothesis was that a conservative approach policy would not be associated with a difference in the rate of death or BPD, compared with using NSAIDs or mechanical closure in extremely premature newborns at ≤29 weeks EGA at birth, including in the subset born at <26 weeks.

9.1.4 Context

Site 1 and 2 are two quaternary care NICUs with a transport team, admitting both inborn and outborn patients. Both are located in the same city and target a similar population of patients in terms of socio-economic status and risk factors. Both sites followed parallel time trends and were similar based on:

(1) clinical practices (example: adoption of new oxygen saturation targets, use of non-invasive continuous positive airway pressure [CPAP] as a primary mode of support with rescue intubation and surfactant, use of pasteurized human milk instead of formula, etc.);

(2) comparable patient populations (comprising cardiac, hypoxic ischemic encephalopathy, surgical and extremely preterm cases);

(3) qualified pool of attending neonatologists, trained at similar university programs;

(4) presence of neonatal nurse practitioners;

(5) part of a university-affiliated institution; both have an affiliated medical school, a general pediatrics training program and a neonatal-perinatal medicine fellowship program;

(6) exposure to institution move (both units moved to a renovated single-room unit during the same time period).

Throughout the study period (2011 to 2017), Site 1, the control centre, recorded 100 to 120 newborns born at ≤29 weeks per year, and continued to use selective NSAIDs and/or ligation to manage this vulnerable population. The newborns at Site 1 were exposed to NSAIDs and/or ligation based on previously published echocardiography criteria (23). Site 2, the exposed centre, with 45 to 55 newborns ≤29 weeks per year, opted to follow a strict conservative management policy with no NSAIDs and/or ligation and with absolutely no "rescue" criteria for treatment, as of September 27, 2013. The similarity of the two institutions in all respects, except for the change in PDA policy, provided a unique opportunity for a "natural experiment" to investigate if premature newborns exposed to a conservative approach to PDA management have outcomes similar to those exposed to selective NSAIDS and/or ligation to accelerate ductal closure. For the purpose of this study, variables were divided by epochs: **epoch 1** included babies born in the years prior to (and including) the adoption of the policy at Site 2 (2011-2013), while **epoch 2** included babies born in the years after the adoption (2014-2017 – up to December 31st, 2017).

9.1.5 Outcomes

The **primary outcome** is a composite outcome of **death or BPD at 36 weeks post-menstrual age (PMA) –** (death at any time before discharge). The rationale behind the primary outcome is that death may be a consequence of the physiological derangements related to the PDA. Theoretically, upon the fall of PVR, blood may rush into the lungs, leading to pulmonary edema and catastrophic pulmonary hemorrhage. Similarly, the steal effect from the cerebral circulation in the context of an immature brain autoregulation, compounded by the hemodynamic instability, may lead to severe IVH. Pulmonary hemorrhage and severe IVH in preterm newborns are associated with early death (in the first 2 weeks of life). The persistence of the PDA may lead to pulmonary edema, which can result in poor pulmonary compliance. This is hypothesized to trigger respiratory failure requiring positive pressure mechanical ventilation to ensure appropriate gas exchange. Mechanical ventilation and oxygen free radicals have been associated to lung injury and BPD. Late death of preterm newborns may be secondary to severe respiratory failure, in the context of severe BPD. Because the diagnosis of BPD occurs, by definition, at 36 weeks PMA (131), death at an earlier corrected age precludes from having this diagnosis.

The **secondary outcomes** are: death, NEC, the combined outcome of death or IVH grade 3 or more (considered as severe IVH), and the combined outcome of severe IVH or early death (within 2 weeks of life – to account for deaths due to pulmonary hemorrhages or re-orientation towards comfort care).

9.1.6 Design of the study

9.1.6.1 Data source and definitions

Data from the CNN registry at each institution were extracted. The CNN was founded in 1995 and collects data on newborns admitted in most NICU across Canada, using standardized definition to allow for collaborative research and quality improvement projects. The CNN produces an annual report about

the current status of outcomes across the country and provides individual sites with their own performance for ongoing monitoring. Perinatal and neonatal data obtained from this registry were based on standardized CNN definitions (132). BPD was defined as any respiratory support or oxygen requirement at 36 weeks PMA. Small for gestational age was diagnosed if birth weight was below the 10th percentile. Early-onset sepsis was defined as a positive bacterial or fungal culture in blood and/or cerebrospinal fluid in the first two days after birth. Late-onset sepsis was defined using the same criteria beyond two days of life. NEC was considered present if stage 2 or higher, according to the Bell's classification (133, 134). IVH was graded according to Papile's classification and considered significant if grade 3 or higher, or if presence of peri-ventricular leukomalacia was detected in a head ultrasound performed during hospitalisation (135). Retinopathy of prematurity (ROP) was defined according to the International Classification for ROP (136). Significant ROP was defined as stage III, IV or V. When ROP was treated, it was with laser photocoagulation or intra-ocular injection of bevacizumab. The Score for Neonatal Acute Physiology, Version II (SNAP-2) was used as a score of neonatal illness severity. This score takes into account physiological disturbances in various organs (i.e. mean blood pressure, lowest temperature, ratio of partial arterial oxygen content on oxygen requirements, lowest serum pH, seizures and urine output) and is calculated in the first 12 hours of admission in the neonatal unit. SNAP-2 is predictive of mortality and has been associated with indicators such as duration of hospitalization, nursing workload and intensity of resources allocated (132, 137). Length of hospitalization was defined as the number of days from birth to discharge from the NICU, among those who survived to discharge.

9.1.6.2 Inclusion and Exclusion criteria

Newborns were included in the study if born at ≤29 weeks and if they were either born or had been transferred within the first 24 hours of life to sites 1 or 2. Exclusion criteria included: death in the first 24 hours of life, presence of a congenital heart defect (except atrial or ventricular septal defect),

significant congenital anomaly or presence of a genetic disorder (refer to Appendix A). The early morbidities attributed to PDA (those that may prompt physicians to intervene to accelerate PDA closure) are: pulmonary hemorrhage (which usually occurs in the first 2 weeks after birth), NEC and severe intraventricular hemorrhage (all of which can re-orient care towards a comfort approach). All the morbidities historically "attributed" to the PDA usually occur after 24 hours of life. As such, this provided the rational to exclude those newborns that died in the first 24 hours.

9.1.6.3 Definition of exposed and control center

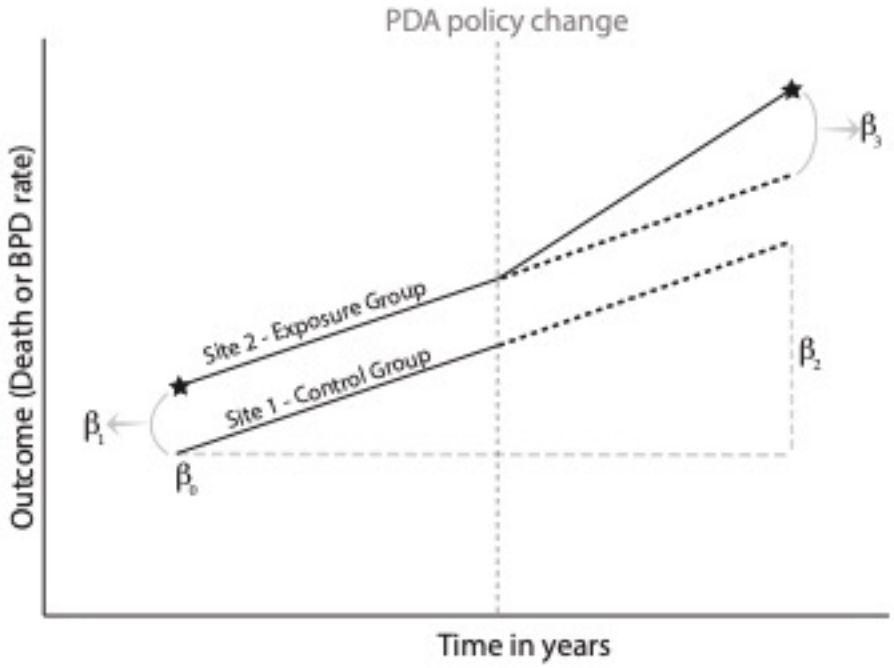
Site 1 (control) continued using NSAIDS and/or ligation throughout the study period, whereas Site 2 (exposed) adopted a strict conservative management policy in September 2013, abandoning NSAIDS or ligation use. The conservative policy was discussed at Site 2 after careful review and presentation of the available literature in the context of a local scientific meeting ("neonatal rounds"), used to review evidence-based medicine practices. The policy was adopted by consensus amongst the neonatologists.

*9.1.6.4 Difference-in-Differences (**Figure** 7)*

The impact of the intervention on the primary and secondary outcomes were evaluated using the difference-in-differences (DID) method (multiple time series design), a quasi-experimental approach derived from the fixed-effects model (138). A DID design emulates a RCT, using a "natural experiment", which is an event such as a policy change within a population, and comparing this group to another similar population that only differs with respect to the event in question (138). This approach allows for better control for confounding by indication, as it avoids comparing individual patients who received NSAIDs/ligation to individual patients who did not. Factors associated with the reasons why a physician chooses a particular management approach are controlled for by the DID design, since policy change serves as a proxy to randomization Assuming that the policy did not impact the outcome of interest, the

control population provides a counterfactual of the outcome to the exposed group, by using the trajectory of the outcome in controls as the trajectory that the exposed group would have in the absence of a policy effect.

Figure 7: Difference in Differences model applied to the context of a change in policy in PDA management.



Legend for Figure 7: Difference in Differences design models the outcome for the exposure to the policy, the epochs and the interaction between both using a regression analysis.

Y** (incidence of the outcome of interest, here death or BPD proportion) = **β₀** + **β₁[Site of exposure] + **β₂***[Epoch pre or post-policy adoption] + **β₃***[Site*Epoch] + **ε** (robust standard errors).*

*Where: **β₀** is the incidence of the outcome before the intervention at Site 1 (control); **β₁** is the difference in the outcome incidence among those exposed to Site 2 (exposure) compared with Site 1 (control) in the pre-policy adoption epoch; **β₂** is the difference in the outcome incidence in the epoch following the adoption of the policy compared with the epoch prior at Site 1 (control); **β₃** is the DID estimator and represents the change in the outcome incidence in the epoch following the adoption of the policy that is specific to Site 2 controlling for shared temporal changes and fixed effects.*

The DID design avoids the underlying assumption of pre-post studies, which assumes no underlying secular trends and no time-varying confounders (138). This design uses a "comparison group that is experiencing the same trends but is not exposed to the policy change" (139). In our case, changes over time include the adoption of common practices in neonatology (different oxygen saturation targets, changing approach to primary mode of respiratory support, etc.) that potentially impact the outcomes of interest (139). By using a control group exposed to similar secular and time trends, the DID model allows to evaluate the impact of a policy change. The underlying assumption is that the trend in the outcomes of interest following the establishment of the policy is accurately represented by the trend experienced by the control group, if the exposed group had not adopted the policy. This assumption is verified by assuming that, if the outcome trends prior to the policy change were "parallel", then they would continue to be parallel after the policy change. Also, it is assumed that the analysed outcomes are impacted by common "shocks" in both groups (events equally affecting the two groups, such as the adoption of certain practices that can influence the outcome – i.e. move to a single-room unit, use of pasteurized human milk instead of formula, etc.) (139). If evaluating the impact of the intervention on the absolute scale (or using an additive model), then the parallel trend assumption can be evaluated visually by comparing rate trajectories over time before the intervention. When evaluating the relative change (i.e. using a multiplicative model), the parallel trends should be evaluated as the natural logarithm of the rates. Hence, rates (and natural logarithm of the rates) are displayed per year (with 95% confidence intervals), to ensure that the parallel trend assumption in the years prior the PDA policy change at Site 2 is respected.

9.1.6.5 Sensitivity analysis for robustness evaluation

Sensitivity analyses are used to evaluate the robustness of the association between the exposure and the outcome in the context of a DID model. We evaluated if there was evidence to support a "*lead effect"*. This is when the outcome of interest may begin to change in anticipation of the start of the policy. Such an effect would suggest that a detected change occurred prior to adoption of the policy (regardless of the

presence or not of that policy). To ensure the absence of a lead effect, we conducted our analysis using a cut-off time for the policy launch set one-year prior to the actual policy introduction. As the policy adopted on September 27th, 2013, we re-ran the DID model using the epochs: *birth years 2011 and 2012* versus *birth years 2013-2017*. Next we evaluated a "lagged" effect. A lag effect is if the policy has an immediate and sustained effect on the outcomes. Indeed, as the adoption of the policy increases in time, the effect of the policy on the outcome of interest should increase. In our case, the DID model was re-run using the epochs: *birth years 2011 to 2014* versus *birth years 2015 to 2017*.

As a further sensitivity analysis, we carried out our DID analysis using a "placebo outcome", i.e., an outcome that is not expected to have been affected by the policy change. This addresses the possibility that the impact of the policy observed for the outcome of interest may have been due to chance. We used the rate of "SNAP-2 score above 20 points" as a "*placebo"* outcome, since it is a severity score describing neonatal illness and based on physiological markers captured in the first 12 hours of admission. These markers are not expected to be affected by PDA policy practice.

Finally, the DID design controls for time-fixed confounders and parallel time varying confounders by design. However, time-varying variables thought to differentially impact the control and exposure groups must be accounted for (138). Hence, we compared variables across sites in each epoch to uncover differences between sites in other parameters that could lead to confounding in time trends. The predictors we examined as having potentially changed over time between sites are all independently associated with mortality and include: gestational age at birth, exposure to antenatal steroids, small for gestational age, and infant sex (36, 140, 141).

9.1.6.6 Statistical analysis

Descriptive summary measures consist of means with standard deviation or median with interquartile range (IQR) for continuous variables, and of counts with proportions for categorical variables. We used Fisher's exact and chi-square tests to assess differences in categorical characteristics

between the two centers, and Student t-test and Wilcoxon-Mann-Whitney test for parametric and non-parametric continuous variables, respectively, between exposed and not-exposed and also between epochs. In the DID models, we modeled the differences in outcomes in the epochs before and after the policy change (transition to a conservative approach) using a generalized linear regression analysis including the exposure (Site 1 versus Site 2), the time indicator (epoch 1 versus epoch 2) and an interaction term between the exposure and time (139).

The linear regression model for the DID represents (**Figure 7**): **Y** (incidence of the outcome of interest, here death or BPD rate) = $\boldsymbol{\beta_0}$ + $\boldsymbol{\beta_1}$*[Site of exposure] + $\boldsymbol{\beta_2}$*[Epoch pre or post-policy adoption] + $\boldsymbol{\beta_3}$*[Site*Epoch] + $\boldsymbol{\varepsilon}$ (robust standard errors). The coefficient of the interaction term is the DID estimator for the policy impact on the outcome of interest. We then evaluated the relative impact of the policy change using a Poisson regression. We chose a Poisson model as opposed to a logistic model, since the outcome under study is not rare (the usual threshold is > 10%), therefore the OR would overestimate the RR (142, 143). We accounted for heteroskedasticity by using robust covariance matrix estimators, also known as *Eicker–Huber–White standard errors* (144-147). We carried out statistical analysis using R (Version 3.4.4) and RStudio (Version 1.0.143).

9.1.6.7 Transfer of data, confidentiality and ethics

A collaboration and data sharing agreement, prepared in conjunction by the two sites contract offices, was signed by collaborators at both the institutions involved in this research project (G.A and A.L.). Data from both sites are anonymized and kept within an Excel spreadsheet under the McGill University Health Centre (MUHC) firewall. Analysis were done at the MUHC. Ethics board approval was obtained from each institution (refer to Appendix B).

9.2 Results

During the study period, 1298 babies were admitted to both NICUs with gestational age at birth ≤29 weeks. Of these, 49 met the exclusion criteria: 9 were admitted after 24 hours, 24 had a major congenital anomaly, and 16 had died in the first few hours after birth. Of the excluded newborns, 18 were born at a gestational age below 26 weeks. The final cohort comprised 1249 infants, including 341 (27.3%) born before 26 weeks. The distribution of gestational age at birth by site and epoch is shown in **Table 2**. Although the absolute number of premature newborns at both sites increased in Epoch 2, the distribution between centres remained the same in each epoch.

Table 2: Population by gestational age at birth by site and epoch

Population by gestational age at birth for ≤29 weeks p = 0.45 for comparisons between % differences of 2 epochs				
	Epoch 1		**Epoch 2**	
Gestational age in weeks	**Site 1 - (n %) n = 383**	**Site 2 – n (%) n = 156**	**Site 1 – n (%) n = 449**	**Site 2 – n (%) n = 261**
23	9 (2.0)	7 (2.7)	18 (4.0)	14 (5.4)
24	21 (5.5)	15 (9.6)	66 (14.7)	39 (14.9)
25	45 (11.7)	22 (14.1)	55 (12.2)	30 (11.5)
26	58 (15.1)	19 (12.2)	61 (20.9)	47 (10.3)
27	81 (21.1)	25 (16.0)	71 (15.8)	32 (12.3)
28	75 (19.6)	41 (26.3)	84 (18.7)	51 (19.5)
29	94 (24.5)	27 (17.3)	94 (20.9)	48 (18.4)
Population by gestational age at birth for <26 weeks p = 0.72 for comparisons between % differences of 2 epochs				
	Epoch 1		**Epoch 2**	
Gestational age in weeks	**Site 1 – n (%) n = 75**	**Site 2 – n (%) n = 44**	**Site 1 – n (%) n = 139**	**Site 2 – n (%) n = 83**
23	9 (12)	7 (15.9)	18 (12.9)	14 (16.9)
24	21 (28)	15 (34.1)	66 (47.5)	39 (47.0)
25	45 (60)	22 (50)	55 (39.6)	30 (36.1)

Legend of Table 2: Distribution of estimated gestational age in weeks at birth per epoch and site. There has been an absolute increase in the number of premature newborns admitted to each unit. The distribution of these age groups remained the same in both units. Importantly, there is no difference in the distribution of those that are the most at risk for morbidities and mortality (the population less than 26 weeks). p-value for the distribution by two-tailed Student t-test.

Demographic and clinical characteristics of the overall (≤ 29 weeks) and < 26 weeks cohorts are shown in **Table 3 and Table 4, respectively**. Average gestational age at birth, birth weight, sex proportions,

antenatal (and post-natal) steroid exposure, rate of caesarian-section and small for gestational age were similar between sites in each epoch. During Epoch 1, newborns at Site 2 were more frequently exposed to surfactant replacement therapy than those at Site 1 (≤ 29 weeks: 76% vs. 58%). Similarly, in the <26 weeks subgroup, more newborns were exposed to surfactant replacement therapy at Site 2 in both Epochs (Epoch 1: 100 vs. 85%; Epoch 2: 90 vs. 81%). Surfactant has been associated with decreased mortality and BPD in previous RCTs (148). Thus, although surfactant administration varied by site, the difference between Sites did not vary in the two epochs. None of the other characteristics examined appeared to differ across sites. When examining the Epoch 1 vs Epoch 2 at each site, none of the variable changed significantly.

Table 3: Demographic information regarding the whole cohort (≤29 weeks)

Table 3 – Demographic information regarding the whole cohort (≤29 weeks)						
	Site 1	**Site 2**		**Site 1**	**Site 2**	
	Epoch 1	**Epoch 1**		**Epoch 2**	**Epoch 2**	
	n=383 (31%)	**n=156 (12%)**	**p-value**	**n=449 (36%)**	**n=261 (21%)**	**p-value**
Gestational Age	27 (1.6)	26.7 (1.8)	0.06	26.6 (1.9)	26.5 (1.9)	0.36
Birth weight	1009 (283)	975 (271)	0.20	952.4 (286.6)	961.1 (283.9)	0.69
Male sex	214 (55.9)	91 (58.3)	0.67	233 (51.9)	136 (52.1)	1.00
Maternal Hypertensive disorder	79 (20.8)	22 (14.4)	0.11	72 (16.2)	38 (14.6)	0.65
Antenatal antibiotic use	226 (59.5)	94 (61.4)	0.75	287 (65.5)	185 (73.1)	0.047
C-Section	237 (62.2)	96 (61.5)	0.96	319 (71.2)	168 (64.4)	0.07
Apgar at 5 minutes < 7	172 (45.4)	4 (41.0)	0.41	205 (46.0)	138 (52.9)	0.09
Outborn	23 (6.0)	18 (11.5)	0.03	50 (11.1)	35 (13.4)	0.44
Singleton	294 (76.8)	113 (72.4)	0.34	318 (70.8)	201 (77.0)	0.08
Rupture of membranes ≥ 24 hours	99 (26.2)	0 (32.3)	0.19	106 (23.9)	89 (34.6)	0.003
Prenatal Care	369 (97.3)	143 (99.3)	0.30	439 (98.0)	251 (98.8)	0.55
Small for gestational age	41 (10.7)	17 (10.9)	1.00	67 (14.9)	19 (7.3)	0.003
SNAPII score >20	102 (26.6)	49 (31.6)	0.29	125 (27.8)	69 (26.7)	0.82
Antenatal steroids	354 (92.9)	139 (89.7)	0.22	400 (89.3)	237 (90.8)	0.61
Surfactant use	222 (58.0)	121 (75.6)	<0.0001	293 (65.3)	165 (63.2)	0.63
Expressed as mean (standard deviation) or count (percentage). SNAPII Score: Score for Neonatal Acute Physiology, Version II (SNAP-2)						

Table 4: Demographic information regarding the cohort <26 weeks

Table 4 – Demographic information regarding the cohort <26 weeks						
	Site 1	**Site 2**		**Site 1**	**Site 2**	
	Epoch 1	**Epoch 1**		**Epoch 2**	**Epoch 2**	
	n=75 (22%)	**n=44 (13%)**	**p-value**	**n=139 (41%)**	**n=83 (24%)**	**p-value**
Gestational Age	24.5 (0.7)	24.3 (0.7)	0.31	24.3 (0.7)	24.2 (0.7)	0.44
Birth weight	718 (119)	732 (102)	0.50	718 (146)	700 (118)	0.35
Male sex	42 (56)	25 (57)	1.00	72 (52)	48 (58)	0.41
Maternal Diabetes	9 (20)	2 (5)	0.049	8 (11)	8 (10)	1.00
Maternal Hypertensive disorder	9 (13)	1 (2)	0.09	13 (10)	8 (10)	1.00
Antenatal antibiotic use	50 (68)	33 (75)	0.53	105 (78)	69 (84)	0.38
C-Section	36 (49)	20 (45)	0.70	89 (64)	52 (63)	0.89
Apgar at 5 minutes < 7	45 (63)	23 (52)	0.33	88 (64)	59 (71)	0.31
Outborn	5 (7)	3 (7)	1.00	17 (12)	8 (10)	0.66
Singleton	56 (75)	31 (70)	0.67	96 (69)	70 (84)	0.01
Rupture of membranes ≥ 24 hours	19 (26)	17 (39)	0.21	39 (29)	37 (46)	0.01
Prenatal Care	70 (96)	42 (100)	0.30	134 (96)	82 (99)	0.41
Small for gestational age	6 (8)	1 (2)	0.26	12 (9)	5 (6)	0.61
SNAPII score >20	42 (56)	25 (57)	1.00	70 (50)	41 (51)	1.00
Antenatal steroids	65 (89)	39 (89)	1.00	120 (86)	76 (92)	0.29
Surfactant use	64 (85)	44 (100)	0.007	113 (81)	75 (90)	0.08
Expressed as mean (standard deviation) or count (percentage). SNAPII Score: Score for Neonatal Acute Physiology, Version II (SNAP-2)						

Neonatal outcomes during hospitalisation are described in **Tables 5 & 6,** for the whole cohort and the <26 weeks sub-cohort, respectively. Use of medical and surgical PDA closure strategies dramatically decreased in Epoch 2 in Site 2, after adoption of the policy. While the use of PDA treatment dramatically decreased at Site 2 in Epoch 2, use of post-natal steroids for treatment of BPD remained stable (36 vs. 34% in ≤29 weeks and 61 vs. 61% in <26 weeks). Furthermore, use of post-natal steroids was similar between sites in each epoch. Site 1 experienced a significant higher rate of BPD at both epochs: (Epoch 1: 42.8 vs. 20.3% and Epoch 2: 46.9 vs. 30% in the overall cohort; 80 vs. 19% in Epoch 1 and 73 vs. 56% in Epoch 2 of the <26 weeks). While the rate of significant retinopathy of prematurity remained relatively stable at both sites, treatment for ROP appeared to decrease in site 2 in epoch 2 (25 vs. 6%) among infants <26 weeks, but the absolute number of patients alive at the time of screening was small (6/24 vs. 3/50).

Table 5: Clinical and outcomes data of the whole cohort (≤29 weeks)

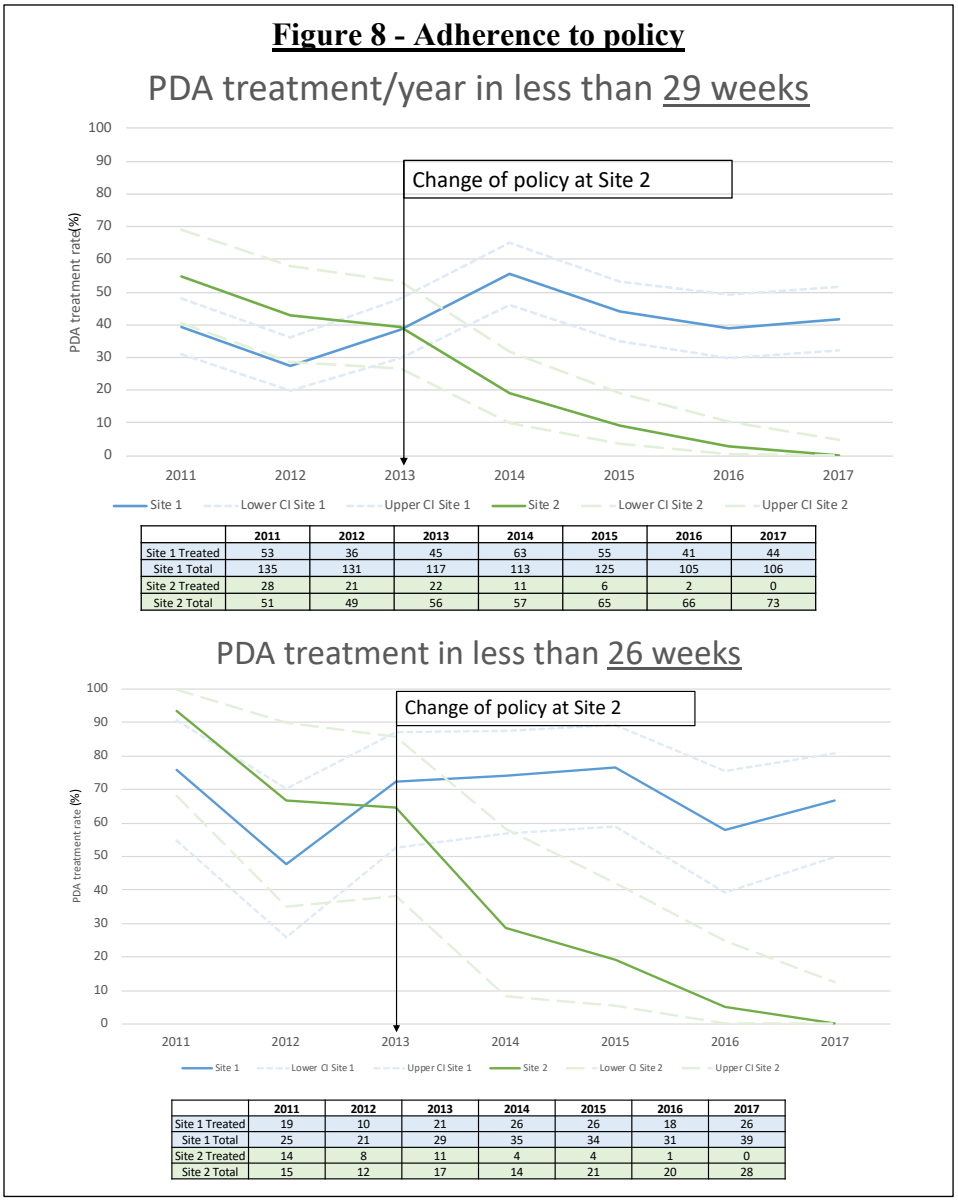
Clinical and outcomes data of the whole cohort (≤29 weeks gestational age)						
	Site 1	**Site 2**		**Site 1**	**Site 2**	
	Epoch 1	**Epoch 1**		**Epoch 2**	**Epoch 2**	
	n=383 (31%)	**n=156 (12%)**	**p-value**	**n=449 (36%)**	**n=261 (21%)**	**p-value**
Neonatal outcomes						
IVH grade 3 or more	44 (11.6)	28 (18.2)	0.06	54 (12.1)	37 (14.5)	0.41
NEC	51 (13.3)	13 (8.3)	0.14	42 (9.4)	11 (4.2)	0.02
Surgical NEC	17 (4.4)	4 (2.6)	0.46	17 (3.8)	4 (1.5)	0.11
ROP stage 3 and more	30 (9.6)	13 (10.1)	1.00	31 (17.1)	5 (5.1)	0.004
ROP treated	11 (9.5)	6 (16.21	0.25	41 (11.0)	19 (8.6)	0.43
BPD	136 (42.8)	28 (20.3)	<0.0001	179 (46.9)	70 (30.0)	<0.0001
Nosocomial infection diagnosed two days after birth (or late onset sepsis)	125 (32.6)	45 (28.8)	0.45	128 (28.5)	4 (28.4)	1.00
Early onset sepsis	6 (1.6)	7 (4.4)	0.06	7 (1.6)	7 (2.7)	0.40
Normal temperature at admission: 36.5-37.5 celcius	95 (50.9)	62 (39.7)	0.02	205 (45.9)	148 (57.4)	0.004
Inhaled steroids exposure	4 (1.0)	1 (0.6)	1.00	6 (1.3)	1 (0.4)	0.43
Post-natal systemic steroids	113 (29.5)	56 (35.9)	0.18	176 (39.2)	89 (34.1)	0.2
Ventilation days	5 (0-25)	6 (2-21)	0.49	5 (1-19)	3 (1-17)	0.17
PDA related management						
Ibuprofen Use	121 (31.6)	71 (45.5)	0.003	192 (42.8)	19 (7.3)	<0.0001
Indomethacin exposure	4 (1.0)	0 (0)	0.33	14 (3.1)	0 (0)	0.009
Ligation	49 (12.8)	8 (5.1)	0.008	23 (5.1)	0 (0)	<0.0001
NSAIDS use or ligation	134 (35.0)	71 (45.5)	0.02	203 (45.2)	19 (7.3)	<0.0001
Outcomes						
Length of stay in alive at discharge	89.4 (43.4)	88.8 (42.3)	0.90	97.5 (40.6)	89.5 (43.8)	0.02
Mortality	76 (19.8)	19 (12.2)	0.046	75 (16.7)	30 (11.5)	0.08
Death or BPD	201 (52)	47 (30)	<0.0001	246 (54.8)	98 (37.5)	<0.0001
Death or NEC	100 (26.1)	30 (19.2)	0.11	99 (22.0)	41 (15.7)	0.051
Death or IVH	98 (25.6)	40 (25.6)	1.00	103 (22.9)	59 (22.6)	0.99
Early Death	33 (8.6)	12 (7.7)	0.86	46 (10.2)	24 (9.2)	0.75
Early Death or IVH	67 (17.5)	36 (23.1)	0.15	81 (18.0)	56 (21.5)	0.31
Expressed as mean (standard deviation), median (inter-quartile range) or count (percentage). BPD (bronchopulmonary dysplasia), IVH (intra-ventricular hemorrhage), NEC (necrotizing enterocolitis – stage 2 or above as per Bell's criteria), NSAIDS (Non-steroidal anti-inflammatory drugs), ROP (retinopathy or prematurity).						

Table 6: Clinical and outcomes data of the <26 weeks gestational age

Clinical and outcomes data of the <26 weeks gestational age						
	Site 1	Site 2		Site 1	Site 2	
	Epoch 1	Epoch 1		Epoch 2	Epoch 2	
	n=75 (22%)	n=44 (13%)	p-value	n=139 (41%)	n=83 (24%)	p-value
Neonatal outcomes						
IVH grade 3 or more	17 (23)	14 (32)	0.34	32 (23)	16 (21)	0.73
NEC	17 (23)	5 (11)	0.15	21 (15)	7 (8)	0.21
Surgical NEC	5 (7)	2 (5)	1.00	10 (7)	3 (4)	0.38
ROP 3 and more	18 (40)	9 (29)	0.46	34 (35)	14 (22)	0.11
ROP treated	10 (29)	6 (25)	1.00	28 (33)	3 (6)	0.0002
BPD	35 (80)	6 (19)	<0.0001	71 (73)	35 (56)	0.03
Nosocomial infection diagnosed two days after birth (or late onset sepsis)	43 (57)	18 (41)	0.09	70 (50)	35 (42)	0.27
Early onset sepsis	3 (4)	2 (5)	1.00	3 (2)	4 (5)	0.43
Normal temperature at admission: 36.5-37.5	5 (47)	16 (36)	0.34	56 (41)	42 (51)	0.16
Inhaled steroids exposure	2 (3)	1 (2)	1.00	2 (1)	0 (0)	0.53
Post-natal systemic steroids	42 (56)	27 (61)	0.7	101 (73)	50 (60)	0.07
Ventilation days	25 (7-43)	18 (8-21)	0.31	23 (10-41)	18 (6-37)	0.19
PDA related management						
Ibuprofen Use	43 (57)	33 (75)	0.07	91 (65)	9 (11)	<0.0001
Indomethacin exposure	3 (4)	0 (0)	0.29	7 (5)	0 (0)	0.047
Ligation	21 (28)	6 (14)	0.11	19 (14)	0 (0)	0.0001
NSAIDS use or ligation	50 (67)	33 (75)	0.41	96 (69)	9 (11)	<0.0001
Outcomes						
Length of stay in alive at discharge	141 (38)	132 (62)	0.28	131 (28)	28 (43)	0.57
Mortality	33 (44)	13 (30)	0.13	47 (34)	21 (25)	0.23
Death or BPD	66 (88)	19 (43)	<0.0001	113 (81)	55 (66)	0.02
Death or NEC	41 (55)	18 (41)	0.18	60 (43)	28 (34)	0.20
Death or IVH	39 (52)	21 (48)	0.71	60 (43)	31 (37)	0.40
Early Death	17 (23)	9 (20)	0.82	31 (22)	16 (19)	0.62
Early Death or IVH	28 (37)	19 (43)	0.56	50 (36)	29 (35)	1.00
Expressed as mean (standard deviation), median (inter-quartile range) or count (percentage). BPD (bronchopulmonary dysplasia), IVH (intra-ventricular hemorrhage), NEC (necrotizing enterocolitis – stage 2 or above as per Bell's criteria), NSAIDS (Non-steroidal anti-inflammatory drugs), ROP (retinopathy or prematurity).						

Figure 8 shows that use of NSAIDS or ligation decreased to 0% at Site 2, while remaining constant at Site 1 for both the ≤29 weeks and the <26 weeks EGA subgroup. Thus, site 2 complied with the policy change. Surgical ligation of PDA decreased at Site 1 from 13% to 5% from Epoch 1 to Epoch 2, and from 5% to 0% at Site 2.

Figure 8: Adherence to the PDA policy – PDA treatment rates (NSAIDS or Ligation)



	2011	2012	2013	2014	2015	2016	2017
Site 1 Treated	53	36	45	63	55	41	44
Site 1 Total	135	131	117	113	125	105	106
Site 2 Treated	28	21	22	11	6	2	0
Site 2 Total	51	49	56	57	65	66	73

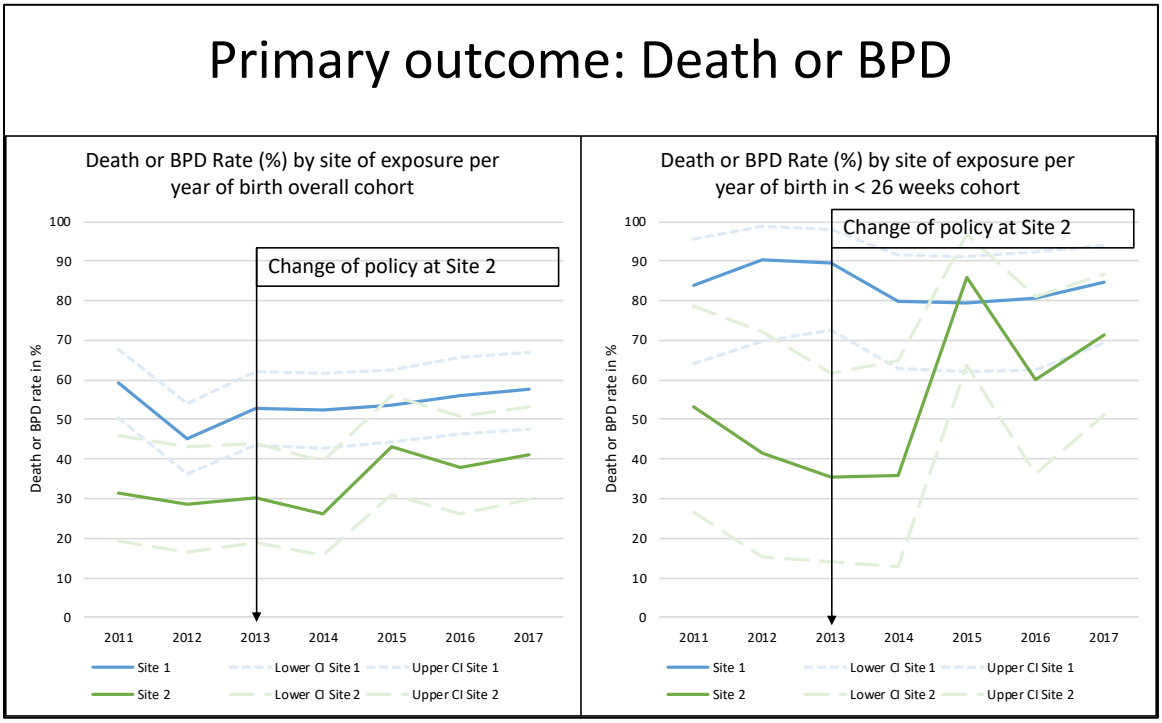
	2011	2012	2013	2014	2015	2016	2017
Site 1 Treated	19	10	21	26	26	18	26
Site 1 Total	25	21	29	35	34	31	39
Site 2 Treated	14	8	11	4	4	1	0
Site 2 Total	15	12	17	14	21	20	28

Legend of Figure 8: Policy was adopted on September 27th, 2013 at Site 2. Following policy adoption, the rate of NSAIDS or ligation at Site 2 (Exposure – Green) dramatically decreased in the overall cohort (as well as in the less than 26 weeks premature newborns) to reach 0% in 2017. The rate of PDA treatments remained stable at Site 1 (Control - Blue).

Primary outcome:

Rates of primary outcome are reported in **Tables 5 and 6.** In the <26 weeks subgroup, the primary outcome occurred in 66/75 (88% of Site 1) patients vs. 18/44 (43% - Site 2) in Epoch 1 and 113/139 (81% - Site 1) vs. 55/83 (66% - Site 2) in Epoch 2. Rates of death or BPD are shown in **Figure 9**. While the rate of death or BPD is higher overall at Site 1, it follows a similar trend throughout the years in the overall cohort. In the <26 weeks, the rate of death or BPD (provided with 95% Confidence Interval [CI]) follows a parallel trend between sites prior to the adoption of the policy at Site 2. There is, however, an increase of the primary outcome in the <26 weeks population at Site 2 following the policy change (**Figure 9)**.

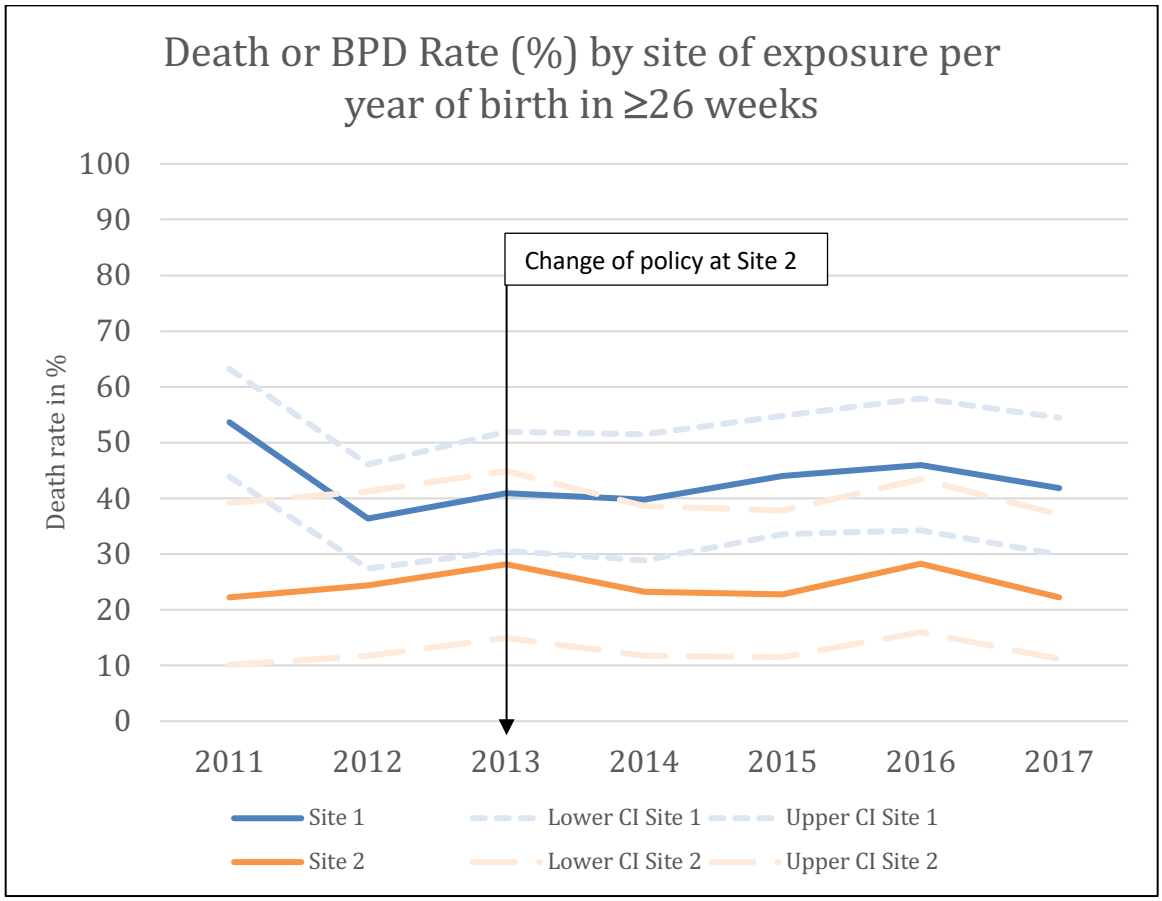
Figure 9: Rates of death and/or BPD (primary outcome)



Legend of Figure 9: Site 1 (Control - Blue), Site 2 (Exposure - Green). The 95% confidence interval for each trend is represented with corresponding dash lines. The death and/or BPD rate was similar between the 2 sites and did not change significantly after the adoption of the policy at Site 2 in the overall cohort. However, there is a graphical increase (at Site 2) in the rate of death and/or BPD after policy adoption at Site 2. Prior to the adoption of the policy, there is a parallel trend (including the 95% CI boundaries) between the exposure and control sites in the overall cohort and in the <26 weeks subgroup for the rates of death and/or BPD.

The rate of death or BPD for the subgroup ≥26 weeks is displayed in **Figure 10**, showing no clear fluctuation in this relatively more mature population after the policy change.

Figure 10: Death or BPD rate by site of exposure per year of birth in babies 26 weeks of gestational age and above.



Legend of Figure 10: Site 1 (Control - Blue), Site 2 (Exposure - Green). The 95% confidence interval for each trend is represented with corresponding dash lines. The death and/or BPD rate was similar between the 2 sites and remained stable throughout the years of the study in the 26 to 29 weeks newborns.

Results of the absolute DID model for the primary outcome of death and/or BPD are presented in **Table 7**. In the absence of identified time-varying differences potentially introducing a confounding effect, the model included: the epoch of exposure, the site of exposure (control vs. exposed) and the interaction between the epoch and the site of exposure. Results are reported for the overall cohort, as well as for the <26 weeks and the ≥26 weeks. Only the interaction term for the <26 weeks subgroup was statistically

significant, with an increase in the occurrence of the primary outcome of 30% (95% Confidence Interval 9 to 50%-point).

Table 7: Primary outcome by Difference-in-Difference model using linear regression

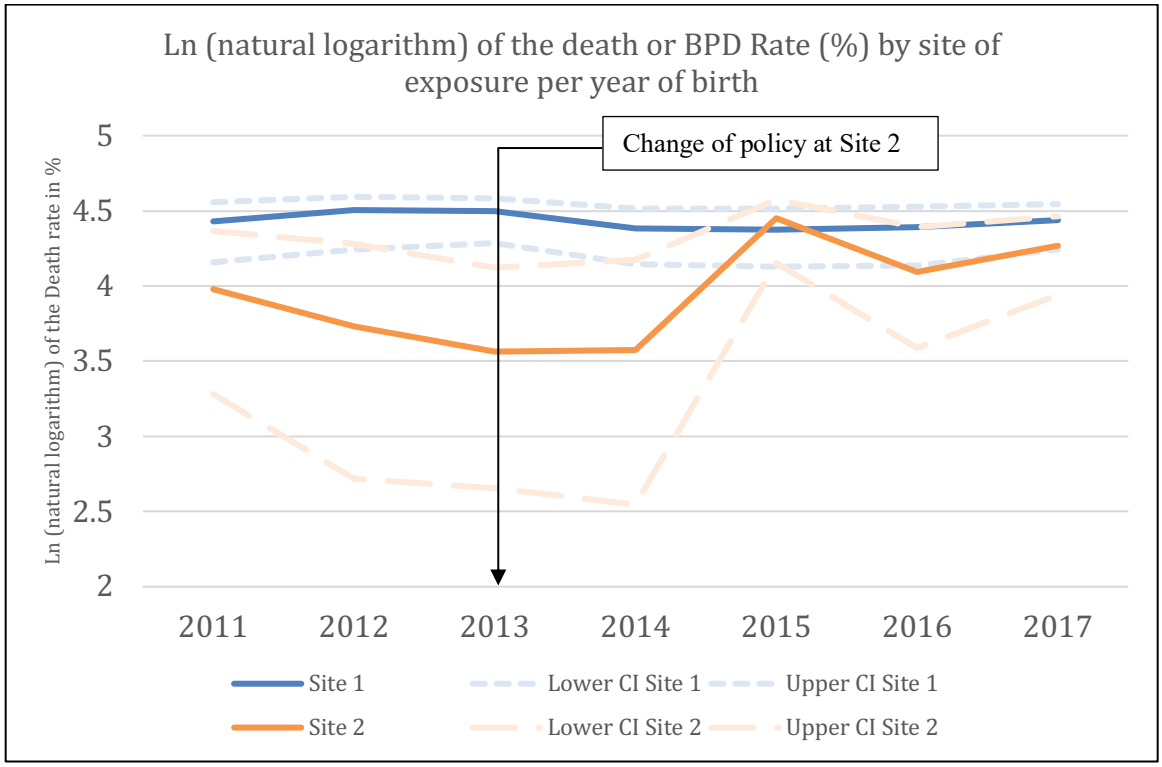
Difference in Difference linear regression for outcome: death or BPD				
	Coefficients Death or BPD	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Cohort ≤29 weeks	Intercept	0.52	0.47 - 0.57	0.03
	Exposed	-0.22	-0.31 – -0.14	0.04
	Epoch	0.02	-0.05 – 0.09	0.03
	Interaction term (DID estimator)	0.05	-0.06 – 0.17	0.06
Cohort <26 weeks	Intercept	0.88	0.81 – 0.95	0.04
	Exposed	-0.45	-0.61 – -0.28	0.08
	Epoch	-0.07	-0.17 – 0.03	0.05
	Interaction term (DID estimator)	**0.30**	**0.09 – 0.50**	**0.10**
Cohort 26 weeks to 29 weeks	Intercept	0.44	0.38 - 0.49	0.03
	Exposed	-0.19	-0.29 - -0.09	0.05
	Epoch	-0.009	-0.09 – 0.07	0.04
	Interaction term (DID estimator)	0.0009	-0.13 – 0.13	0.07

*Legend of Table 7: DID (with linear regression) with the primary outcome of death and/or BPD modeled for the exposure to the site, epoch (2011-2013 vs. 2014-2017) and interaction between site and epoch. The interaction term between Epoch and Site Exposure is not significantly associated with the primary outcome in the 26 weeks to 29 weeks gestational age at birth newborns. The interaction term between Epoch and Site Exposure is significantly associated with the primary outcome in the less than 26 weeks gestational age at birth newborns (β interaction term estimator of 30% increase [95% CI 11-49%] in point estimate for the incidence of the death and/or BPD outcome). Standard errors were computed using the robust method (robust covariance matrix estimators). Hence, the model is represented as: BPD or Death incidence = $\beta_0 + \beta_1$ *Exposed to Site 2 + β_2 *Epoch 2 + β_3 * Site 2 and Epoch 2.*

<u>Interpretation of the results for the <26 weeks population model output:</u>
Site 1 in Epoch 1 → incidence of Death and/or BPD is 0.88 (88%)
Site 1 in Epoch 2 → incidence is 0.88 - 0.07 = 0.81 (81%)
Site 2 in Epoch 1 → incidence is 0.88 - 0.45 = 0.43 (43%)
Site 2 in Epoch 2 → incidence is 0.88 - 0.45 – 0.07 + 0.30 = 0.66 (66 %)

The increase in BPD or death in the <26 weeks population at Site 2 on the natural logarithm scale is shown in **Figure 11**. On the logarithmic scale, the assumption of parallel outcome rates prior to adoption of the policy between the 2 sites appears fulfilled.

Figure 11: Natural logarithm of rate of death or BPD by site and year of birth



Legend of Figure 11: Graphical presentation of the natural logarithm of the rate of primary outcome of death or BPD per year of birth and by Site (Site 1[Blue]- Control; Site 2 [Green] – Exposure group). The 95% confidence interval for each trend is represented with corresponding dash lines. The parallel assumption prior to policy in September 2013 at Site 2 is verified. Hence, the DID model can be used using a Poisson regression model. The increase natural log of the rate of death or BPD at Site 2 after policy adoption is visually appreciated.

We additionally evaluated the impact of the policy on the relative scale by DID (**Table 8**). Again, only the interaction term for the subgroup < 26 weeks was significantly associated with the primary outcome of death and/or BPD (relative risk 1.66; 95% Confidence Interval: 1.13 - 2.46). Thus, being exposed to Site 2 in Epoch 2 resulted in a 66% higher risk of the primary outcome among infants born <26 weeks.

Table 8: Primary outcome by DID model using Poisson regression

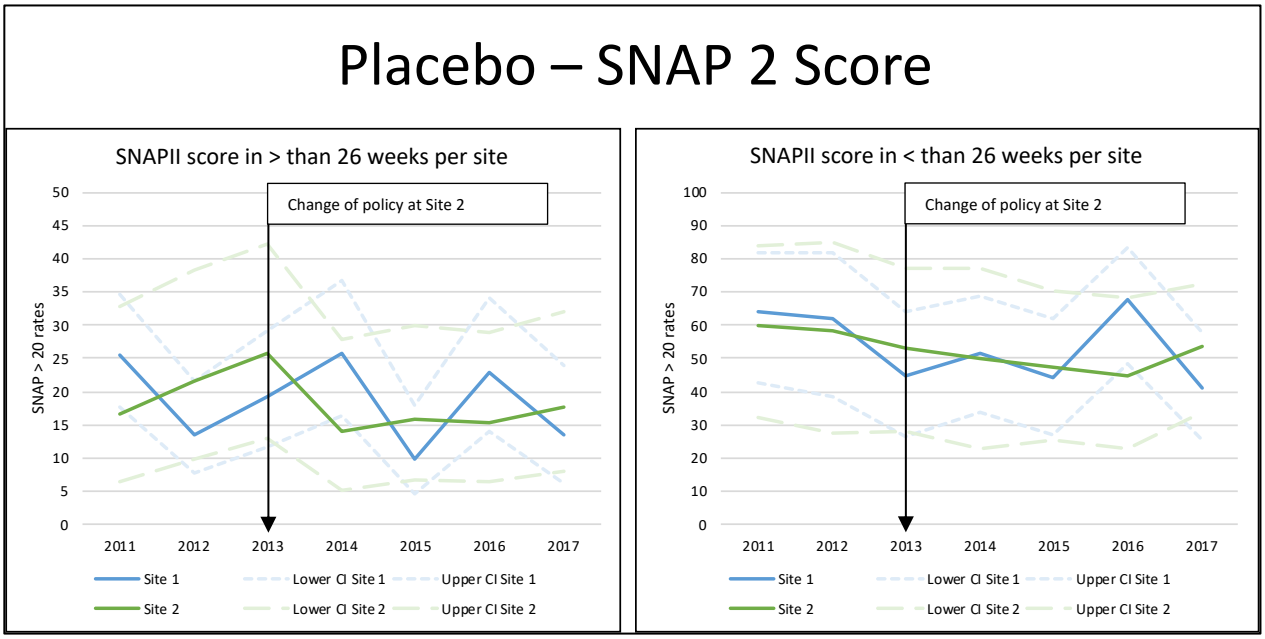
Difference in Difference Poisson regression for outcome: death or BPD				
	Death or BPD	**Relative Risk (RR)**	**95%CI**	**Standard Error (Robust)**
Cohort ≤29 weeks	Intercept	0.52	0.48 - 0.58	0.05
	Exposed	0.57	0.44 - 0.74	0.13
	Epoch	1.04	0.92 - 1.19	0.06
	Interaction term	1.19	0.87 - 1.60	0.16
Cohort <26 weeks	Intercept	0.88	0.81 - 0.96	0.04
	Exposed	0.49	0.34 - 0.70	0.18
	Epoch	0.92	0.82 - 1.04	0.06
	Interaction term	**1.66**	**1.13 - 2.46**	**0.20**
Cohort 26 weeks to 29 weeks	Intercept	0.44	0.39 - 0.50	0.06
	Exposed	0.57	0.40 - 0.80	0.18
	Epoch	0.98	0.82 - 1.17	0.09
	Interaction term	0.99	0.63 - 1.55	0.23

Legend of Table 8: DID regression using Poisson with the primary outcome of death and/or BPD modeled for the exposure to the site, epoch (2011-2013 vs. 2014-2017) and interaction between site and epoch. The interaction term between Epoch and Site Exposure is not significantly associated with the primary outcome in the 26 weeks to 29 weeks gestational age at birth newborns. The interaction term between Epoch and Site Exposure is significantly associated with the primary outcome in the less than 26 weeks gestational age at birth newborns (relative risk of 1.66 [95% CI 1.12 – 2.46]). Standard errors were computed using the robust method (robust covariance matrix estimators).

Sensitivity analyses

Rates of newborns with a SNAP-2 score above 20 remained stable in both sites and were similar between epochs (**Figure 12**). **Table 9** shows the results of the DID model using a linear regression for the placebo (SNAP-2 score >20) in the overall cohort and the <26 weeks sub-cohort. As expected, the interaction terms were not significant, indicating that the adoption of the policy did not affect the proportion of patients with a SNAP-2 score >20.

Figure 12 – Placebo (Rate of SNAP-2 score above 20)



Legend of Figure 12: Site 1 (Control - Blue), Site 2 (Exposure - Green). The 95% confidence interval for each trend is represented with corresponding dash lines. The rate of those with a SNAP-2 score above 20 were similar between the 2 sites and did not change significantly after the adoption of the policy at Site 2.

Table 9: Difference-in-Difference linear regression model using the Placebo (SNAP-2 score) as the outcome

Coefficients **Death or BPD ≤29 weeks**	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Intercept	0.27	0.22 – 0.31	0.02
Exposed	0.05	-0.04 – 0.14	0.04
Epoch	0.01	-0.05 – 0.07	0.03
Interaction term	-0.06	-0.17 – 0.05	0.06

Coefficients **Death or BPD <26 weeks**	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Intercept	0.56	0.45 – 0.67	0.06
Exposed	0.01	-0.18 – 0.19	0.09
Epoch	-0.06	-0.20 – 0.08	0.07
Interaction term	0.001	-0.23 – 0.23	0.12

Legend of Table 9: DID regression with the placebo (in our case the rate of SNAP-2 score >20) as the outcome modeled for the exposure to site, epoch (2011-2013 vs. 2014-2017) and interaction between site and epoch. The interaction term between Epoch and Site Exposure is not, as expected significantly associated with the placebo outcome and, as such, the policy at Site 2 did not "impact" the distribution of SNAP-2 scores.

For the "*lead effect*" sensitivity analysis (changing the epochs as: Epoch 1 [2011-2012] and Epoch 2 [2013-2017]), we used a DID linear regression model, shown in **Table 10**. The results suggest that the outcome of interest (death or BPD) did not start changing in anticipation of the policy adoption, as the interaction term was not different from 0.

Table 10: Primary outcome by DID model using linear regression evaluating for a lead effect (using a model with epochs defined as: Epoch 1- 2011-2012 and Epoch 2 - 2013-2017):

≤29 weeks	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Intercept	0.52	0.46-0.58	0.03
Exposed	-0.22	-0.33 – -0.11	0.06
Epoch	0.02	-0.05 - 0.09	0.04
Interaction term	0.04	-0.09 – 0.17	0.06
<26 weeks	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Intercept	0.87	0.77 – 0.97	0.05
Exposed	-0.39	-0.60 - -0.17	0.11
Epoch	-0.04	-0.16 – 0.07	0.06
Interaction term	0.17	-0.07 – 0.41	0.12

Legend of Table 10: DID regression with the primary outcome of death and/or BPD modeled for the exposure to the site, epoch (2011-2012 vs. 2013-2017) and interaction between site and epoch. The interaction term between Epoch and Site Exposure is not significantly associated with the primary outcome in the overall cohort and in the <26 weeks subgroup. Hence, there is no evidence of a lead effect – primary outcome change in anticipation to the policy adoption.

Results of the "*lag effect*" sensitivity analysis (with epochs redefined as: Epoch 1 [2011-2014] and Epoch 2 [2015-2017]) are presented in **Table 11**. According to this DID model, the interaction term for the <26 weeks population reached statistical significance with a stronger association (interaction term point estimate of 0.35; 95% Confidence Interval 0.15-0.54), compared with the original model using the pre-determined Epochs (0.30 point-estimate [95% CI 0.11-0.49]). As expected, there is a lag effect. Indeed, as adherence to the policy increases, the robustness of the association between the policy change and the outcome of death and/or BPD increased.

Table 11: Primary outcome by DID model using linear regression evaluating for a lag effect (using a model with epochs defined as: Epoch 1- 2011-2014 and Epoch 2 - 2015-2017):

≤29 weeks	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Intercept	0.52	0.48 – 0.57	0.02
Exposed	-0.23	-0.31 – 0.16	0.04
Epoch	0.03	-0.04 – 0.10	0.04
Interaction term	0.08	-0.03 – 0.20	0.06
<26 weeks	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Intercept	0.85	0.79 – 0.92	0.03
Exposed	-0.44	-0.58 – -0.30	0.07
Epoch	-0.03	-0.14 – 0.06	0.05
Interaction term	**0.35**	**0.15-0.54**	**0.10**

Legend of Table 11: DID regression with the primary outcome of death and/or BPD modeled for the exposure to the site, epoch (2011-2012 vs. 2013-2017) and interaction between site and epoch. The interaction term between Epoch and Site Exposure is not significantly associated with the primary outcome in the overall cohort. The interaction term between Epoch and Site Exposure is significantly associated with the primary outcome in <26 weeks subgroup.

Secondary outcomes:

Rates for the secondary outcomes (death, NEC, "early death or severe IVH" and "death or severe IVH") are displayed in **Figures 13 to 16** for the overall cohort and for the <26 weeks sub-cohort. None of the graphs for the secondary outcomes suggested a noticeable impact following adoption of the policy at Site 2. The interaction terms did not reach statistical significance for any of the secondary outcomes (death, early death or severe IVH, death or severe IVH and NEC); results of the DID (linear) models are presented in **Tables 12 to 15**.

Figure 13 – Rates for severe IVH and/or death:

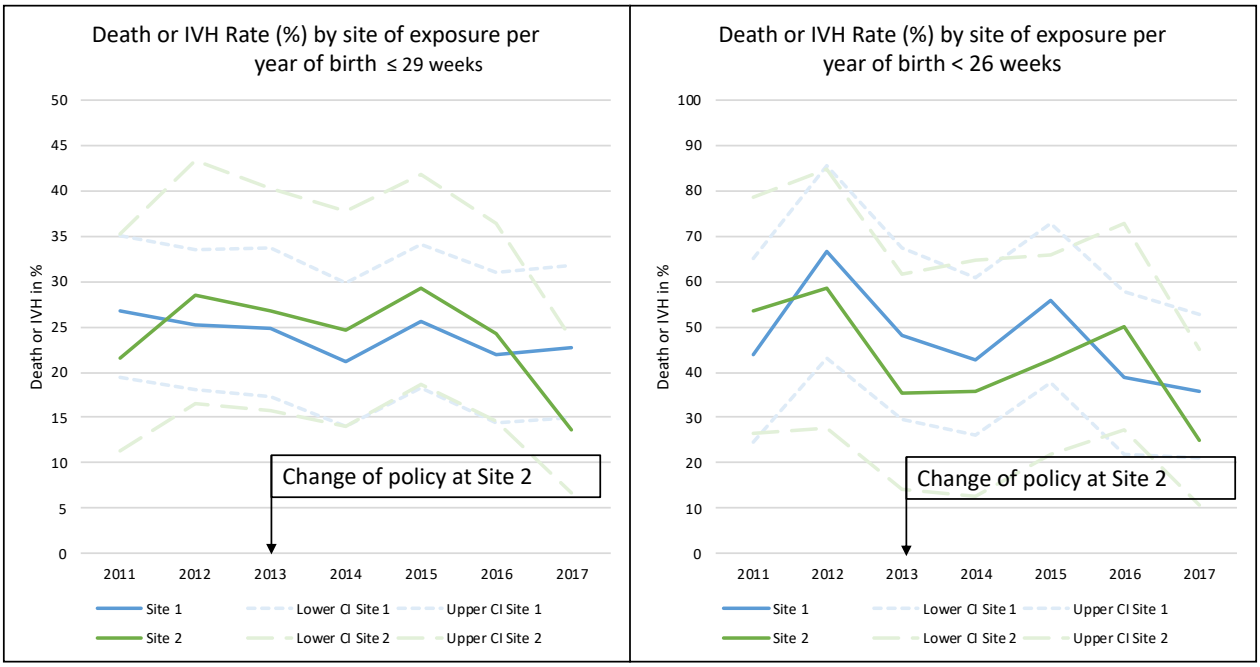
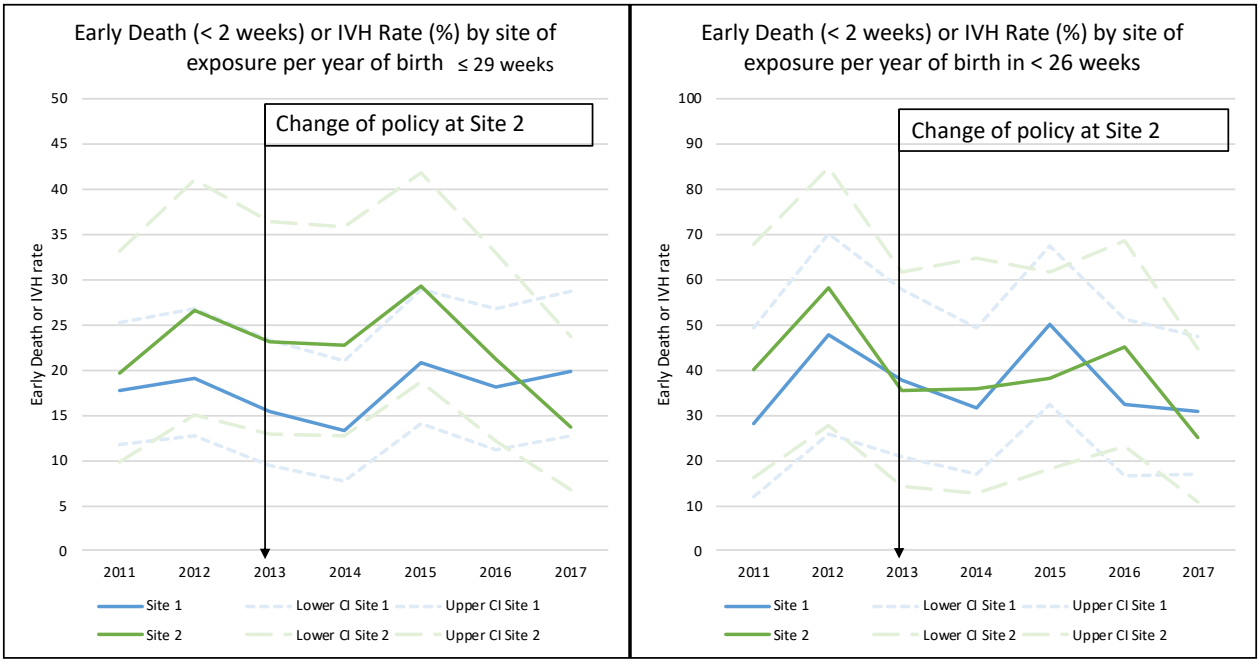


Figure 14 – Rates for Severe IVH and/or early death:



Legend of Figure 13 and 14: The secondary outcomes are graphically presented per year of birth and site. There are no graphical differences between sites in the trends appreciated after policy change at site 2 for any of the secondary outcomes.

Figure 15 – Death rates per Site and per year of birth before discharge:

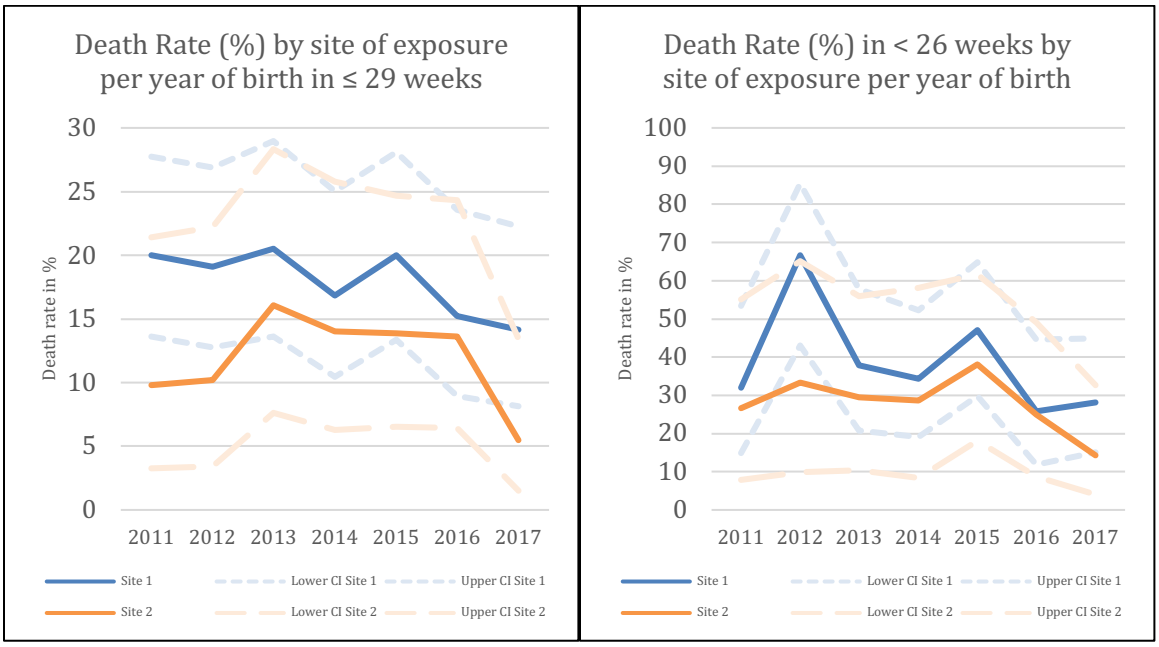
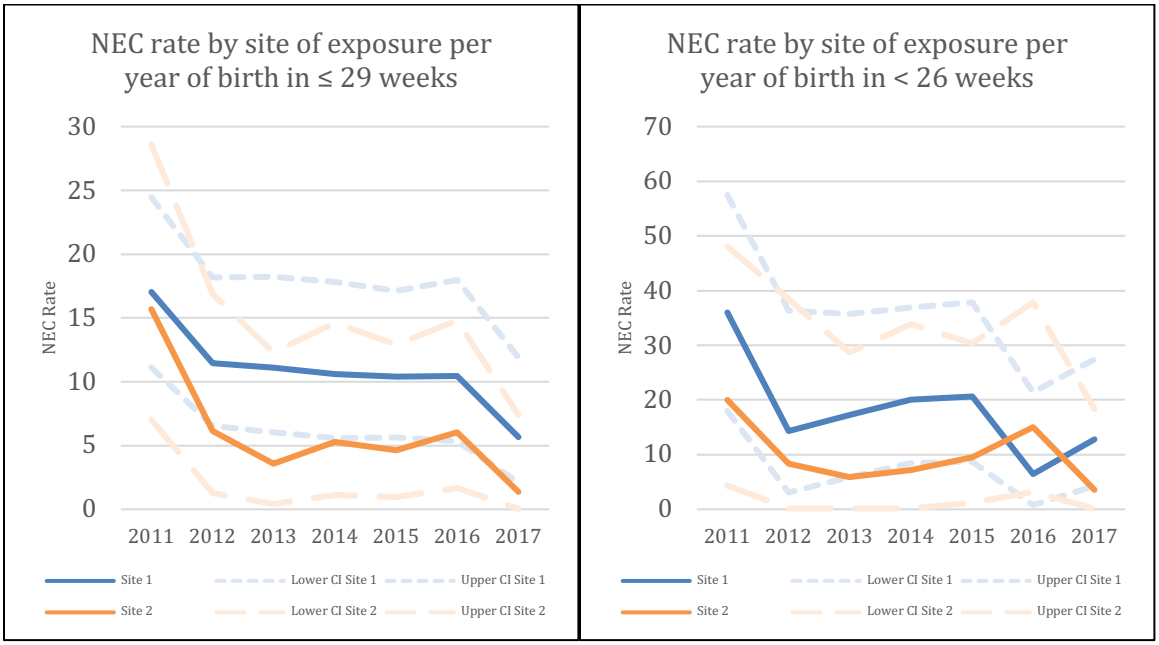


Figure 16 – Rates for NEC:



Legend of Figure 15 and 16: The secondary outcomes are graphically presented per year of birth and site. There are no graphical differences between sites in the trends appreciated after policy change at site 2 for any of the secondary outcomes.

Table 12: Difference in Difference linear regression for the secondary outcome of death

Difference in Difference linear regression for outcome: death				
	Coefficients Death or BPD	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Cohort ≤29 weeks	Intercept	0.20	0.16 – 0.24	0.02
	Exposed	-0.08	-0.14 – -0.01	0.03
	Epoch	-0.03	-0.08 – 0.02	0.03
	Interaction term	0.02	-0.06 – 0.11	0.04
Cohort <26 weeks	Intercept	0.43	0.34 – 0.53	0.05
	Exposed	-0.08	-0.23 – 0.07	0.08
	Epoch	-0.10	-0.22 – 0.02	0.06
	Interaction term	-0.004	-0.20 – 0.19	0.10

Table 13: Difference in Difference linear regression for the secondary outcome of early death or severe IVH

Difference in Difference linear regression for outcome: early death or severe IVH				
	Coefficients Death or BPD	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Cohort ≤29 weeks	Intercept	0.17	0.14 – 0.21	0.02
	Exposed	0.06	-0.02 – 0.13	0.04
	Epoch	0.01	-0.05 – 0.06	0.03
	Interaction term	-0.02	-0.12 – 0.08	0.05
Cohort <26 weeks	Intercept	0.42	0.33 – 0.51	0.05
	Exposed	0.06	-0.09 – 0.21	0.08
	Epoch	-0.06	-0.18 – 0.06	0.06
	Interaction term	-0.07	-0.27 – 0.13	0.10

Table 14: Difference in Difference linear regression for the secondary outcome of death before discharge or severe IVH

Difference in Difference linear regression for outcome: death before discharge or severe IVH				
	Coefficients Death or BPD	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Cohort ≤29 weeks	Intercept	-0.26	0.21 – 0.30	0.02
	Exposed	0.001	-0.08 – 0.08	0.04
	Epoch	-0.03	-0.09 – 0.03	0.03
	Interaction term	-0.004	-0.11 – 0.10	0.04
Cohort <26 weeks	Intercept	0.53	0.44 – 0.62	0.05
	Exposed	-0.01	-0.16 – 0.15	0.08
	Epoch	-0.10	-0.22 – 0.03	0.06
	Interaction term	-0.05	-0.25 – 0.15	0.10

Table 15: Difference in Difference linear regression for the secondary outcome of NEC

Difference in Difference linear regression for outcome: NEC stage 2 or above				
	Coefficients Death or BPD	**Point Estimate (β)**	**95%CI**	**Standard Error (Robust)**
Cohort ≤29 weeks	Intercept	0.13	0.10 – 0.17	0.02
	Exposed	-0.05	-0.11 – 0.01	0.03
	Epoch	-0.04	-0.08 – 0.00	0.02
	Interaction term	-0.002	-0.07 – 0.06	0.03
Cohort <26 weeks	Intercept	-0.23	0.15 – 0.30	0.04
	Exposed	-0.12	-0.23 – -0.01	0.06
	Epoch	-0.08	-0.17 – 0.02	0.05
	Interaction term	0.05	-0.09 – 0.19	0.07

10 Discussion

In this study, we examined the impact of a PDA non-intervention policy on the composite outcome of death or BPD. The analysis included 1249 newborns ≤29 weeks born in 2011 to 2017 and admitted at two comparable sites, including 341 born <26 weeks EGA. After taking into account time-invariant differences between sites and secular trends, we saw no change in death or BPD after policy change in the entire cohort of newborns. However, death or BPD increased significantly among infants born at <26 weeks. The distribution of gestational age at birth between groups in each epoch were similar. Adherence to policy indicated that use of NSAIDs and ligation declined progressively to 0% at Site 2, the exposure group, and remained stable at Site 1, the control group. Our sensitivity analyses corroborated the finding that, among newborns <26 weeks EGA at birth, the policy of non-intervention was associated with an increase in the primary outcome of death or BPD.

While there have been a few reports of premature newborns managed conservatively (9-11, 149), this is the largest cohort of newborns evaluated using a DID model, best suited to avoid the inherent assumptions of pre-post studies (no time-varying confounders extrinsic to the analyzed intervention) and the confounding by indication typically present in observational comparative studies.

10.1 The case for spontaneous closure

Historically, case series regarding an association between ductal ligation and improvement in respiratory clinical status, suggested that interventions accelerating PDA closure may lead to a decline in the rate of death or BPD in vulnerable premature newborns (2, 150-152). Following these reports, publications described the role of prostaglandins in maintaining the patency of the ductus in patients with congenital heart defects (153) and the induction of its closure using NSAIDs (154). However, as highlighted in a recent meta-analysis by Benitz & Bhombal (2) and the AAP statement by the same first author recommending against the use of NSAIDs in premature infants during their first 2 weeks of life (1), no

evidence of benefit for death or BPD has emerged from the more than 50 trials that have been carried out. Since the release of these recommendations, publications (9-11) describing cohorts of premature newborns non-exposed or partly exposed to NSAIDs have reported a high rate of spontaneous closure of the PDA before hospital discharge.

Rolland *et al.* described a cohort of 91 premature newborns <29 weeks, who survived their first 72 hours of life and who were managed without NSAIDs to describe rate of spontaneous closure (10). PDA was found to still be open in 40% of infants <26 weeks, compared with 20% of the $26^{0/7}$–$27^{6/7}$ weeks, but this cohort did not include infants <24 weeks, and only 31 were <26 weeks. Death or BPD occurred in 52% of the overall cohort. The authors mention that they could not exclude that the conservative approach may have resulted in increased mortality, since some patients died from pulmonary hemorrhages and severe IVH, complications that are often "attributed" to the PDA in premature newborns. Our findings suggest that a conservative management policy was, indeed, associated with a rise in death or BPD in the <26 weeks population (which, compared to Rolland *et al.*, included very vulnerable patients <24 weeks). Pulmonary hemorrhages leading to mortality are catastrophic but rare events, hypothesized to be secondary to the excessive pulmonary blood flow and pressure via the PDA. In our population, early death (sometimes associated with death secondary to pulmonary hemorrhages) or severe IVH did not change following the adoption of the conservative policy. It is plausible that, with a population of only 83 newborns <26 weeks exposed to the conservative policy (epoch 2, site 2), the power was not sufficient to evaluate for these outcomes individually. The aforementioned placebo-controlled RCT evaluating early indomethacin treatment (based on ductal size by echocardiography), in a cohort <29 weeks, demonstrated a significant reduction in pulmonary hemorrhages within the first 72 hours of life in the treatment group (23). The overall number of pulmonary hemorrhages was similar between groups, but there was a high open-label use of indomethacin after 72 hours of life in the placebo arm. The trial ended prematurely due to the sudden unavailability of indomethacin in the Australian market, which led to insufficient recruitment (92 out of

the anticipated 340), thus limiting further analyses (including stratification by gestational age). In this cohort, there was no significant difference in the use of oxygen or respiratory support at 36 weeks PMA (indomethacin group: 25% [11/44] vs. placebo group: 31% [15/48]). The authors did not provide the numbers for the combined outcome of death or BPD (as some of the newborns who died might have had BPD), or the breakdown by gestational age.

Another retrospective analysis of a cohort of 195 newborns <29 weeks managed conservatively, and who had survived to discharge, described the rate of spontaneous closure of the PDA (9). Those born <25 weeks were exposed to an open PDA for much longer (average of 56 days at closure [range 24-200]), compared with infants who had been born at 25-26 weeks (41 days [range 11-70]) and ≥27 weeks (36 days post-natal [range 7 to 70]). The authors defined a hemodynamically significant PDA (hsPDA) as ≥2 mm in the context of respiratory support requirements. The majority of the <25-week babies were found to have a hsPDA (93% [50/54]). In the overall cohort, the authors did not find an association between the duration per week of exposure to hsPDA and the outcome of BPD (odds ratio of 1.27 [95% CI: 0.96 – 1.68, p=0.09]). BPD occurred in 37% of patients with a hsPDA compared with 21% without, and those with a hsPDA were much younger (24.8 ± 1.3 vs. 26.7 ± 1.2 weeks). Although spontaneous closure was observed in the majority of infants in this cohort, these results indicate that the most premature newborns are those with the most long-lasting patency of the ductus and with an increased likelihood for a hsPDA. Thus, it is possible that, with a greater sample size, the study would have found a significant association between duration of exposure to a hsPDA and BPD, specifically in the very premature infants (authors did not stratify by EGA). Our results indicate that a conservative management did not impact the outcomes of the ≥26 weeks, who generally have a lower frequency of hsPDA and earlier closure of PDA. However, with long-lasting exposure to an open hsPDA, the lower EGA newborns may have increased risk for significant lung injury explaining the finding in our cohort. Similarly, the meta-analysis by Benitz *et al.* mentioned that the subgroup of

extremely premature newborns, who may have the most significant ductal shunting, have not been well captured within the included trials and may (or not) benefit from early closure (2).

The latest available report describing the natural history of PDA in 297 premature newborns (≤1500 grams) also described an increased duration of ductal patency in the <26 weeks subgroup with an average of 71 days (95% CI: 51–91) (11). These babies were partly exposed to NSAIDs or ligation (6% [17/297]) and 15% of those managed conservatively (43/280) had an open ductus at hospital discharge. Only 48 newborns <26 weeks were included and 32% (15/48) of them had open ductus at hospital discharge (compared with 12% [28/232] among the ≥26 weeks). In the overall cohort, BPD was found in 19% of those with a patent ductus at discharge, vs. 10% in those who had a spontaneous closure (p=0.123). Again, it is possible that, with a larger population of babies and by stratifying for those born <26 weeks, the outcome of BPD would have reached statistical significance. Although most newborns ≥26 weeks will have spontaneous closure of the PDA, the majority of babies in the category that is considered to be the most immature (<26 weeks) is exposed to a prolonged patency and its potential respiratory and physiological effects. These factors may explain why we found an increase in death or BPD in our <26 weeks subgroup managed without NSAIDs or ligation.

10.2 PDA approach and adverse outcomes

Beyond observational studies describing the natural history of PDA, recent publications have analyzed the differences in outcomes based on exposure to various PDA management strategies. These reports must be interpreted with caution, due to the likely presence of confounding by indication. An analysis of infants born at 400 to 999 grams in 16 neonatal units of the Brazilian Neonatal Research Network reported an association between the conservative approach and the combined outcome of death or BPD (pharmacological approach: Odds Ratio 0.29, 95% CI: 0.14 to 0.62) (155). This cohort of patients included newborns <33 weeks and, as such, had some relatively

more mature babies born with intra-uterine growth restriction compared with the reports described above. This retrospective analysis comprised newborns who had a PDA confirmed by echocardiography and categorized in 3 groups: conservative management (n=187), NSAIDs use (n=205) and surgical ligation (n=102). Death or BPD was found in 72% (134/187) in the conservative group, compared with 58% (119/205) in the NSAIDs group. These results may support the finding in our study indicating that the introduction of a conservative approach in the very vulnerable premature newborns may be associated with a rise in death or BPD.

The impact of screening for PDA has also been evaluated in the context of the EPIPAGE 2 cohort, which is a population-based prospective study carried out in 68 French neonatal units in 2011 (156). Premature infants were categorized according to whether or not they were screened by echocardiography before 72 hours of life. The authors included 1513 patients <29 weeks in their analysis. Death rate in those screened was lower (14.2% vs. 18.5%; Odds ratio of 0.73 - 95% CI: 0.54 to 0.98), with a number needed to test estimated at 23. Similarly, Hagadorn *et al.* (157) attempted to evaluate the impact of PDA management using the California Perinatal Quality Care Collaborative database, which collects data on >90% of infants 400 to 1500 grams born in California. Authors included, in their analysis, 32 094 infants born between 2008 and 2015. Among infants born 400 to 749 grams (which usually corresponds to infants born <26 weeks EGA), each percentage-point reduction in unit-specific proportion of NSAIDs or ligation use was associated with a significant dose-response increase of 0.21%-point increase (95% CI: 0.06 - 0.33 %) in adjusted mortality. Although the secondary outcome of "absolute rate of death" did not reach statistical significance in our model, the results of these studies support the concern that we raise regarding the safety of a conservative management policy in the most premature newborns and suggests that some of these babies may benefit from selective PDA treatment.

Furthermore, regarding later introduction of NSAIDs administration, the impact of treating after the first week of life has also been evaluated in an observational prospective study recruiting

newborns <28 weeks (n=397) (158). A cohort exposed to indomethacin prophylaxis (n=247), from 2005 to 2011, was compared with infants not treated until 8 days of life (n=150), from 2011 to 2016. In the <26 weeks, permanent closure was achieved in 2 days (25–75th percentile: 2–5 days) during the prophylactic indomethacin epoch and in 25 days (25–75th percentile: 11–47 days) during the later treatment epoch. The authors reported a significant increase in death or BPD in the later treatment epoch (RR=0.784 [95%CI: 0.624–0.945]), which supports the concept that longer duration of patency is associated with increased risk of death or BPD. One must interpret these results with caution due to the inherent biases introduced by a pre-post study design. However, the association of death or BPD with the more conservative approach to PDA management supports our findings in <26 weeks subgroup.

10.3 The conservative treatment – equipoise regarding the extreme premature

The recent PDA-TOLERATE trial was a multicenter (17 sites) RCT that recruited infants born at $23^{0/7}$ to $27^{6/7}$ weeks with a PDA confirmed by echocardiography at 6 to 14 days of post-natal life (159). Infants were randomized to early treatment (indomethacin, ibuprofen, or acetaminophen depending on clinician's or unit's preference) or to conservative management (no placebo). The study was unblinded and excluded newborns who had either died (n=187) or were treated for PDA in the first 7 days of life. Although reasons for exclusions were not specified, 729 infants (some of whom might have been treated) were excluded due to the absence of a moderate-to-large PDA at study entry. In the trial, specified "rescue" criteria (inotrope-dependent hypotension, persistent oliguria, prolonged use of gavage and requirement for respiratory support at specific post-natal ages) allowed for administration of PDA treatment in the conservative management arm. Indeed, the authors postulated that some patients had such important ductus that it warranted treatment (even in the conservative arm), based on the aforementioned criteria – which describe symptoms that the authors attributed to the effect of the PDA (they write that *"our study investigators felt there were certain conditions that justified rescue PDA*

treatment even in infants assigned to the conservative group" (159)). Furthermore, 181 newborns were excluded due to "lack of equipoise" by the physician taking care of the patient (the physician wanting to treat in 86% of cases and not wanting to treat in the rest). The final cohort included 202 patients (only 11% of all the <28 weeks cohort identified initially). Conservative management was applied to 49% (98 patients), of whom half (n=48) received "rescue" treatment. They were compared with 104 infants in the treatment arm. The authors did not find any major differences between both groups. In the <26 weeks, death or BPD was found in 75% (conservative) compared with 69% ("early" treatment), not reaching statistical significance. The use of "rescue" criteria in the PDA-TOLERATE RCT raises concerns about the equipoise regarding treatment in those newborns with large and persistent PDA, who generally are the newborns <26 weeks who were underrepresented in the meta-analysis that supported the AAP recommendations. The PDA-TOLERATE investigators also published the results of the 181 newborns comprising the "lack of equipoise" group (160) and found that these infants were of lower EGA (25.8 ± 1.2 vs. 25.5 ± 1.2 weeks; p=0.02) and had increased need for respiratory support (compared with the newborns included in the RCT). Yet, these infants had a lower death rate than the infants included in the study (RCT group: 14% vs. "Lack of equipoise" group: 3%). Furthermore, infants that were treated before day 6 had a significantly lower rate of BPD (OR 0.28; 95% CI: 0.11-0.68) and of the combined outcome of death or BPD (OR 0.26; 95% CI: 0.11-0.63) (160). Babies born at <26 weeks comprised 69% of the group treated earlier (day 5 of life or less) and 58% of the group treated after day 6 of life (p=0.01). These results suggest that the more preterm newborns treated early had decreased rates of death or BPD, in agreement with our findings of increased adverse outcome following the adoption of the conservative management policy in our study.

10.4 Strengths and limitations of our study

We used a DID model to evaluate a policy of non-intervention for the PDA adopted at a quaternary care institution in 2013. This quasi-experimental design uses pre- and post-intervention

trends, comparing them to a similar population not exposed to the policy change. Strengths of the study included that our data fitted with the model used and the parallel trend assumption of the outcomes was verified. Also, the two centers have strong similarities and the definition and collection of variables are standardized. No evidence of violations of the assumptions were found. All sensitivity analysis pointed towards the same direction of effect between conservative management and a rise in the primary outcome in the <26 weeks subgroup. This design is quasi-experimental and avoids confounding by indication, as well as inherent assumptions of pre-post studies. Although only two centres were included in our study (due to their similarities and availability of the data), this is a relatively large population for a study in the premature population. The results are limited by the small population for the subgroup <26 weeks. The cumulative dose of steroids and/or NSAIDs per patient was not available. Neither site had an official protocol for PDA screening in place during the entire study period. Hence, it is difficult to ascertain the incidence of a PDA and of a hemodynamically significant PDA, as well as the time to closure of the PDA in our cohort. Further granular information about the severity of the respiratory illness and the causes of death in our population were not available. As such, the severity of BPD may have changed over time and is not captured by this study.

10.5 Future directions

Recent studies have evaluated the role of acetaminophen on the ductus arteriosus, in the hope of avoiding the side-effects associated with NSAIDs in preterm newborns. Indeed, a meta-analysis of 8 RCTs found that acetaminophen had similar efficacy compared with indomethacin and ibuprofen to achieve ductal closure in premature newborns, with fewer side effects (decreased gastro-intestinal bleed and decreased serum creatinine concentration) (161). Future studies should evaluate if the selective use of acetaminophen, based on echocardiographic assessment of the PDA in the <26 weeks premature newborns, leads to improved outcomes with a more favorable side-effect profile (compared with NSAIDs). Furthermore, current trials are evaluating the use of the Amplatzer Piccolo Occluder for the catheter-based closure of the PDA in newborns beyond 72 hours of life and at a weight above 700 grams

(162, 163). Future studies should evaluate if the conservative management policy is associated with an increase in pulmonary hypertension at 36 weeks PMA or in pulmonary hemorrhages leading to mortality. These variables were not recorded in our local databases, due to the lack of consensus regarding their definition. Also, the long-term impact on neurodevelopment of a conservative management policy should be further investigated.

11 Conclusion

Premature newborns are at high risk of mortality and various morbidities. Although the PDA has been associated with some of the adverse outcomes of prematurity, previous studies have failed to show an improvement with the use of NSAIDs or ligation. Current guidelines recommend against the use of treatment of premature newborns in the first two weeks of life, regardless of gestational age at birth.

Our study of infants born premature 29 weeks and less, based on a quasi-experimental model emulating a RCT, indicated that a conservative management policy of the PDA was associated with a rise in death or BPD in infants born at less than 26 weeks, but not in the overall cohort. Our results indicate that abandoning the use of NSAIDs or ligation in the population born at $26^{0/7}$ weeks or more is not associated with a rise in adverse outcomes. Thus, our results do not support the practice of attempting PDA closure in this more mature cohort of newborns but suggests that it may be beneficial in infants born <26 weeks. As our study included only 341 <26 weeks, our results should be further corroborated in this more vulnerable subpopulation.

12 References

1. Benitz WE, Committee on F, Newborn AAoP. Patent Ductus Arteriosus in Preterm Infants. *Pediatrics* 2016; 137.
2. Benitz WE, Bhombal S. The use of non-steroidal anti-inflammatory drugs for patent ductus arteriosus closure in preterm infants. *Seminars in fetal & neonatal medicine* 2017; 22: 302-307.
3. Prescott S, Keim-Malpass J. Patent Ductus Arteriosus in the Preterm Infant: Diagnostic and Treatment Options. *Adv Neonatal Care* 2017; 17: 10-18.
4. Steinhorn RH. Diagnosis and treatment of pulmonary hypertension in infancy. *Early human development* 2013; 89: 865-874.
5. Mathew B, Lakshminrusimha S. Persistent Pulmonary Hypertension in the Newborn. *Children (Basel)* 2017; 4: 63.
6. Schneider DJ. The patent ductus arteriosus in term infants, children, and adults. *Semin Perinatol* 2012; 36: 146-153.
7. Gournay V. The ductus arteriosus: physiology, regulation, and functional and congenital anomalies. *Arch Cardiovasc Dis* 2011; 104: 578-585.
8. Semberova J, Sirc J, Miletin J, Kucera J, Berka I, Sebkova S, O'Sullivan S, Franklin O, Stranak Z. Spontaneous closure of patent ductus arteriosus in infants≤ 1500 g. *Pediatrics* 2017: e20164258.
9. Sung SI, Chang YS, Kim J, Choi JH, Ahn SY, Park WS. Natural evolution of ductus arteriosus with noninterventional conservative management in extremely preterm infants born at 23-28 weeks of gestation. *PLoS One* 2019; 14: e0212256.
10. Rolland A, Shankar-Aguilera S, Diomande D, Zupan-Simunek V, Boileau P. Natural evolution of patent ductus arteriosus in the extremely preterm infant. *Archives of disease in childhood Fetal and neonatal edition* 2015; 100: F55-58.
11. Semberova J, Sirc J, Miletin J, Kucera J, Berka I, Sebkova S, O'Sullivan S, Franklin O, Stranak Z. Spontaneous Closure of Patent Ductus Arteriosus in Infants </=1500 g. *Pediatrics* 2017; 140.
12. Hajj H, Dagle JM. Genetics of patent ductus arteriosus susceptibility and treatment. Seminars in perinatology: Elsevier; 2012. p. 98-104.
13. Freudenthal FP, Heath A, Villanueva J, Mendes J, Vicente X, von Alvensleben I, Echazu G, Navarro J, Lang N, Kozlik-Feldmann R. Chronic hypobaric hypoxia, patent arterial duct and a new interventional technique to close it. *Cardiology in the Young* 2012; 22: 128-135.
14. Hoffman JI, Kaplan S. The incidence of congenital heart disease. *Journal of the American college of cardiology* 2002; 39: 1890-1900.
15. Lloyd TR, Beekman RH. Clinically silent patent ductus arteriosus. *American heart journal* 1994; 127: 1664.
16. Nora JJ. Multifactorial inheritance hypothesis for the etiology of congenital heart diseases. *Circulation* 1968; 38: 604-617.
17. Gournay V. The ductus arteriosus: physiology, regulation, and functional and congenital anomalies. *Archives of cardiovascular diseases* 2011; 104: 578-585.
18. Krichenko A, Benson LN, Burrows P, Möes C, McLaughlin P, Freedom RM. Angiographic classification of the isolated, persistently patent ductus arteriosus and implications for percutaneous catheter occlusion. *The American journal of cardiology* 1989; 63: 877-880.
19. El-Khuffash A, James AT, Corcoran JD, Dicker P, Franklin O, Elsayed YN, Ting JY, Sehgal A, Malikiwi A, Harabor A, Soraisham AS, McNamara PJ. A Patent Ductus Arteriosus Severity Score Predicts Chronic Lung Disease or Death before Discharge. *J Pediatr* 2015; 167: 1354-1361.e1352.
20. Fink D, El-Khuffash A, McNamara PJ, Nitzan I, Hammerman C. Tale of Two Patent Ductus Arteriosus Severity Scores: Similarities and Differences. *Am J Perinatol* 2018; 35: 55-58.

21. Urquhart DS, Nicholl RM. How good is clinical examination at detecting a significant patent ductus arteriosus in the preterm neonate? *Archives of disease in childhood* 2003; 88: 85-86.
22. Philip R, Nathaniel Johnson J, Naik R, Kimura D, Boston U, Chilakala S, Hendrickson B, Rush Waller B, Sathanandam S. Effect of patent ductus arteriosus on pulmonary vascular disease. *Congenit Heart Dis* 2019; 14: 37-41.
23. Kluckow M, Jeffery M, Gill A, Evans N. A randomised placebo-controlled trial of early treatment of the patent ductus arteriosus. *Archives of disease in childhood Fetal and neonatal edition* 2014; 99: F99-f104.
24. Jain A, Shah PS. Diagnosis, Evaluation, and Management of Patent Ductus Arteriosus in Preterm Neonates. *JAMA Pediatr* 2015; 169: 863-872.
25. Clyman RI, Mauray F, Heymann MA, Roman C. Cardiovascular effects of patent ductus arteriosus in preterm lambs with respiratory distress. *The Journal of pediatrics* 1987; 111: 579-587.
26. Shimada S, Kasai T, Konishi M, Fujiwara T. Effects of patent ductus arteriosus on left ventricular output and organ blood flows in preterm infants with respiratory distress syndrome treated with surfactant. *The Journal of pediatrics* 1994; 125: 270-277.
27. Lemmers PM, Benders MJ, D'Ascenzo R, Zethof J, Alderliesten T, Kersbergen KJ, Isgum I, de Vries LS, Groenendaal F, van Bel F. Patent Ductus Arteriosus and Brain Volume. *Pediatrics* 2016; 137.
28. Chock VY, Rose LA, Mante JV, Punn R. Near-infrared spectroscopy for detection of a significant patent ductus arteriosus. *Pediatr Res* 2016; 80: 675-680.
29. Dix L, Molenschot M, Breur J, de Vries W, Vijlbrief D, Groenendaal F, van Bel F, Lemmers P. Cerebral oxygenation and echocardiographic parameters in preterm neonates with a patent ductus arteriosus: an observational study. *Arch Dis Child Fetal Neonatal Ed* 2016.
30. Martin CG, Snider AR, Katz SM, Peabody JL, Brady JP. Abnormal cerebral blood flow patterns in preterm infants with a large patent ductus arteriosus. *The Journal of pediatrics* 1982; 101: 587-593.
31. Lemmers PM, Toet MC, van Bel F. Impact of patent ductus arteriosus and subsequent therapy with indomethacin on cerebral oxygenation in preterm infants. *Pediatrics* 2008; 121: 142-147.
32. Laughon M, O'Shea MT, Allred EN, Bose C, Kuban K, Van Marter LJ, Ehrenkranz RA, Leviton A. Chronic lung disease and developmental delay at 2 years of age in children born before 28 weeks' gestation. *Pediatrics* 2009; 124: 637-648.
33. Altit G, Dancea A, Renaud C, Perreault T, Lands LC, Sant'Anna G. Pathophysiology, screening and diagnosis of pulmonary hypertension in infants with bronchopulmonary dysplasia-a review of the literature. *Paediatric respiratory reviews* 2017; 23: 16-26.
34. Shah P, Yoon EW, Chan P. Annual Report 2014. *The Canadian Neonatal Network* 2014: 15.
35. Lemyre B, Moore G. Counselling and management for anticipated extremely preterm birth. *Paediatrics & child health* 2017; 22: 334-341.
36. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, Laptook AR, Sanchez PJ, Van Meurs KP, Wyckoff M, Das A, Hale EC, Ball MB, Newman NS, Schibler K, Poindexter BB, Kennedy KA, Cotten CM, Watterberg KL, D'Angio CT, DeMauro SB, Truog WE, Devaskar U, Higgins RD. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. *Jama* 2015; 314: 1039-1051.
37. (CNN) TCNN. Annual Report. 2015, 2016, 2017.
38. Society CC. Childhood leukemia. 2019.
39. Harrison MS, Goldenberg RL. Global burden of prematurity. *Seminars in fetal & neonatal medicine* 2016; 21: 74-79.
40. Baraldi E, Filippone M. Chronic lung disease after premature birth. *N Engl J Med* 2007; 357: 1946-1955.

41. Mirza H, Ziegler J, Ford S, Padbury J, Tucker R, Laptook A. Pulmonary hypertension in preterm infants: prevalence and association with bronchopulmonary dysplasia. *J Pediatr* 2014; 165: 909-914 e901.
42. Altit G, Dancea A, Renaud C, Perreault T, Lands LC, Sant'Anna G. Pathophysiology, screening and diagnosis of pulmonary hypertension in infants with bronchopulmonary dysplasia - A review of the literature. *Paediatric respiratory reviews* 2017; 23: 16-26.
43. Abman SH, Hansmann G, Archer SL, Ivy DD, Adatia I, Chung WK, Hanna BD, Rosenzweig EB, Raj JU, Cornfield D. Pediatric pulmonary hypertension. *Circulation* 2015; 132: 2037-2099.
44. Patel RM, Kandefer S, Walsh MC, Bell EF, Carlo WA, Laptook AR, Sánchez PJ, Shankaran S, Van Meurs KP, Ball MB. Causes and Timing of Death in Extremely Premature Infants from 2000 through 2011. *New England Journal of Medicine* 2015; 372: 331-340.
45. Vollsæter M, Clemm HH, Satrell E, Eide GE, Røksund OD, Markestad T, Halvorsen T. Adult respiratory outcomes of extreme preterm birth. A regional cohort study. *Annals of the American Thoracic Society* 2015; 12: 313-322.
46. Lazdam M, de la Horra A, Pitcher A, Mannie Z, Diesch J, Trevitt C, Kylintireas I, Contractor H, Singhal A, Lucas A, Neubauer S, Kharbanda R, Alp N, Kelly B, Leeson P. Elevated blood pressure in offspring born premature to hypertensive pregnancy: is endothelial dysfunction the underlying vascular mechanism? *Hypertension* 2010; 56: 159-165.
47. Alvarez-Fuente M, Arruza L, Muro M, Zozaya C, Avila A, Lopez-Ortego P, Gonzalez-Armengod C, Torrent A, Gavilan JL, Del Cerro MJ. The economic impact of prematurity and bronchopulmonary dysplasia. *European journal of pediatrics* 2017; 176: 1587-1593.
48. Groves AM, Kuschel CA, Knight DB, Skinner JR. Does retrograde diastolic flow in the descending aorta signify impaired systemic perfusion in preterm infants? *Pediatric research* 2008; 63: 89-94.
49. Dollberg S, Lusky A, Reichman B, Network IN. Patent ductus arteriosus, indomethacin and necrotizing enterocolitis in very low birth weight infants: a population-based study. *Journal of pediatric gastroenterology and nutrition* 2005; 40: 184-188.
50. Cassady G, Crouse DT, Kirklin JW, Strange MJ, Joiner CH, Godoy G, Odrezin GT, Cutter GR, Kirklin JK, Pacifico AD, et al. A randomized, controlled trial of very early prophylactic ligation of the ductus arteriosus in babies who weighed 1000 g or less at birth. *N Engl J Med* 1989; 320: 1511-1516.
51. de Freitas Martins F, Ibarra Rios D, MH FR, Javed H, Weisz D, Jain A, de Andrade Lopes JM, McNamara PJ. Relationship of Patent Ductus Arteriosus Size to Echocardiographic Markers of Shunt Volume. *J Pediatr* 2018; 202: 50-55.e53.
52. Schena F, Francescato G, Cappelleri A, Picciolli I, Mayer A, Mosca F, Fumagalli M. Association between Hemodynamically Significant Patent Ductus Arteriosus and Bronchopulmonary Dysplasia. *The Journal of pediatrics* 2015; 166: 1488-1492.
53. Vincent RN, Bauser-Heaton H. Transcatheter Echocardiographic-Guided Closure of Patent Ductus Arteriosus in Extremely Premature Newborns. *JACC Cardiovascular interventions* 2016; 9: 2438-2439.
54. Neumann R, Schulzke SM, Buhrer C. Oral ibuprofen versus intravenous ibuprofen or intravenous indomethacin for the treatment of patent ductus arteriosus in preterm infants: a systematic review and meta-analysis. *Neonatology* 2012; 102: 9-15.
55. Pezzati M, Vangi V, Biagiotti R, Bertini G, Cianciulli D, Rubaltelli FF. Effects of indomethacin and ibuprofen on mesenteric and renal blood flow in preterm infants with patent ductus arteriosus. *The Journal of pediatrics* 1999; 135: 733-738.
56. van Bel F, Guit GL, Schipper J, van de Bor M, Baan J. Indomethacin-induced changes in renal blood flow velocity waveform in premature infants investigated with color Doppler imaging. *The Journal of pediatrics* 1991; 118: 621-626.

57. Van Bel F, Van de Bor M, Stijnen T, Baan J, Ruys JH. Cerebral blood flow velocity changes in preterm infants after a single dose of indomethacin: duration of its effect. *Pediatrics* 1989; 84: 802-807.
58. Stavel M, Wong J, Cieslak Z, Sherlock R, Claveau M, Shah PS. Effect of prophylactic indomethacin administration and early feeding on spontaneous intestinal perforation in extremely low-birth-weight infants. *Journal of perinatology : official journal of the California Perinatal Association* 2017; 37: 188-193.
59. Achanti B, Yeh TF, Pildes RS. Indomethacin therapy in infants with advanced postnatal age and patent ductus arteriosus. *Clinical and investigative medicine Medecine clinique et experimentale* 1986; 9: 250-253.
60. McCarthy JS, Zies LG, Gelband H. Age-dependent closure of the patent ductus arteriosus by indomethacin. *Pediatrics* 1978; 62: 706-712.
61. Ohlsson A, Walia R, Shah SS. Ibuprofen for the treatment of patent ductus arteriosus in preterm or low birth weight (or both) infants. *The Cochrane database of systematic reviews* 2015: CD003481.
62. Mirza H, Oh W, Laptook A, Vohr B, Tucker R, Stonestreet BS. Indomethacin prophylaxis to prevent intraventricular hemorrhage: association between incidence and timing of drug administration. *The Journal of pediatrics* 2013; 163: 706-710 e701.
63. Ment LR, Oh W, Ehrenkranz RA, Philip AG, Vohr B, Allan W, Duncan CC, Scott DT, Taylor KJ, Katz KH, et al. Low-dose indomethacin and prevention of intraventricular hemorrhage: a multicenter randomized trial. *Pediatrics* 1994; 93: 543-550.
64. Schmidt B, Asztalos EV, Roberts RS, Robertson CM, Sauve RS, Whitfield MF. Impact of bronchopulmonary dysplasia, brain injury, and severe retinopathy on the outcome of extremely low-birth-weight infants at 18 months: results from the trial of indomethacin prophylaxis in preterms. *Jama* 2003; 289: 1124-1129.
65. Schmidt B, Davis P, Moddemann D, Ohlsson A, Roberts RS, Saigal S, Solimano A, Vincer M, Wright LL. Long-term effects of indomethacin prophylaxis in extremely-low-birth-weight infants. *N Engl J Med* 2001; 344: 1966-1972.
66. Benitz W. Learning to live with patency of the ductus arteriosus in preterm infants. *Journal of Perinatology* 2011; 31: S42.
67. Benitz WE. Treatment of persistent patent ductus arteriosus in preterm infants: time to accept the null hypothesis? *Journal of perinatology : official journal of the California Perinatal Association* 2010; 30: 241-252.
68. Benitz WE. Patent ductus arteriosus: to treat or not to treat? *Archives of disease in childhood Fetal and neonatal edition* 2012; 97: F80-82.
69. Clyman RI, Saha S, Jobe A, Oh W. Indomethacin prophylaxis for preterm infants: the impact of 2 multicentered randomized controlled trials on clinical practice. *The Journal of pediatrics* 2007; 150: 46-50.e42.
70. Nelin TD, Pena E, Giacomazzi T, Lee S, Logan JW, Moallem M, Bapat R, Shepherd EG, Nelin LD. Outcomes following indomethacin prophylaxis in extremely preterm infants in an all-referral NICU. *Journal of perinatology : official journal of the California Perinatal Association* 2017; 37: 932-937.
71. Altit G, Basso O, Grandi SM, Yang S. Letter to the editor. *Journal of perinatology : official journal of the California Perinatal Association* 2018.
72. Mitra S, Florez ID, Tamayo ME, Mbuagbaw L, Vanniyasingam T, Veroniki AA, Zea AM, Zhang Y, Sadeghirad B, Thabane L. Association of Placebo, Indomethacin, Ibuprofen, and Acetaminophen With Closure of Hemodynamically Significant Patent Ductus Arteriosus in Preterm Infants: A Systematic Review and Meta-analysis. *Jama* 2018; 319: 1221-1238.

73. Gersony WM, Peckham GJ, Ellison RC, Miettinen OS, Nadas AS. Effects of indomethacin in premature infants with patent ductus arteriosus: results of a national collaborative study. *J Pediatr* 1983; 102: 895-906.
74. Alfaleh K, Smyth JA, Roberts RS, Solimano A, Asztalos EV, Schmidt B, Trial of Indomethacin Prophylaxis in Preterms I. Prevention and 18-month outcomes of serious pulmonary hemorrhage in extremely low birth weight infants: results from the trial of indomethacin prophylaxis in preterms. *Pediatrics* 2008; 121: e233-238.
75. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, Hale EC, Newman NS, Schibler K, Carlo WA. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics* 2010: peds. 2009-2959.
76. Gudmundsdottir A, Johansson S, Hakansson S, Norman M, Kallen K, Bonamy AK. Timing of pharmacological treatment for patent ductus arteriosus and risk of secondary surgery, death or bronchopulmonary dysplasia: a population-based cohort study of extremely preterm infants. *Neonatology* 2015; 107: 87-92.
77. Kaur S, Stritzke A, Soraisham A. DOES THE TIMING OF MEDICAL TREATMENT OF PATENT DUCTUS ARTERIOSUS (PDA) HAVE AN IMPACT ON THE SUCCESS OF TREATMENT IN PRETERM INFANTS? *Paediatrics & Child Health* 2018; 23: e26.
78. Yang CZ, Lee J. Factors affecting successful closure of hemodynamically significant patent ductus arteriosus with indomethacin in extremely low birth weight infants. *World J Pediatr* 2008; 4: 91-96.
79. Hammerman C, Bin-Nun A, Markovitch E, Schimmel MS, Kaplan M, Fink D. Ductal closure with paracetamol: a surprising new approach to patent ductus arteriosus treatment. *Pediatrics* 2011; 128: e1618-1621.
80. El-Khuffash A, Jain A, Corcoran D, Shah PS, Hooper CW, Brown N, Poole SD, Shelton EL, Milne GL, Reese J, McNamara PJ. Efficacy of paracetamol on patent ductus arteriosus closure may be dose dependent: evidence from human and murine studies. *Pediatric research* 2014; 76: 238-244.
81. Pharande P, Watson H, Tan K, Sehgal A. Oral Paracetamol for Patent Ductus Arteriosus Rescue Closure. *Pediatric cardiology* 2017.
82. Oncel MY, Yurttutan S, Erdeve O, Uras N, Altug N, Oguz SS, Canpolat FE, Dilmen U. Oral paracetamol versus oral ibuprofen in the management of patent ductus arteriosus in preterm infants: a randomized controlled trial. *The Journal of pediatrics* 2014; 164: 510-514 e511.
83. Bardanzellu F, Neroni P, Dessi A, Fanos V. Paracetamol in Patent Ductus Arteriosus Treatment: Efficacious and Safe? *BioMed research international* 2017; 2017: 1438038.
84. Guimaraes AFM, Araujo FDR, Meira ZMA, Tonelli HAF, Duarte GG, Ribeiro LC, Rezende GQM, Castilho SRT. Acetaminophen in low doses for closure of the ductus arteriosus of the premature. *Ann Pediatr Cardiol* 2019; 12: 97-102.
85. Le J, Gales MA, Gales BJ. Acetaminophen for patent ductus arteriosus. *Ann Pharmacother* 2015; 49: 241-246.
86. Luecke CM, Liviskie CJ, Zeller BN, Vesoulis ZA, McPherson C. Acetaminophen for Patent Ductus Arteriosus in Extremely Low-Birth-Weight Neonates. *J Pediatr Pharmacol Ther* 2017; 22: 461-466.
87. Ohlsson A, Shah PS. Paracetamol (acetaminophen) for patent ductus arteriosus in preterm or low birth weight infants. *Cochrane Database Syst Rev* 2018; 4: CD010061.
88. Perez KM, Laughon MM. What is new for patent ductus arteriosus management in premature infants in 2015? *Curr Opin Pediatr* 2015; 27: 158-164.
89. Terrin G, Conte F, Oncel MY, Scipione A, McNamara PJ, Simons S, Sinha R, Erdeve O, Tekgunduz KS, Dogan M, Kessel I, Hammerman C, Nadir E, Yurttutan S, Jasani B, Alan S, Manguso F, De Curtis M. Paracetamol for the treatment of patent ductus arteriosus in preterm neonates: a

systematic review and meta-analysis. *Archives of disease in childhood Fetal and neonatal edition* 2016; 101: F127-136.
90. Vaidya R, Wilson D, Paris Y, Madore L, Singh R. Use of acetaminophen for patent ductus arteriosus treatment: a single center experience. *J Matern Fetal Neonatal Med* 2019: 1-7.
91. Weisz DE, Martins FF, Nield LE, El-Khuffash A, Jain A, McNamara PJ. Acetaminophen to avoid surgical ligation in extremely low gestational age neonates with persistent hemodynamically significant patent ductus arteriosus. *J Perinatol* 2016; 36: 649-653.
92. Roclawski M, Sabiniewicz R, Potaz P, Smoczynski A, Pankowski R, Mazurek T, Daibo B. Scoliosis in patients with aortic coarctation and patent ductus arteriosus: does standard posterolateral thoracotomy play a role in the development of the lateral curve of the spine? *Pediatric cardiology* 2009; 30: 941-945.
93. Clement WA, El-Hakim H, Phillipos EZ, Cote JJ. Unilateral vocal cord paralysis following patent ductus arteriosus ligation in extremely low-birth-weight infants. *Archives of otolaryngology--head & neck surgery* 2008; 134: 28-33.
94. El-Khuffash AF, Jain A, McNamara PJ. Ligation of the patent ductus arteriosus in preterm infants: understanding the physiology. *The Journal of pediatrics* 2013; 162: 1100-1106.
95. El-Khuffash A, Weisz DE, McNamara PJ. Reflections of the changes in patent ductus arteriosus management during the last 10 years. *Archives of disease in childhood Fetal and neonatal edition* 2016; 101: F474-478.
96. Weisz DE, Mirea L, Resende MHF, Ly L, Church PT, Kelly E, Kim SJ, Jain A, McNamara PJ, Shah PS. Outcomes of Surgical Ligation after Unsuccessful Pharmacotherapy for Patent Ductus Arteriosus in Neonates Born Extremely Preterm. *J Pediatr* 2018; 195: 292-296.e293.
97. Morville P, Akhavi A. Transcatheter closure of hemodynamic significant patent ductus arteriosus in 32 premature infants by amplatzer ductal occluder additional size-ADOIIAS. *Catheterization and cardiovascular interventions : official journal of the Society for Cardiac Angiography & Interventions* 2017; 90: 612-617.
98. Pamukcu O, Tuncay A, Narin N, Baykan A, Korkmaz L, Argun M, Ozyurt A, Sunkak S, Uzum K. Patent Ductus Arteriosus closure in preterms less than 2kg: Surgery versus transcatheter. *International journal of cardiology* 2017.
99. Fowlie PW, Davis PG, McGuire W. Prophylactic intravenous indomethacin for preventing mortality and morbidity in preterm infants. *The Cochrane database of systematic reviews* 2010: CD000174.
100. Watterberg KL, Gerdes JS, Cole CH, Aucott SW, Thilo EH, Mammel MC, Couser RJ, Garland JS, Rozycki HJ, Leach CL, Backstrom C, Shaffer ML. Prophylaxis of early adrenal insufficiency to prevent bronchopulmonary dysplasia: a multicenter trial. *Pediatrics* 2004; 114: 1649-1657.
101. Peltoniemi O, Kari MA, Heinonen K, Saarela T, Nikolajev K, Andersson S, Voutilainen R, Hallman M. Pretreatment cortisol values may predict responses to hydrocortisone administration for the prevention of bronchopulmonary dysplasia in high-risk infants. *The Journal of pediatrics* 2005; 146: 632-637.
102. Vincer M, Allen A, Evans J, Nwaesei C, Stinson D, Rees E, Fraser A. Early intravenous indomethacin prolongs respiratory support in very low birth weight infants. *Acta paediatrica Scandinavica* 1987; 76: 894-897.
103. Yaseen H, al Umran K, Ali H, Rustum M, Darwich M, al-Faraidy A. Effects of early indomethacin administration on oxygenation and surfactant requirement in low birth weight infants. *Journal of tropical pediatrics* 1997; 43: 42-46.
104. Van Overmeire B, Van de Broek H, Van Laer P, Weyler J, Vanhaesebrouck P. Early versus late indomethacin treatment for patent ductus arteriosus in premature infants with respiratory distress syndrome. *The Journal of pediatrics* 2001; 138: 205-211.

105. Tauzin L, Joubert C, Noel AC, Bouissou A, Moulies ME. Effect of persistent patent ductus arteriosus on mortality and morbidity in very low-birthweight infants. *Acta Paediatrica* 2012; 101: 419-423.
106. Amendolia B, Lynn M, Bhat V, Ritz SB, Aghai ZH. Severe pulmonary hypertension with therapeutic L-lysine ibuprofen in 2 preterm neonates. *Pediatrics* 2012; 129: e1360-1363.
107. Bellini C, Campone F, Serra G. Pulmonary hypertension following L-lysine ibuprofen therapy in a preterm infant with patent ductus arteriosus. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2006; 174: 1843-1844.
108. Kluckow M, Evans N. Early echocardiographic prediction of symptomatic patent ductus arteriosus in preterm infants undergoing mechanical ventilation. *The Journal of pediatrics* 1995; 127: 774-779.
109. Wyllie J. Treatment of patent ductus arteriosus. *Seminars in neonatology : SN* 2003; 8: 425-432.
110. Kluckow M, Evans N. Superior vena cava flow in newborn infants: a novel marker of systemic blood flow. *Archives of disease in childhood Fetal and neonatal edition* 2000; 82: F182-187.
111. De Buyst J, Rakza T, Pennaforte T, Johansson AB, Storme L. Hemodynamic effects of fluid restriction in preterm infants with significant patent ductus arteriosus. *The Journal of pediatrics* 2012; 161: 404-408.
112. de Waal KA, Evans N, Osborn DA, Kluckow M. Cardiorespiratory effects of changes in end expiratory pressure in ventilated newborns. *Archives of disease in childhood Fetal and neonatal edition* 2007; 92: F444-448.
113. Fajardo MF, Claure N, Swaminathan S, Sattar S, Vasquez A, D'Ugard C, Bancalari E. Effect of positive end-expiratory pressure on ductal shunting and systemic blood flow in preterm infants with patent ductus arteriosus. *Neonatology* 2014; 105: 9-13.
114. Dikshit K, Vyden JK, Forrester JS, Chatterjee K, Prakash R, Swan H. Renal and extrarenal hemodynamic effects of furosemide in congestive heart failure after acute myocardial infarction. *New England Journal of Medicine* 1973; 288: 1087-1090.
115. Sulyok E, Varga F, Nemeth M, Tenyi I, Csaba IF, Ertl T, Gyory E. Furosemide-induced alterations in the electrolyte status, the function of renin-angiotensin-aldosterone system, and the urinary excretion of prostaglandins in newborn infants. *Pediatric research* 1980; 14: 765-768.
116. Green TP, Thompson TR, Johnson DE, Lock JE. Furosemide promotes patent ductus arteriosus in premature infants with the respiratory-distress syndrome. *The New England journal of medicine* 1983; 308: 743-748.
117. Doyle LW, Davis PG, Morley CJ, McPhee A, Carlin JB. Low-dose dexamethasone facilitates extubation among chronically ventilator-dependent infants: a multicenter, international, randomized, controlled trial. *Pediatrics* 2006; 117: 75-83.
118. Halliday HL. Update on postnatal steroids. *Neonatology* 2017; 111: 415-422.
119. Doyle LW, Cheong JL. Postnatal corticosteroids to prevent or treat bronchopulmonary dysplasia–Who might benefit? Seminars in Fetal and Neonatal Medicine: Elsevier; 2017. p. 290-295.
120. Cheong JL, Burnett AC, Lee KJ, Roberts G, Thompson DK, Wood SJ, Connelly A, Anderson PJ, Doyle LW. Association between postnatal dexamethasone for treatment of bronchopulmonary dysplasia and brain volumes at adolescence in infants born very preterm. *The Journal of pediatrics* 2014; 164: 737-743.e731.
121. Morales P, Rastogi A, Bez ML, Akintorin SM, Pyati S, Andes SM, Pildes RS. Effect of dexamethasone therapy on the neonatal ductus arteriosus. *Pediatric cardiology* 1998; 19: 225-229.
122. Takami T, Momma K, Imamura S. Increased constriction of the ductus arteriosus by dexamethasone, indomethacin, and rofecoxib in fetal rats. *Circulation journal : official journal of the Japanese Circulation Society* 2005; 69: 354-358.

123. Laughon MM, Simmons MA, Bose CL. Patency of the ductus arteriosus in the premature infant: is it pathologic? Should it be treated? *Current opinion in pediatrics* 2004; 16: 146-151.
124. Benitz WE. Learning to live with patency of the ductus arteriosus in preterm infants. *Journal of perinatology : official journal of the California Perinatal Association* 2011; 31 Suppl 1: S42-48.
125. Clyman RI, Chorne N. Patent ductus arteriosus: evidence for and against treatment. *The Journal of pediatrics* 2007; 150: 216-219.
126. Chock VY, Punn R, Oza A, Benitz WE, Van Meurs KP, Whittemore AS, Behzadian F, Silverman NH. Predictors of bronchopulmonary dysplasia or death in premature infants with a patent ductus arteriosus. *Pediatr Res* 2014; 75: 570-575.
127. Benitz WE. Patent ductus arteriosus: to treat or not to treat? *Archives of disease in childhood-fetal and neonatal edition* 2012; 97: F80-F82.
128. Janz-Robinson EM, Badawi N, Walker K, Bajuk B, Abdel-Latif ME, Bowen J, Sedgley S, Carlisle H, Smith J, Craven P. Neurodevelopmental outcomes of premature infants treated for patent ductus arteriosus: a population-based cohort study. *The Journal of pediatrics* 2015; 167: 1025-1032. e1023.
129. Jhaveri N, Moon-Grady A, Clyman RI. Early surgical ligation versus a conservative approach for management of patent ductus arteriosus that fails to close after indomethacin treatment. *J Pediatr* 2010; 157: 381-387, 387 e381.
130. Kaempf JW, Wu YX, Kaempf AJ, Kaempf AM, Wang L, Grunkemeier G. What happens when the patent ductus arteriosus is treated less aggressively in very low birth weight infants? *Journal of perinatology : official journal of the California Perinatal Association* 2012; 32: 344-348.
131. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001; 163: 1723-1729.
132. CNN. Patient log - Admission screen definitions - Canadian Neonatal Network - Abstractor's Manual. 2012 January 16, 2012 Available from: http://www.canadianneonatalnetwork.org/Portal/LinkClick.aspx?fileticket=I3jnvN9fGfE%3D&tabid=69.
133. Neu J. Necrotizing enterocolitis: the search for a unifying pathogenic theory leading to prevention. *Pediatric clinics of North America* 1996; 43: 409-432.
134. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, Brotherton T. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Annals of surgery* 1978; 187: 1.
135. Papile L-A, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *The Journal of pediatrics* 1978; 92: 529-534.
136. Prematurity ICftCoRo. The international classification of retinopathy of prematurity revisited. *Archives of ophthalmology* 2005; 123: 991.
137. Richardson DK, Corcoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: simplified newborn illness severity and mortality risk scores. *The Journal of pediatrics* 2001; 138: 92-100.
138. Saeed S, Moodie EEM, Strumpf EC, Klein MB. Evaluating the impact of health policies: using a difference-in-differences approach. *Int J Public Health* 2019; 64: 637-642.
139. Dimick JB, Ryan AM. Methods for evaluating changes in health care policy: the difference-in-differences approach. *Jama* 2014; 312: 2401-2402.
140. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, Hale EC, Newman NS, Schibler K, Carlo WA, Kennedy KA, Poindexter BB, Finer NN, Ehrenkranz RA, Duara S, Sanchez PJ, O'Shea TM, Goldberg RN, Van Meurs KP, Faix RG, Phelps DL, Frantz ID, 3rd, Watterberg KL, Saha S, Das A, Higgins RD, Eunice Kennedy Shriver National Institute of Child H, Human Development Neonatal Research N. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics* 2010; 126: 443-456.

141. Garfinkle J, Yoon EW, Alvaro R, Nwaesei C, Claveau M, Lee SK, Shah PS. Trends in sex-specific differences in outcomes in extreme preterms: progress or natural barriers? *Archives of disease in childhood Fetal and neonatal edition* 2019.
142. Yelland LN, Salter AB, Ryan P. Performance of the modified Poisson regression approach for estimating relative risks from clustered prospective data. *American journal of epidemiology* 2011; 174: 984-992.
143. Zou G. A modified poisson regression approach to prospective studies with binary data. *American journal of epidemiology* 2004; 159: 702-706.
144. Lechner M. The estimation of causal effects by difference-in-difference methods. *Foundations and Trends® in Econometrics* 2011; 4: 165-224.
145. White H. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *econometrica* 1980; 48: 817-838.
146. Huber PJ. The behavior of maximum likelihood estimates under nonstandard conditions. Proceedings of the fifth Berkeley symposium on mathematical statistics and probability: University of California Press; 1967. p. 221-233.
147. Eicker F. Limit theorems for regressions with unequal and dependent errors. Proceedings of the fifth Berkeley symposium on mathematical statistics and probability; 1967. p. 59-82.
148. Soll R. Synthetic surfactant for respiratory distress syndrome in preterm infants. *Cochrane Database of Systematic Reviews* 1998.
149. Letshwiti JB, Semberova J, Pichova K, Dempsey EM, Franklin OM, Miletin J. A conservative treatment of patent ductus arteriosus in very low birth weight infants. *Early Hum Dev* 2017; 104: 45-49.
150. Burnard ED. The Cardiac Murmur in Relation to Symptoms in the Newborn. *British Medical Journal* 1959; 1: 134-138.
151. Decancq H. Repair of patent ductus arteriosus in a 1,417 gm infant. *American Journal of Diseases of Children* 1963; 106: 402-410.
152. EDMUNDS JR LH, GREGORY GA, HEYMANN MA, KITTERMAN JA, RUDOLPH AM, TOOLEY WH. Surgical closure of the ductus arteriosus in premature infants. *Circulation* 1973; 48: 856-863.
153. Elliott RB, Starling MB, Neutze JM. Medical manipulation of the ductus arteriosus. *Lancet* 1975; 1: 140-142.
154. Friedman WF, Hirschklau MJ, Printz MP, Pitlick PT, Kirkpatrick SE. Pharmacologic closure of patent ductus arteriosus in the premature infant. *N Engl J Med* 1976; 295: 526-529.
155. Sadeck LS, Leone CR, Procianoy RS, Guinsburg R, Marba ST, Martinez FE, Rugolo LM, Moreira ME, Fiori RM, Ferrari LL, Menezes JA, Venzon PS, Abdallah VQ, Duarte JL, Nunes MV, Anchieta LM, Alves Filho N. Effects of therapeutic approach on the neonatal evolution of very low birth weight infants with patent ductus arteriosus. *J Pediatr (Rio J)* 2014; 90: 616-623.
156. Roze JC, Cambonie G, Marchand-Martin L, Gournay V, Durrmeyer X, Durox M, Storme L, Porcher R, Ancel PY, Hemodynamic ESG. Association Between Early Screening for Patent Ductus Arteriosus and In-Hospital Mortality Among Extremely Preterm Infants. *Jama* 2015; 313: 2441-2448.
157. Hagadorn JI, Bennett MV, Brownell EA, Payton KSE, Benitz WE, Lee HC. Covariation of Neonatal Intensive Care Unit-Level Patent Ductus Arteriosus Management and In-Neonatal Intensive Care Unit Outcomes Following Preterm Birth. *J Pediatr* 2018; 203: 225-233 e221.
158. Liebowitz M, Clyman RI. Prophylactic Indomethacin Compared with Delayed Conservative Management of the Patent Ductus Arteriosus in Extremely Preterm Infants: Effects on Neonatal Outcomes. *J Pediatr* 2017; 187: 119-126 e111.
159. Clyman RI, Liebowitz M, Kaempf J, Erdeve O, Bulbul A, Hakansson S, Lindqvist J, Farooqi A, Katheria A, Sauberan J, Singh J, Nelson K, Wickremasinghe A, Dong L, Hassinger DC, Aucott

SW, Hayashi M, Heuchan AM, Carey WA, Derrick M, Fernandez E, Sankar M, Leone T, Perez J, Serize A. PDA-TOLERATE Trial: An Exploratory Randomized Controlled Trial of Treatment of Moderate-to-Large Patent Ductus Arteriosus at 1 Week of Age. *J Pediatr* 2019; 205: 41-48.e46.

160. Liebowitz M, Katheria A, Sauberan J, Singh J, Nelson K, Hassinger DC, Aucott SW, Kaempf J, Kimball A, Fernandez E, Carey WA, Perez J, Serize A, Wickremasinghe A, Dong L, Derrick M, Wolf IS, Heuchan AM, Sankar M, Bulbul A, Clyman RI. Lack of Equipoise in the PDA-TOLERATE Trial: A Comparison of Eligible Infants Enrolled in the Trial and Those Treated Outside the Trial. *J Pediatr* 2019; 213: 222-226.e222.
161. Jasani B, Weisz DE, McNamara PJ. Evidence-based use of acetaminophen for hemodynamically significant ductus arteriosus in preterm infants. *Seminars in perinatology* 2018; 42: 243-252.
162. Food U, Administration D. Premarket Approval (PMA) of the AMPLATZER Piccolo Occluder. *Atlanta: US FOOD and Drug Adminisration* 2019.
163. Backes CH, Giesinger RE, Rivera BK, Berman DP, Smith CV, Cua CL, Kelleher KJ, McNamara PJ, Slaughter JL. Percutaneous Closure of the Patent Ductus Arteriosus in Very Low Weight Infants: Considerations Following US Food and Drug Administration Approval of a Novel Device. *The Journal of pediatrics* 2019; 213: 218-221.

13 Appendix A: Congenital anomalies excluded:

Diagnosis	ICD10 - Number
Anencephaly	Q00
Encephalocele	Q01
Spina Bifida	Q05
Spinal Cord Anomaly Other Than Spina Bifida	Q06
Other Congenital Malformations Of The Nervous System	Q07
Anophthalomos, Microphthalmos And Macrophthalmos	Q11
Double Outlet Right Ventricle	Q20.1
Transposition Of The Great Vessels (Tgv)	Q20.3
Atrioventricular Septal Defect	Q21.2
Tetralogy Of Fallot	Q21.3
Pulmonary Valve Stenosis	Q22.1
Hypoplastic Left Heart Syndrome	Q23.4
Total Anomalous Pulmonary Venous Connection	Q26.2
Coarctation Of The Aorta	Q25.1
Congenital Malformations Of The Larynx	Q31
Congenital Malformations Of The Trachea And Bronchus	Q32
Congenital Malformations Of The Lung	Q33
Congenital Cystic Lung	Q33.0
Sequestration of The Lung	Q33.2
Atresia Of Oesophagus Without Fistula	Q39.0
Atresia Of Oesophagus With Tracheo-Oesophageal Fistula	Q39.1
Congenital Absence, Atresia And Stenosis Of The Small Intestine	Q41
Congenital Absence, Atresia And Stenosis Of The Duodenum	Q41.0
Congenital Absence, Atresia And Stenosis Of The Jejunum	Q41.1
Congenital Absence, Atresia and Stenosis of The Anus (Imperforate Anus)	Q42.3
Indeterminate Sex And Pseudohermaphroditism	Q56
Renal Agenesis And Other Defects Of The Kidney	Q60
Congenital Posterior Urethral Valves	Q64.2
Craniosynostosis	Q75.0
Congenital Diaphragmatic Hernia	Q79.0
Exomphalos	Q79.2
Gastroschisis	Q79.3
Congenital Ichthyosis	Q80
Epidermolysis Bullosa	Q81
Neurocutaneous Syndromes	Q85
Fetal Alcohol Syndrome (Dysmorphic)	Q86.0
Situs Inversus	Q89.3
Down'S Syndrome	Q90
Edwards' Syndrome Or Trisomy 18	Q91.3
Patau Syndrome Or Trisomy 13	Q91.7
Other Trisomies And Parial Trisomies Of The Autosomes Not Elsewhere Classified	Q92
Monosomies And Deletions From The Autosomes Not Elsewhereclassified	Q93
Balanced Rearrangements And Structural Markers Not Elsewhere Classified	Q95
Turner's Syndrome	Q96
Other Sex Chromosome Abnormalities, Female Phenotype Not Elsewhere Classified	Q97
Other Sex Chromosome Abnormalities, Male Phenotype Not Elsewhere Classified	Q98

14 Appendix B : Ethics Board Approval

Approvals (PDF attached)

Centre universitaire de santé McGill | McGill University Health Centre

2019-10-02

Dr. Gabriel Altit

email: gabalt@gmail.com

Re: Authorisation to conduct your research study at the MUHC
Objet: Autorisation de réaliser votre projet de recherche au CUSM

Titre du projet: Issues reliées à diverses stratégies de prise en charge du canal artériel chez l'extrême prématuré
Project Title: Neonatal outcomes of extremely premature infants comparing patent ductus arteriosus management approaches

CÉR évaluateur / Reviewing REB: CER CUSM / MUHC REB
Numéro de dossier CER CUSM / MUHC REB File Number: MP-37-2019-4876 / Neonatal outcomes of extremely premature infants comparing patent ductus arteriosus management approaches
Date d'approbation éthique / REB Approval Date: 2019-09-30
Personne contacte au CER CUSM / MUHC REB contact person:

Pediatrics Panel (PED)
Elizabeth Craven
elizabeth.craven@muhc.mcgill.ca

***** La version française suit *****

Dr. Altit,

We are pleased to allow you to carry out the research project, identified above, under the auspices of the MUHC.

This authorization allows you to perform research at the MUHC.

By granting this authorization, our institution recognizes the ethical review that was done by the REB mentioned above.

- This REB is the Reviewing REB for this project in accordance with the MSSS *Cadre de référence des établissements publics du RSSS pour l'autorisation d'une recherche menée dans plus d'un établissement* (le Cadre de référence) (the MSSS Framework);
- This REB confirmed on the date of REB approval, see above, the positive outcome of the scientific and ethics reviews of the project; and
- This REB approved the network version of the consent form used in French and English for this research. If the Reviewing REB determines that changes to the network version of the consent form affect the ethical acceptability of the project it

may suspend its ethical approval for the institution.

We acknowledge receipt of the consent form that you prepared for our institution from the network version. A copy of this consent form and of this authorization will be forwarded to Reviewing REB.

Our institution also recognizes that the study mentioned above has received all the required institutional approvals, namely:

- Contracts
- Use of pediatric resources
- Access to pediatric health records

This authorization is given on condition that you commit to:

- Respecting the provisions of the MSSS framework relevant to your research project;
- Complying with the MUHC regulatory framework (April 2016) for research involving humans, including the identification of research participants;
- Using the version of the documents relating to the research approved by the Reviewing REB, to which only administrative changes have been made and communicated to the Reviewing REB; and
- Meeting the requirements set by the Reviewing REB for ongoing ethical oversight of research.

Please note that we will not communicate with the sponsors. This responsibility belongs to the researcher concerned in accordance with good clinical practice.

The authorization given to you to realize the research project under the auspices of our institution will be renewed without further proceedings on the date specified by the REB assessor's decision to renew its ethical approval for this research.

Please contact the MUHC REB coordinator mentioned above for any questions regarding this authorization or its renewal or about administrative changes that have been made to the version of the documents relating to the research approved by the Reviewing REB.

Please do not hesitate to contact me during the conduct of the study at our institution, if necessary. You can also seek the advice from our REB by contacting the MUHC REB Panel mentioned above to obtain the support needed.

Lastly, we ask you to refer to both study numbers assigned to your research project by our institution and by the Reviewing REB when discussing the study.

Sincerely,

Sheldon Levy (see signature below)
for:
Marie Hirtle, LL.B. LL.M.
Mandated Person
McGill University Health Centre

cc. Vincent Lajoie
MUHC REB Co-Chair

Docteur Altit,

Il nous fait plaisir de vous autoriser à réaliser la recherche identifiée en titre et sous les auspices du CUSM.

Nous vous écrivons pour confirmer que l'étude susmentionnée a reçu toutes les approbations institutionnelles requises, à savoir:

- Les contrats
- Utilisation des ressources adultes
- Accès aux dossiers de santé adultes

Cette autorisation vous permet de réaliser la recherche au CUSM.

Pour vous donner cette autorisation, notre établissement reconnaît l'examen éthique effectué par le CER évaluateur mentionné ci-haut.

- Ce CER agit comme CER évaluateur pour ce projet, conformément au *Cadre de référence des établissements publics du RSSS pour l'autorisation d'une recherche menée dans plus d'un établissement* (le Cadre de référence) ;
- Ce CER a confirmé le résultat positif de l'examen éthique et scientifique du projet à la date d'approbation éthique mentionnée ci-haut ; et
- Ce CER a approuvé la version réseau du formulaire de consentement en français et en anglais utilisé pour cette recherche.

Nous accusons réception du formulaire de consentement que vous avez préparé pour notre établissement à partir de la version réseau et nous le joindrons à la copie de cette autorisation qui sera transmise au CER évaluateur.

Cette autorisation vous est donnée à condition que vous vous engagiez à:

- Respecter les dispositions du Cadre de référence se rapportant à votre recherche;
- Respecter le cadre réglementaire de notre établissement sur les activités de recherche, notamment pour l'identification des participants à la recherche;
- Utiliser les versions des documents se rapportant à la recherche approuvées par le CER évaluateur, les seuls changements apportés, si c'est le cas, étant d'ordre administratif et identifiés de façon à ce que le CER évaluateur puisse en prendre connaissance ; et
- Respecter les exigences fixées par le CER évaluateur pour le suivi éthique de la recherche.

Veuillez noter que nous ne communiquerons pas avec les commanditaires. Cette responsabilité appartient au chercheur concerné en vertu des bonnes pratiques cliniques.

L'autorisation qui vous est donnée ici de réaliser la recherche sous les auspices du CUSM sera renouvelée sans autre procédure à la date indiquée par le CER évaluateur dans sa

décision de renouveler son approbation éthique de cette recherche.

Pour toute question relative à cette autorisation ou à son renouvellement ou au sujet de changements d'ordre administratifs qui auraient été apportés à la version des documents se rapportant à la recherche approuvée par le CER évaluateur, veuillez communiquer avec le coordinateur de CER mentionné en rubrique.

Je vous invite à entrer en communication avec moi pendant le déroulement de cette recherche dans notre établissement, si besoin est. Vous pouvez aussi solliciter l'appui de notre CER en vous adressant au Panel du CER CUSM mentionné ci-haut pour obtenir les conseils et le soutien voulu.

En terminant, veuillez toujours mentionner dans votre correspondance au sujet de cette recherche le numéro attribué à votre demande par notre établissement ainsi que le numéro attribué au projet de recherche par le CER évaluateur.

En espérant le tout à votre entière satisfaction.

Cordialement,

Sheldon Levy
MUHC REB Coordinator
for MUHC REB Co-chair mentioned above

Le 4 octobre 2019

Docteure Anie Lapointe
CHU Sainte-Justine

Objet	Autorisation de réaliser la recherche
	MEO-37-2019-2125 Issues néonatales des nouveau-nés extrêmement prématurés en comparant deux approches médicales du canal artériel. Cheurcheur principal au CÉR Éval: Babriel Altit Cochercheurs: Sahar Saeed; Martine Claveau; Gilles Paradis; Marc Beltempo; Olga Basso Numéro de dossier CÉR évaluateur CUSM: MP-37-2019-4876

Bonjour,

Il nous fait plaisir de vous autoriser à réaliser la recherche identifiée en titre dans notre établissement et/ou sous ses auspices.

Cette autorisation vous est accordée sur la foi des documents que vous avez déposés auprès de notre établissement afin de compléter l'examen de convenance ainsi que la lettre du CER évaluateur. Si ce CER vous informe pendant le déroulement de cette recherche d'une décision négative portant sur l'acceptabilité éthique de cette recherche, vous devrez considérer que la présente autorisation de réaliser la recherche dans notre établissement est, de ce fait, révoquée à la date que porte l'avis du CER évaluateur.

Notre établissement a reçu une copie de la version finale des documents se rapportant à la recherche, approuvée par le CER évaluateur.

Cette autorisation de réaliser la recherche suppose également que vous vous engagez :

1) à vous conformer aux demandes du CER évaluateur, notamment pour le suivi éthique continu de la recherche;
2) à rendre compte au CER évaluateur et à la signataire de la présente autorisation du déroulement du projet, des actes de votre équipe de recherche, s'il en est une, ainsi que du respect des règles de l'éthique de la recherche;
3) à respecter les moyens relatifs au suivi continu qui ont été fixés par le CER évaluateur;
4) à conserver les dossiers de recherche pendant la période fixée par le CER évaluateur, après la fin du projet, afin de permettre leur éventuelle vérification;
5) à respecter les modalités arrêtées au regard du mécanisme d'identification des sujets de recherche dans notre établissement, à savoir la tenue à jour et la conservation de la liste à jour des participants de recherche recrutés dans notre établissement. Cette liste devra nous être fournie sur demande.

La présente autorisation peut être suspendue ou révoquée par notre établissement en cas de non-respect des conditions établies. Le CER évaluateur en sera alors informé.

Vous consentez également à ce que notre établissement communique aux autorités compétentes des renseignements personnels qui sont nominatifs au sens de la loi en présence d'un cas avéré de manquement à la conduite responsable en recherche de votre part lors de la réalisation de cette recherche.

En terminant, je vous demanderais de toujours mentionner dans votre correspondance au sujet de cette recherche le numéro attribué à votre demande par notre établissement ainsi que le numéro attribué au projet de recherche par le CER évaluateur.

Veuillez accepter mes sincères salutations.

Marc Girard, M.D.
Directeur des services professionnels (DAMU)
Personne formellement mandatée au CHU Sainte-Justine pour autoriser la réalisation des projets de rercherche