Project Title:

- Pilot non-inferiority study investigating daily versus every-other-day dosing of oral iron in premature infants

Principal Investigator and Co-Investigator Information

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Research Question/Hypothesis

- Research Question: Will preterm neonates with estimated gestational age of 26 0/7 to 32 6/7 gestation who are supplemented with iron on an every-other-day (EOD) dosing, compared to those who are supplemented with iron daily, have the same incidence of achieving iron replete status (reticulocyte-Hb >29) at 36 weeks post-menstrual age (PMA)?
- Hypothesis: We hypothesize that at the same dosage in oral form, there will be no difference in the incidence of preterm neonates achieving iron replete status as measured by reticulocyte hemoglobin (Ret-Hb) between EOD and daily iron supplementation.

Objectives

- Primary objective: determine if daily versus EOD oral iron at the same dose per kilogram per day will achieve similar incidence of iron replete status at 36 weeks PMA.
- Secondary objective:
    - Characterize Ret-Hb levels in preterm infants
    - Identify the number of blood transfusions received between enrollment and 36 weeks' PMA between two groups
    - Determine prevalence of bronchopulmonary dysplasia between two groups
    - Identify the number with sepsis (defined as a positive blood or cerebrospinal fluid culture that was treated with antibiotics for at least 7 days) between two groups
    - Identify the number with necrotizing enterocolitis (NEC)/gastrointestinal perforation (medical NEC defined as ≥stage II Bell's criteria between two groups
    - Characterize growth (assessed by length gain at 36 weeks' PMA or at discharge if discharged before 36 weeks) between two groups

Background to Research Questions

- Iron is an important component of hemoglobin, and an essential part of erythropoiesis. It is also a necessary micronutrient for rapidly proliferating and differentiating cells and tissues especially in the brain.[1] Iron deficiency in infancy has been associated with anemia and impaired neurodevelopmental outcomes that extend into childhood. Premature infants are at highest risk for iron deficiency because they are deprived of the iron accretion that occurs in the third trimester of pregnancy, are born with lower iron stores compared to their term counterparts, and have increased utilization and depletion of iron stores with their rapid growth rate.[2,3]

- While there is no biomarker that serves as a gold standard for iron status in premature infants, hemoglobin (Hgb) levels and mean corpuscular volume (MCV) have been previously used, though they are late markers of iron deficiency.[1,4] Ferritin, an acute phase reactant, has also been used as biochemical marker of iron deficiency, but is often abnormally elevated during inflammatory states. Obtaining a combination of ferritin and a complete blood count for Hgb and MCV levels to characterize iron stores requires additional blood volume and predisposes the neonate to iatrogenic blood loss from frequent phlebotomy.[5] Iron-limited erythropoiesis can be better recognized by Ret-Hb even in preterm neonates <29 weeks' gestation, and can result from the same sample obtained to evaluate Hgb and MCV.[1,4,6] Ret-Hb levels >29 pg have been considered as iron-replete.[1]
- In older populations, EOD iron supplementation is as effective as daily iron supplementation in the treatment of iron deficiency anemia, with studies revealing significantly fewer gastrointestinal side effects in those who are on EOD iron.[7–10] Adults regulate their iron status through a feedback pathway involving hepcidin whereby iron-sufficient individuals will have upregulated hepcidin, which leads to decreased iron absorption and availability. Recent studies have revealed that pediatric patients and premature neonates regulate iron absorption through hepcidin in a similar fashion.[11,12] A randomized control trial in Kenya found that consecutive day iron dosing increases hepcidin and decreases iron absorption, compared with EOD dosing in pediatric patients as young as 5 months of age.[12] Though the regulation of iron status through hepcidin has been studied in extremely premature neonates, the clinical effect of EOD dosing of iron has not yet been examined in this population.

METHODOLOGY

Study design

- This is a non-inferiority, blinded, randomized control trial designed to investigate if EOD iron is comparable to daily iron dosing in achieving iron replete status by Ret-Hb measurements in premature infants.

Intervention

- The control group / current standard for iron supplementation in neonates is detailed as follows: after the infant achieves full enteral feeds, the infant is started on 6 mg/kg of oral iron daily supplementation. Though the American Academy of Pediatrics and World Health Recommendation recommend 2 – 4 mg/kg of supplemental iron for premature infants, studies examining the effects of higher dose of enteral iron supplementation (3.4 mg/kg to 7.1 mg/kg per day) revealed no differences between the lower versus higher dosages on morbidity (sepsis, necrotizing enterocolitis, or enteral feeding intolerance) and mortality outcomes.[13] The dose of 6 mg/kg of enteral iron was chosen based on the aforementioned recommendations with evidence of its safety, while minimizing the need to increase the enteral iron dosage if an infant were to be started on ESAs where a dose of 6 mg/kg of enteral iron supplementation is the standard practice.[14] Phlebotomy to obtain a complete blood count, reticulocyte count, and reticulocyte hemoglobin count is pursued the Monday after iron supplementation is started and every 2 weeks thereafter to monitor hematocrit or hemoglobin levels and iron status.

- The intervention group for EOD iron supplementation in neonates is as follows: after the infant achieves full enteral feeds, the infant is started on 6mg/kg of oral iron supplementation administered every other day. Phlebotomy to obtain a complete blood count, reticulocyte count, and reticulocyte hemoglobin count is pursued the Monday after starting iron supplementation and every 2weeks thereafter to weeks to monitor hematocrit or hemoglobin levels and iron status. There is literature revealing that neonates even <29 weeks' gestation absorb and regulate iron similar to adults.[11,12] There is literature revealing that there is no difference between EOD and daily iron supplementation in older pediatric populations and adults in improving hemoglobin levels, with one study in Switzerland revealing that EOD iron reduced iron deficiency in the longer term and triggered fewer gastrointestinal side effects in adult women.[7–10] Given the evidence that premature infants regulate their iron status similarly to older infants and adults, and the current evidence of EOD iron in older children and adults, this intervention, compared with our control, will help use address our primary question to determine if EOD iron is non-inferior to daily iron.
- Phlebotomy to obtain a complete blood count, reticulocyte count, and reticulocyte hemoglobin count may be pursued sooner than the 2-week mark based on physician judgment and the clinical status of the infant.
- Parents or guardians of infants who meet inclusion criteria will be consented prior to enrollment into the study. Following written informed consent, eligible participants will be randomized by simple randomization to the EOD arm or daily arm by computer generated sequence of random numbers. Allocation will be concealed using sequentially numbered opaque sealed envelopes which will be unknown to the investigators. The random sequence will be generated by a statistician who will not be directly involved in the care of patients.
- All study-related materials will be destroyed after official closure has been confirmed by the IRB.

Setting

- The study will be conducted in the neonatal intensive care unit at two sites: CHRISTUS Children's and Brooke Army Medical Center.

Expected Start Date

- Upon IRB approval

Duration of Study

- We anticipate the duration of the study begin upon IRB approval date and patient accrual will continue for until we have our target number of patients that meet inclusion criteria based on sample size analysis. We estimate this to be achievable within a 12-24-month period. Data analysis is anticipated to be completed within three months from the date of final patient accrual.

Study Subjects

- The population to be studied include premature infants who completed 26 0/7 to 32 6/7 weeks' gestation. We will include premature infants who are on full enteral feeds and are started on oral iron

therapy. Infants with known congenital anomalies or chromosomal abnormalities (such as Trisomy 18 or Trisomy 21), conditions that affect iron metabolism (such as thalassemia or hemochromatosis), bleeding disorders or coagulopathy, and received iron parenterally will be excluded from the study.

- There will be need for a written consent from the patient's' parent or legal guardian. A copy of the written consent will be included in the appendix.
- Our target population are neonates, which fall under the category of special subject population. Literature on the benefits of every other day iron dosing compared with daily iron dosing is already available in older pediatric patients and adults. There have been no studies investigating their benefits in the neonatal population, despite this population being the most vulnerable to iron deficiency and its consequences, as well as emerging evidence that neonate regular iron metabolism similar to adults.

Equipment/Supplies/Services

- For data accrual, the study will not require additional equipment, supplies, or services that are not already typically rendered in the care of a preterm neonate. Both CHRISTUS and BAMC use SYSMEX XN2000 to run laboratory tests that will be pertinent to this study. Therefore, no additional equipment, supply, or service will be required that is not already part of standard of care of a premature neonate.
- For data collection and management, our study requires the use of REDCap, which will be provided by CHRISTUS.

Data Collection

- The electronic medical records of CHRISTUS and Brooke Army Medical Center (BAMC) will be queried for patients who meet inclusion criteria as described above and consented for participation in the trial. Patient demographic information will be collected, along with other variables as outlined below:
    - Maternal Information
        - Age
        - Race / ethnicity: non-Hispanic White, non-Hispanic Black, Hispanic, Asian/Pacific Islander, Native
        - For patients at BAMC:
            - Mother Active Duty, Non-Active Duty, Retired
            - Rank of Active Duty: Enlisted versus Officer
        - Past medical history such as anemia, diabetes, mood disorder, hypertension, and more
        - Prenatal laboratory history: result of hepatitis B, syphilis, HIV, GBS status, blood type, rubella and varicella immunity status, gonorrhea and chlamydia status
        - Medication history to include use of antidepressants, anticonvulsants, insulin, aspirin, iron supplementation
        - Complications during delivery such as postpartum hemorrhage (>500 mL vaginal delivery, >1000mL c-section), magnesium around labor/delivery time-period, required pRBC transfusion
        - Blood type, antibody status

        - Hemoglobin, hematocrit results prior to delivery
    - Neonatal Information
        - Gestational age at birth
        - Birth order (first born, not first born)
        - Weight at birth
        - Delivery method: vaginal versus cesarean section
        - Instrumentation at delivery: vacuum assisted, forceps assisted, none
        - Race / ethnicity: non-Hispanic White, non-Hispanic Black, Hispanic, Other, Asian/Pacific Islander, Native
        - Phototherapy requirement
        - Blood type, DAT status
        - Delayed cord clamping
        - Hemoglobin, hematocrit, mean corpuscular volume at:
            - T0= first lab draw following starting iron
            - T1 = 2 weeks following T0
            - T#= every 2 week intervals until 36 weeks PMA or discharge
        - reticulocyte count, reticulocyte hemoglobin at:
            - T0= first lab draw following starting iron
            - T1 = 2 weeks following T0
            - T#= every 2 week intervals until 36 weeks PMA or discharge
        - Change in hematocrit, hemoglobin, retic count, retic hemoglobin between end of trial and T0
        - Ferritin, Serum TIBC, and iron levels at 36 weeks PMA
        - Darbepoetin / EPO use
        - Number of pRBC transfusions
        - Neonatal morbidities documented in the EMR which includes include anemia defined by unit guidelines based on the neonate's clinical status and corrected gestational age, necrotizing enterocolitis, sepsis, chronic lung disease, intubation, death, feeding difficulties
        - Neonatal severity scoring (NSOFA) at start of trial (T0) and every 2 weeks thereafter (T#)
        - Type of feed and calories from T0 to T#
        - Feeding volume at T0 to T#
        - Weekly growth parameters T0 to T# (weight, length, and head circumference)
        - Type of respiratory support T0 to T#
        - Blood draw volume T0 to T#
        - Transfusions from T0 to T#
        - Presence of specific diagnosis: constipation, feeding intolerance, infectious screen, clinical sepsis
- Data collected will include MRN; however, this will not be used in the analysis. All data will be deidentified before they are entered into REDCap for effective safeguard of information. Personnel

identified in this protocol will be responsible for collecting the data and will be the only persons who will have access to the de-identified data inputted on REDCap for further management and analysis.

Data Analysis

- A sample size determination was conducted using Riskcalc.org (Chow S-C, Shao J, Wang H, Lokhnygina Y. Sample Size Calculations in Clinical Research. Third ed: Chapman and Hall/CRC; 2017.) and confirmed by Dr. Jay Aden (BAMC Statistician). Sample size estimates are for type 1 error of 5% % ($\alpha = 0.05$), power (1-β) of 80%, ratio of 1:1 of intervention to control. The effect size is determined by estimating the proportion of infants with Ret-Hgb >29 pg to be similar at 80% between daily versus EOD iron, with a margin of 20%. It was determined that a sample size of 100 patients would be needed for this study (50 for intervention, and 50 for control).

- A statistician who is blinded to the study allocation will analyze the data. All statistical analyses will be performed with IBM SPSS Statistics (Armonk, NY: IBM Corp) and JASP Team (2022). JASP, Version 0.16.1 (Netherlands: JASP Team). Descriptive statistics will be performed for all variables. Categorical data will be reported as percentages, and continuous data will be reported as medians with interquartile range or means with standard deviation. The two groups will be compared according to the intention to treat with the use of Student's t-tests, Mann-Whitney-U tests, chi-square analysis based on the outcome being evaluated. All p-values will be one-sided, with a statistical significance level of <0.05. Covariates to include gestational age, in-born versus out-born infants, ESA use, receipt of pRBC transfusion will be controlled for using a logistic regression model and will be reported with an odds ratio (OR) and 95% confidence intervals (CI). A medical monitor who is not involved in the study will be assigned prior to the initial enrollment of the trial to monitor the data periodically (every 3 months) to ensure the safety of the participants and the scientific integrity of the study. An interim analysis will be performed at halfway to target enrollment.

Data Management

- Collected data will be entered into and managed within REDCap for effective safeguard of information. No physical or hard copies of the data will be made available. The information will be kept confidential by de-identifying the data before it is entered into REDCap. Standard confidentiality measures will be taken. All members of the study team are updated with their CITI/HIPAA training. Results will presented in table as well as boxplots.

Identified Risks/Ethical Considerations

- The risks associated with iron deficiency include anemia (and associated symptoms to include tachycardia, tachypnea, increased oxygen requirement, increased apnea/bradycardia/desaturation events which can lead to unnecessary evaluations for sepsis and antibiotic administration), long-term neurodevelopmental impairment, increased risk for necrotizing enterocolitis, packed red blood cell (pRBC) transfusion and transfusion-related risks, as well as delayed growth. The overall risk of iron deficiency will be mitigated by monitoring the complete blood count and reticulocyte count for hemoglobin/hematocrit levels and Ret-Hb levels every two weeks. There is no evidence to suggest EOD

iron dosing will result in worse outcomes compared to daily iron dosing. However, outcomes will be monitored every three months to ensure there are no significant adverse outcomes which would warrant study cessation.

- Current practice for frequency of phlebotomy varies by institution but the recommended frequency of lab draws in this study are within normal standards for preterm infants of this gestational age.
- A medical monitor who is not involved in the study will be assigned prior to the initial enrollment of the trial to monitor the data periodically (every 3 months) to ensure the safety of the participants and the scientific integrity of the study.

Benefits of Proposed Research to the Subjects and Others (significance of your study)

- This will give us insight if EOD iron is equivalent to daily iron supplements. This may also benefit hospital costs as it can help reduce total iron utilization for the duration of the neonate's hospitalization. This study may also help researchers and clinicians gain insight if EOD iron is as efficacious as daily iron in achieving iron replete status in premature infants with the potential of minimizing the side effects of oral iron intake. Participants will not receive compensation for participating in this trial.

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ATTACHMENTS

- **Data collection spreadsheet**
- **Consent Form**