University of Chicago Medical Center Research Protocol

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Study Title: Antibiotic stewardship and protection of the developing preterm infant microbiome

ABSTRACT:

Fear of early-onset sepsis (EOS) has led to nearly universal initiation of empiric antibiotics at birth in preterm infants. While the risk of morbidity and mortality from EOS is high, the frequency is low at 0.98 per 1000 live births (Stoll et al 2011). Evidence shows that there are significant risks involved with prolonged antibiotic therapy, including necrotizing enterocolitis, invasive fungal infection, and even death. Furthermore, antibiotics alter the microbiome, which is increasingly being recognized as a key determinant of health and disease. The development of the microbiome begins at birth; the very time empiric antibiotics are begun in the majority of preterm infants. We hypothesize that antibiotic stewardship principles can be used to limit empiric antibiotic use at birth in preterm infants, thus leading to protection of the developing microbiome and improved clinical outcomes.

I. Aim and Hypotheses

Preterm infants are frequently started on empiric antibiotics at birth due to the risk of early onset sepsis (EOS). While the risk of morbidity and mortality from EOS is high, the frequency is low at 0.98 per 1000 live births (Stoll et al 2011). There are risks involved with antibiotic therapy including disseminated fungal infection, neonatal necrotizing enterocolitis, and alteration of the gastrointestinal microbiome (Nash et al 2009). We hypothesize that antibiotic stewardship principles can be used to limit empiric antibiotic use at birth in preterm infants, thus leading to protection of the developing microbiome and improved clinical outcomes.

Aim 1: Implement an antibiotic stewardship program to limit initial empiric antibiotic administration in a pilot study group of 30 preterm patients, 28-34 weeks gestation.

Aim 2: Evaluate outcomes in infants who do, or do not, receive empiric antibiotics at birth. Blood culture results, clinical outcomes, length of stay, total days of antibiotics received during NICU admission, and microbiome development from birth until hospital discharge will be examined.

II. Background and Rationale

A. Background:

Greater than 12% of infants in the United States are born premature and may begin their lives within the neonatal intensive care unit (Matthews et al). Within this population, the evaluation and treatment for early-onset sepsis (EOS) is an ongoing controversy. EOS remains the leading cause of morbidity and mortality in neonates with a rate of 0.98 per 1000 live births (Stoll et al). Infants admitted to NICUs are frequently started on empiric antibiotics due to the risk of EOS. Although the CDC has released algorithms for the evaluation of EOS for all newborns, without a lower gestational age limit indicated to which they apply, clinical experience has shown that nearly all premature infants receive empiric antibiotics (Verani et al 2010). Recent algorithms by the AAPs Committee on the Fetus & Newborn recommend discontinuing empiric therapy at 48 hours with negative cultures in preterm infants (Polin et al 2012). However, although it has been shown that discontinuing antibiotics when cultures are negative in asymptomatic ELBW infants does not compromise clinical outcomes, they often receive prolonged antibiotic treatment courses (Cordero & Ayers 2003). Furthermore, Kuppala et al (2011), studied very low birth weight (VLBW) infants, and found that within their population, 16% did not receive empiric antibiotics. For those who were treated, each day of initial empiric antibiotic therapy significantly increased the odds of developing late-onset sepsis (LOS), NEC or death, and for infants who had received any initial empiric antibiotic exposure, the number needed to harm was 3.

Within our unit, there were 794 admissions in 2014. Of these, 196 were within our study's target age range of 28-34 6/7 weeks of gestation at birth. While there were no positive blood cultures at birth within this gestational age range, only 6 of these 196 patients did not receive empiric antibiotics at birth. Additionally, of the 190 that were treated, 43 were treated beyond the standard 48-hour rule-out-sepsis recommendations.

Recently, there has been a growing body literature describing the effects of antibiotics on the gastrointestinal microbiome. In a study of 26 patients in 2009, Tanaka et al. found that the neonates that has received either oral or intravenous antibiotics during the first 4 days of life had less gut microbial diversity, as well as an attenuation in colonization with *Bifidobacterium* and an increase in colonization with *Enterococcus.* Additionally, a study by Fouhy et al showed that infants exposed to the empiric antibiotics ampicillin and gentamicin shortly after birth tend to develop microbiomes with higher proportions of *Proteobacteria*, *Actinobacteria*, and *Lactobacillus* than the unexposed infants for up to a month following the treatment course.

These changes within the microbiome may be especially important within the premature population, where Wang et al (2009) found that infants who developed necrotizing enterocolitis (NEC) had significantly more days of antibiotics prior to disease onset, as well

as differences in their gastrointestinal microbiome, having less microbial diversity and an increased abundance of *Gammaproteobacter*. Similarly, Cotton et al (2009) has also shown that prolonged antibiotic therapy (>5 days) was associated with increased odds of NEC or death. In earlier studies, Cotton et al (2006) linked prolonged broad spectrum antibiotic therapy with increased risk for invasive fungal infections. Beyond the immediate neonatal timeframe, gastrointestinal microbial dysbiosis has also been linked to multiple diseases affecting long term health, including inflammatory bowel disease (IBD), obesity, asthma and atopy (Arietta et al 2014).

Due to the effects of the microbiome health and disease, as well as growing concerns for antibiotic resistance, there has been a growing recognition for the need for antimicrobial stewardship. According to the Infections Diseases Society of America (IDSA), antimicrobial stewardship includes interventions targeted toward the improvement and monitoring of appropriate antimicrobial use by selecting the most optimal drug regimen, including the type of drug used, the dose, the duration of therapy and the route of administration. While studies have found that, in general, antibiotic stewardship is not commonly used within pediatric hospitals (Hersh et al 2009), Comer Children's Hospital has a strong, multidisciplinary antimicrobial stewardship team with an active presence within the NICU. These collaborations are necessary to promote proper and safe use of antibiotics, as Grohskoph et al (2005) found that as many as 47% if infants within NICUs were on at least one antibiotic, and Clark et al (2006) found that antibiotics are the most prescribed medications within NICUs. Within our NICU at Comer, 30-40% of patients are on at least one antibiotic on a given day (Nash et al 2014). While there is less published literature on the presence of antibiotic resistant organisms (AROs) within NICUs, de Man et al (2000) found that infants treated with cefotaxime as initial empiric therapy were 18 times as likely to grow resistant organisms.

Limiting initial empiric antibiotic therapy is one way to promote healthy gastrointestinal microbiome development, as well as limit the presence of AROs. While there is little data supporting the withholding of initial antibiotics in neonates, the administration of empiric antibiotics has been questioned for decades. In 1961, William Blanc questioned the safety of empiric antibiotics, stating "The diagnosis of congenital and early neonatal infections is difficult, and the systemic prophylactic administration of antibiotics is potentially harmful. Its dangers have to be weighed against the risk of infection." More recently, Tagare et al (2010) found that in low risk preterm neonates there was no evidence that routine empiric antibiotic use had a protective effect in clinical sepsis, further questioning the immediate use of antibiotics at birth.

B. Rationale:

Evaluation and treatment for EOS is an ongoing controversy in neonatology. While the risk of morbidity and mortality from EOS is high, the frequency is low at 0.98 per 1000 live births (Stoll et al 2011). Although the CDC has released algorithms for the evaluation of EOS for all newborns, there are no specific algorithms for preterm infants, and clinical

experience has shown that nearly all premature infants receive empiric antibiotics (Verani et al 2010). Recently, there has been growing literature on the detrimental effects of antibiotics on the development of the gastrointestinal microbiome. Important in the premature population, Wang et al (2009) found that infants who developed NEC had significantly more days of antibiotics prior to disease onset, as well as differences in their microbiome. Cotton et al (2009) has also shown that prolonged antibiotic therapy (>5 days) was associated with increased odds of NEC or death. Additionally, the microbiome has been linked with long-term health outcomes, such as asthma, obesity and IBD. With this growing body of evidence suggesting potential harm associated with empiric antibiotic exposure by preventing healthy microbialization, and the growing numbers of infections with antibiotic resistant organisms (Bizzarro & Gallagher 2007), it is critical to develop guidelines to limit antibiotic use in premature infants, and study the effect on clinical outcomes and the microbiome development.

III. Research Plan

Methods:

Through literature review and expert input, inclusion and exclusion criteria for randomization have been developed and reviewed to allow preterm infants, with a low risk of infection, to be closely observed and monitored, rather than receive routine empiric antibiotics on admission to the NICU.

Inclusion Criteria for randomization:

1) Infant must be born between the gestational ages of 28 0/7 weeks and 34 6/7 weeks
   -AND-
2) Infant must be considered to have a low risk of infection by one of the following criteria:
   a. Delivered for maternal indications (Cesarean section or induction of labor for maternal health, including HELLP, pre-eclampsia, placental abruption, history of IUFD/abruption, multiple gestation requiring preterm delivery, etc)
      -OR-
   b. Delivered due to preterm labor to a mother without the diagnosis of chorioamnionitis/maternal fever or prolonged rupture of membranes >18 hours

Exclusion Criteria:

1) Signs of clinical illness within the first 3 hours of life (Escobar et al 2014):
   a. 5-minute Apgar <5
   b. Requiring vasoactive drugs
   c. Seizures
   d. Significant respiratory distress requiring supplemental oxygen >40%
2) I:T Ratio of >0.2 on initial CBC
3) Congenital anomalies, including renal anomalies requiring serum antibiotic level monitoring.

30 patients who meet inclusion criteria will be enrolled for randomization. Parents of infants that may be eligible will be consented prior to the birth if the infant. If the infant meets the inclusion criteria at the time of birth, they will be randomized into one of the study groups. Parents who consent will be notified whether or not their child met inclusion criteria and were randomized in the study. They can choose to withdraw their child from the study at any time.

If infants are not determined to be low-risk at the time of birth, and do not meet the inclusion criteria for randomization, they will remain in the study for stool sample analysis and data collection. Their medical management will be ordered by the primary care team, including the possible administration for antibiotics at birth.

The study design will be a randomized, double-blinded study where all consented infants who meet the inclusion criteria will undergo a standard limited sepsis evaluation at birth, consisting of a blood culture and complete blood count with differential, as recommended in the CDC guidelines for prevention of GBS sepsis. Following evaluation, infants will be randomized. The control group of infants will receive current standard of care empiric antibiotic coverage with ampicillin and gentamicin. The infants in the intervention group will receive placebo at the same dosing intervals of the routine antibiotic coverage. Antibiotics, or placebo, will be discontinued after 48 hours, at which time the blood culture would be reported as negative at 48 hours, consistent with the standard of care rule out sepsis evaluation. Each treatment group, control and intervention, will have 15 study participants. We will further aim to have half in each treatment group to be under the gestational age of 32 weeks and half older than 32 weeks. Multiples will be randomized to the same treatment group. We will consent up to 80 patients in order to have 30 that will meet randomization criteria.

If at any time during the initial 48 hour trial period an infant shows clinical signs of sepsis, as defined below, or the initial blood culture becomes positive, the infant will be un-blinded. Repeat evaluation will be performed, and antibiotics will either be initiated for those in the intervention group, or appropriately broadened, per the discretion of the medical team, for those already receiving antibiotics. These patients will remain in the study for intention-to-treat analysis

Signs of clinical sepsis for which to unblind infant from the study and repeat evaluation (Hofer et al 2012, Bekhof et al 2013)

-Temperature Instability (Rectal, >38.5C, <36.0C or >1.5C change in 3 hours)
-Increased respiratory support of more than 20% increase in oxygen
-Pale/dusky skin tones
-Prolonged capillary refill time
-Lethargy
-Development of Seizures
-Requiring vasoactive medications for cardiovascular support
-Other signs of illness per the discretion of the managing team

Throughout their entire hospitalization, all infants will receive routine neonatal care per the medical team managing the patient.

During the entire hospitalization of the patients, daily stool samples will be collected from the infant diaper as available from spontaneous defecation, for analysis of the fetal microbiota through 16S rRNA gene-based analysis, as previously described by Wang, et al (2009). Through this stool analysis, the development of the fetal microbiome from birth through discharge will be examined and compared between the infants who do, or do not, receive empiric antibiotics at birth. Some of the stool samples will also be shared with the investigators at the University of Guelph in Ontario, Canada for further analysis, including identification of certain metabolites and antibiotic levels. The samples that are sent will be deidentified. Parents may be contacted when the infants are 18 months of life to answer a short health questionnaire about hospitalizations and chronic illnesses, as well as to provide stool samples for further analysis to determine if the differences persist once they are outside of the hospital environment. All supplies for stool samples and return postage will be provided to the parents. Parents will receive a small monetary compensation ($15 gift card) for returning the questionnaire and stool sample.

In addition to analyzing the microbiome, blood culture results, clinical outcomes (chronic lung disease, retinopathy of prematurity, intraventricular hemorrhage, death, NEC), length of stay, initiation and type of feeds, feeding tolerance and total days of antibiotic use will be examined through review of the EMR.

Monitoring:

The study team will report all events in accordance with IRB policies and procedures. Events not meeting reporting criteria will be provided at the time of continuing review.

All enrolled infants will be continuously monitored for signs of infection during the first 48 hours of life, as well as through the remainder of their hospitalization due to their admission in an ICU setting. Because of the small size of this pilot study, if there is any adverse event or safety concern, it will be immediately analyzed.

In addition, we will meet to evaluate for adverse events and safety for every 3 patients that are enrolled into the randomization portion of the study (weekly may be impractical due to the varied response in consent among our patient population - there may be many weeks without new study participants). This will result in 10 meetings throughout the course of the pilot study. We will also invite the NICU's medical director to participate in these meetings to ensure patient safety.

We will not be performing an interim efficacy analysis. This is due to the small sample size and single site design, as well as their prematurity of our study population which requires prolonged hospitalization. The study subjects will be continuously and closely monitored due to their gestational age and hospitalization within an intensive care setting. If at any time a patient becomes ill during the initial 48 hours, they will become unblinded. If it is a

repeated pattern that only those patients who are receiving placebo are becoming ill, the safety of the study will be reevaluated by the study team.

If three patients from this group develop sepsis in the first 48 hours, the trial will end.

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