Asphyxiated Newborns Treated with Therapeutic Hypothermia
What are the Determinants
and Predictors of Adverse Outcome?

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Table of contents

Summary

Birth asphyxia and subsequent neonatal encephalopathy remain a significant cause of mortality and long-term disability in term newborns. Currently, therapeutic hypothermia is the only available treatment that may reduce the mortality and long-term impairments by preventing the development of brain injury in asphyxiated newborns. However, the benefit of hypothermia is limited, especially in severe cases, and some asphyxiated newborns still die and/or develop brain injury despite this treatment. Therefore, it remains crucial to understand why these patients develop adverse outcome despite treatment, and which risk factors place them at higher risk of developing adverse outcome. The purpose of this thesis is to investigate the maternal and perinatal determinants and/or predictors of adverse outcome (defined as death and/or brain injury) in asphyxiated newborns treated with hypothermia. To reach this goal, I evaluated some maternal, delivery, and perinatal characteristics in a local cohort of asphyxiated newborns treated with hypothermia from the neonatal intensive care unit of the Montreal Children's Hospital (Chapter 2), and in a national neonatal cohort from Canadian Neonatal Network database (Chapter 3). In our local cohort, when looking at maternal risk factors, results show that primiparity, prolonged duration of labor, along with the severity of initial asphyxia event placed asphyxiated newborns at higher risk of adverse outcome. In the national cohort, when looking at perinatal risk factors, the severity of initial asphyxia event was confirmed as a significant risk factor: in addition, hypotension requiring inotrope use, and renal failure were two other independent predictors of adverse outcome. This research allowed a better understanding of the determinants and predictors of adverse outcome in asphyxiated newborns treated with hypothermia. Future studies are required to investigate how an improved management of these risk factors may decrease the incidence of adverse outcome in asphyxiated newborns treated with hypothermia.

Résumé

L'asphyxie à la naissance et l'encéphalopathie néonatale qui en découle sont une cause importante de mortalité et d'invalidité à long terme chez les nouveaux-nés nés à terme. À l'heure actuelle, l'hypothermie est le seul traitement disponible qui peut réduire la mortalité et les invalidités à long terme en empêchant le développement de lésions cérébrales chez les nouveau-nés qui ont souffert d'asphyxie à la naissance. Cependant, les bénéfices du traitement par hypothermie sont limités, particulièrement pour les cas sévères, et de nombreux nouveaux-nés qui ont souffert d'asphyxie à la naissance meurent ou développent des lésions cérébrales quand même malgré le traitement par hypothermie. Par conséquent, il est important de comprendre pourquoi ces nouveau-nés sont plus à risque de mourir ou développer des lésions cérébrales malgré le traitement.

L'objectif de cette thèse est d'identifier les facteurs de risque maternels et périnataux qui prédisposent les nouveau-nés qui ont soufferts d'asphyxie à la naissance et qui ont été traités avec l'hypothermie à mourir et/ou développer des lésions cérébrales. Pour atteindre cet objectif, j'ai évalué certaines facteurs de risque maternelles et perinatales dans une cohorte locale de nouveau-nés ayant souffert d'asphyxie à la naissance et traités avec l'hypothermie à l'unité de soins intensifs néonatals de l'Hôpital de Montréal pour enfants (chapitre 2) et dans une cohorte nationale néonatale issue d'une base de données du Réseau Néonatal Canadien (Canadian Neonatal Network, chapitre 3).

Dans la cohorte locale, en regardant les facteurs de risque maternels, la primiparité, ainsi qu'un travail prolongé pendant l'accouchement, augmentaient le risque de mourir et/ou développer des lésions cérébrales chez les nouveau-nés qui ont souffert d'asphyxie à la naissance, en plus de la gravité de l'asphyxie initiale. Dans la cohorte nationale, en regardant les facteurs de

risque périnataux, l'hypotension nécessitant l'utilisation d'inotrope et l'insuffisance rénale étaient deux autres facteurs de risque significatifs, en plus de la gravité de l'asphyxie initiale. Cette recherche a permis de mettre en évidence des facteurs de risque chez les nouveaux-nés ayant souffert d'asphyxie à la naissance, qui les prédisposent à un futur plus sombre malgré le traitement par hypothermie. D'autres études de recherche seront nécessaires, afin de déterminer dans quelle mesure un meilleur contrôle de ces facteurs de risques permettrait de diminuer l'incidence de mortalité et d'invalidité à long terme chez les nouveau-nés ayant souffert d'asphyxie à la naissance et soignés avec l'hypothermie.

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Authors' contribution

The research that was conducted during my Msc's study have led to two manuscripts that are included with this thesis and will be submitted to related journals. Brain magnetic resonance imaging results that are used as the outcome in Chapter 2 were assessed by Dr. Christine Saint-Martin, who is a neuroradiologist at the Montreal Children's Hospital. Dr. Richard Brown, an obstetrician from the Royal Victoria Hospital, also gave us some advices regarding the important maternal risk factors to look at in this population.

Introduction

Despite advances in neonatal care, birth asphyxia and subsequent neonatal encephalopathy (NE) in term newborns remain a serious condition causing significant mortality (i.e., 23% of all neonatal death worldwide) and long-term morbidity, including cerebral palsy and other neurodevelopmental disabilities (Ferriero, 2004; Bryce et al., 2005; Fatemi et al., 2009; Al-Macki N et al., 2009). Currently, therapeutic hypothermia is the only available treatment providing neuroprotection in asphyxiated newborns (Gluckman et al., 2005; Shankaran et al., 2005; Edwards et al., 2010). It has been proven that either whole body cooling or selective head cooling significantly reduces the occurrence of poor outcomes (death and disability) and increase the number of healthy survivors (Edwards et al., 2010, Azzopardi et al., 2009; Jacobs et al., 2013). However, the success of this therapy is limited and many patients treated with hypothermia still encounter adverse outcome (van Bel & Groenendaal, 2016). Therefore, it remains important to understand why induced hypothermia is effective in preventing brain injury and improving neurological outcome in some but not all asphyxiated newborns (Gluckman et al., 2005; Rutherford et al., 2010). It is of utmost importance to identify those patients who will develop brain injury despite this therapy.

The hypothesis of this study is that some maternal, delivery, and perinatal characteristics place asphyxiated newborns at higher risk of dying and/or developing brain injury despite hypothermia. The first objective of my MSc thesis is to study the possible association between some maternal characteristics and adverse outcome (i.e., death and/or brain injury) in a local cohort of asphyxiated newborns treated with hypothermia (Chapter 2). The second objective of my MSC thesis is to study the possible association between some maternal, delivery and

perinatal characteristics adverse outcome (i.e., death and/or brain injury) in a national cohort of asphyxiated newborns treated with hypothermia (Chapter 3).

Chapter 1

Literature review

1.1 Birth Asphyxia

Birth asphyxia is a disorder of impaired circulation and/or oxygenation in newborns around the time of birth (Low et al., 1999). The incidence of birth asphyxia depends on which part of the world the infants are born. In developed countries, birth asphyxia happens in 1-6 per 1000 live births, while in developing countries the number is 5-10 per 1000 live births (Shireen et al. 2009). During the intrapartum period, any events that interrupt the maternal-fetal circulation and/or oxygenation may result in fetal distress. The causes of these circulatory interruptions are multiple and not always clear. They can be transient carrying less likelihood for adverse outcome (Low, 1997; Spencer, 1997), or they can be persistent, such as uterine rupture, placental abruptio, and cord prolapse (Wintermark, 2011).

1.2. Neonatal encephalopathy and neurological impairment

1.2.1. The relationship between birth asphyxia and neonatal encephalopathy

Neonatal encephalopathy (NE) is a clinically defined syndrome of impaired brain function in the earliest days of life in newborns, presenting with difficulty in initiation and maintenance of respiration, depression of muscle tone and reflexes, subnormal level of consciousness, and often accompanied with seizures (Nelson & Leviton, 1991). NE may result from many conditions including birth asphyxia, perinatal stroke, maternal/neonatal infection, cerebral malformation and genetic disorders (Liston et al., 2007; Nelson & Leviton, 1991).

1.2.2. Brain damage and long-term neurological impairments following birth asphyxia

Common mechanisms of brain injury following birth asphyxia include excitotoxicity, inflammation, and oxidative stress by free radicals (Lai & Yang., 2011; Shankaran, 2012). At the cellular level, birth asphyxia leads to a two-phase energy failure. The primary phase follows the reduction in blood flow and oxygen supply with a fall in ATP, failure of Na+/K+ pump, depolarization of cells, lactic acidosis, release of excitatory amino acids, calcium entry into the cell and, if severe, cell necrosis (Ferriero, 2004; Shalak & Perlman, 2004; Gunn & Bennet, 2009). The secondary phase of energy failure develops at 12h to 36 h after the initial event, and may last 7 to 14 days after the initial event, with initiation of apoptosis, mitochondrial failure, cytotoxic edema, accumulation of excitatory amino acids and release of free radicals terminating in cell death (Peliowski-Davidovich, 2012; Ferriero, 2004; Shalak & Perlman, 2004; Gunn & Bennet, 2009).

1.3. Magnetic resonance imaging (MRI) in assessing brain injury

Magnetic resonance imaging is the most often used tool to assess the severity of brain injury in asphyxiated newborns (Barkovich et al., 1998; Miller et al., 2005; Lawrence & Inder, 2008). Compared to other neuroimaging strategies, such as head ultrasonography and computed tomography (CT), MRI provides more detailed imaging allowing for better characterization of the extent of the brain injury (Miller et al., 2005). MRI performed around day 10 of life has been demonstrated as the gold standard to diagnose brain injury and to predict neurodevelopmental outcome in the term asphyxiated newborns (Barkovich et al., 1998; Miller et al., 2005; Lawrence & Inder, 2008; Wintermark, 2012).

The resulting brain injury depends upon the nature of the initial event (Liston et al., 2007). Term asphyxiated newborns typically display one of three different patterns of injury: i.e., basal

ganglia injury, watershed injury, or near-total injury. Before the cooling era, several scoring systems were validated to grade the severity of the brain injury depicted on MRIs performed in asphyxiated newborns during the perinatal period. One of those MRI scoring systems described by Barkovich et al. (Barkovich et al., 1998) was demonstrated as accurate in predicting neuromotor and cognitive outcome at 3 and 12 months. This system (BG/W) scores the brain injury in the basal ganglia and thalamus (BG), and the cortex and watershed area (W), as follows: 0, normal; 1, abnormal signal in basal ganglia or thalamus; 2, abnormal signal in cortex; 3, abnormal signal in cortex and basal nuclei; 4, abnormal signal in entire cortex and basal nuclei.

1.4. Maternal, delivery, and perinatal characteristics in predicting brain injury

The association between antenatal and perinatal risk factors and adverse outcome has been previously investigated in asphyxiated newborns (Cowan, et al., 2003; Klinger et al., 2005; Miller et al., 2005; Selewski et al., 2013). Results of the different studies were not always similar. For example, parity and labor duration were reported to be associated with more severe neonatal acidosis and lower Apgar scores (Altman et al., 2015; Barton et al., 1991), whereas their impact on developing brain injury in asphyxiated newborns was never clearly proven. In addition, the impact of these factors in causing brain injury in asphyxiated newborns treated with hypothermia is not yet clarified. It remains thus important to investigate the association between these maternal, delivery and perinatal risk factors and the development of adverse outcome in asphyxiated newborns treated with hypothermia.

1.5. Therapeutic strategies

Currently, the only available neuroprotective treatment for asphyxiated infants is therapeutic hypothermia, which can be conducted as whole body cooling or selective head cooling resulting

in a reduction of temperature to 33.5°C for 72 hours (Shankaran et al., 2005; Peliowski-Davidovich, 2012; Thoresen, 2010). Proven beneficial effect of hypothermia includes prevention of apoptosis, reduced inflammation, decreased loss of high-energy phosphates, reduced oxygen consumption, reduced release of nitric oxide, glutamate, free radicals and excitatory amino acid neurotransmitters, and the induction of genes that reduce neuronal death (Ferriero, 2004; Jacobs et al., 2013; Peliowski-Davidovich, 2012). The therapeutic window to start hypothermia is believed to be within the first 6 hours of life, which corresponds to the time when normalization of oxidative metabolism takes place after resuscitation (Peliowski-Davidovich, 2012). To be eligible for hypothermia, asphyxiated newborns should meet the clinical and physiologic criteria (criteria A), as well as the neurologic criteria (criteria B). Criteria A includes the presence of the following items: (1) Apgar score < 5 at 10 min of age; (2) continued need for ventilation and resuscitation at 10 min of age; (3) cord pH ≤ 7.0 or base deficit ≤ − 16 mEq/L, or an arterial pH ≤ 7.0 or base deficit or base deficit ≤ − 16 mEq/L on a gas performed within 60 minutes of birth. Criteria B include the demonstration of moderate or severe encephalopathy demonstrated by complete neurological examinations and/or amplitude-integrated electroencephalogram (Mosalli, R, 2012; Peliowski-Davidovich, 2012). The success of hypothermia is limited, especially in newborns with severe encephalopathy (Gluckman et al., 2005; Rutherford et al., 2010). Some asphyxiated newborns treated with hypothermia still die and/or develop brain injury. (Wintermark, 2011). The reasons why brain injury continues to develop despite hypothermia treatment remain unclear, and further investigations on potential risk factors are still needed.

Chapter 2

Impact of maternal, delivery, perinatal characteristics on asphyxiated newborns treated with hypothermia from a local cohort

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Status of the Article: Prepared for submitting to a journal

Foreword: This manuscript is providing the results of the first project that I have worked on during my master's studies. It describes a clinical study that was conducted on a retrospective cohort of term asphyxiated newborns admitted to the NICU of Montreal Children's Hospital for therapeutic hypothermia. Some maternal, delivery, and perinatal characteristics were compared between newborns developing or not adverse outcome. The results from this study demonstrates that primiparity, prolonged duration of labor and neonatal acidosis place asphyxiated newborns at higher risk of developing adverse outcome despite hypothermia.

Maternal risk factors for adverse outcome

in asphyxiated newborns treated with hypothermia:

Parity and labor duration matter

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2.1. Abstract

Background: Birth asphyxia remaines a frequent cause of perinatal mortality and long-term neurodevelopmental sequelae, despite the introduction of therapeutic hypothermia. Some maternal characteristics may predispose these newborns to worst outcome.

Objective: To investigate the possible association between some maternal factors and the development of adverse outcome in asphyxiated newborns treated with hypothermia.

Design/Methods: We conducted a retrospective cohort study of asphyxiated newborns treated with hypothermia from 2008 to 2015. Maternal characteristics including parity and labor duration were collected. Adverse outcome was defined as death and/or evidence of brain injury; the severity of brain injury was evaluated using brain magnetic resonance imaging. Maternal characteristics were compared between asphyxiated newborns developing adverse outcome and those who did not.

Results: Asphyxiated newborns born from mothers who did not have a previous child had significantly more risk to develop adverse outcome (61%), compared to newborns born from mothers who had already one (19%) or more children (20%) ($p = 0.002$). Longer duration of vaginal delivery was also associated with more risk of adverse outcome ($p = 0.03$). Labor duration beyond 10 hours in mothers who did not have a previous child seemed to place the asphyxiated newborns at increased risk to develop adverse outcome despite hypothermia treatment.

Conclusions: Mothers who did not have a previous child, especially when they are experiencing prolonged duration of labor, are at higher risk to have an asphyxiated newborn developing adverse outcome despite therapeutic hypothermia. Labor duration beyond 10 hours in these women should be closely monitored.

2.2. Introduction

Birth asphyxia remains a serious condition in newborns (Bryce et al., 2005), responsible for significant perinatal mortality and long-term sequelae, despite the introduction of therapeutic hypothermia (Azzopardi et al., 2014; Shankaran et al., 2012; Simbruner et al., 2010). The development of neurological sequelae still appears in 65% of the asphyxiated newborns treated with hypothermia, compared to 78% in the non-treated newborns (Edwards et al., 2010; Jacobs, 2013; Shah, 2010; van Bel et al., 2016).

Previous studies in a general population of newborns have highlighted that some maternal characteristics may predispose the newborns to adverse outcome (Cowan et al., 2003; Klinger et al., 2005; Miller et al., 2005; Selewski et al., 2013). For example, parity and labor duration have been reported to be associated with more severe neonatal acidosis and lower Agpar scores (Altman et al., 2015; Barton et al., 1991). However, these factors have not been specifically studied in a population of asphyxiated newborns treated with hypothermia, and their impact on developing brain injury was never investigated. It remains thus important to better understand the possible impact of these maternal risk factors in asphyxiated newborns treated with hypothermia.

We hypothesized that some maternal characteristics may place the asphyxiated newborns treated with hypothermia at increased risk of death and/or brain injury. Thus, the objective of this

study was to assess the association between several maternal characteristics and the development of adverse outcome in asphyxiated newborns treated with hypothermia.

2.3. Methods

2.3.1. Study design

We conducted a retrospective observational cohort study of asphyxiated newborns admitted to our neonatal intensive care unit from 2008 to 2015 and treated with hypothermia. They all met the following criteria (Shankaran et al., 2005): (1) gestational age ≥ 36 weeks and birth weight ≥ 1800 grams; (2) evidence of fetal distress, i.e., a history of an acute perinatal event, cord pH ≤ 7.0 or base deficit ≤ − 16 mEq/L; (3) evidence of neonatal distress, such as an Apgar score ≤ 5 at 10 min, postnatal blood gas pH obtained within the first hour of life ≤ 7.0 or base deficit ≤ − 16 mEq/L, or a continued need for ventilation initiated at birth and continued for at least 10 min; (4) evidence of moderate to severe neonatal encephalopathy revealed by an abnormal neurological exam and/or an amplitude-integrated electroencephalogram. Eligible patients received whole-body cooling to an esophageal temperature of 33.5 °C initiated within the first 6 h of life, continued for 72 h, and then they were slowly rewarmed. The retrospectively collected database was approved by the institutional research ethics board who waived the need for informed consent, since the data were collected from reviewing charts without any additional testing of the newborns.

2.3.2. Variables and outcome

The general characteristics of the newborns were collected, including gestational age, birth weight, gender, Apgar score at 10 minutes, arterial cord pH, and initial blood gas pH. As per

standard cooling protocols at our institution, the severity of the neonatal encephalopathy was assessed on admission according to the modified Sarnat score on admission (Shankaran et al., 2005; Sarnat & Sarnat 1976).

In addition, maternal characteristics, including maternal age, parity, maternal diabetes, maternal hypertension, mode of delivery, time of rupture of maternal membranes, labor duration, and sentinel events, were recorded. Parity was defined as the number of viable offspring including the asphyxiated newborn. Maternal diabetes included both gestational diabetes as well as maternal diabetes prior to conception. Maternal hypertension included both gestational hypertension and hypertension that was preexisting before current pregnancy. Mode of delivery included vaginal delivery vs cesarean section. Rupture of maternal membranes (either artificial or natural) were recorded with regards to onset of labor, and classified into three groups: i.e., < 12 hours, 12-24 hours and > 24 hours. Labor duration was defined as the interval between the onset of labor and the time of birth; the onset of labor was defined as the time when the painful uterine contractions initiated regularly and/or the time of the rupture of maternal membranes (O'Driscoll, 1985; Crowther et al., 1989). Sentinel events at the time of birth, including shoulder dystocia, cord prolapse, uterine rupture, placenta praevia, vasa praevia, velamentous cord insertion, nuchal cord and placenta abruption, were collected. Clinical chorioamnionitis was defined as maternal fever (>38°C) and/or at least 2 symptoms of uterine tenderness, fetal tachycardia (>160 beats per minute) or maternal tachycardia (>120 beats per minute), maternal leukocytosis (total blood leukocyte count > 15,000 cells/mm^3) and foul-smelling amniotic fluid.

For the purpose of this analysis, adverse outcome was defined as death within the perinatal period and/or evidence of brain injury on brain magnetic resonance imaging. As per the current standard protocol in our neonatal intensive care unit for evaluating brain injury in these

newborns, a brain magnetic resonance imaging was performed after the hypothermia treatment was complete, usually around day 10 of life. Neuroradiologists, who were blind to the clinical condition of the newborns, interpreted the brain MRI, and reported the presence and extent of any brain injury as per a previously described MRI scoring system (Barkovich et al., 1998): i.e., a score of 0 for "no brain injury," a score of 1 for "abnormal signal in basal ganglia or thalamus", a score of 2 for "abnormal signal in cortex", a score of 3 for "abnormal signal in cortex and basal nuclei (basal ganglia or thalami)", and a score of 4 for "abnormal signal in entire cortex and basal nuclei".

2.3.3. Statistical analysis

Descriptive statistics including the mean and standard deviation or standard error for continuous variables and frequency distributions for categorical variables were produced for all variables. The differences between asphyxiated newborns treated with hypothermia (i) developing adverse outcome and (ii) not developing adverse outcome were assessed for statistical significance using Mann-Whitney U tests for continuous variables or Chi-square tests for categorical variables. Labor duration was further compared by parity. A Two-way ANOVA was used to assess the interactional effect of parity and labor duration on the risk of developing adverse outcome. A p value less than 0.05 was considered as statistically significant

2.4. Results

Two hundred and fifteen asphyxiated newborns received treatment with therapeutic hypothermia during the study period (**Figure 1**). Of these, 15% (33/215) died within the perinatal period. All the newborns who survived had a brain MRI; 55% (101/182) did not develop brain

injury, and 45% (81/182) developed brain injury despite hypothermia, including 26% (21/81) abnormal signal in basal ganglia or thalamus, 32% (26/81) abnormal signal in cortex, 30% (24/81) abnormal signal in cortex and basal nuclei, and 12% (10/81) abnormal signal in entire cortex and basal nuclei. Thus, for the remaining analysis, 53% (114/215) newborns were considered as having adverse outcome (dead and/or brain injury) and 47% (101/215) were not (no brain injury) (**Figure 1**).

General characteristics (i.e., birth weight, gestational age, and gender) of the newborns were similar between both groups. Newborns developing adverse outcome had worst primary adaptation, as demonstrated by worst Apgar score at 10 minutes ($p = 0.007$), as well as worst acidosis on cord pH ($p = 0.002$) and on the first postnatal gas within the first hour of life ($p = 0.001$); they also had more severe encephalopathy on admission ($p < 0.001$) (**Table 1**).

A majority of asphyxiated newborns treated with hypothermia (51%) were born from mothers who did not have a previous child; 29% were born from mothers who already had a previous child, and 20% were born from multiparous mothers. When comparing maternal characteristics between asphyxiated newborns treated with hypothermia developing or not adverse outcome, newborns born from primiparous women were at increased risk to develop adverse outcome (61%) ($p = 0.002$), compared to those born from mothers who already had a previous child (19%), and those born from multiparous mothers (20%) (**Table 1**). Other maternal characteristics, including maternal age, and incidence of maternal diabetes and maternal hypertension, were not different between the groups.

Approximately half the newborns were born by vaginal delivery and the other half by cesarean section. Forty-three percent of the vaginal deliveries were assisted with instruments (i.e., forceps and/or ventouse), and the majority (94%) of cesarean sections were emergent. When

comparing maternal characteristics between asphyxiated newborns treated with hypothermia developing or not adverse outcome (**Table 1**), the mode of delivery type was not associated with higher risk of adverse outcome in this cohort. Duration of rupture of maternal membranes was also not different between the groups. Labor duration in the newborns born following vaginal delivery was significantly longer in the newborns developing adverse outcome (11.98 ± 5.41 hours, $p = 0.027$) compared to those who did not (9.65 ± 5.84 hours) (**Table 2**). Incidence of chorioamnionitis was not different between the groups.

A majority of asphyxiated newborns treated with hypothermia (53%) were born in the context of at least one sentinel event. The three most common ones were nuchal cord (20%), placenta abruptio (15%), and shoulder dystocia (13%). The incidences of the different sentinel events were not different between the groups, except uterine rupture that was slightly more frequent in those developing adverse outcome (6% vs 1%, $p = 0.046$) (**Table 2**).

When comparing maternal characteristics according to parity (**Table 3**), labor duration was significantly longer in newborns born from mothers who did not have a previous child ($p = 0.03$), compared to newborns born from mothers who already had a previous child. A two-Factor ANOVA analysis revealed a significant interaction between parity and labor duration in determining the development of adverse outcome ($p = 0.04$) (**Figure 2**). Labor duration beyond 10 hours in mothers who did not have a previous child seemed to place the asphyxiated newborns at increased risk to develop adverse outcome despite hypothermia treatment.

2.5. Discussion

National statistics released by the Canadian government estimates that 47% newborns are born from mothers who did not have a previous child, 38% are born from mothers who already

had a previous child, and 15% are born from multiparous mothers (2011 Census of Population, Statistics Canada Catalogue no. 98-312-XCB2011019). In our study, the majority (51%) of asphyxiated newborns treated with hypothermia were born from mothers who did not have a previous child, which represents a higher incidence of such newborns than in the general population, suggesting that primiparity may be a risk factor for birth asphyxia in itself. In addition, in our study, primiparity was one of the highlighted maternal factors placing the asphyxiated newborns treated with hypothermia at risk of developing an adverse outcome. To our knowledge, only one previous study suggested that parity might be a risk factor for neonatal adverse outcome in a general population of newborns (Barton et al., 1991). They demonstrated that intrapartum scalp acidosis and neonatal seizures were significantly more frequent in primigravidae compared to multigravidae (Barton et al., 1991), but did not provide further information on brain injury and outcome of the newborns.

Longer labor duration was another of the highlighted maternal factors placing the asphyxiated newborns treated with hypothermia at risk of developing an adverse outcome. It is commonly described that, if left unaided, most women usually finish labor within 10 hours in total (Cunningham, 2014); however, this was not true for most of the primiparous women in our cohort. Previously, the American College of Obstetricians and Gynecologist has emitted guidelines recommending the avoidance of prolonged duration of second stage of labor in pregnant women (ACOG Practice Bulletin: Operative Vaginal Delivery, 2000). However, no clear guidelines currently exist about the total duration of labor in pregnant women. Our data suggest that labor duration beyond 10 hours in primiparous women, who will deliver vaginally, should be closely monitored. From the literature, most previous studies discussing the possible impact of prolonged labor duration (Altman, & Lydon-Rochelle, 2006; Altman et al., 2015; Janni

et al., 2002; Laughon et al., 2014; Menticoglou at al., 1995) have concentrated on the second stage of labor and the maternal consequences and only discussed neonatal outcome, such as fetal acidosis, low 5-minute Apgar score, neonatal seizures, or admission to the neonatal intensive care unit, but not other outcome such as death and/or brain injury. In addition, in most of these studies, the numbers of newborns with adverse outcome were small compared to the included population, limiting the detection of a reliable effect of prolonged labor on newborns (Altman & Lydon-Rochelle, 2006; Janni et al., 2002; Menticoglou et al., 1995).

Interestingly, labor duration was highly correlated with parity in our study, and, if both were present, the asphyxiated newborns treated with hypothermia seemed even more at risk to develop an adverse outcome. Other studies have previously demonstrated such association and its possible risk for the newborns. A retrospective study of a national cohort of Swedish pregnant primiparous women who delivered singleton by vaginal delivery highlighted the association between prolonged second stage of labor of 3 hours or more and low 5-min Apgar scores (Altman et al., 2015). Another large retrospective study of American pregnant women reported an absolute increased risk of birth asphyxia in primiparous women having prolonged second stage of labor with an epidural (Laughon et al., 2014). However, both studies did not take into account the first stage of labor when evaluating the impact of labor duration, and did not comment on the outcome of the asphyxiated newborns, especially on the development of brain injury. While it is known that primiparous women usually have longer labor duration of labor compared to multiparous women (Cunningham, 2014), it is not known beyond which time this prolonged labor duration becomes always associated with adverse outcome. Our study seems to suggest that labor duration beyond 10 hours in mothers who did not have a previous child seemed to place the asphyxiated newborns at increased risk to develop adverse outcome despite

hypothermia treatment. Further studies, optimally in large national or multinational prospective cohorts, needs to investigate whether a stricter monitoring of labor beyond 10 hours in mothers who did not have a previous child may reduce the incidence of newborns developing adverse outcome.

Interestingly, several of the other investigated maternal factors were more frequent in this cohort of newborns in comparison to the incidence described in various literature on the general neonatal population. Though these factors did not seem to place asphyxiated newborns at higher risk of developing adverse outcome. During the study period, more male newborns (male/female=1.31) were treated with hypothermia, when compared to the ratio of gender reported in the general neonatal population (male/female=1.05) (Joel et al., 2012). Maternal and neonatal infections also had a higher incidence in our cohort compared to the incidence reported in the general neonatal population, including clinical chorioamnionitis (23% versus 0.9-10.5%) (KOH et al.,1979; Soper et al., 1996), early onset neonatal sepsis (2% versus 1-4.6‰) (Sgro et al., 2011) and neonatal meningitis (3% versus 0.4‰) (Croxen & Finaly, 2010; Grover et al., 1961). Sentinel events were also more frequently documented in this cohort, compared to the incidence in the general neonatal population, including shoulder dystocia (13% versus 0.1-3%) (Gherman et al.,2006), uterine rupture (4% versus 0.4-0.7%) (Landon et al., 2004; Guise et al., 2004), placenta praevia (3% versus 0.3-0.6%) (Crane et al., 1999), vasa praevia (2% versus 1 per 2000/5000 delivery) (Hasegawa et al., 2012; Lee, W., et al., 2000), placenta abruptio (15% versus 1%) (Ananth & Wilcox, 2001). Only uterine rupture was significantly more frequent in those developing adverse outcome.

The strength of the present study was the study of these maternal risk factors for the first time to our knowledge in a large population of asphyxiated newborns. Previous studies that have looked at these factors in a general neonatal population only included a very limited number of asphyxiated newborns. The main limitation of the present study was the lack of neurodevelopmental follow-up studies for our patients. Future studies should investigate how these early maternal risk factors influence long-term neurodevelopmental impairments. Another limitation of this study was that labor duration was not always clearly documented. In addition, we could unfortunately not run a separate analysis looking at the impact of the duration of the first stage of labor vs the duration of the second stage of labor, as the differences between the two stages were not always clearly specified in the charts. However, since uterine contractions during labor may decrease uteroplacental blood flow resulting in reduced blood and oxygen delivery to the fetus (Liston et al., 2007), we believe that both prolonged first stage and second stage of labor may have an impact. Most healthy fetuses probably tolerate well the reduction in uteroplacental blood flow caused by uterine contractions and have no adverse effects. However, it may be different for newborns prone to asphyxia, for example those with sentinel events.

In conclusion, mothers who did not have a previous child, especially when they present with prolonged duration of labor, are at higher risk to have an asphyxiated newborn developing adverse outcome despite hypothermia. Further studies need to investigate how the management of these women could be improved to reduce the incidence of death and/or brain injury in their newborns. In the meantime, labor duration beyond 10 hours in these women should probably be

2.6. Figures

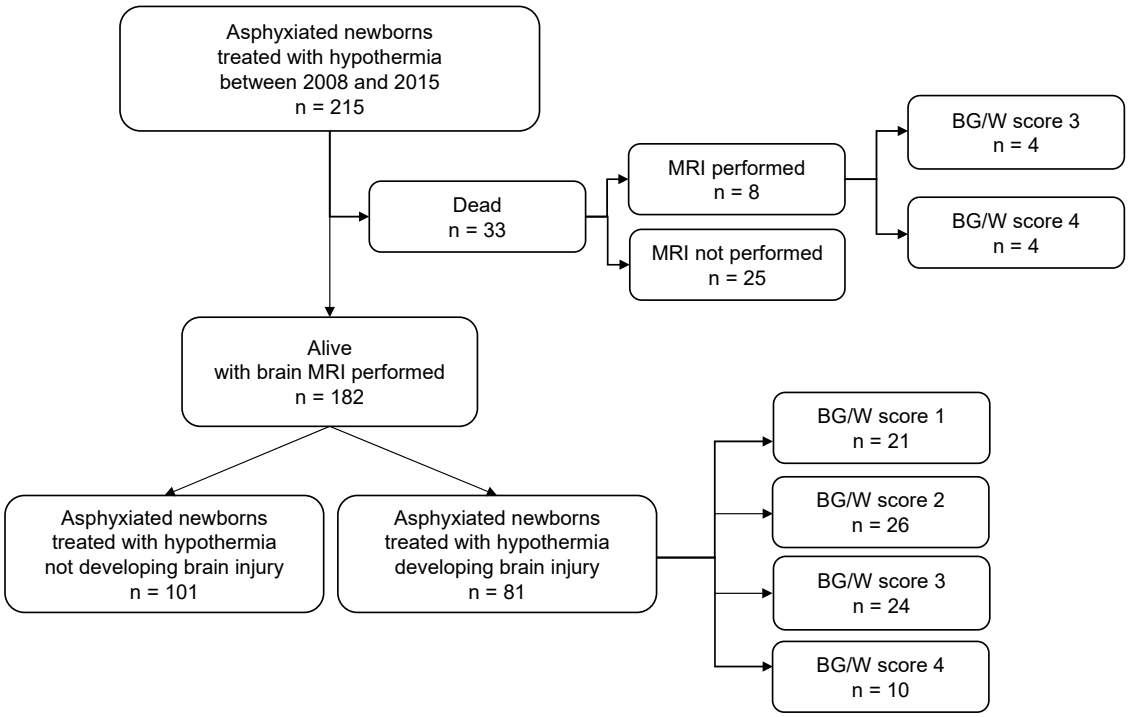

Figure 1: During the study period, 215 asphyxiated newborns were treated with hypothermia. 53% (114/215) developed adverse outcome

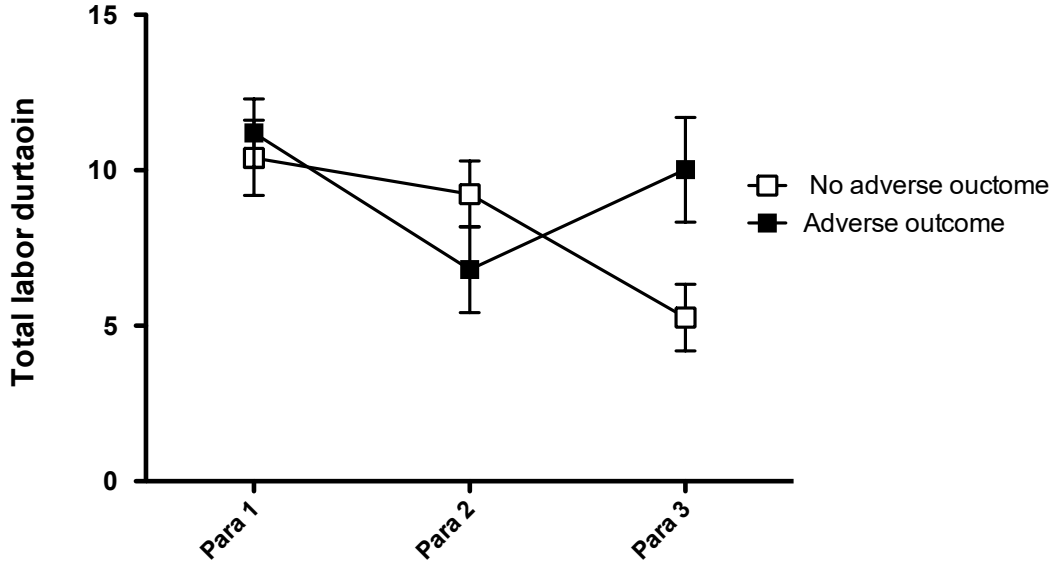

Figure 2: Adverse outcome by duration of labor & parity in asphyxiated newborns treated with hypothermia: Total labor duration was counted as the hours between onset of labor and time of birth. Mean ± SEM. Parity was defined as the number of viable offspring including the one born in the duration of this study period. *p<0.05 was considered statistically significant.

2.7. Tables

Table 1

TABLE 1: General characteristics of the asphyxiated newborns treated with hypothermia, categorized by the presence or not of adverse outcome.

	All newborns (n = 215)	**Newborns not developing adverse outcome** (n =101)	**Newborns developing adverse outcome** (n = 114)	***p* value**
General characteristics				
Birth weight (g), mean ± SD	3377 ± 634	3460 ± 681	3303 ± 581	*0.07*
Gestational age (w), mean ± SD	39 ±1.47	39.15 ± 1.54	39.00 ± 1.55	*0.14*
Gender	Male/female 1.31			*0.22*
Female, n (%)	93 (43)	42 (42)	51 (45)	
Male, n (%)	122 (57)	59 (58)	63 (55)	
Apgar score ≤ 5 at 10 minutes, n(%)	127 (59)	50 (50)	77 (68)	*0.007*
Arterial cord pH, mean ± SD	6.99 ± 0.19	7.03 ± 0.17	6.95 ± 0.20	*0.002*
Initial postnatal blood gas pH, mean ± SD	7.04 ± 0.17	7.08 ± 0.15	7.00 ± 0.20	*0.001*
Initial severity of encephalopathy on admission				*< 0.001*
Mild, n (%)	29 (13)	18 (18)	11(10)	
Moderate, n (%)	144 (67)	78 (77)	66 (58)	
Severe, n (%)	42 (20)	5 (5)	37 (32)	

Adverse outcome was defined as death within the perinatal period and/or evidence of brain injury on brain magnetic resonance imaging.

Table 2

TABLE 1: Maternal characteristics of the asphyxiated newborns treated with hypothermia, categorized by the presence or not of adverse outcome.

	All newborns (n = 215)	**Newborns not developing adverse outcome** (n =101)	**Newborns developing adverse outcome** (n = 114)	***p* value**
Maternal characteristics				
Maternal age, mean ± SD	30.48 ± 5.52	30.17 ± 5.87	30.77 ± 5.19	*0.43*
Parity				*0.002*
1, n (%)	110 (51)	41 (41)	69 (61)	
2, n (%)	63 (29)	41 (41)	22 (19)	
≥ 3, n (%)	42 (20)	19 (18)	23 (20)	
Maternal diabetes, n (%)	34 (16)	14 (14)	20 (18)	*0.46*
Maternal hypertension, n (%)	19 (9)	6 (6)	13 (11)	*0.15*
Mode of delivery				
Vaginal, n (%)	111 (52)	56 (55)	55 (48)	*0.29*
Vaginal with instruments, n (%)	48/111 (43)	24/56 (43)	24/55 (44)	*0.93*
Cesarean section, n (%)	104 (48)	45 (45)	59 (52)	*0.01*
Emergent cesarean section, n (%)	98/104 (94)	39/45 (87)	59/59 (100)	*0.01*
Rupture of maternal membranes				
<12 hours, n (%)	146 (72)	66 (69)	80 (75)	*0.40*
12-24 hours, n(%)	40 (20)	20 (21)	20 (19)	
>24 hours, n(%)	16 (8)	10 (10)	6 (6)	
Duration of labor				
- All newborns Available, n (%)	171 (80)	83 (82)	88 (77)	
Total duration in all newborns (hours), mean ± SD	9.56 ± 7.03	8.96 ± 6.47	10.14 ± 7.52	*0.41*
- Newborns born vaginally Available, n (%)	99/111 (89)	53/56 (95)	46/55 (84)	
Total duration of labor in newborns born vaginally (hours), mean ± SD	10.73 ± 5.74	9.65 ± 5.84	11.98 ± 5.41	*0.027*
- Newborns born by emergent cesarean section				

Available, n (%)	67/98 (68)	27/39 (69)	40/59 (68)	
Total duration of labor in newborns born by emergent cesarean section (hours), mean ± SD	8.27 ± 8.60	8.01 ± 7.74	8.03 ± 9.06	*0.79*
Clinical chorioamnionitis, n (%)	50 (23)	27 (27)	24 (21)	*0.42*
At least 1 sentinel event at birth, n (%)	113 (53)	52 (51)	61 (54)	*0.77*
- In newborns born vaginally, n (%)	65/111 (59)	35/56 (63)	30/55 (55)	*0.40*
- In newborns born by emergent cesarean section, n (%)	46/98 (47)	15/39 (38)	31/59 (53)	*0.15*
Nuchal cord, n (%)	42(20)	22(22)	20(28)	*0.43*
Placenta abruptio, n (%)	32 (15)	15 (15)	17 (15)	*0.99*
Shoulder dystocia, n (%)	29 (13)	16 (16)	13(11)	*0.34*
Cord prolapse, n (%)	13 (6)	6 (6)	7 (6)	*0.95*
Uterine rupture, n (%)	8 (4)	1 (1)	7 (6)	*0.046*
Placenta praevia, n (%)	7 (3)	5 (5)	2 (2)	*0.19*
Vasa praevia, n (%)	4 (2)	2 (2)	2 (2)	*0.90*
Velamentous cord insertion, n (%)	2 (1)	0 (0)	2 (2)	*0.18*

Adverse outcome was defined as death within the perinatal period and/or evidence of brain injury on brain magnetic resonance imaging.

Table 3

TABLE 3: Labor duration by Parity in asphyxiated newborns treated with hypothermia, categorized by parity.

	Parity 1 (n=110)	**Parity 2** (n =63)	**Parity ≥ 3** (n = 42)	***p* value**
Mode of delivery Vaginal, n (%)	58 (53)	32 (51)	21(50)	*0.94*
Vaginal with instruments, n (%)	26/58 (45)	14/32 (44)	8/21 (38)	*0.87*
Cesarean section, n (%)	52 (47)	31 (49)	21 (50)	0.94
Emergent cesarean section, n (%)	48/52 (92)	29/31 (94)	21/21 (100)	*0.43*
Labor duration Available in # patients	88 (80)	48 (76)	35 (83)	
Labor duration, (hours), mean ± SD	10.78 ± 7.60	8.43 ± 5.91	7.85 ± 6.51	*0.03*
At least 1 sentinel event at birth, n (%)	54 (49)	38 (60)	21 (50)	*0.44*

Chapter 3

Impact of maternal, delivery, perinatal characteristics on asphyxiated newborns treated with hypothermia from a national cohort

Authors: Eric Hongbo Xu, Martine Claveau, Pia Wintermark, on behalf of the Canadian Neonatal Network (CNN)

Status of the Article: Prepared for submitting to a journal

Foreword: This manuscript is providing the results of the second project of my master's studies. After investigating the impact of some maternal, delivery, and perinatal characteristics in our local cohort, I was interested to see if similar findings can be found in a national cohort of these newborns. Therefore, the access of CNN database was requested and permitted. This study included all admissions to CNN tertiary-level NICUs throughout Canada. The number of newborns with neonatal encephalopathy treated with hypothermia was defined. Maternal, delivery, and perinatal characteristics were compared between newborns developing or not adverse outcome. The results from this study demonstrated that birth asphyxia remains a significant problem in Canada. Hypotension requiring inotrope use, renal failure during hospital stay, in addition to prolonged low Apgar scores, were the main predictors of adverse outcome.

Asphyxiated newborns treated with hypothermia across Canada:

Incidence and determinants of adverse outcome

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Short title: Adverse outcome despite hypothermia.

Key words: birth asphyxia; hypoxic-ischemic encephalopathy; brain injury; magnetic resonance imaging.

Abbreviations: CNN, Canadian Neonatal Network; MRI, magnetic resonance imaging; HIE, hypoxic-ischemic encephalopathy; NICUs, neonatal intensive care units.

Conflict of interest: This manuscript has been contributed to, seen and approved by all the authors. There is no conflict of interest. All the authors fulfill the authorship credit requirements

3.1. Abstract

Background: Despite improvements in neonatal care and the introduction of therapeutic hypothermia, the prevalence of mortality and morbidity related to birth asphyxia remain elevated.

Objective: To define the incidence of admission for birth asphyxia to tertiary-level neonatal intensive care units (NICUs) across Canada, and to investigate birth and postnatal risk factors for adverse outcome despite therapeutic hypothermia.

Methods: This retrospective observational cohort study examined the newborns born with a gestational age ≥ 36 weeks and a birth weight ≥ 1800 g with a diagnosis of hypoxic-ischemic encephalopathy (HIE), who were admitted to the Canadian Neonatal Networks (CNN) tertiary-level NICUs between January 2010 and December 2014. Adverse outcome was defined as evidence of injury on brain magnetic resonance imaging (MRI) exams or death without MRI being performed. Birth and postnatal characteristics were compared between asphyxiated newborns treated with hypothermia who developed adverse outcome and those who did not.

Results: During the study period, the incidence of newborns with a diagnosis of HIE represented 1.143 per 1000 births in Canada, and 6.7% of the admissions with a gestational age ≥ 36 weeks and a birth weight ≥ 1800g to tertiary-level NICUs. Among them, 52% were treated with hypothermia, and 40% of those developed brain injury or died without MRI being performed. A lower Apgar score at 10 minutes was associated with a higher risk of adverse outcome (OR [95% CI]: 1.18 [1.10-1.26] per unit of decrease). In addition, hypotension requiring inotrope use (OR [95% CI]: 1.87 [1.38-2.55]) and renal failure (OR [95% CI]: 2.03 [1.49-2.76]) during the hospital

stay were both independent predictors of increased risk of adverse outcome. Birth weight, intrauterine growth retardation, and gender did not have significant impact on the development of an adverse outcome.

Conclusion: Birth asphyxia remains a significant problem in Canada, and many newborns still die and/or develop brain injury despite hypothermia treatment. Hypotension requiring inotrope use and renal failure during the hospital stay, in addition to prolonged low Apgar score at birth, were the main predictors of adverse outcome.

3.2. Introduction

Despite improvements in neonatal care, birth asphyxia in term newborns remains a serious condition causing significant mortality (i.e., 23% of all neonatal deaths worldwide) (Bryce et al., 2005; Lawn et al., 2005) and long-term morbidity, including cerebral palsy and intellectual disabilities (De Vries & Jongmans, 2010; Polat et al., 2013). The global incidence of birth asphyxia varies between 1.3% and 6.6%, depending on which part of the world the newborn was born (Maulik & Darmstadt, 2007; Shah, 1991).

The only available treatment for this condition is hypothermia (i.e., cooling the whole body to an esophageal temperature of 33.5°C initiated within the first 6 hours of life and continued for 72 hours) that may prevent the development of brain injury (Edwards et al., 2010; Jacobs, 2013; Shah, 2010). It has now become the standard care in all tertiary-level neonatal intensive care units (NICUs) across Canada since 2010 (Peliowski-Davidovich, 2012). Despite the overall improvement in reducing neonatal mortality and morbidity, the success of this treatment is limited, especially in those asphyxiated newborns with severe encephalopathy on admission (Gluckman et al., 2005; Rutherford et al., 2010). It remains important to understand why brain injury develops in asphyxiated newborns despite hypothermia treatment (Barks, 2008; Levene, 2010).

We hypothesize that some perinatal characteristics permit to explain why the asphyxiated newborns treated with hypothermia develop brain injury. By accessing a national perinatal-neonatal database, the first objective of this study was to define the incidence of admission for birth asphyxia to tertiary-level neonatal intensive care units (NICUs) across Canada. The second objective was to investigate maternal, birth and postnatal risk factors for adverse outcome despite therapeutic hypothermia in this population of newborns.

3.3 Methods

3.3.1. Study design

The current study was a retrospective observational cohort study, analyzing prespecified data from the Canadian Neonatal Network (CNN) database. The CNN is a national perinatal-neonatal database including data from 29 of the 30 tertiary-level neonatal intensive care units (NICUs) in Canada, covering approximately 95% of tertiary-level neonatal intensive care units nationally. Data were abstracted from infant medical records at each site according to standardized definitions (Canadian Neonatal Network. Canadian Neonatal Network Abstractor's Manual, 2014) and electronically transmitted to the CNN coordinating center as previously described (Lee, S. K., 2000). Data collection at each site was approved by the respective institutional research ethics boards. The retrospective evaluation of de-identified data for this study was approved by the local institutional research ethics board and by the CNN Executive Committee.

3.3.2. Study population

Newborns with a gestational age of 36 weeks or more and a birth weight of 1800g or more and admitted with a diagnosis of hypoxic-ischemic encephalopathy (HIE) to one of the Canadian Neonatal Network tertiary-level NICUs between January 2010 and December 2014 were included in this study. Newborns were diagnosed with HIE when they presented with encephalopathy in addition to the two following conditions: (1) documented evidence of an acute perinatal event, such as fetal distress, cord prolapse, uterine rupture, reduced fetal movements, abruptio, antepartum hemorrhage or emergency cesarean section due to fetal distress; and (2)

evidence of intrapartum hypoxia, with at least one of the following item: (a) Apgar score ≤ 5 at 10 minutes, (b) mechanical ventilation or resuscitation within 10 minutes, and/or (c) cord pH < 7.00 or infant arterial pH < 7.00 or base deficit ≤ -12 within 60 minutes of birth. Degree of encephalopathy was defined as: (1) mild (stage 1), if the newborns displayed irritability, jitteriness, hyperalertness; (2) moderate (stage 2), if the newborns displayed lethargy, hyperreflexia, miosis, bradycardia, seizures, abnormal tone, weak suck and Moro reflex; (3) severe (stage 3), if the newborns presented with stupor, flaccidity, small to mid-position pupils which react poorly to light, decreased stretch reflexes, hypothermia and absent Moro reflex; and (4) not available, if the severity of encephalopathy could not be ascertained clearly from the infant medical records. The highest grading of encephalopathy at any time during the NICU stay was recorded into the database. Timing to initiation of hypothermia treatment was also recorded. Newborns with major congenital anomalies were excluded.

3.3.3. Variables and outcome

Study variables were defined according to the CNN manual (Canadian Neonatal Network. Canadian Neonatal Network Abstractor's Manual, 2014). Maternal, birth and postnatal characteristics of the newborns included in our cohort were analyzed. Maternal characteristics included maternal age, parity, maternal diabetes, and maternal hypertension. Parity was defined as the number of viable offspring in previous pregnancies. Maternal diabetes included both gestational diabetes as well as maternal diabetes prior to conception. Maternal hypertension included both gestational hypertension and hypertension that was preexisting before current pregnancy. Birth characteristics included the general characteristics of the newborns, such as birth weight, gestational age, and gender. Gestational age was calculated based on the best available information: date of conception via in-vitro fertilization pregnancy, early ultrasound,

date of last menstrual period, and/or neonatal assessment at birth. Mode of delivery included vaginal delivery vs cesarean section. Rupture of maternal membranes (either artificial or natural) were recorded with regards to onset of labor and classified into four groups: i.e., < 24 hours, 24 hours to 1 week, > 1 week between rupture of membranes and time of birth, or not available. Clinical chorioamnionitis was recorded if the mother presented with fever ≥ 38.4°C within 24 hours before birth, uterine tenderness and/or leukocytosis > 15000/mm^3, if the chart mentioned chorioamnionitis, and/or if the pathology report confirmed the diagnosis. Resuscitation details that the infant received in the first 30 minutes after birth included whether the newborns received positive pressure ventilation via endotracheal tube, and/or chest compression for more than 30 seconds. Seizures were recorded if witnessed by 2 or more clinicians and antiepileptics treatment was ordered and/or if diagnosed by electroencephalography and/or amplitude-integrated electroencephalography. Hypotension requiring inotropes, including dopamine, dobutamine, epinephrine, norepinephrine, and/or phenylephrine, was recorded; the use of epinephrine for initial resuscitation was not considered in this diagnosis. Persistent pulmonary hypertension was defined by clinical and echocardiographic evidence of pulmonary hypertension, and the use of nitric oxide. Renal failure was defined as urine output < 0.5ml/kg/hour and/or rising creatinine > 100 mmol/L at any time within the first 72 hours of life. Early onset sepsis was diagnosed if a pathogen was detected in the blood within the first 7 days of life. Meningitis was diagnosed if a pathogen was detected in the cerebrospinal fluid.

For the purpose of this analysis, adverse outcome was defined as death without MRI being performed and/or evidence of brain injury on brain magnetic resonance imaging (MRI) exams. Presence of brain injury was noted if injury to the white matter (watershed injury) and/or basal ganglia injury and/or grey matter injury and/or diffusion changes were present.

3.3.4. Statistical analysis

Descriptive statistical methods were used to summarize the study cohort. Birth and postnatal characteristics were compared between asphyxiated newborns treated with hypothermia who developed adverse outcome and those who did not, using the chi-square test for categorical variables and Student t-test for continuous variables. General characteristics (gestational age, birth weight, sex) and variables for which a trend for statistical significance ($p < 0.05$) were then be included in a multiple logistic regression analysis to identify the independent predictors of adverse outcome. Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). A two-sided significance level of *0.05* was used without adjustment for multiple comparisons.

3.4. Results

During the study period, 71105 newborns were admitted to one of the CNN tertiary-level NICUs (**Figure 3**). Among all the admitted newborns, 32700 had a gestational age greater or equal to 36 weeks and a birth weight greater or equal to 1800 grams; and 6.7% (2187/32700) were admitted with a diagnosis of HIE. According to the statistics published by the Canadian government, 1912601 infants were born across Canada during the same study period (Statistics Canada, CANSIM, table 051-0004 and Catalogue no. 91-215-X, 2015: http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/demo04a-eng.htm); newborns diagnosed with HIE and admitted to one of the CNN tertiary-level NICUs represented thus 1.143‰ (95% CI: 1.096 - 1.191‰) of all births in Canada.

Among the newborns diagnosed with HIE, 48% (814/2187) were not treated with hypothermia, and 52% (1144/2187) were treated with hypothermia (**Figure 3**). Among the

asphyxiated newborns who were not treated with hypothermia, 56% (453/814) had mild encephalopathy, 18% (144/814) had moderate encephalopathy, and 7% (60/814) had severe encephalopathy; severity of encephalopathy was not available in 19% (157/814). Reasons for not cooling newborns diagnosed with moderate or severe HIE included delayed transfer (n = 69), unit policy (n = 56), extreme condition (n=28), health care preference (n = 15), head traumatism or intracranial hemorrhage (n = 7), parental request (n = 1), and/or the reason was not available (n=46).

Among the asphyxiated newborns treated with hypothermia, 49% (565/1144) had moderate encephalopathy, 25% (290/1144) had severe encephalopathy, and 19% (221/1144) had mild encephalopathy; severity of encephalopathy was not available in 6% (68/1144). Among those newborns treated with hypothermia, 86% (983/1144) survived, and 14% (161/1144) died. Age at the time of death was 7.1 ± 8.1 days (range: 1-57); causes of death were not recorded in the database. Brain MRI results were available in 89% (877/983) asphyxiated newborns treated with hypothermia who survived and in 52% (83/161) of those who died. Exact timing of the brain MRIs was not recorded in the database. Reasons for not performing brain MRIs, especially in those newborns who survived, were not recorded; of note, among the newborns who survived but did not have a brain MRI, 44% (45/102) had moderate encephalopathy, 30% (31/102) had mild encephalopathy, 13% (13/102) had severe encephalopathy, and severity of encephalopathy was not available in 13% (13/102). For those who died without available brain MRI results (n = 78), age at the time of death was 3.2 ± 1.8 days (range: 1-9). Among the asphyxiated newborns treated with hypothermia with available brain MRI results, 60% (578/960) did not demonstrate brain injury at the time of the MRI, and 40% (382/960) demonstrated brain injury. Thus, for the

remaining analysis, 460 newborns were considered as having adverse outcome (dead without imaging and/or brain injury) and 578 were not (no brain injury on imaging) (**Figure 3**).

When comparing the asphyxiated newborns treated with hypothermia developing or not an adverse outcome (**Table 4**), maternal characteristics, including maternal age, parity, and incidence of maternal diabetes and maternal hypertension, were not different between the groups. Perinatal baseline characteristics (i.e., birth weight, gestational age, incidence of small for gestational age, and gender) were similar between both groups. Newborns developing adverse outcome were more often delivered by cesarean section ($p = 0.005$). Their birth were more often preceded by a rupture of membranes greater than 24 hours ($p = 0.04$); however, the incidence of chorioamnionitis were not different between the two groups. Newborns developing adverse outcome had worst primary adaptation, as demonstrated by worst Apgar score at 10 minutes ($p < 0.0001$), as well as higher incidence of positive pressure ventilation via endotracheal tube ($p < 0.0001$) and chest compression for more than 30 seconds ($p < 0.0001$). Newborns developing adverse outcome presented with more severe encephalopathy on admission ($p < 0.0001$) and had more often seizures ($p < 0.0001$) during their hospitalization. Time to initiation of hypothermia treatment was not different between the two groups. In addition, newborns developing adverse outcome presented more often with hypotension requiring inotrope use ($p < 0.0001$), persistent pulmonary hypertension ($p = 0.0003$) and renal failure ($p < 0.0001$) during their hospitalization in the neonatal intensive care unit; however, the incidence of early onset sepsis and meningitis was not different between the two groups.

A multivariate analysis showed that when adjusting for general characteristics (i.e., gestational age, small for gestational age, male gender) and significantly different variables between the two groups (i.e., Apgar score at 10 minutes, hypotension requiring inotrope use,

persistent pulmonary hypertension and renal failure), both inotrope use and renal failure were identified as independent predictors of adverse outcome associated with an increased odds of adverse outcome (respectively, odds ratio [95% CI]: 1.87 [1.38-2.55] for inotrope use, $p < 0.001$, and 2.03 [1.49-2.76] for renal failure, $p < 0.001$) (**Table 5**). A lower Apgar score at 10 minutes was also significantly associated with a higher risk of adverse outcome (odds ratio [95% CI]: 1.18 [1.10-1.26], $p < 0.001$) (**Table 5**). The remaining variables that were examined did not have significant impact on developing an adverse outcome.

3.5. Discussion

Birth asphyxia remains thus a significant problem in Canada, with an incidence similar to the one previously described in developed countries (Maulik & Darmstadt, 2007). It represents a non-negligible number (i.e., 6.7%) of newborns with a gestational age ≥ 36 weeks and a birth weight ≥ 1800g admitted to the Canadian Neonatal Networks (CNN) tertiary-level NICUs. This number may even be a little bit underestimated, as CNN database covers approximately 95% of tertiary-level neonatal intensive care units nationally (1/30 tertiary-level NICUs across Canada not included), and as newborns deemed too sick to be transported to tertiary-level NICUs may not have been referred from the birth hospital and thus may not have been accounted for in the database. Despite improvements in neonatal care and the introduction of therapeutic hypothermia, our study confirmed that many asphyxiated newborns still die and/or develop brain injury.

A significant proportion of newborns diagnosed with HIE were not cooled in our study. Most of the newborns with HIE who were not cooled had an initial mild encephalopathy. However, still a quite large proportion (i.e., 25%) of asphyxiated newborns with moderate or severe encephalopathy were also not cooled in this population. Delayed transfer was the most

frequent reason not to cool them, and reflect the fact that some newborns are still not recognized within the adequate timeframe to benefit from hypothermia and/or they arrive beyond the 6-hours window in the tertiary-level NICUs. It probably also reflects that many units across Canada still do not systematically offer passive cooling on transport. However, passive cooling during transport has been demonstrated to be safely performed with minimal equipment on transport (Chaudhary et al., 2013; Kendall et al., 2010) and should thus strongly be considered, at least for those newborns whose admission in the tertiary-level NICUs will be delayed beyond first 6 hours of life because of long transportation time. A delay in referral should not prevent offering hypothermia treatment to these newborns (Khurshid et al., 2011). More concerning reasons not to cool newborns with moderate or severe encephalopathy included unit policy and health care preference, as hypothermia treatment is now the standard care in all tertiary-level NICUs across Canada since 2010 and eligibility criteria are pretty well defined. Even if this treatment is proven to be less successful in the newborns with severe encephalopathy (Gluckman et al., 2005; Rutherford et al., 2010), it should still be offered to them as it is still of benefit in some of them. Starting the cooling for the newborns with severe encephalopathy would not prevent a reassessment of the encephalopathy during the first days of life and any potential discussion for withdrawal of care may still happen if deemed necessary (Wilkinson et al., 2011). Parental request not to cool should also be dealt with by giving more explanations to the parents about the benefits of this treatment and should not constitute an excuse not to cool anymore, as the evidence for the benefits of hypothermia treatment are clearly demonstrated (Edwards et al., 2010; Jacobs, 2013; Shah, 2010).

Alternatively, interestingly, a quite large proportion (19%) of asphyxiated newborns with mild encephalopathy were cooled, even if the current guidelines only recommend hypothermia

treatment for moderate and severe encephalopathy (Shankaran et al., 2005). This may related to the fact that some tertiary-level NICUs used amplitude-integrated electroencephalogram in addition to the clinical assessment to evaluate the initial degree of encephalopathy during the study period, and it has been previously demonstrated that some newborns with mild encephalopathy by clinical assessment may correspond to moderate encephalopathy by amplitude-integrated electroencephalogram (Gagne-Lorange et al., 2016). The aEEG coupled with an early neurological exam has been shown to improve the prediction of the term newborns at risk for persistent encephalopathy (Shalak et al., 2003). The practice to cool newborns with mild encephalopathy may also reflect that unit policy to cool or not these newborns may differ across Canadian tertiary-level NICUs; and health care preference may also probably intervene in the decision. Some literature suggests that some newborns from this population may benefit from cooling (DuPont et al., 2013; Gagne-Lorange et al., 2016; Massaro et al., 2015; Odd et al., 2009; Van Handel et al., 2010; Walsh et al., 2017). The current study did not permit to evaluate whether cooling this population might be of benefit to reduce the development of brain injury, as brain MRIs were not systematically performed in this population.

As expected, asphyxiated newborns developing adverse outcome despite hypothermia treatment had worst primary adaptation, with worst Apgar score, with more often active resuscitation, and with more severe encephalopathy, suggesting a worst initial asphyxial event. They were also born more often by cesarean section, probably related to an increased incidence of sentinel events in these newborns. Their births were more often preceded by a rupture of membranes greater than 24 hours; however, the incidence of chorioamnionitis, easly onset sepsis and meningitis were not different between the two groups. These results suggest that these newborns were born from mothers who probably experienced a longer duration of labor (Gross

et al, 2009), which has been suggested as a risk factor for adverse outcome (Altman et al., 2015; Laughton et al., 2014).

Hypotension requiring inotrope use and renal failure were the two other independent predictors of adverse outcome. Other studies have pointed out these two neonatal complications as additional risk factors contributing to brain injury and/or outcome (Al Balushi et al., 2017; Gupta et al., 2016; Sarkar et al., 2014; Selewski et al., 2013). The initial asphyxial event already compromised the cerebral blood flow autoregulation, and so hypotension may further impair this ability to autoregulate cerebral blood flow (Chalak et al., 2014; Rosenberg, 1988). Hypotension in the context of asphyxia has been shown to further decrease cerebral blood flow and cerebral oxygen delivery, increase cerebral fractional oxygen extraction, and decrease oxygen consumption (Chalak et al., 2014; Peng et al., 2015; Tsuji et al., 1998). Ultimately, hypotensive episodes combined with asphyxia have been shown to lead to decreased cerebral blood flow and impaired cerebral vasoregulation, leading to more severe brain injury (Chalak et al., 2014; Tsuji et al., 1998), and hypotension was associated with an increased risk of (severe) brain injury in aspxhyiated newborn treated with hypothermia (Al Balushi et al., 2017). In addition, renal failure has been shown to have a negative impact on multiple body organs (Doi & Rabb, 2016), especially in the context of perinatal asphyxia. Newborns with acute kidney injury in the context of asphyxia have been demonstrated to have higher overall mortality, to stay longer in the neonatal intensive care unit and to require prolonged mechanical ventilation (Selewski et al., 2013). Newborns with acute kidney injury also developed more often brain injury on brain imaging performed after cooling (Sarkar et al., 2014). Further prospective studies on the optimal management to treat hypotension and to prevent renal failure in term asphyxiated newborns treated with hypothermia are warranted. In the meantime, a strict monitoring of blood pressure

and its prompt correction and fluid management on a daily basis based on end-organs damages in each of these newborns should probably be targeted to maintain homeostasis as much as possible, and prevent ongoing brain injury.

Practice to perform brain MRI in all asphyxiated newborns treated with hypothermia is not uniform across Canada, as demonstrated by the fact that 11% of the asphyxiated newborns treated with hypothermia who survived did not have brain imaging. Reasons for not performing brain MRIs, especially in the surviving newborns, were unfortunately not recorded in the database. The degree of encephalopathy may explain partially why brain MRI was not performed in some of them, especially those with mild encephalopathy; however, it did not permit to explain why it was not performed in those with moderate or severe encephalopathy who survived (57%). We can speculate that a reassuring neurological exam before NICU discharge may be another reason why brain MRI was not performed, as this has been demonstrated as a predictor of death or disability (Shankaran et al., 2012); however, some brain injury may not always be symptomatic yet at time of discharge from the NICU and brain MRI may still be useful in those newborns to document the injury. Unfortunately, also the exact timing of the brain MRIs was not recorded into the database. Patterns of injury detected by brain MRI vary considerably during the first 2 weeks of life of asphyxiated newborns (Boudes et al., 2015; Chakkarapani it al., 2016), and the prognostic value of brain MRI findings following birth asphyxia may be affected by the postnatal age at the time of the MRI scan. Since early diffusion abnormalities normalize over time, and injury may not be completely visible on T1- and T2-weighted imaging before day 7 of life, MRIs performed between day 4 and 6 of life may be falsely reassuring. Current recommendations are to perform brain MRI in those newborns between day 7 and 21 of life (American College of Obstetricians and Gynecologists' Task Force on Neonatal Encephalopathy.

Obstet Gynecol 2014). Developing specific guidelines for systematically imaging all the newborns with HIE treated with hypothermia across Canada would provide a more definitive answer regarding the incidence of brain injury in these newborns.

The main limitation of this study is that long-term neurodevelopment was not included as a measure of adverse outcome. Follow-up data are up to now only collected through CNN for the preterm newborns across Canada, but unfortunately not yet for the term asphyxiated newborns treated with hypothermia. Such follow-up of large cohorts would be helpful to better understand how early determinants of adverse outcome unfolds into neurodevelopmental impairments. In this study, brain injury was used as a short-term outcome in addition to death, as brain injury has been shown to predict neurodevelopment in those newborns (Charron et al., 2016; Shankaran et al., 2015). Another limitation of the study was that different scoring systems are used across Canada to evaluate the initial degree of encephalopathy in asphyxiated newborns, including the modified Sarnat scoring system (Shankaran et al., 2005; Sarnat & Sarnat 1976) and the Thompson score (Thompson et al., 1997). This may have introduced some variations in the grading on encephalopathy and may have impacted our results.

In conclusion, birth asphyxia remains a significant problem in Canada, and many newborns still died and/or developed brain injury despite hypothermia treatment. Resuscitation measures at birth, as well as hypotension and renal failure during the hospital stay, were the main predictors of adverse outcome. A strict monitoring of blood pressure and its prompt correction, as well as an individualized fluid management to prevent renal failure, should be targeted for those newborns to maintain as much as possible homeostasis and to prevent ongoing brain injury. General guidelines should be developed at a national level to assess the initial degree of encephalopathy, to image the brain injury, and to follow up the neurodevelopment of these

asphyxiated newborns treated with hypothermia, so to better understand how initial treatments may impact outcome in these newborns.

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3.7. Figures

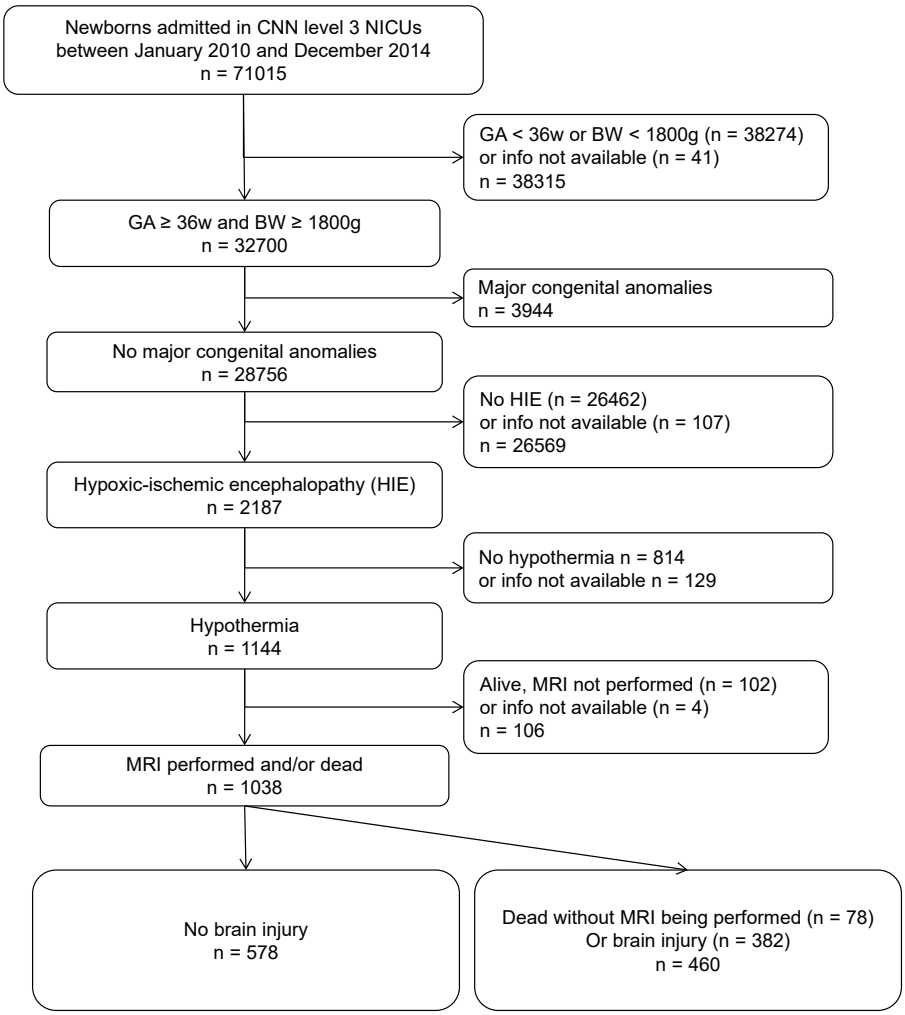

Figure 3: During the study period, 1114 asphyxiated newborns were treated with hypothermia. 41% (460/1114) developed adverse outcome

3.8. Tables

Table 4.

TABLE 1: Clinical characteristics of asphyxiated newborns treated with hypothermia developing or not adverse outcome.

Clinical variables	Asphyxiated newborns treated with hypothermia not developing adverse outcome (n = 578)	Asphyxiated newborns treated with hypothermia developing adverse outcome (n = 460)	*p* value
Maternal characteristics			
Maternal age, mean (SD)	30.0 (5.6)	29.9 (5.5)	*0.81*
Parity			*0.51*
0, n (%)	339 (59)	281 (61)	
1, n (%)	136 (23)	110 (24)	
2, n (%)	58 (10)	34 (7)	
> 3, n (%)	33 (6)	25 (5)	
Info not available, n (%)	12 (2)	10 (2)	
Maternal diabetes, n (%)	65 (11)	55 (12)	*0.65*
Maternal hypertension, n (%)	61 (11)	41 (9)	*0.45*
Birth characteristics			
Birth weight, mean (SD)	3408 (618)	3369 (581)	*0.30*
Gestational age, mean (SD)	39.1 (1.5)	39.1 (1.5)	*0.86*
Small for gestational age (< 10^{th} percentile), n (%)	87 (15)	74 (16)	*0.34*
Male, n (%)	336 (58)	255 (55)	*0.38*
Cesarean section, n (%)	260 (45)	248 (54)	*0.005*
Rupture of membranes			*0.04*
< 24 hours, n (%)	521 (90)	391 (85)	
24 hour – 1 week, n (%)	26 (5)	36 (8)	
> 1 week, n (%)	2 (1)	1 (1)	
Chorioamnionitis, n (%)	44 (8)	32 (7.0)	*0.89*
Apgar score at 10 minutes			
Available in # patients	517	411	
Median (IQR)	5 (4, 6)	4 (2, 6)	*< 0.0001*
Positive pressure ventilation via	376 (65)	367 (80)	*< 0.0001*

endotracheal tube, n (%)			
Chest compression > 30 seconds, n (%)	175 (30)	245 (53)	*< 0.0001*
Postnatal characteristics			
Highest grading of encephalopathy at any time during the NICU stay			*< 0.0001*
Mild, n (%)	152 (26)	37 (8)	
Moderate, n (%)	345 (60)	175 (38)	
Severe, n (%)	51 (9)	224 (49)	
Not available, n (%)	30 (5)	24 (5)	
Time to initiation of hypothermia treatment (hours), mean (SD)	4.2 (5)	4.7 (6)	*0.15*
Seizures, n (%)	230 (40)	331 (72)	*< 0.0001*
Hypotension requiring inotrope use, n (%)	181 (31)	256 (56)	*< 0.0001*
Persistent pulmonary hypertension, n (%)	89 (15)	112 (24)	*0.0003*
Renal failure, n (%)	124 (21)	197 (43)	*< 0.0001*
Early onset sepsis, n (%)	1 (1)	3 (1)	*0.33*
Meningitis, n (%)	0 (0.0)	1 (0.2)	*1.00*

Adverse outcome was defined as death without MRI being performed or evidence of brain injury on brain MRI.
Abbreviations: IQR, interquartile range; SD, standard-deviation.

Table 5.

TABLE 2: Multivariate logistic regression to identify the independent predictors of adverse outcome

Parameters	Odds ratio	95% confidence interval for OR		*p* value
Birth weight (gram)	1.00	1.00	1.00	*0.18*
Small for gestational age	0.97	0.61	1.52	*0.88*
Male	1.02	0.77	1.35	*0.90*
Apgar at 10 minutes *	1.18	1.10	1.26	*< 0.001*
Hypotension requiring inotrope use	1.87	1.38	2.55	*< 0.001*
Persistent pulmonary hypertension	1.27	0.88	1.85	*0.21*
Renal failure	2.03	1.49	2.76	*< 0.001*

* Per unit of decrease

Conclusion

Birth asphyxia remains a significant problem in Canada and many asphyxiated newborns still die and/or develop brain injury despite receiving therapeutic hypothermia. Results from the two projects of my MSc thesis demonstrated that specific maternal, delivery, and perinatal factors increased the risk of developing adverse outcome, including death and/or brain injury, in asphyxiated newborns treated with hypothermia.

A retrospective review of the data from our local cohort revealed that mothers, who did not have a pervious child, especially when they presented with prolonged duration of labor, were at higher risk to have an asphyxiated newborn who developed adverse outcome. My results suggest that labor duration beyond 10 hours in primiparous woman undergoing vaginal delivery should be closely monitored. Future study should investigate if monitoring closely the duration of labor in primiparous women may reduce the incidence of death and/or brain injury in their newborns.

A retrospective review of the data from the CNN database highlighted the current management of birth asphyxia throughout Canada. There is wide variability of management for these patients across Canada, including how the severity of encephalopathy is initially assessed and how the severity of brain injury is later evaluated. Many asphyxiated newborns with moderate and/or severe encephalopathy in this country still did not receive hypothermia treatment, many of them for reasons such as transport delay or NICU policy that are modifiable. Future guidelines should be developed at a national level to ensure therapeutic hypothermia may benefit all eligible candidates and to optimize the management of these newborns. The results of this study also demonstrated that hypothermia requiring inotrope use and renal failure, in addition to severity of the initial asphyxial event, were independent predictors of adverse

outcome in asphyxiated newborns treated with hypothermia. Further prospective studies on the optimal management to treat hypotension and to prevent renal failure in term asphyxiated newborns treated with hypothermia are warranted.

The major limitation of this thesis was that unfortunately we could not compare the same risk factors at the local level and at the national level, because the same items were not collected in both database. Another limitation of this thesis was the lack of long-term follow-up data in both cohorts, limiting our ability to better understand how early determinants of adverse outcome unfold into later neurodevelopmental impairments. Moreover, the retrospective design of both studies limits the interpretation of causal relationship between early determinants and adverse outcome. For future work, it would be beneficial to prospectively study how these early determinants and/or predictors correlate with long-term follow up in a large population of asphyxiated newborns treated with hypothermia.

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