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### Magnesium Exposure in Very Preterm Neonates and Adverse Gastrointestinal Outcomes

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MAGNESIUM EXPOSURE IN VERY PRETERM NEONATES AND ADVERSE  
GASTROINTESTINAL OUTCOMES

A Thesis Presented to  
The Faculty of the School of Medicine  
Yale University

In Candidacy for the Degree of  
Master of Medical Science

June 2020

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## List of Abbreviations

ACOG – American College of Obstetricians and Gynecologists  
ANOVA – analysis of variance  
CCMC – Connecticut Children’s Medical Center  
CI – confidence interval  
ELBW – extremely low birth weight  
EMR – electronic medical record  
FI – feeding intolerance  
GA – gestational age  
IRB – Institutional Review Board  
IVH – intraventricular hemorrhage  
NEC – necrotizing enterocolitis  
NICU – neonatal intensive care unit  
NNT – number needed to treat  
NSAID – non-steroidal anti-inflammatory drug  
PDA – patent ductus arteriosus  
PHI – protected health information  
RCT – randomized controlled trial  
RR – relative risk  
SGA – small for gestational age  
SIP – spontaneous intestinal perforation  
SMA – superior mesenteric artery  
VLBW – very low birth weight  
WIH – Women and Infants Hospital  
YNHCH – Yale New Haven Children’s Hospital

## List of Definitions

Preterm – less than 37 weeks’ gestation at the time of delivery  
Very preterm – less than 32 weeks’ gestation at the time of delivery  
Extremely preterm – less than 28 weeks’ gestation at the time of delivery  
Very low birth weight – birth weight less than 1500g  
Extremely low birth weight – birth weight less than 1000g

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## **Abstract**

Preterm birth is the leading cause of perinatal morbidity and mortality, especially among neonates born less than 32 weeks' gestation. Magnesium sulfate is the standard treatment for seizure prevention in preeclampsia and for fetal neuroprotection in mothers at risk for preterm delivery. However, the consequences of antenatal magnesium exposure on the very preterm neonate's gastrointestinal tract are not fully established. **This study will determine whether elevated magnesium levels in very preterm neonates are associated with adverse gastrointestinal outcomes from birth to 4 months.**

Specifically, using a prospective cohort design, we will measure magnesium in umbilical cord blood of very preterm neonates at the time of delivery and determine whether elevated levels are associated with feeding intolerance, necrotizing enterocolitis, or spontaneous intestinal perforation. This study may provide evidence for the use of umbilical cord magnesium concentration as a screening tool for risk for adverse gastrointestinal outcomes among very preterm neonates.

## **CHAPTER 1: INTRODUCTION**

### **1.1 Background**

#### **1.1.1 Problem of Prematurity**

Preterm birth is common, comprising 10.02% of live births in the United States in 2018.<sup>1</sup> With improvements in neonatal intensive care over the last three decades such as the use of antenatal corticosteroids and antibiotics, human viability is now approximately 22-24 weeks' gestation in high-income, developed countries.<sup>2,3</sup> Despite these advances in neonatal care, preterm birth is still the leading cause of perinatal morbidity and mortality among non-anomalous newborns in the United States.<sup>4</sup> Per a secondary analysis of 115,502 neonates born from 2008-2011, very preterm neonates have a 7.34% risk of mortality with a 28.14% risk of developing a major morbidity. These morbidities include pulmonary hypertension, intraventricular hemorrhage (IVH) grade III/IV, stage II/III necrotizing enterocolitis (NEC), hypoxic-ischemic encephalopathy, seizures, and bronchopulmonary dysplasia.<sup>4</sup> It is important to discover ways to decrease the development and improve the management of these and other morbidities as they have immediate and long-lasting effects on the neonate.

#### **1.1.2 Exposure of the Premature Neonate to Antenatal Magnesium Sulfate**

The use of magnesium sulfate in prenatal management has become increasingly prevalent over the past few decades. It is now standard of care for seizure treatment in maternal eclampsia, seizure prevention in maternal preeclampsia, and fetal neuroprotection in women at risk of delivering preterm at <32 weeks' gestational age (GA).<sup>5,6</sup> Additionally, some institutions use magnesium sulfate as a tocolytic agent for short-term prolongation of pregnancy to allow for antenatal corticosteroid administration,

although studies have proven there is no benefit to its use and there may be increased risk of fetal mortality.<sup>7</sup> As such, it is common for the very preterm neonate to have had antenatal magnesium sulfate exposure.

A 2009 systematic review of five randomized controlled trials (RCTs) revealed that antenatal magnesium sulfate therapy reduces the risk of cerebral palsy and gross motor dysfunction in the very preterm neonate without increasing pediatric mortality.<sup>6</sup> This finding was an important breakthrough for the medical community and was sufficient to bring magnesium sulfate into mainstream use for fetal neuroprotection. However, the studies in the systematic review included the use of antenatal magnesium for varying indications, and maternal magnesium sulfate regimens differed in dosing and timing.<sup>6</sup> Thus, a number of questions regarding the use as well as the risks of antenatal magnesium sulfate remained unanswered. Observational studies have since suggested that antenatal magnesium sulfate therapy could be associated with neonatal gastrointestinal morbidities such as feeding intolerance (FI), NEC, and spontaneous intestinal perforation (SIP).<sup>8-10</sup> Most of these studies, however, fail to account for different amounts of magnesium exposure.

### **1.1.3 Magnesium Sulfate Therapy and Neonatal Magnesium Levels**

Much of the existing literature evaluates neonates who have been exposed to antenatal magnesium sulfate as one large cohort. As the ideal dose and timing of antenatal magnesium sulfate has not been standardized, and as the speed of labor and delivery differs, each mother-fetus dyad may receive a different amount of magnesium sulfate, resulting in a different final neonatal magnesium concentration.<sup>11</sup> Additionally, a number of other dyad characteristics can impact neonatal magnesium concentration such



as maternal BMI, multiple gestation pregnancies, and maternal and neonatal renal function.<sup>12,13</sup> The result is a spectrum of neonatal magnesium concentrations in the cohort of neonates exposed to antenatal magnesium sulfate. Thus, it is imperative that a future study clarifying the association of antenatal magnesium sulfate and adverse gastrointestinal outcomes accounts for neonatal magnesium concentration.

#### **1.1.4 Feeding Intolerance**

Feeding intolerance, or difficulty digesting enteral feeds, is common among very preterm neonates and is one of the adverse outcomes that could be associated with exposure to magnesium sulfate. A retrospective study by Belden et al. provides support for this association. In this study, neonates  $\geq 24$  weeks' GA who had FI were found to have been exposed to a larger total dose of antenatal magnesium sulfate than neonates who did not have FI (70.4 +/- 52.3g vs 47.4 +/- 40.1g; p=0.04).<sup>8</sup>

Neonatal FI can be a benign condition related to an immature gastrointestinal tract; however, it can also lead to suboptimal nutrition and prolonged use of intravenous nutrition which increases the risk of neonatal cholestasis and septicemia. Additionally, FI may lead to additional laboratory testing and diagnostic imaging, affect length of stay in the hospital, and subsequently increase healthcare cost.<sup>14,15</sup>

FI is a clinical diagnosis based on signs and symptoms which include abdominal distension, emesis, gastric residuals, and episodes of apnea, bradycardia, and oxygen desaturation.<sup>14,16</sup> As these signs and symptoms overlap with stage I NEC in Bell's modified staging criteria, FI can suggest underlying NEC.<sup>14,17</sup> Primary management of FI involves reducing enteral feeding volumes, delaying enteral feeding advancement, temporarily discontinuing enteral feeds, or switching the composition of feeds.<sup>14</sup>

If an association between elevated magnesium and neonatal FI exists as observational studies suggest, then elevated neonatal magnesium level could provide an explanation for benign FI and decrease unnecessary testing and healthcare cost in a neonate with signs of FI who is otherwise well-appearing. It could also create an opportunity for early recognition of FI to reduce adverse consequences outlined above.

### **1.1.5 Necrotizing Enterocolitis and Spontaneous Intestinal Perforation**

In addition to FI, NEC and SIP are gastrointestinal morbidities that impact very preterm neonates and could be linked to antenatal magnesium exposure.<sup>9,10</sup> NEC is the most common life-threatening surgical emergency in neonates.<sup>16,17</sup> A 2018 systematic review reveals a 6.8% incidence of NEC among very preterm neonates in the United States.<sup>18</sup> The most severe cases of NEC may result in death or the need for surgical bowel resection leading to short bowel syndrome, growth failure, cholestasis, or liver failure.<sup>17</sup> Other complications include intestinal stricture formation, respiratory and cardiac insufficiency, later-onset neurodevelopmental injury, and sepsis.<sup>17,19</sup>

The exact pathophysiology of NEC is unknown and is likely multifactorial; however, it presents most often with the initiation or progression of enteral feeds.<sup>16</sup> NEC may result from an abnormal balance of gut microbiota followed by intestinal injury, which activates an inflammatory immune response and leads to intestinal necrosis, allowing gas-forming organisms to invade the bowel.<sup>16,17,20</sup> The most important risk factor for the development of NEC is prematurity, especially for neonates who are very low birth weight (VLBW) or small for gestational age (SGA).<sup>17</sup> Another risk factor may be anemia requiring packed red blood cell transfusion. This may be due to decreased oxygen delivery and increased oxygen requirement associated with feeding, followed by

a reperfusion-type intestinal injury as a result of transfusion.<sup>17,21</sup> Finally, human milk has been shown to be an important protective factor against the development of NEC.<sup>17,22</sup>

Although SIP is prevalent in a similar patient population and manifests with similar clinical signs, it has recently been recognized as a distinct clinical entity from NEC.<sup>23,24</sup> Postnatal indomethacin and exogenous glucocorticoids have been found to be synergistic risk factors for SIP.<sup>24</sup> The pathophysiology of SIP is thought to involve decreased intestinal perfusion or thinning of the intestinal lining leading to perforation.<sup>10,23</sup> Although NEC and SIP can only be truly differentiated intraoperatively, physical examination and radiographic findings can support clinical diagnoses.<sup>10</sup> Per a 2014 prospective cohort of 177,618 VLBW neonates, SIP has an estimated mortality rate of 19% as compared to 38% for NEC.<sup>25</sup>

As has been outlined, NEC and SIP most commonly affect very preterm neonates, and lead to severe outcomes including death or disability. Identifying neonates who are particularly at risk for developing these diseases is paramount. Wertheimer et al. notes that recognizing NEC [and SIP] early in its progression may improve outcomes, as it allows for early medical or surgical treatment.<sup>17,26</sup> Therefore, it is critical to clarify if elevated neonatal magnesium concentration as a result of antenatal magnesium sulfate therapy is associated with increased risk for NEC or SIP. This could give clinicians the opportunity for increased monitoring and early intervention to reduce associated morbidity, mortality, and cost of NEC and SIP.

## **1.2 Statement of the Problem**

Antenatal magnesium sulfate is effective in preventing and treating seizures in mothers with preeclampsia and eclampsia, respectively, and reducing the rate of cerebral

palsy in very preterm neonates. However, it is unclear whether elevated neonatal magnesium concentration as a result of antenatal magnesium sulfate therapy is associated with feeding intolerance and other adverse gastrointestinal outcomes including NEC and SIP. Much of the literature surrounding this question is retrospective and compounded by problematic study design including inefficient power to draw conclusions, failure to account for neonatal magnesium concentration, and lack of representation of extremely preterm neonates. Nevertheless, these limited studies suggest that increased dose of antenatal magnesium sulfate, and presumably increased neonatal magnesium concentration, could be associated with neonatal feeding intolerance.

### **1.3 Goals and Objectives**

To establish if magnesium concentration in umbilical cord blood at the time of delivery is associated with feeding intolerance and other adverse gastrointestinal outcomes, including necrotizing enterocolitis and spontaneous intestinal perforation, in very preterm neonates.

### **1.4 Hypothesis**

The incidence rate of feeding intolerance as measured by time to full enteral feed in very preterm neonates is different among neonates in the middle and high terciles of magnesium concentration as compared to the low tercile of magnesium concentration in umbilical cord blood at the time of delivery after controlling for confounding.

### **1.5 Definitions**

Time to full enteral feed – the time it takes to reach an enteral intake of 150 mL/kg/day

Very preterm – less than 32 weeks' gestational age at the time of delivery by best obstetrical dating

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## **CHAPTER 2: REVIEW OF THE LITERATURE**

### **2.1 Introduction: Search Criteria**

During the period of August 2019 to June 2020, we conducted repeated searches of PubMed, Ovid Medline, Scopus, and Cochrane Library databases with the assistance of the librarians at the Yale School of Medicine. Primary searches were conducted using combinations of the MeSH terms “infant, premature,” “magnesium sulfate,” and key words “feeding intolerance” or “feeding tolerance.” Additional search terms included “nutrition, enteral,” “enterocolitis, necrotizing,” “intestinal perforation,” “first stool,” and “first feed.” We also examined the reference lists of all studies to further identify relevant papers. We included pertinent clinical studies, systematic reviews, and meta-analyses, with preference given to articles published in the past 10 years. All articles were written in the English language.

This literature search demonstrates the uncertainty surrounding the association of antenatal magnesium sulfate and adverse gastrointestinal outcomes such as feeding intolerance in very preterm neonates. By analyzing pertinent studies and reviewing their limitations, we will demonstrate how our prospective observational study will help to fill the current gaps in research in a way that is novel, feasible, and realistic.

### **2.2 Review of Empirical Studies**

#### **2.2.1 The Use of Antenatal Magnesium Sulfate**

Magnesium sulfate is the medication of choice to prevent and treat seizures in mothers with preeclampsia and eclampsia, respectively.<sup>1,2</sup> In a 2010 systematic review of fifteen RCTs, magnesium sulfate was found to reduce the risk of seizures by more than 50% compared to placebo and to be superior to both phenytoin and nimodipine for this

purpose (relative risk (RR), 0.41; 95% confidence interval (CI), 0.29-0.58; number needed to treat (NNT), 100).<sup>1</sup> Antenatal magnesium sulfate is also indicated for fetal neuroprotection in women at risk for preterm birth at less than 32-34 weeks' gestation. This role was primarily established by five landmark studies published between 2002 and 2008.<sup>3-7</sup> A 2009 Cochrane Review of these five trials revealed that antenatal magnesium sulfate therapy significantly reduces the risk of cerebral palsy in the infant born at less than 32 weeks' gestation (RR, 0.68; 95% CI, 0.54-0.87; five trials; 6145 infants). The NNT to prevent cerebral palsy in one baby is 63 (95% CI, 43-155) without an increase in mortality or morbidities.<sup>8</sup> Following these studies, the World Health Organization has recommended the use of magnesium sulfate for fetal neuroprotection when delivery is expected at less than 32 weeks' gestation and the American College of Obstetricians and Gynecologists (ACOG) has stated that physicians can elect to use magnesium sulfate for this indication.<sup>9,10</sup>

Although antenatal magnesium sulfate therapy for neuroprotection of very preterm neonates is now standard practice, there remain uncertainties about associated morbidities and the ideal therapeutic regimen. Potential adverse neonatal outcomes associated with antenatal magnesium sulfate use described throughout the literature include but are not limited to, IVH<sup>11</sup>, hypotonia<sup>12</sup>, respiratory depression<sup>8,13</sup>, bronchopulmonary dysplasia<sup>14</sup>, patent ductus arteriosus (PDA)<sup>15</sup>, hypotension<sup>14</sup>, feeding intolerance<sup>16</sup>, necrotizing enterocolitis<sup>14</sup>, and spontaneous intestinal perforation.<sup>17</sup> Gastrointestinal outcomes such as FI, NEC, and SIP require further evaluation as existing studies are incomplete.

### **2.2.2 Potential Mechanisms of Action of Magnesium on the Neonatal Bowel**



Of the potential adverse neonatal outcomes associated with antenatal magnesium sulfate therapy, gastrointestinal problems are biologically plausible and have been the subject of numerous studies. There are two predominant physiologic theories on how magnesium impacts the bowel and could lead to FI, NEC, SIP, or other adverse gastrointestinal outcomes. First, magnesium antagonizes calcium in smooth muscle cells which reduces contractility leading to reduced GI motility. Hypomotility could lead to increased intraluminal pressure via increased water absorption, stool plug formation, and bacterial growth, which can eventually damage the immature intestine and lead to rupture.<sup>16,18</sup> Second, magnesium causes vasodilation which could alter intestinal blood flow and impact tolerance to enteral feeds.<sup>19,20</sup> A compounding factor is that magnesium sulfate is renally eliminated. Therefore, the immature renal function of preterm neonates can potentiate and prolong the effects of magnesium.<sup>16</sup>

Observational studies have investigated whether antenatal magnesium sulfate exposure effects neonatal intestinal blood flow, as physiologic mechanisms suggest it could. A 2011 retrospective cohort study measured peak mean end-diastolic velocities in the superior mesenteric artery (SMA) of 56 neonates born <37 weeks' GA who weighed <2500g at birth.<sup>19</sup> The mean SMA blood flow velocity was similar in neonates with and without magnesium sulfate exposure; however, there was a negative correlation between SMA blood flow velocity and the number of hours between birth and time of measurement in the magnesium-exposed group that was not appreciated in the unexposed group ( $r=0.38$ ;  $p=0.852$ ). These findings suggest that magnesium could be exerting an effect on intestinal blood flow velocity in the immediate hours after birth.<sup>19</sup>

A 2015 prospective study measured daily Doppler flow measurements of the SMA in the first five postnatal days in 50 birth weight and GA-matched neonates who were exposed and non-exposed to antenatal magnesium sulfate and were born at 26-34 weeks' GA.<sup>21</sup> Although blood flow velocities did not differ between the two groups, there was a trend toward increasing blood flow over time in the non-exposed group that did not occur in the exposed group (non-exposed,  $p < 0.001$ ; exposed,  $p = 0.29$ ). This finding could be due to the vasodilatory effects of magnesium. As magnesium levels decreased over the five days causing vasodilation to decrease, blood flow velocity did not increase in the exposed group to the degree that it did in the non-exposed group.<sup>21</sup> Overall, these two studies suggest that magnesium could contribute to adverse gastrointestinal outcomes by attenuating the increase in intestinal blood flow velocity seen in neonates who have not been exposed to antenatal magnesium sulfate.

As noted above, the immature renal function of preterm neonates can potentiate and prolong the physiologic effects of elevated magnesium concentration.<sup>16</sup> Renal magnesium clearance in a neonate, especially a preterm neonate, is decreased during the first few days of life, resulting in elevated magnesium levels that persist for longer periods of time after delivery than in the mother.<sup>22</sup> For example, the half-life of magnesium sulfate in women with normal renal function is four hours while the half-life of magnesium in neonates exposed to antenatal magnesium sulfate is greater than forty hours.<sup>23,24</sup> Maternal magnesium toxicity appears to be concentration-dependent: a loss of reflexes occurs when serum magnesium concentration is above 8.5 mg/dL and respiratory paralysis occurs when serum magnesium concentration is greater than 12 mg/dL.<sup>23</sup> Similarly, adverse neonatal gastrointestinal effects such as reduced GI motility and

altered intestinal blood flow may be dependent on neonatal serum magnesium levels. In fact, Pryde and Mittendorf have hypothesized that magnesium has a therapeutic window in the neonate where a concentration too low fails to provide neuroprotection and a concentration too high is associated with poor outcomes in a dose-related fashion.<sup>7,25,26</sup>

### **2.2.3 Antenatal Magnesium Sulfate and Adverse Effect: Feeding Intolerance**

Despite the plausible physiologic mechanism by which elevated neonatal magnesium levels can adversely affect bowel function, observational studies investigating a possible association between antenatal magnesium sulfate therapy and neonatal feeding intolerance have shown conflicting results.

Data from a 2017 single-center retrospective observational study suggest an association exists between elevated neonatal magnesium level and enteral feeding intolerance. In this study, cumulative dose of maternal magnesium sulfate was compared to incidence of enteral FI in 83 neonates  $\geq 24$  weeks' gestational age.<sup>16</sup> Neonates found to have FI were exposed to larger cumulative magnesium sulfate doses than those who did not have FI (70.4 +/- 52.3 vs 47.4 +/- 40.1g; p=0.04), with those exposed to greater than 80g being more likely to develop FI (44% vs 22%; p=0.04). Due to differences in maternal and neonatal baseline characteristics between neonates with and without FI, a multivariate logistic regression was performed which found that the strongest predictors of FI were cumulative maternal magnesium sulfate dose and gestational age.<sup>16</sup> The data from this study are retrospective and need to be confirmed by a prospective study. Additionally, cumulative magnesium sulfate dose was used as a marker of neonatal magnesium sulfate exposure, but neonatal serum magnesium concentration was not measured.<sup>16</sup> Although an increased total dose of maternal magnesium sulfate therapy may

be associated with an increased neonatal magnesium concentration, neonatal serum magnesium concentration should be measured to determine its association with FI.<sup>27,28</sup>

Other retrospective and prospective observational studies have evaluated FI or time to full enteral feed (our measure of FI) as a secondary outcome and were underpowered to see an association even when, in some cases, raw data suggest one. A 2015 prospective study discussed above compared SMA blood flow in neonates who were exposed to antenatal magnesium sulfate to weight and age-matched neonates who were not exposed. Secondary outcomes included time to reach full feeds, first meconium passage, and presence of FI defined as gastric residuals >50% on consecutive feedings, abdominal distension, or vomiting that resulted in failure to make the daily increments in feeding.<sup>21</sup> No secondary outcomes reached statistical significance although 6 neonates (24%) in the magnesium-exposed group were feeding intolerant while only 2 neonates (8%) in the unexposed group were feeding intolerant ( $p=0.12$ ).<sup>21</sup> The study was limited by its small sample size. Additionally, the average GA of neonates in the study was 31 weeks, so the study did not address a possible effect of magnesium exposure on FI in neonates born at lower GAs.

A 2011 study evaluated clinical outcomes in neonates born at 24-32 weeks' gestation who were either exposed to antenatal magnesium sulfate for neuroprotection ( $n=289$ ) or not exposed ( $n=186$ ).<sup>22</sup> Time to reach full enteral feeds, a secondary outcome, was delayed in neonates exposed to magnesium sulfate compared to those unexposed ( $36.6 \pm 30.7$  vs  $29.9 \pm 29$  days;  $p=0.03$ ). After correcting for GA and birth weight, this delay was no longer significant. The exposed group was further divided into four groups by serum magnesium levels obtained in the first 24 hours of life:  $<3$  mg/dL,  $3-4.3$

mg/dL, 4.3-<5.5 mg/dL, and  $\geq 5.5$  mg/dL. Time to reach full enteral feed was not associated with increasing magnesium concentration in these groups (35 +/- 25, 37 +/- 28, 39 +/- 39, 35 +/- 26 days; p=0.85) although the large standard deviations suggest great variability in outcomes, decreasing our confidence in these results.<sup>22</sup>

The same group performed a 2017 retrospective observational study that correlated serum magnesium levels obtained within the first 48 hours of life of 304 neonates born 24-34 weeks' GA with immediate neonatal outcomes.<sup>29</sup> Similar to the 2011 study, the 225 neonates who were exposed to magnesium sulfate took longer to achieve full feeds than the 63 neonates who were not exposed (16 days (10-27.5) vs 10 days (6-17); p <0.01), where values are expressed as medians (Q1:25% to Q3:75%). Data for time to full feed were missing for 16 neonates. As in their prior study, exposed neonates were further stratified based on magnesium concentrations: Group 1 <2.5 mg/dL (n=55), Group 2  $\geq 2.5$ -4.5 mg/dL (n=154), Group 3  $\geq 4.5$  mg/dL (n=17). Increasing neonatal magnesium concentrations were associated with longer time taken to achieve full feeds (13 (9-13.5), 18 (11-27), and 32 (17.5-45.5) days, respectively; p <0.01) where values are expressed as medians. A regression analysis was performed to control for birth weight and multiple gestation using Group 2 as a reference, which rendered this result not statistically significant. The probability of a type II error ( $\beta$ ) was 0.02 with a 95% CI (-0.06-0.09) for Group 1 and -0.02 (-0.14-0.09) for Group 3.<sup>29</sup>

As mentioned, these two studies were underpowered to see an association between magnesium concentration and time to full feed. Although their retrospective design was successful in generating the hypothesis that an association could exist, a larger prospective cohort study is needed. Additionally, magnesium concentrations were

obtained from serum up to 24 or 48 hours after birth, which introduced information bias as data were not extracted at the same point in time and likely do not represent each patient's maximum magnesium concentration after birth.<sup>22,29</sup> Finally, the number of neonates in Groups 1 through 3 of the 2017 study were dissimilar. Only 17 neonates were in Group 3 as compared to 154 neonates in Group 2, the reference group, which could have resulted in an inaccurate representation of adverse outcomes in this group and decreased the likelihood of finding an association.<sup>29</sup>

Finally, a 2011 retrospective study mentioned above evaluating intestinal blood flow in neonates <37 weeks' GA exposed and unexposed to antenatal magnesium sulfate additionally measured time to full enteral feed as a secondary outcome.<sup>19</sup> They found no difference in days to achieve full enteral feeding volumes between neonates exposed to antenatal magnesium sulfate compared to neonates unexposed (14.5 +/- 11.4 vs 16.5 +/- 13.6 days; p=0.58), arguing against an association between antenatal magnesium sulfate and neonatal FI.<sup>19</sup>

Although only one observational study described above found that increased antenatal magnesium sulfate dose was significantly associated with FI in very preterm neonates, the raw data of additional studies suggest that an association could exist between elevated neonatal magnesium and FI. By performing a larger prospective study with a primary outcome of feeding intolerance and measuring neonatal magnesium levels at the time of delivery, our study will be able to clarify this association.

#### **2.2.4 Antenatal Magnesium Sulfate and Adverse Effects: NEC and SIP**

Multiple RCTs have looked at the incidence of necrotizing enterocolitis in preterm neonates that have been exposed to antenatal magnesium sulfate. Data from three

of the landmark neuroprotection RCTs discussed above showed no statistical difference in the incidence of NEC among those exposed to magnesium sulfate and those exposed to placebo.<sup>3,4,30</sup> A subgroup analysis of these three studies in a 2009 meta-analysis of antenatal magnesium sulfate for the prevention of cerebral palsy in infants less than 34 weeks' gestation revealed that neonates exposed to antenatal magnesium sulfate had a higher incidence of NEC (155 of 2169, or 7.1%) than those unexposed (131 of 2218, or 5.9%), although the result did not quite achieve statistical significance (RR, 1.23; 95% CI, 0.98-1.54).<sup>14</sup> The three RCTs included in this evaluation all had a Modified Jadad score of 8 which indicates the highest quality of methods including successful randomization, appropriate double blinding and concealment, and >95% follow-up of fetuses.<sup>14</sup>

There have been multiple secondary analyses of one of these studies, the 2008 Maternal Fetal Medicine Units Beneficial Effects of Antenatal Magnesium (MFMU BEAM) trial, a multicenter RCT.<sup>4</sup> A 2016 secondary analysis of the de-identified data set demonstrated a significant association between antenatal magnesium sulfate and the composite of severe NEC or death in neonates born less than 26 weeks' gestation after controlling for confounders such as GA and SGA (AOR, 1.90; 95% CI, 1.12-3.22; p=0.017).<sup>31</sup> The association was not noted in a larger group of 697 neonates with gestational ages up to 27.9 weeks. A secondary analysis published in 2019 included 648 neonates born between 24 to less than 32 weeks' gestation who had cord blood magnesium levels drawn at birth.<sup>32</sup> The aim of this study was to determine the effects of antenatal magnesium sulfate on non-neurologic neonatal outcomes including severe NEC with respect to cord blood magnesium level. Neonates were divided into quintiles of

magnesium cord blood levels and outcomes were compared between the highest quintile ( $\geq 2.9$  mg/dL) and the lowest quintile ( $\leq 1.5$  mg/dL). There was a significant increase in the rate of NEC in the highest vs the lowest quintile, (6.6% vs 2.8%; OR, 2.41; 95% CI, 1.11-5.24;  $p=0.02$ ) but this was no longer significant after multivariate logistic regression adjusted for GA, birth weight, and treatment group (AOR, 1.56; 95% CI, 0.51-5.58).<sup>32</sup>

As secondary analyses of a quality RCT, both studies have strong internal validities with low information bias. However, there are several limitations of such analyses. First, secondary analyses are limited by variables collected by the parent study. For example, the specifics of care in the neonatal intensive care unit (NICU) are unknown.<sup>31</sup> Second, there could be overlap of SIP in the diagnosis of NEC as this was not a contemporary cohort and only recently have NEC and SIP been well-distinguished.<sup>31</sup> Additionally, 31% of neonates were missing cord blood magnesium levels in the 2019 study which could have impacted findings. Furthermore, the majority of neonates in this study were 30-32 weeks' GA so an association between magnesium and NEC may have been missed in neonates of lower gestational ages.<sup>32</sup>

There have been fewer investigations into the relationship between antenatal magnesium sulfate and neonatal spontaneous intestinal perforation. A 2014 single-center prospective cohort study evaluated the association between antenatal magnesium sulfate for neuroprotection and SIP among 155 extremely low birth weight (ELBW) neonates.<sup>17</sup> Both gestational age (OR, 6.0; 95% CI, 2.4-18.2) and total magnesium sulfate dose (OR, 9.3; 95% CI, 1.04-104.6) were associated with SIP and mortality with a marked increase in SIP once maternal magnesium exposure reached 100g. Overall, there was increased SIP and mortality in neonates born less than 25 weeks' gestation. After multivariate



analysis, the administration of postnatal hydrocortisone was also independently associated with the risk of SIP or death ( $p=0.021$ ).<sup>17</sup> The authors discuss a synergistic effect of hypomotility from magnesium and submucosal thinning from hydrocortisone resulting in SIP. Although this study is limited in its small sample size and its inability to control for possible covariates in local management, it suggests the need for further investigation into the relationship between magnesium exposure and SIP, especially among neonates born <25 weeks' GA.<sup>17</sup>

Conversely, two large retrospective cohort studies have found no statistically significant association between antenatal magnesium sulfate exposure and SIP. First, a 2017 multicenter retrospective cohort study evaluated the relationship between antenatal magnesium sulfate and SIP in 28,035 ELBW infants.<sup>33</sup> Out of 11,789 infants exposed to antenatal magnesium sulfate, 2.9% developed SIP, and out of 16,246 unexposed infants, 2.3% developed SIP (AOR, 1.08; 95% CI, 0.91-1.29). This study has strong external validity as it took place at many diverse study centers and its large number of participants allowed control for multiple confounders. Limitations include the retrospective design and lack of information recorded about antenatal magnesium therapy including dose, timing, and indication for its use. Neonatal magnesium levels were not routinely measured, so no association between magnesium level and risk of SIP could be assessed. Additionally, if an infant was transferred outside of the study network, outcome data were not recorded.<sup>33</sup>

Second, a 2017 population-based retrospective cohort study evaluated the association between antenatal magnesium sulfate and either NEC or SIP among 4,355 neonates born less than 28 weeks' gestation.<sup>34</sup> Similar to the previous study, this study

found no difference in the odds of NEC (AOR, 0.92; 95% CI, 0.75-1.14; p=0.45) or SIP (AOR, 1.05; 95% CI, 0.75-1.48; p=0.75) between neonates exposed and unexposed to antenatal magnesium sulfate after adjusting for multiple confounders. This study is strengthened by its large sample size and ability to control for risk factors for SIP.<sup>34</sup> It suffers from the same limitations as the previous study, including retrospective design and the fact that neonatal magnesium concentrations were not routinely measured.

Antenatal magnesium sulfate exposure has even been shown to have possible beneficial effects on the neonatal gastrointestinal tract. One 2019 retrospective observational study evaluated 302 inborn neonates  $\leq 28$  weeks or  $\leq 1000$ g and found that antenatal magnesium sulfate exerted a protective effect in which every 10g increase in cumulative maternal dose correlated with an 18.9% decrease in SIP, NEC or death prior to discharge, especially among neonates who were SGA.<sup>35</sup> Although this study is limited by its retrospective single center design, it is unique in its finding and further highlights the need to clarify the relationship between magnesium, NEC and SIP.

In a 2019 review, Bhawan Deep Garg evaluated the risks and benefits of antenatal magnesium sulfate in very preterm neonates using published systematic reviews, meta-analyses, RCTs, and observational studies.<sup>18</sup> He concluded that there is not enough evidence to prove that magnesium sulfate is associated with gastrointestinal complications. Thus, it should continue to be used according to protocol with high suspicion for GI complications in extremely preterm neonates.<sup>18</sup> Our review reveals that evidence needed to clarify the relationship between antenatal magnesium exposure and NEC or SIP would need to overcome the limitations of the above studies. Thus, a future study would require prospective investigation of a contemporary cohort of neonates,

especially those of lower GA, with consideration of neonatal magnesium concentration and minimal missing data.

### **2.2.5 Antenatal Magnesium Sulfate and Parameters of Gastrointestinal Function**

Three other parameters that may reflect an influence of magnesium exposure on gastrointestinal function in preterm neonates are time to first enteral feed, time to first stool, and number of abdominal X-rays in the first 30 days of life. Thus, they will be evaluated as exploratory secondary outcomes in our proposed study.

The timing of first enteral feed in very preterm and VLBW neonates may be related to feeding tolerance. Early research suggested that early enteral feeds could increase the risk of NEC.<sup>36</sup> However, early enteral feeding aids the development of the gastrointestinal tract, and holding feeds for greater than 72 hours can lead to intestinal atrophy and loss of function. Holding feeds could then contribute to FI when enteral feeds are introduced.<sup>37</sup> A 2014 systematic review of nine RCTs (n=1106 infants) found that delayed introduction of enteral feeds led to delay in time to reach full enteral feed (reported median differences 2-4 days), but there was no difference in risk of NEC or all-cause mortality between early and late enteral feeding groups.<sup>36</sup> We believe it is important for our study to note any difference in time to first feed between our groups of patients with different magnesium concentrations and how it could contribute to delay in full enteral feed and FI.

Time to first stool is often used as a representation of gastrointestinal motility.<sup>38</sup> There are mixed data regarding the association between elevated magnesium and delayed time to first stool. In a 2017 retrospective study discussed above, time to first stool was evaluated as a secondary outcome and compared between a group of neonates with FI

and higher total dose of antenatal magnesium sulfate and a group of neonates without FI who were found to have received a lower total dose of antenatal magnesium sulfate.<sup>16</sup> Time to first stool was found to be significantly longer in the feeding intolerance group (3.4 vs 1.8 days;  $p < 0.05$ ). Delayed first stool suggests that decreased gastrointestinal motility could be a contributing factor to feeding intolerance secondary to magnesium sulfate exposure.<sup>16</sup> A 1982 prospective cohort study (n=56) found a similar result. Fifty percent of neonates born <36 weeks' gestation to hypertensive mothers treated with magnesium sulfate had delayed stooling >24 hours compared to only 21% of age-matched, unexposed controls.<sup>38</sup> Alternatively, a number of prospective and retrospective observational studies have found no difference in time to first stool between neonates exposed to antenatal magnesium sulfate and those unexposed.<sup>12,21,39</sup> To the best of our knowledge, there are no studies that compare neonatal magnesium concentration at the time of delivery to time to first stool.

The number of abdominal x-rays performed during the first month of life can be used as a surrogate assessment for clinician concern about feeding intolerance or other gastrointestinal pathology. Abdominal x-rays are often obtained in the setting of neonatal feeding intolerance and are used to diagnose and monitor the progression of gastrointestinal disease.<sup>40</sup> Additionally, this outcome is worth exploration as it is a source of neonatal radiation exposure and significant healthcare cost.

### **2.2.6 Determinants of Neonatal Magnesium Concentration**

The landmark studies that brought maternal magnesium sulfate into clinical use for fetal neuroprotection used different loading doses, maintenance infusion rates, and durations of maternal magnesium sulfate therapy.<sup>8</sup> As no dosing regimen was proven to

be superior, ACOG recommended that clinicians base their treatment guidelines on one of the larger trials.<sup>9</sup> Importantly, the relationship between maternal magnesium sulfate therapy (dose, duration, and timing of treatment), maternal serum magnesium concentration, and neonatal serum magnesium concentration is unclear. All three factors, as well as the delayed renal clearance of magnesium and persistence of elevated concentrations described earlier, may impact neonatal well-being.

Magnesium ions cross the placenta readily by either passive or active facilitated transport.<sup>15</sup> In neonates born at 24-34 weeks' GA, Narasimhulu et al. found that maternal magnesium concentration predicts neonatal magnesium concentration obtained in the first 48 hours of life ( $r=0.72$ ,  $p<0.001$ ). They also found that total maternal magnesium sulfate dose ( $r=0.66$ ;  $p<0.0001$ ) and duration of therapy ( $r=0.70$ ,  $p<0.0001$ ) predict neonatal magnesium concentration.<sup>29</sup> They suggest that total maternal dose or duration of therapy should be thought of as a surrogate for fetal exposure rather than maternal magnesium concentration, which may be more representative of maternal renal clearance of magnesium sulfate.<sup>29</sup> Other studies have confirmed that total maternal magnesium sulfate dose and duration of therapy predict neonatal magnesium concentration.<sup>27,28</sup> By using 4.5 mg/dL as the magnesium concentration beyond which neonates may experience increased morbidity and mortality,<sup>22</sup> García Alonso et al. predicted that maternal doses  $\leq 20$  g of antenatal magnesium sulfate given continuously over 16 hours are safe.<sup>28</sup> In summary, these studies all suggest that total maternal magnesium dose is the best predictor of serum magnesium concentration in neonates.<sup>28</sup>

Other factors may contribute to the final serum magnesium concentration in preterm neonates. These include maternal BMI<sup>29</sup>, maternal albumin level<sup>29</sup>, multiple

gestation pregnancies<sup>29</sup>, neonatal GA<sup>28,41</sup>, neonatal birth weight<sup>28,29,41</sup>, neonatal renal function<sup>42</sup>, and neonatal nutrition<sup>42</sup>. The timing of maternal magnesium therapy may be a final important consideration. A 2016 secondary analysis of the 2008 MFMU BEAM Trial found that therapy less than 12 hours prior to delivery was associated with reduced odds of neonatal cerebral palsy at 2 years compared to therapy greater than or equal to 12 hours prior to delivery (AOR, 0.41; 95% CI, 0.18-0.91; p=0.03).<sup>4,43</sup> The multitude of factors which can impact the concentration of magnesium in a neonate make it clear that a study attempting to assess the effect of magnesium on neonatal FI, NEC, or SIP must take into account neonatal magnesium concentration.

### **2.3 Confounding Variables**

The relationship between neonatal magnesium concentration and gastrointestinal outcomes is difficult to isolate as there are a multitude of potential confounding variables identified in the literature that could influence this relationship. Variables include maternal factors such as age, BMI, and race, perinatal factors such as reason for magnesium sulfate therapy, hospital of delivery, antenatal steroids, prolonged rupture of membranes, mode of delivery, and multiple gestations, and neonatal factors such as GA, birth weight, sex, SGA, 5-minute Apgar score, feeding with human milk, sepsis, postnatal steroids, postnatal indomethacin for IVH prophylaxis, and postnatal NSAID treatment for PDA. Although many of these variables have only been shown in studies to relate to the risk of NEC, they may also relate to the risk of feeding intolerance.

#### **2.3.1 Maternal Variables**

Studies suggest that maternal variables can impact magnesium concentration. In a retrospective study discussed above, higher maternal BMI was found to be associated

with lower neonatal magnesium concentration ( $\beta = -0.29$ ,  $p < 0.001$ ). Maternal age and race were not correlated with neonatal magnesium concentration.<sup>29</sup>

In studies conducted in the United States, black race has been associated with increased risk for developing NEC whereas maternal age, BMI, and maternal education have not been associated with NEC.<sup>44,45</sup> Thus, it is important to consider maternal age, BMI, and race as these factors could potentially confound results.

### **2.3.2 Perinatal Variables**

A retrospective study discussed above found that multiple gestation pregnancies were associated with lower magnesium concentrations ( $\beta = -0.14$ ;  $p = 0.02$ ) and maternal preeclampsia was associated with higher neonatal magnesium concentration ( $\beta = 0.19$ ,  $p = 0.003$ ).<sup>29</sup> Factors that have been associated with increased risk for NEC include preeclampsia in mothers of neonates born  $< 29$  weeks' GA and premature rupture of membranes.<sup>44,46,47</sup> A Cochrane Systematic Review found that the use of antenatal steroids reduces the relative risk of NEC by 50% (RR, 0.50; 95% CI, 0.32-0.78; ten trials; 4702 infants).<sup>48</sup> Additionally, there are conflicting data regarding Cesarean delivery and risk for NEC, thus this factor must be considered.<sup>44</sup> Finally, one study found that antenatal steroid use shortens the time to first stool.<sup>39</sup> We will also consider hospital of delivery as slight differences in care may lead to a difference in outcomes.

### **2.3.3 Neonatal Variables**

The most common covariates identified in the literature are gestational age and birth weight of the neonate. There are increased rates of FI, NEC, and SIP with both lower GA and with lower birth weight.<sup>49,50</sup> Additionally, lower GA and lower birth

weight are associated with higher magnesium concentration.<sup>41</sup> Therefore, it is important to include both of these neonatal characteristics as covariates.

In addition to GA and birth weight, several other potential neonatal covariates have been identified that may increase or decrease the risk of our primary or secondary outcomes. Neonates who are SGA, have a low Apgar score, or who develop sepsis are at increased risk for developing NEC.<sup>44,47,51</sup> A 2017 systematic review reveals that feeding with human milk can decrease both FI and NEC.<sup>52</sup> Postnatal indomethacin and postnatal steroids are co-risk factors for the development of SIP.<sup>53</sup>

Two NSAIDs, indomethacin and ibuprofen, are used to treat PDAs in preterm infants and are known to have gastrointestinal side effects. Indomethacin decreases intestinal blood flow, and both Indomethacin and Ibuprofen decrease intestinal mucosal barrier function.<sup>37</sup> These effects could lead to bacterial colonization when enteral feeding is introduced, so enteral feeding is frequently delayed while neonates are receiving these medications. Delay in feeding advance regimens, however, could increase the risk of FI when enteral feeds are re-introduced.<sup>37</sup> Therefore, postnatal NSAID use must be closely monitored as a potential covariate.

## **2.4 Relevant Methodology**

This portion of the literature review includes a review of relevant methodology to the proposed study. A more detailed explanation of the proposed study methods can be found in Chapter 3.

### **2.4.1 Study Design and Setting**

The proposed study will be a multicenter prospective observational study examining whether there is a difference in FI among very preterm neonates divided into



terciles based on magnesium concentration in their umbilical cord blood at the time of delivery. Although a randomized controlled trial is the gold standard study design, we selected a prospective cohort design as we could not meet the equipoise principle required of an RCT.<sup>54</sup> Since the use of antenatal magnesium sulfate is now standard of care for fetal neuroprotection in neonates <32 weeks' gestation, it would be unethical to assign mothers to not receive antenatal magnesium sulfate. In addition, our primary exposure is neonatal magnesium concentration at the time of delivery, which cannot be randomized.

The study centers include the three Level IV NICUs in Connecticut and Rhode Island. These centers were selected because they have the highest capability of caring for critically ill newborns; thus, they deliver the highest volume of the desired population, very preterm neonates, to fulfill our study's sample size.<sup>55</sup> Additionally, it is unlikely that neonates will be transferred to another hospital for specialty care, increasing the likelihood that we will have a complete data set.

Given the degree of prematurity of our study population, all neonates are admitted to the NICU at the time of delivery. We plan to follow neonates from the time of birth until the time of discharge from the hospital or up until four months of their hospital stay. Based on prior studies, our outcomes of FI, NEC, and SIP occur by around one month of life.<sup>29,31,33,40</sup> We will follow each infant for an additional three months to ensure that we capture all measures of our primary and secondary outcomes.

#### **2.4.2 Sampling for Study Population**

In terms of sample selection, convenience sampling carries its own risk of selection bias; however, given the relatively rare nature of birth at <32 weeks' GA, we

will need to use convenience sampling in order to recruit our necessary sample size. This is consistent with previous studies with similar populations.<sup>15,17,28</sup>

### **2.4.3 Selection Criteria**

The inclusion and exclusion criteria for the proposed study have been designed to be consistent with the literature and to allow the results of this study to be generalizable to very preterm neonates who are exposed to antenatal magnesium sulfate. Inclusion criteria include being born at <32 weeks' gestation at one of our study centers with antenatal exposure to magnesium sulfate for any purpose. Exclusion criteria include neonates born at  $\geq 32$  weeks' gestation, outborn neonates, and neonates who have not been exposed to antenatal magnesium sulfate. Additional exclusion criteria include neonates with major congenital malformations or neonates with chromosomal abnormalities as defined in prior studies.<sup>16,17,27,29,31-34</sup> These two criteria would introduce significant confounding that we would be unable to control for.

### **2.4.4 Exposure**

Our primary exposure will be magnesium concentration in umbilical cord blood at the time of delivery. As this measurement is made at birth, it is the most accurate representation of magnesium sulfate exposure and defines the highest neonatal magnesium concentration.<sup>13</sup>

We plan to divide our primary exposure into three groups by terciles of magnesium concentration since data regarding average magnesium concentration in neonates exposed to magnesium sulfate are varied. Rigo et al. performed a meta-analysis in 2017 of 47 eligible studies comprised of 992 preterm and term neonates and found that the average neonatal serum magnesium level at birth was 3.13 mg/dL (95% CI, 1.22-

5.05).<sup>42</sup> This evaluation included preterm and term neonates, and there was high variability in the included studies ( $I^2 = 99.1\%$ ;  $p < 0.001$ ).<sup>42</sup> While defining groups by upper and lower limits of magnesium concentration would most efficiently group similar concentrations, it could result in groups with uneven numbers of subjects that are underpowered to detect a difference in outcomes. Additionally, we do not feel confident assigning concentration cut-offs to define groups due to the variability of average magnesium concentration seen above. By dividing groups by terciles of magnesium concentration, we increase the likelihood of having an equivalent number of neonates in each group for final analyses.

#### **2.4.5 Primary Outcome**

The primary outcome for the proposed study will be FI measured as time to full enteral feed (150 ml/kg/day). Throughout the literature, there are a number of ways to define FI, none of which have been validated as superior. For example, one study defined FI as “presence of gastric residuals greater than 50% on consecutive feedings, abdominal distention, or vomiting that resulted in failure to make daily increments in feeding,”<sup>21</sup> while another study defined FI as a composite outcome of deviations from the research institution’s standard NICU feeding protocol consisting of deviation in time to initiation of enteral feeds, time to non-trophic enteral feeds, or time to full enteral feeds.<sup>16</sup> As the incidence of feeding intolerance will likely be high in our very preterm population, using a definition that is operationalized as time to an event will allow us to compare the severity of FI between groups instead of simply looking at its presence or absence. We believe that the signs of FI included in other definitions of FI such as gastric residuals >50% on consecutive feedings, abdominal distention, and significant emesis will be

captured by our measurement, as they should lead to a delay in the time to full enteral feed. For example, Yale New Haven Children's Hospital's (YNHCH) NICU feeding protocol, which will be adopted by all three study centers, states that if signs of FI are present, an abdominal exam should be performed and feedings should be held for at least six hours, pending further evaluation.

#### **2.4.6 Secondary Outcomes**

NEC and SIP will be defined as in reviewed studies to keep outcomes consistent and generalizable.<sup>29,31,34,35,56</sup>

#### **2.4.7 Confounders**

As noted above, there are a number of variables that could affect both magnesium concentration as well as the incidence of our primary and secondary outcomes. We plan to integrate as many covariates as possible to limit their effect. As modeled in reviewed studies, we will operate under the assumption that if factors that influence the frequency of our outcomes are not statistically significantly different between the three study groups, then their effect will cancel one another out. Possible confounders that are statistically different between groups on univariate analysis will be adjusted for in regression models to determine if they are significant covariates.<sup>28,29,34,35,41</sup> It is likely that many characteristics will be similar within each group. For example, all mothers at risk for very preterm delivery should receive antenatal steroids; therefore, the percent of neonates exposed to antenatal steroids should be similar among the three groups.

We plan on controlling for gestational age by stratifying our data analysis into neonates <25 weeks', 25-27 and 6/7 weeks', and 28-31 6/7 weeks' GA as performed in prior studies.<sup>15,34</sup> Additionally, multiple studies have noted that more data are needed for

extremely preterm neonates.<sup>18,32</sup> By stratifying our analysis, we will be able to evaluate the association between magnesium concentration in neonates with lower GA and gastrointestinal outcomes more clearly.

Of note, we expect increased total dose and duration of maternal magnesium to be correlated with umbilical cord magnesium concentration, as multiple studies have noted above.<sup>27-29</sup> These influence the independent variable directly rather than confound outcomes. Thus, they do not need to be analyzed in a subgroup analysis.

#### **2.4.8 Sample Size and Statistical Significance**

Though many studies discussed above evaluated feeding intolerance or time to full enteral feed as a secondary outcome, few studied it as a primary outcome. Therefore, most studies were not powered to see an effect on FI and could not be used to determine an expected effect size. One study that measured time to full enteral feed in days found the median survival to be 13, 18, and 32 days for neonates with low ( $<2.5$  mg/dL), medium ( $\geq 2.5-4.5$  mg/dL), and high ( $\geq 4.5$  mg/dL) serum magnesium concentrations, respectively.<sup>29</sup> Two differences between this study and our proposed study are important. First, our study utilizes umbilical cord blood at the time of delivery rather than serum blood after delivery. Due to the long half-life of magnesium in neonates, we assume that our magnesium levels will be similar or slightly higher than the levels obtained in this study.<sup>24</sup> Second, our study divides neonates into terciles in order to have an even number of neonates in each group, regardless of the average magnesium concentration. If there is a relatively small range of magnesium concentrations in our study, the between group differences will not be as large as in this study where groups were created by defined concentration cut-offs.<sup>29</sup> Even so, by only using median survival data from low and

medium groups in our calculation, we have increased the confidence that we will at least be able to detect a difference between our low and high tercile groups.

Although our systematic literature review has led us to evaluate the adverse effects of elevated neonatal magnesium, we cannot rule out that magnesium sulfate could exert a protective effect on the intestine of SGA infants, as one study has suggested above.<sup>35</sup> Thus, we have utilized a two-tailed calculation so that we will be able to detect a difference, if it exists, in either direction. The reviewed literature is typically powered based on the specific primary outcome measure to an alpha of 0.05 and beta of 0.20, which we will follow. Our sample size is limited by the number of inborn neonates <32 weeks' GA at our three study centers so we needed to work backwards from the fixed sample size and median survival discussed above to identify the hazard rate we would be powered to detect. Our final crude sample size is 486 (162 neonates per tercile) which includes 120 neonates to control for four covariates (10 per group per covariate). Our adjusted analysis will be able to detect a hazard rate as small as 0.68. Our sample size calculation can be found in its entirety in Appendix D.

## **2.5 Conclusion**

Multiple studies have looked at the potential associations of antenatal magnesium exposure and neonatal outcomes, particularly FI, NEC, and SIP. However, these studies have several shortcomings. Most studies compare antenatal magnesium sulfate exposure to lack of exposure and do not measure neonatal magnesium concentration. We have shown that there are many factors that may affect neonatal magnesium levels, so studies that only record maternal treatment but do not assess neonatal magnesium level may not accurately assess the effect of magnesium on neonatal FI and risk of NEC or SIP. While a

few studies have measured neonatal magnesium levels, only one study utilized measurements from umbilical cord blood, which most accurately measures the neonate's greatest magnesium level.<sup>32</sup> Finally, many of the studies enrolled only small numbers of patients, under-enrolled extremely preterm neonates, were retrospective, or did not adequately control for confounding factors. In summary, our proposed study will fill in significant gaps regarding the important question of whether maternal magnesium therapy and subsequent elevation in neonatal magnesium concentration is associated with FI, NEC, or SIP. It will do so by recruiting a large sample at multiple study centers with appropriate demographic representation, measuring neonatal umbilical cord magnesium concentration at the time of delivery, following neonates prospectively, and conducting a statistical analysis that considers a multitude of covariates.

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## **CHAPTER 3: STUDY METHODS**

### **3.1 Study Design**

The proposed study will be a multicenter prospective cohort trial to analyze the association between magnesium concentration in umbilical cord blood of very preterm neonates and feeding intolerance from birth until discharge from the NICU or until four months of life. A consent form to participate in the study for self and child will be reviewed and signed. Next, an intake survey as well as chart review will extract maternal demographic data and features of maternal magnesium sulfate administration. Neonates born at less than 32 weeks' gestation will have an umbilical cord blood sample taken at the time of delivery which will be analyzed for magnesium concentration. Neonates will be followed prospectively until NICU discharge or up to 4 months, and numerous parameters related to primary and secondary outcomes will be collected via chart review. There are no interventions related to this study and there will be no additional sample collection or follow-up.

### **3.2 Study Population and Sampling**

The source population is pregnant women delivering within the YNHCH, Connecticut Children's Medical Center (CCMC) or Women and Infants Hospital (WIH) of Rhode Island. The selection for the study population is derived from mothers given antenatal magnesium sulfate who deliver a newborn at less than 32 weeks' GA. We will not limit subjects based on indication for magnesium sulfate therapy e.g. eclampsia treatment, seizure prevention in preeclampsia, fetal neuroprotection, tocolysis, or a combination. Exclusion criteria consist of neonates born at  $\geq 32$  weeks' GA, outborn neonates, neonates who have not been exposed to antenatal magnesium sulfate, neonates

with major congenital malformations, and neonates with chromosomal abnormalities. Eligibility criteria are summarized in Table 1.

Because very preterm delivery occurs relatively infrequently, we will utilize convenience sampling to select all consented neonates who meet inclusion criteria and are free of exclusion criteria.

**Table 1.** *Eligibility Criteria*

<b>Inclusion Criteria</b>	<b>Exclusion Criteria</b>
Neonate born < 32 weeks' gestation	Neonate born $\geq$ 32 weeks' gestation
Antenatal magnesium sulfate exposure	No antenatal magnesium sulfate exposure
Inborn	Outborn
	Major congenital malformation
	Chromosomal abnormality

### **3.3 Recruitment**

Recruitment will occur at the Level IV NICUs in Connecticut and Rhode Island: YNHCH, CCMC, and WIH. We plan to recruit pregnant mothers at risk for preterm delivery who are delivering at the three recruitment centers over a twenty-month period. Each site will be assigned a research assistant who will provide information about the study to clinicians and subjects, enroll eligible patients, and obtain informed consent. As delivering mothers can present at any time of day including weekends, we will train the fellows at YNHCH and WIH to obtain consent for study participants who present when our research assistants are not present. Fellows are involved in all deliveries <32 weeks' gestation and they are present in the hospital 24 hours per day and on weekends. As CCMC does not have fellows, we will train the equivalent in-house team leader.

### **3.4 Subject Protection and Confidentiality**

The study will be conducted pending review by each institution's Institutional Review Board (IRB). For example, the Human Investigation Committee of Yale

University School of Medicine and the Yale New Haven Health System must approve the trial to be conducted at YNHCH. All study personnel will complete Health Insurance Portability and Accountability (HIPAA) training and Yale Human Subjects Protection training. Study personnel will access all participant electronic medical records (EMRs) on university-approved, encrypted, and secure electronic devices. Protected health information (PHI) not in electronic form will be stored within a locked cabinet in the locked office of the principal investigator, to which only direct research staff will have access. All PHI will be disposed of in a secure manner after the study is completed.

All mothers will be required to grant written, informed consent in order to participate in the study. Consent for the neonate will be given by the mother. Consent is necessary as identifiable information will be collected throughout the course of the study. A clinical research assistant, trained fellow, or equivalent will explain the consent form and participants will have the opportunity to ask questions and discuss concerns prior to providing consent. The consent form contains a study description, duration of participation, and potential risks and benefits of the study. It will be available in English and Spanish with translation to other languages if needed. Interpreter services will be utilized as needed for Spanish and other languages. For those who are unable to read, informed consent will be obtained after an oral presentation with a third-party present to ensure all information is read and accurately represented. An example of the informed consent form can be found in Appendix A.

### **3.5 Study Variables and Measures**

#### **3.5.1 Independent Variable**

The independent variable and primary exposure of interest in our proposed study is umbilical cord magnesium concentration, which will be operationalized into three terciles. The terciles will be created by rank ordering neonates' magnesium levels and then dividing the patients into three groups (low, medium, and high magnesium concentrations) with an equal number of participants in each group.

Samples will be obtained by collecting blood from the umbilical vein into a serum separator tube immediately after delivery and freezing within 12 hours at  $-70^{\circ}\text{C}$ . Samples will then be sent to the laboratory and total serum magnesium will be measured using a chemistry analyzer.<sup>1</sup>

### 3.5.2 Primary Dependent Variable

The primary dependent variable is feeding intolerance, which will be assessed by the time it takes for a neonate to reach full enteral feed (150 mL/kg/day). Any neonate born  $\leq 1250\text{g}$  who reaches full feed past eight days is feeding intolerant per YNHCH's NICU feeding protocol. The longer time it takes for a neonate to reach full feed, the more feeding intolerant he or she will be considered. Thus, FI will be operationalized as time to an event with the aim of determining incidence rates.

YNHCH's feeding protocol will be adopted by all three study centers pending minor modifications after conferring with CCMC and WIH. YNHCH's feeding advance regimen can be seen in Table 2.

**Table 2.** *YNHCH's Enteral Feeding Advance Regimen for Neonates  $\leq 1250\text{g}$ .*

	<b>Birth weight <math>\leq 450\text{g}</math></b>	<b>Birth weight 451-550g</b>	<b>Birth weight 551-650g</b>	<b>Birth weight 651-750g</b>	<b>Birth weight 751-850g</b>	<b>Birth weight 851-950g</b>	<b>Birth weight 951-1050g</b>	<b>Birth weight 1051-1150g</b>	<b>Birth weight 1151-1250g</b>
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<b>Day 1 Non-nutritive feeds (24 mL/kg/day)</b>	1.6 mL q4h	2.0 mL q4h	2.4 mL q4h	3.0 mL q4h	3.2 mL q4h	3.6 mL q4h	4.0 mL q4h	4.4 mL q4h	5.0 mL q4h
<b>Day 2 Non-nutritive feeds (24 mL/kg/day)</b>	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above
<b>Day 3 Non-nutritive feeds (24 mL/kg/day)</b>	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above
<b>Day 4 (48 mL/kg/day)</b>	3.0 mL q4h	4.0 mL q4h	5.0 mL q4h	6.0 mL q4h	6.4 mL q4h	7.2 mL q4h	8.0 mL q4h	8.8 mL q4h	10 mL q4h
<b>Day 5 (72 mL/kg/day)</b>	2.4 mL q2h	3.0 mL q2h	3.6 mL q2h	4.2 mL q2h	4.8 mL q2h	5.4 mL q2h	6.0 mL q2h	6.6 mL q2h	7.2 mL q2h
<b>Day 6 (96 mL/kg/day)</b>	3.2 mL q2h	4.0 mL q2h	4.8 mL q2h	5.6 mL q2h	6.4 mL q2h	7.2 mL q2h	8.0 mL q2h	8.8 mL q2h	9.6 mL q2h
<b>Day 7 (120 mL/kg/day)</b>	4.0 mL q2h	5.0 mL q2h	6.0 mL q2h	7.0 mL q2h	8.0 mL q2h	9.0 mL q2h	10.0 mL q2h	11.0 mL q2h	12.0 mL q2h
<b>Day 8 (144 mL/kg/day)</b>	4.8 mL q2h	6.0 mL q2h	7.2 mL q2h	8.4 mL q2h	9.6 mL q2h	10.0 mL q2h	12.0 mL q2h	13.2 mL q2h	14.4 mL q2h

### 3.5.3 Secondary Dependent Variables

There are five secondary outcomes of interest: (1) necrotizing enterocolitis, (2) spontaneous intestinal perforation, (3) time to initiation of first feed (not counting buccal swabs or oral immunotherapy), (4) time to first stool, and (5) number of abdominal X-rays in the first 30 days of life.



NEC will be assessed with the Modified Bell's Staging Criteria and will be operationalized as a dichotomous variable (yes/no) where yes is classified as stage II or III NEC.<sup>2</sup> The Modified Bell's Staging Criteria were adapted from Bell's original criteria in 1986 and remain the most validated way to classify NEC.<sup>3</sup> The Modified Bell's Staging Criteria can be found in Table 3.

SIP will be diagnosed by (1) radiological evidence of perforation in the absence of a) clinical features of NEC, b) radiological features of intestinal ischemia e.g. fixed dilated bowel loops or pneumatosis intestinalis, or (2) intra-operative surgical report and/or histopathology assessment indicating a perforation located in the ileum and on the anti-mesenteric border.<sup>4,5</sup> SIP will be operationalized as a dichotomous variable (yes/no).

Secondary outcomes 3-5 are exploratory to further evaluate additional indicators of the adverse effect of magnesium on neonatal gastrointestinal function. Time to first feed and time to first stool will be operationalized as time to an event in hours with the aim of determining incidence rates. Number of X-rays during the first 30 days of life will be operationalized as a continuous variable.

**Table 3.** Modified Bell's Staging Criteria for NEC as adapted from <sup>2</sup>.

<b>Bell Stage</b>	<b>Systemic Signs</b>	<b>Gastrointestinal Signs</b>	<b>Radiographic Signs</b>
<b>IA</b> Suspected NEC	Apnea, bradycardia, temperature instability, lethargy	Gastric residuals, fecal occult blood, mild abdominal distension	Normal gas pattern or mild ileus
<b>IB</b> Suspected NEC	Same as IA	Bright red blood from rectum	Same as IA
<b>IIA</b> Definite NEC, mildly ill	Same as IA	Same as IA and IB, plus absent bowel sounds +/- abdominal tenderness	Ileus gas pattern with ≥1 dilated loops and focal pneumatosis intestinalis
<b>IIB</b> Definite NEC, moderately ill	Same as IA, plus mild thrombocytopenia,	Same as IIA, plus definite abdominal tenderness, +/-	Same as IIA, plus portal venous gas +/- ascites

	mild metabolic acidosis	abdominal cellulitis or palpable bowel loops	
<b>IIIA</b> Advanced NEC, severely ill, bowel intact	Same as IIB, plus hypotension, bradycardia severe apnea, mixed acidosis, DIC, neutropenia	Same as IIB, plus signs of generalized peritonitis, worsening tenderness and distension	Same as IIB, plus definite ascites
<b>IIIB</b> Advanced NEC, severely ill, bowel perforated	Same as IIIA, plus shock, deterioration in vital signs	Same as IIIA	Same as IIB, plus pneumoperitoneum

### 3.5.4 Potential Confounding and Explanatory Variables

Potential covariates which include maternal, perinatal, and neonatal baseline characteristics as identified in the literature review will be compared between the three study groups by univariate analysis. Statistically significant variables will be adjusted for in regression models to determine if they are significant covariates for each outcome. A summary of baseline characteristics and how we plan to test them on univariate analysis can be found in Table 4. We will control for the confounder of GA at delivery by stratifying data into neonates born <25 weeks, 25-27 and 6/7 weeks, and 28-31 and 6/7 weeks. Birth weight and additional covariates identified will be analyzed with subgroup analyses.

**Table 4.** Maternal, Perinatal, and Neonatal Baseline Characteristics

	Low Tercile	Medium Tercile	High Tercile	p-value
<b>Maternal Characteristics</b>				
Age (years)	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	ANOVA
Body mass index	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	ANOVA
Race:				
White	n (%)	n (%)	n (%)	Chi-square
Black	n (%)	n (%)	n (%)	Chi-square

Other	n (%)	n (%)	n (%)	Chi-square
<b>Perinatal Characteristics</b>				
Magnesium sulfate total dose (g)	mean ± SD	mean ± SD	mean ± SD	ANOVA
Magnesium sulfate total duration (hours)	mean ± SD	mean ± SD	mean ± SD	ANOVA
Length of rupture of membranes (hours)	mean ± SD	mean ± SD	mean ± SD	ANOVA
Indication for magnesium sulfate				
Maternal preeclampsia or eclampsia	n (%)	n (%)	n (%)	Chi-square
Fetal neuroprotection	n (%)	n (%)	n (%)	Chi-square
Tocolysis	n (%)	n (%)	n (%)	Chi-square
Hospital of delivery				
YNHCH	n (%)	n (%)	n (%)	Chi-square
WIH	n (%)	n (%)	n (%)	Chi-square
CCMC	n (%)	n (%)	n (%)	Chi-square
Cesarean delivery	n (%)	n (%)	n (%)	Chi-square
Antenatal steroids	n (%)	n (%)	n (%)	Chi-square
Multiple gestation	n (%)	n (%)	n (%)	Chi-square
<b>Neonatal Characteristics</b>				
Umbilical Cord Mg Concentration (mg/dL)	mean ± SD	mean ± SD	mean ± SD	ANOVA
GA (weeks)	mean ± SD	mean ± SD	mean ± SD	ANOVA
Birth weight (g)	mean ± SD	mean ± SD	mean ± SD	ANOVA
Male sex	n (%)	n (%)	n (%)	Chi-square
SGA	n (%)	n (%)	n (%)	Chi-square
Apgar score <7 at 5 minutes	n (%)	n (%)	n (%)	Chi-square
Human milk feeding				
All	n (%)	n (%)	n (%)	Chi-square
Partial	n (%)	n (%)	n (%)	Chi-square
None	n (%)	n (%)	n (%)	Chi-square
Sepsis	n (%)	n (%)	n (%)	Chi-square
Postnatal steroids	n (%)	n (%)	n (%)	Chi-square
Postnatal NSAID for treatment of PDA	n (%)	n (%)	n (%)	Chi-square
Postnatal Indomethacin for IVH	n (%)	n (%)	n (%)	Chi-square

prophylaxis				
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### **3.6 Additional Methodology Considerations**

Maternal magnesium sulfate regimen will be based off protocols at respective institutions. At YNHCH, women who receive magnesium sulfate but do not deliver within 12 hours are retreated. As outlined in the literature review, we expect increasing total dose of magnesium sulfate to be associated with increased neonatal cord blood magnesium concentration. As this is on the causal pathway, it is not considered to be a covariate. Total dose of magnesium sulfate will be calculated by adding the loading dose to the product of the infusion dose and duration.

### **3.7 Blinding of Exposure and Outcome**

Other than the fellows or equivalents obtaining consent, NICU teams including attendings and nursing staff will be unaware of a neonate's umbilical cord magnesium concentration and thus will not know neonatal groupings. They will also be unaware of the outcomes of interest to avoid hypervigilance leading to a change in management. Additionally, there will be minimal contact between study investigators and clinical teams to prevent influencing care.

### **3.8 Data Collection**

After consent is obtained, mothers will fill out an intake survey which will assess maternal demographics (age, race, parity, body mass index during the first visit for the present pregnancy), comorbidities, serum creatinine, and pregnancy complications. Surveys will be administered by a research assistant during the initial recruitment of pregnant mothers. Any information unknown to the mother will be elicited from the maternal EMR. An example of the intake survey can be found in Appendix B.

Neonatal baseline characteristics and outcome data will be obtained from neonatal EMRs and recorded into a data collection sheet. An example of the data collection sheet can be found in Appendix C. For completeness, we will extract data at the time of delivery, at one week, at two weeks, at one month, and at discharge from the NICU or four months after delivery (whichever comes first). If necessary, study personnel can clarify clinical questions regarding outcome data with the care team in near real-time. We have chosen to obtain outcome data via chart review because we believe that having study personnel round on patients to collect data regarding outcomes could influence care. Adherence is not applicable to our study as there are no interventions after initial umbilical cord sample is drawn.

### **3.9 Sample Size Calculation**

The main goal of the proposed study is to test the two-sided null hypothesis that there is no difference in incidence rate of FI (time to full enteral feed defined as 150 mL/kg/day) in the low tercile as compared to medium and high terciles of magnesium concentration from 0-4 months among very preterm neonates. Our calculation was performed using the *Power and Precision 4 Software* (Biostat, Inc) under the assumption that a log-rank test could be used, as FI is operationalized as time to an event.

Our sample size is limited by the number of inborn neonates <32 weeks' GA at our three study centers so we needed to work backwards from our fixed sample size to identify the hazard rate we would be powered to detect. Per data collected by Yale New Haven Health, approximately 130 neonates <32 weeks' gestation are born each year at Yale New Haven Hospital. Per data sent by respective faculties, there were 156 neonates <32 weeks' gestation inborn at WIH and 75 neonates <32 weeks' gestation inborn at

CCMC in 2018. In total, we estimate that approximately 601 very preterm neonates will be inborn at our study centers during the twenty-month period we have designated for subject accrual. With the help of an obstetric fellow at Yale, we estimate that 90%, or 541 neonates, should receive antenatal magnesium sulfate and thus be available for recruitment to our study. Of those available, we estimate a 90% success rate of recruitment secondary to the low risk and lack of intervention associated with our study. Therefore, our feasible starting sample size is 486 neonates.

From our starting sample size, we anticipate the need to account for mortality and to control for covariates. To account for mortality, we will only include neonates that survive past six weeks in our data analyses as this is long enough to be at risk for our primary and secondary outcomes. We estimate 10% mortality of our study population prior to six weeks based on data from 2013-2018 at YNHCH revealing 88% average survival to discharge (assuming some of these deaths will occur past 6 weeks). To control for four covariates, we will require ten extra neonates per group per covariate (totaling 120 neonates to control for confounding). Thus, we will start with 162 neonates per tercile for our crude analysis and 122 neonates per tercile for our adjusted analysis, noting a drop rate of 0.10 due to mortality prior to six weeks. For the adjusted analysis, we will be powered to detect a hazard rate as small as 0.68 between the low and medium terciles for a two-sided test with criterion for significance ( $\alpha$ ) = 0.05 and power of 81%. This is based on the median survival published by a similarly designed study by Narasimhulu et al.<sup>6</sup> The total calculation can be found in Appendix D.

There is no additional follow-up or data collection other than chart review after initial umbilical cord sample is taken. In addition, neonates born at <32 weeks' of

gestation are infrequently transferred out of the Level IV NICUs where we will conduct the study and, if so, are usually transferred to a within-network hospital using the same EMR as the Level IV NICU so data can continue to be collected. We therefore do not need to correct for loss to follow-up. If the sample size requirement has been met prior to twenty months, additional subjects may continue to be recruited to strengthen the study analysis.

### **3.10 Statistical Analysis**

First, baseline maternal, perinatal, and neonatal characteristics among the three study groups (low, medium, high terciles of umbilical cord magnesium concentration) will be compared using chi-square or Fisher's exact test for categorical variables and analysis of variance (ANOVA) test for continuous variables, as appropriate. Continuous endpoints will be expressed as medians and IQRs or as means  $\pm$  SD. Categorical endpoints will be summarized as frequencies and proportions.

Second, we will test our unadjusted main hypothesis by creating Kaplan-Meier survival curves and using log-rank tests to perform time to full enteral feed analyses for each group. For our adjusted analysis, we will stratify based on gestational age with a stratified Cox model. We will also use Cox proportional-hazards regressions to identify and control for additional covariates.

Third, we will analyze secondary outcomes. For NEC and SIP, we will compare incidence proportions of dichotomous outcomes with chi-square tests for our unadjusted analysis and multivariate adjustment through multiple logistic regression to control for confounders in our adjusted analysis. Time to first feed and time to first stool will be analyzed using the Kaplan-Meier method and log-rank test followed by Cox

proportional-hazards regression model to control for confounding. For number of X-rays in one month we will compare means between the three terciles with ANOVA. We will perform this particular analysis after excluding neonates who develop NEC and SIP, as they will typically receive many X-rays which would obscure the results regarding feeding tolerance in neonates without NEC or SIP. A p value of <0.05 will be considered statistically significant. All analyses will be performed using SPSS software v22.0 (SPSS Inc., Chicago, IL).

### **3.11 Timeline and Resources**

Pending IRB approval, we will recruit pregnant women at imminent risk for preterm delivery over twenty months. All mothers included in the study must have been administered antenatal magnesium sulfate and must deliver a neonate at <32 weeks' gestation within the first twenty months of the study. From the point of delivery, we will follow each neonate forward until discharge from the NICU or, if not yet discharged, for a total of four months. The study will be completed within two years. We will then interpret results and report our findings.

Proposed study personnel include:

- One principal investigator and one co-investigator to oversee all operations: Dr. Steven Peterec and Giavanna Chirico, PA-SII
- Three research assistants, one for each study center, trained for recruitment, obtaining informed consent, and intake survey delivery
- YNHCH and WIH neonatal-perinatal fellows and CCMC equivalent, trained for recruitment, obtaining informed consent, and intake survey delivery throughout the night and on weekends (when research assistants are not available)



- Three collaborating maternal-fetal medicine fellows, or equivalent, one at each center, to coordinate and oversee cord blood collection and temporary storage
- One physician associate student, Giavanna Chirico, for chart review, data organization, and writing
- One data analyst to perform the necessary statistical analyses once data collection has concluded

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## CHAPTER 4: CONCLUSION

### 4.1 Advantages

Our study has several major strengths. First, the prospective design allows us to analyze a diverse group of high-risk neonates in a real-world setting and to identify outcomes as they occur in real-time. Although an RCT is the ideal study design to determine causation, antenatal magnesium sulfate is the standard of care for fetal neuroprotection in our study population so randomizing women to not receive magnesium would be unethical. However, our study design allows us to evaluate the effect of magnesium exposure without randomization by dividing neonates into terciles based on magnesium sulfate level in cord blood.

Next, NICU teams do not know the magnesium concentration of each neonate or the specific parameters we are interested in related to our outcomes, which minimizes information bias and improves the internal validity of our study. We have further strengthened internal validity by including an extra 120 neonates solely to control for confounding variables.

Our study design is both feasible and ethical. By using umbilical cord blood rather than neonatal blood drawn after delivery, we will not expose the neonate to additional risks such as phlebotomy blood loss which could result in iatrogenic anemia and the need for blood transfusions, as well as pain or risk of infection from drawing a blood sample.<sup>1,2</sup> Additionally, by only drawing a sample of umbilical cord blood at the time of delivery and not requiring any other blood samples or follow-up appointments, we minimize loss to follow-up. Other than privacy, there is no risk of participation to the neonate, and thus negligible ethical concerns.

Finally, we have improved the external validity and generalizability of our study by utilizing multiple study centers. We also include the use of antenatal magnesium sulfate for any indication and women at risk for preterm birth for any indication which further increases the generalizability of our study and minimizes selection bias.

#### **4.2 Limitations**

Despite significant attention to study methodology, we acknowledge that this study has potential limitations. Our sample size is limited as there are only a certain number of neonates born <32 weeks' gestation at our three study centers. Having a larger sample size would increase our power to detect an association and would allow us to control for more covariates. Although having multiple study centers increases our sample size and generalizability, it introduces the potential for information bias secondary to variations in obstetric and neonatal care across study centers. In order to minimize the aforementioned variability, all study centers will adopt YNHCH's feeding protocol after conferring and making any minor modifications that may be needed.

We foresee two problems that could occur related to our exposure of interest and primary outcome. First, we may obtain a narrow range of umbilical cord magnesium concentrations. If this is the case, our low, medium, and high terciles will not be significantly different from one another, and it will be unlikely that we will see a difference in outcomes even if an association between elevated magnesium and our outcomes does exist. Second, our primary outcome, FI, has no definitive diagnostic test. We chose time to full enteral feed as our measure of FI as it will capture any delay in feeding due to signs and symptoms of FI; however, there are events that delay feeding for reasons unrelated to FI. For example, YNHCH's feeding protocol states to maintain or

reduce feeding volumes when a neonate is found to have a hemodynamically significant PDA and is treated with Indomethacin. In this scenario, our neonate may appear to be “feeding intolerant” when that may not be the case. We hope that should this scenario arise it will occur evenly in the three groups and effectively cancel the impact on our primary outcome.

Lastly, we recognize that the feeding protocol that our study centers will adopt follows advancement of enteral feed volumes by 24 mL/kg/day which will impact the time it takes to reach full feed.<sup>3</sup> As all subjects will be following the same feeding regimen, conclusions regarding an association between magnesium and time to full feed will still be accurate, but the exact number of days to reach full feed should not be used to estimate effect size in future studies where centers could have slower or faster advancement of enteral feeds.

#### **4.3 Clinical Significance**

As all neonates born very preterm should have antenatal magnesium sulfate exposure, the safety of this treatment is paramount. In current practice, administration of maternal magnesium sulfate is interrupted when signs of maternal magnesium toxicity, such as depressed reflexes, are present on exam or when maternal magnesium concentration is above the recommended therapeutic range.<sup>4</sup> We practice under the assumption that maternal clinical symptoms or magnesium levels can be monitored to assure fetal clinical safety because it is not reasonable to obtain fetal magnesium levels and it is not standard care to obtain neonatal magnesium levels. A fetus can have a magnesium concentration outside of a safe range as his or her mother continues to receive a magnesium sulfate infusion. Since the gastrointestinal outcomes discussed are already

known to be associated with prematurity, this can lead to the dangerous assumption that neonates born to mothers without overt signs of magnesium toxicity are developing complications due solely to their prematurity and not in part to their elevated magnesium levels as a result of their magnesium exposure.

If this study identifies an association between high cord magnesium concentrations and feeding intolerance, then neonatal cord or early postnatal magnesium levels could be used to identify neonates at increased risk for feeding intolerance. By increasing a clinician's index of suspicion, there would be opportunity for better monitoring, earlier recognition and interventions to decrease the negative consequences of FI. Additionally, knowing a neonate had magnesium exposure may allow a clinician to avoid unnecessary testing in the setting of feeding intolerance when the neonate otherwise appears well. If this study identifies an association between high cord magnesium concentrations and NEC or SIP, then neonatal cord or early postnatal magnesium levels could be used to identify neonates at increased risk for these devastating neonatal morbidities and possibly allow their prevention through modification of care practice. Finally, clarifying the relationship between high cord magnesium concentrations and adverse gastrointestinal outcomes could elucidate whether changes need to be made to the maternal magnesium sulfate treatment regimen to prevent elevated neonatal magnesium concentrations in the first place.

Alternatively, identifying that there is no association between elevated magnesium and FI, NEC, or SIP could be equally as beneficial. Our study, which avoids some of the limitations of existing studies, could demonstrate that magnesium level is not associated with increased risk for these gastrointestinal outcomes. Feeding problems in a

newborn exposed to magnesium therapy would not erroneously be attributed to magnesium level, making it less likely that other underlying pathology is missed. It would also increase our confidence in the safety of antenatal magnesium sulfate for very preterm neonates.

We recognize that observational studies alone cannot be the basis for changing clinical practice. Nevertheless, if we document an association between elevated magnesium levels and neonatal gastrointestinal problems, it could help to create opportunities for early intervention and mitigation of the effects of FI, NEC, and SIP in very preterm neonates. If no association is identified, our study would benefit the scientific community by clarifying data that have long been unclear or conflicting. Either result will reveal important information about the safety, or lack thereof, of antenatal magnesium exposure on the very preterm neonate's gastrointestinal tract.

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## APPENDICES

### Appendix A: Parental Consent Form

310 PR. 1: Informed Consent in Research Involving Children

**Title of Study:** Magnesium Exposure in Very Preterm Neonates and Adverse Gastrointestinal Outcomes

**Principal Investigator:** Steven Peterec, M.D.

**Affiliation:** Yale University School of Medicine and Yale New Haven Health System

#### Invitation to Participate and Study Purpose:

We are inviting you and your child to participate in a research study designed to look at the relationship between magnesium concentration in the blood and gastrointestinal outcomes in preterm infants. You and your child have been asked to participate because he/she will be born preterm, you are being treated with magnesium, and this will result in your child being exposed to antenatal magnesium sulfate. We plan to study over 500 newborns born within Yale New Haven Children's Hospital, Connecticut Children's Medical Center, and Women and Infants Hospital of Rhode Island to study this question. Newborns will be followed until discharge from the NICU or up until four months of their life.

In order to decide whether you wish yourself and your child to be a part of this research study, please read this form which provides detailed information about the study. Next a member of our research team will discuss the purpose, procedures, risks, and benefits of the study with you so that you can make an informed decision. Once you are confident that you understand the study, you will be asked if you wish for yourself and your child to participate; if so, you will be asked to sign this form.

#### Description of Study and Procedures Used:

- We will ask you to fill out a survey which will include a number of questions about your medical, social, and demographic history to get an accurate picture of your baby's exposures and environment. If you are unable to answer any questions, we will review your medical record to see if it provides an answer.
- We will draw a sample of blood from the umbilical vein during your delivery which will be analyzed to determine the concentration of magnesium. The umbilical vein is a blood vessel in the umbilical cord. When your baby is delivered the cord is clamped and cut; we will obtain the blood from the part of the umbilical cord that is attached to the placenta and is usually discarded after delivery. We will not draw the blood from the part of the cord still attached to your baby after delivery, nor from anywhere else from your baby.
- We will then review your baby's medical record at delivery, 1 week, 2 weeks, 1 month, and either at the time of discharge from the Newborn Intensive Care Unit or at 4 months to determine baseline characteristics of your child as well as parameters relating to the gastrointestinal outcomes we are analyzing, particularly related to how well your baby tolerates feeds and whether your baby has any problems associated with the intestines.

- The only procedure we will be performing is drawing the blood sample at the time of delivery. There will be no further time commitment or procedures for you or your child to partake in.

You will be told of any findings that develop during the course of your child's participation in this study that may affect your willingness to continue to participate.

### **Risks and Inconveniences**

There are no physical risks associated with this study. Although we will make every effort to safeguard you and your child's information (as we will describe below), there is the risk of loss of confidentiality.

### **Expected Benefits**

This study aims to clarify whether elevated magnesium concentration in the preterm infant, as a result of maternal magnesium sulfate treatment, is associated with feeding intolerance and other gastrointestinal outcomes. While you and your baby are unlikely to receive any direct benefit as a result of this research, you may help to advance the medical community's understanding of the risks associated with elevated magnesium concentration and improve the care of future mothers and their babies.

### **Economic Considerations**

There will be no costs associated with you or your child's participation in this research study. There will also be no paid reward for participation in this study.

### **Confidentiality of Information**

Any identifiable information that is obtained throughout this study will remain confidential and will only be disclosed as required by United States, Connecticut, or Rhode Island State law. Only the researchers involved in this study and those responsible for research oversight (such as representatives from the Yale University Human Research Protection Program and members of the Institutional Review Boards at the hospital where you are delivering your baby), will have access to any identifiable information that we collect. These individuals are required to keep all information confidential as well. When the results of the research are published or discussed, no information will be included that would reveal your child's identity unless your specific permission for this activity is obtained.

We will protect your information by only accessing electronic medical records on university-approved, encrypted, and secure electronic devices. All healthcare providers and research staff are subject to the Health Insurance Portability and Accountability Act (HIPAA) and thus are required to protect the privacy of your information. Our data collection sheets will be stored within a locked cabinet within the locked office of the principal investigator. Information related to you and your baby and the blood sample collected as part of the research will not be used or distributed for future research studies.

### **Research Subjects' Rights**

You are free to choose not to have yourself and your child participate and doing so will not result in penalty or loss of benefits that you or your baby is otherwise entitled

to (such as your child's health care outside the study). If you do choose to allow yourself and your child to participate in this study, you may withdraw yourself and your child from the study at any time with no penalty or loss of benefits. You can do so by calling or sending written notice to the Principal Investigator, Dr. Steven Peterec, at 1 Park Street New Haven, CT, 06504. When you withdraw your permission, no new personal health information will be gathered after that date. Information that has already been gathered may still be used until the end of the research study to ensure the integrity of the study and/or study oversight. Refusing to participate or withdrawing from the study will not have any effect on you or your child's relationship with your own doctors or within the Yale New Haven Health System, Connecticut Children's Medical Center, or Women and Infants Hospital of Rhode Island.

### Questions

We have used technical terms in this form. Please feel free to ask about anything you do not understand. Consider your options as long as you feel necessary before making a decision.

### Authorization

I have read (or someone has read to me) this form, and I have decided to allow myself and my child to participate in the project described above. Its general purposes, the details of mine and my child's involvement, the possible risks and inconveniences, and the possible benefits have been explained to my satisfaction.

By signing this form, I give permission to the researches to use information about myself and my child for the purposes described in this form. By refusing to give permission, I understand that I or my child will not be able to participate in this research study. My signature also indicates that I have received a copy of this permission form.

Name of Child: \_\_\_\_\_

Signature of Parent: \_\_\_\_\_ Date: \_\_\_\_\_

Name of Person Obtaining Consent (Print): \_\_\_\_\_

Signature of Person Obtaining Consent: \_\_\_\_\_ Date: \_\_\_\_\_

If you have further questions about this project or if you have a research-related problem, you may contact the Principle Investigator at his office. [Dr. Peterec at 203-688-2320]

If after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at 203-432-5919.

If you have questions about your rights as a research participant, or you have complaints about this research, you can contact the Yale Institutional Review Boards at 203-785-4688 or email [hrpp@yale.edu](mailto:hrpp@yale.edu).

## Appendix B: Maternal Intake Survey

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Medical Record Number: \_\_\_\_\_

Hospital: \_\_\_\_\_

Date of Birth: \_\_\_\_\_

Weight: \_\_\_\_\_

Height: \_\_\_\_\_

Race: \_\_\_\_\_

Serum Creatinine: \_\_\_\_\_

Past Medical History: \_\_\_\_\_

---

Have you ever been diagnosed with elevated blood pressure or hypertension?

Have you ever been diagnosed with diabetes mellitus?

### Have you had any of the following with your current pregnancy?

Gestational diabetes?

Preeclampsia?

Eclampsia?

Multiple gestation (twins or more)?

Other? \_\_\_\_\_

### Past Obstetric History:

What number pregnancy is this?

How many living children do you have?

Have you had any complications with prior pregnancies?

## Appendix C: Data Collection Sheet

Name: \_\_\_\_\_ MRN: \_\_\_\_\_

Name of mother: \_\_\_\_\_ Mother MRN: \_\_\_\_\_

Date of Birth: \_\_\_\_\_ Hospital: \_\_\_\_\_

Gestational age at delivery: \_\_\_\_\_

Birth weight: \_\_\_\_\_

Umbilical cord magnesium concentration: \_\_\_\_\_

### Perinatal Factors:

Antenatal Magnesium Sulfate Exposure:

Total dose (g): \_\_\_\_\_

Total duration (hours): \_\_\_\_\_

Indication for magnesium sulfate: \_\_\_\_\_

Antenatal steroids (y/n): \_\_\_\_\_

Length of rupture of membranes (hours): \_\_\_\_\_

Mode of delivery (vaginal, cesarean): \_\_\_\_\_

Multiple or single gestation: \_\_\_\_\_

### Neonatal and Postnatal Factors:

Sex: \_\_\_\_\_

SGA (y/n): \_\_\_\_\_

Apgar score at 5-minutes: \_\_\_\_\_

Human milk feeds (all, partial, none): \_\_\_\_\_

Postnatal Indomethacin for IVH prophylaxis (y/n): \_\_\_\_\_

Postnatal NSAID treatment for PDA (y/n): \_\_\_\_\_

Postnatal steroids

Hydrocortisone (y/n): \_\_\_\_\_

Dexamethasone (y/n): \_\_\_\_\_

Sepsis (y/n): \_\_\_\_\_

**Outcomes:**

Time to full enteral feed (150 mL/kg/day): \_\_\_\_\_

Necrotizing enterocolitis stage II or III (yes/no): \_\_\_\_\_

Spontaneous intestinal perforation (yes/no): \_\_\_\_\_

Time to first feed (hours): \_\_\_\_\_

Time to first stool (hours): \_\_\_\_\_

Number of abdominal X-rays in first 30 days of life: \_\_\_\_\_

\*Data collection terminates in the cases of neonatal death, discharge from the NICU, or 4 months after delivery.

## Appendix D: Sample Size Calculation

Group	Duration (Intervals)			Sample Size		Treatment Effect			Attrition
	Accrual Period (mo)	Follow up (mo)	Total duration (mo)	N per interval	Total subjects	Hazard Rate	Median Survival (mo)	24 Interval Survival	Drop Rate Per Interval
Control - Low Tercile	20	4	24	6.1	122	1.61	0.43	0.00	0.10
Middle Tercile				6.1	122	1.10	0.63	0.00	
				12.2	244	<b>0.68</b>			
<b>Alpha = 0.05, Tails = 2</b>							<b>Power = 81%</b>		

In this chart, we calculate that we are powered to detect a hazard rate of 0.68 for our primary outcome between the low and middle tercile groups. This calculation was made using a two-tailed test with an alpha = 0.05 and 122 neonates per group, the fixed number of neonates we have available per tercile after controlling for confounding. Data for median survival in months between the two groups were estimated from Narasimhulu et al., where neonates in the low magnesium group took a median of 13 days to reach full feed and neonates in the medium magnesium group took a median of 18 days to reach full feed.<sup>1</sup> Drop rate is 0.10 as we estimate 10% mortality for our study population. Interval survival was estimated to be 0 as we expect all surviving subjects to eventually reach full enteral feed in the allotted 4 months. Including the high tercile group, which was not required for this calculation, we will have 366 total subjects for our adjusted analysis. We have accounted for 120 additional neonates to control for confounding, so our total sample size is 486 neonates.

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