Published in final edited form as:

Early Hum Dev. 2012 August ; 88(8): 583–587. doi:10.1016/j.earlhumdev.2011.12.030.

Association between Neonatal Iron Overload and Early Human Brain Development in Premature Infants

Sanjiv B. Amin, MD, MS[#], Gary Myers, MD^{*}, and Hongyue Wang, PhD^{**}

[#]Department of Pediatrics, Division of Neonatology, The University of Rochester School of Medicine and Dentistry

^{*}Department of Pediatrics, Division of Pediatric Neurology, The University of Rochester School of Medicine and Dentistry

^{**}Department of Biostatistics, The University of Rochester School of Medicine and Dentistry

Abstract

Background—Emerging evidence suggests that excess iron may be detrimental for brain development. However, little is known regarding the association between neonatal iron overload and subsequent neurodevelopment during infancy in vulnerable premature infants.

Aims—To evaluate the association between neonatal iron overload and neurodevelopment in premature infants.

Study Design—Prospective cohort study

Subjects—24–32 weeks gestational age infants who had serum ferritin (SF) measured at 34–35 weeks post-menstrual age (PMA) and did not meet exclusion criteria: SF < 76 ng/ml, toxoplasmosis, syphilis, rubella, cytomegalovirus, herpes infections, chromosomal disorders, or cranio-facial anomalies were eligible. In addition, infants with sepsis or elevated C-reactive protein within 10 days before their SF measurement were excluded.

Outcome Measures—Infants were evaluated for neurodevelopmental outcome at 8–12 months of age and were deemed to have neurodevelopmental impairment if they had one or more of the following: mental developmental index < 70, abnormal neurological examination, bilateral blindness, bilateral deafness, or required occupational, physical, or speech therapy.

Results—95 infants were studied. 70 had normal iron status (SF 76–400 ng/ml) while 25 were deemed to have iron overload (SF >400 ng/ml) at 34–35 weeks PMA. There was a marginal increase in neurodevelopmental impairment among infants with iron overload compared to infants with normal iron status (64% vs. 41%, $p = 0.05$). However, after controlling for confounders, iron overload was not associated with neurodevelopmental impairment (Adjusted OR 0.71, 95% CI, 0.21 – 2.5).

Conclusion—Modest neonatal iron overload is not associated with neurodevelopmental impairment during infancy in premature infants.

Corresponding Author: Sanjiv B Amin, MD, MS, Department of Pediatrics, University of Rochester, PO Box 651, 601 Elmwood Avenue, Rochester, NY-14642, Tel: 585-275-2972, Fax: 585-461-3614, Sanjiv_amin@urmc.rochester.edu.

CONFLICT OF INTEREST STATEMENT

The research was partly supported by Grant Number UL1 RR 024160 from the National Center for Research Resources.

Keywords

premature infants; neurodevelopmental impairment; oxidative stress; iron overload; latent iron deficiency

INTRODUCTION

The human fetal brain undergoes a rapid period of development during the last trimester of pregnancy. During this critical period of brain development, iron is essential for myriad brain developmental processes.(1–3) Recent studies have demonstrated that latent iron deficiency during the perinatal period is associated with abnormal short-term and long-term neurological outcomes.(4–7) Although iron is essential for brain development, emerging evidence also suggests that excess iron may be detrimental for brain development.(8–10) Moreover, excess non-protein-bound iron (iron overload) was recently implicated in the pathogenesis of hypoxic ischemic brain injury and periventricular white matter injury in neonates; and several neurodegenerative disorders in adults.(10–13) The deleterious effects of iron overload on the brain are thought to be primarily mediated by the production of free oxygen radicals via Fenton and Haber-Weiss reaction.(10, 13, 14)

Although liver biopsy, an invasive test, is the gold standard method for the diagnosis of iron overload, serum ferritin (SF) concentration, a less invasive biochemical test, is commonly used as a surrogate measure to evaluate and treat iron overload in older children. (15) Iron overload or elevated SF concentrations have not been described with enteral supplementation of up to 18 mg/kg/day elemental iron for premature infants.(16–18) However, iron overload is commonly seen after multiple erythrocyte transfusions during the neonatal period in premature infants.(15, 19) Despite its common occurrence, little is known regarding the effects of iron overload on brain development during the critical period of perinatal brain development in premature infants.(15, 19) Based on the available literature and biological plausibility, we hypothesized that neonatal iron overload might be associated with abnormal neurodevelopmental outcome during infancy in premature infants. We evaluated the association between neonatal iron overload and neurodevelopmental outcome assessed at 8–12 months of age in infants born at 24 to 32 weeks gestational age (GA).

METHODS

Study Design

We conducted a prospective cohort study in premature infants comparing neurodevelopmental outcome during infancy between those with modest iron overload (SF > 400 ng/ml or >898.8 pmol/L; to convert ng/ml to pmol/L multiply by 2.247) and those with normal iron status (SF 76–400 ng/ml or 170.7–898.8 pmol/L) identified at 34–35 weeks post-menstrual age (PMA). SF concentrations are normally elevated in neonates with the 97th percentile for normal SF concentration described previously in similar subject population being ~400 ng/ml.(20, 21) We, therefore, selected a SF concentration of >400 ng/ml as a cut-off level to define iron overload. We chose the lower limit of normal iron status as 76 ng/ml because latent iron deficiency (SF < 76 ng/ml) during the neonatal period may be associated with abnormal short-term and long-term neurodevelopmental outcome.(7, 22) We were particularly interested in neurodevelopmental outcome at 8–12 months of age because early neurodevelopmental evaluation forms the basis for children's long-term neurodevelopment and is more likely to be influenced by perinatal factors such as neonatal iron overload.(23) Moreover, socio-economic and educational factors are less likely to influence neurodevelopmental outcomes during infancy.(23, 24) The study was approved by

the local institutional research review board. Parental consent was obtained for each subject enrolled in the study.

Participants

This was a nested prospective study including 24–32 weeks GA infants participating in the primary neurological outcome study between 2008 and 2009 and on full enteral feeds for more than a week before SF measurement at 34–35 weeks PMA. Infants with craniofacial anomalies, chromosomal disorders, or TORCH (toxoplasmosis, other infections, rubella, cytomegalovirus infection and herpes simplex) infections were excluded from the primary study. For the nested study, infants with latent iron deficiency (defined as a SF of <76 ng/ml) at 34–35 weeks PMA were excluded. In addition, infants with sepsis (clinical or culture-proven) or with c-reactive protein (CRP) > 5 mg/L within 10 days before SF measurement at 34–35 weeks PMA were also excluded.(25) GA was determined by the best estimate of first trimester ultrasound, maternal history, and physical assessment of the infant by the attending neonatologist.

Exposure Measures

For each infant, SF was measured at 34–35 weeks PMA using 0.5 ml of blood drawn in a serum separation tube (Becton Dickinson, Franklin lakes, NJ, USA).. The SF was measured using the chemiluminescence immunoassay method at the institutional chemistry laboratory. Infants were classified into 2 groups of iron status depending on SF concentration at 34–35 weeks PMA: 1) Infants with SF >400 ng/ml (iron overload, Group I) and 2) Infants with SF 76–400 ng/ml (normal iron status, Group II). The Neonatal Intensive Care Unit policy follows the recommendations of the American Academy of Pediatrics and supplements premature infants with a total of 2 mg/kg/day elemental iron.(26) Most infant formulas and human milk supplemented with four packs of human milk fortifier provide ~ 2 mg/kg/day elemental iron on a full enteral feeding volume of 150 ml/kg/day.(19) The unit policy is to give erythrocyte transfusion when clinically indicated as per restrictive erythrocyte transfusion guidelines.(27)

Outcome Measures

Each infant had neurodevelopmental evaluation performed at 8–12 months of age (6–10 months corrected age). The neurodevelopmental evaluation included a neurological examination by a Pediatric neurologist, and a cognitive evaluation by a certified Psychologist. The Psychologist administered the Bayley Scale of Infant Development (BSID III). On the BSID III, the Mental Developmental Index (cognitive function) has a mean score of 100 with a standard deviation (SD) of ± 15 . Infants with score < 70 (2 SDs below the mean) were considered to have abnormal cognitive function. The neurological assessment included an evaluation of tone, strength, reflexes, angles, and posture. Neurological abnormality was defined as abnormal muscle tone in at least 1 extremity and abnormal control of movement and posture. Based on the overall evaluation and depending on the specific neurological and developmental findings at 8–12 months, recommendations were made for referral to an Early Intervention Program. If the findings indicated concerns about speech or motor development then children were referred to speech, occupational, or physical therapy as appropriate. We defined neurodevelopmental impairment (NDI) as an infant with one or more of the following findings: neurological abnormality, abnormal cognitive function, blindness (no functional vision in either eye), severe bilateral sensorineural hearing loss (requiring bilateral hearing aids) or one requiring speech, occupational, or physical therapy.

Covariates

Data on demographics, potential confounders, and outcomes were prospectively collected. Head ultrasounds were performed during the first week, at the end of second week, and at 6 weeks after birth. A diagnosis of intraventricular hemorrhage (IVH) was based on the head ultrasound findings following the Papille classification system as reported by a Pediatric Radiologist. Periventricular leukomalacia (PVL) was defined based on the head ultrasound findings reported by a Pediatric Radiologist. Chronic lung disease (CLD) was defined based on an oxygen requirement at 36 weeks PMA age. Necrotizing enterocolitis was defined based on X-ray findings of pneumatosis and/or free air in the peritoneum as reported by a Pediatric Radiologist.

Statistical analyses

Statistical analyses were performed using Stata 10 (Stata Corporation, College Station, TX). Two sample t-test (or Wilcoxon rank sum tests for non-normally distributed variables) and Chi-square (or Fisher Exact test) were used to compare continuous and categorical variables, respectively. An alpha of 0.05 was considered statistically significant. Bivariate analyses were performed to identify the clinical variables associated with iron overload. Bivariate analyses were also performed to evaluate the association between covariates and NDI in subjects unexposed to iron overload. Finally, multiple logistic regression model was fitted to evaluate the association between iron overload and NDI. Variables significantly associated with the outcome or iron status ($p < 0.1$) were considered as possible confounders and were included in building the regression model. Log likelihood ratio test was performed to evaluate the inclusion of potential confounders (identified based on bivariate analyses) in the regression model. Covariates that did not make a statistically significant contribution to the model were removed from the model using backward selection method. The final model was evaluated for a fitness using Pearson and Deviance statistics.

Sample size calculation

An approximate sample size was determined based on earlier findings of neurodevelopmental outcome in a similar population.(28) Based on earlier findings of 35% incidence of NDI (composite outcome) in a similar population and assuming the prevalence ratio of infants with iron overload and normal iron status to be ~1:3 ratio among 24–32 weeks GA premature infants, 22 subjects with iron overload and 66 subjects with normal iron status (1:3 ratio) would achieve 85% power to detect a two-fold increase in the proportion of 24–32 weeks GA infants with NDI at a two-sided α level of 0.05.

RESULTS

A total of 140 infants between 24 and 32 weeks GA were enrolled in the primary study. Among these, 132 infants were on full enteral feeds for more than a week before SF measurement at 34–35 weeks PMA and were eligible for the study. Of the 132 subjects, 30 (22%) infants had latent iron deficiency, 77 (59%) infants had normal iron status, and 25 (19%) were deemed to have iron overload. Of these 132 subjects, 31 infants were excluded (30 with SF < 76 ng/ml and one with sepsis before SF measurement at 34–35 weeks PMA). Of the 101 infants remaining, 95 had neurodevelopmental assessment performed between 8 and 12 months of age. The mean GA of the 95 infants who had a neurodevelopmental assessment performed and the 6 who did not was similar (27.6 vs. 28.6 weeks, respectively, $p = 0.3$). Among those who had a neurodevelopmental assessment, 70 infants had normal iron status and 25 infants had iron overload at 34–35 weeks PMA.

There were no significant differences between the two groups in demographic and perinatal factors except for GA and birth weight (Table 1). Infants with iron overload were

significantly more premature and had a lower birth weight compared to infants with normal iron status. There was a significant difference in some of the neonatal characteristics between the two groups (Table 2). Infants with iron overload at 34–35 weeks PMA had prior history of an increased frequency of hypotension (requiring vasopressors), patent ductus arteriosus, sepsis, and IVH. Compared to infants with normal iron status, infants with iron overload had a lower degree of unconjugated hyperbilirubinemia during the first two weeks after birth and received increased cumulative amount of erythrocyte transfusions prior to assessment of iron status at 34–35 weeks PMA. Infants with iron overload also had an increased incidence of CLD at 36 weeks PMA and a higher proportion received postnatal dexamethasone prior to assessment of iron status at 34–35 weeks PMA (Table 2). The CRP concentrations measured at the time of the assessment of iron status were normal among study infants with no significant difference ($p = 0.4$) in CRP concentration between the infants with iron load status (Mean $\pm$ SD, 1.2 ± 1.3 mg/L) and infants with normal iron status (Mean $\pm$ SD, 0.9 ± 1.1 mg/L). All study infants were started on iron supplementation before the assessment of iron status. There was significant difference between the two groups in the postnatal day of life when iron supplementation was initiated with infants with iron overload status were started on iron supplementation later (Mean $\pm$ SD, 44 ± 19 days) than infants with normal iron status (Mean $\pm$ SD, 21 ± 16 days). Infants from both the groups were discharged home on 2 mg/kg/day elemental iron with recommendation to be continued during the first year.

There was no significant difference in median head circumference (45 cm vs. 45 cm, $p = 0.9$), median height (64 cm vs. 67 cm, $p = 0.2$) and median weight (7.62 kg vs. 7.84 kg, $p = 0.7$) at 8 and 12 months of age between infants with iron overload and infants with normal iron status, respectively. Among the 95 cohort infants, 45 were deemed to have NDI on evaluation. No infants had bilateral blindness or deafness. The two groups were similar in their mean cognitive scores and need for physical, speech, or occupational therapy (Table 3). Fewer infants with normal iron status had NDI compared to infants with iron overload, but the difference was not statistically significant ($p = 0.05$). Among clinical variables, birth weight, *in-utero* exposure to illicit drug usage, patent ductus arteriosus, respiratory distress syndrome, hypotension, CLD, PVL, sepsis, and use of postnatal dexamethasone were all associated with NDI in subjects not exposed to iron overload on bivariate analyses.

Clinical variables significantly associated with the outcome or iron status ($p < 0.1$) were considered as potential confounders and were included in the regression model building. None of the variables were identified as effect modifiers for the association between iron status and outcome measures. Of these potential confounders, birth weight, postnatal dexamethasone usage, *in-utero* exposure to illicit drug usage, and CLD were selected using log likelihood ratio tests, and were included in the final logistic regression model. On controlling for confounding variables (Table 3), iron overload was not significantly associated with abnormal cognitive function (Adjusted odds ratio [OR] 2.6, 95% CI 0.1 – 81.8), need for speech, physical or occupational therapy (Adjusted OR 0.43, 95% CI 0.12–1.6), neurological abnormality (Adjusted OR 1.6, 95% CI 0.37–6.7), or NDI (Adjusted OR 0.71, 95% CI, 0.21 – 2.5).

DISCUSSION

We studied iron overload in premature infants because iron is essential for brain development, the brain undergoes rapid growth during the perinatal period, and iron has recently been implicated in the pathogenesis of neonatal hypoxic ischemic encephalopathy and in several neurodegenerative disorders in adults.(11–14) In this study we did not find any evidence that modest neonatal iron overload in premature infants, defined as a SF> 400 ng/ml at 34–35 weeks PMA, is associated with abnormal neurodevelopment in the first year

of life. We believe that this is the first reported study that evaluated the association between modest neonatal iron overload and neurodevelopmental outcome in premature infants.

In premature infants, iron overload usually develops because of either inherent defects in iron metabolism or secondary to multiple erythrocyte transfusions. (19, 29) An increase in plasma non-transferrin bound or free iron has been previously demonstrated after erythrocyte transfusions in premature infants.(30) Excess iron is initially sequestered and stored in tissues as ferritin. Even if excess iron is initially sequestered and stored in tissues as ferritin, iron can be released from its storage form during oxidative stress.(31) When excess free iron is released, it can be toxic to several organs by causing oxidative stress through generation of cytotoxic free oxygen radicals via the Fenton and Haber-Weiss reaction.(10, 14) Oxidative stress is usually seen when generation of cytotoxic free oxygen radicals exceeds the cellular capacity to detoxify free oxygen radicals. Recently, oxidative stress has been implicated in the pathogenesis of white matter injury which is considered the primary mechanism for NDI in premature infants.(9, 13) Despite emerging evidence and biological plausibility of adverse neurological effects from iron overload, an indirect indicator of the risk to produce free radicals, our findings do not suggest that modest iron overload is associated with NDI during infancy in premature infants. Although we did not measure oxidative stress, our finding that iron overload is not associated with NDI is suggestive that the degree of iron overload status seen among our study subjects was insufficient to cause significant oxidative stress that may be necessary to cause NDI. Several previous studies have failed to demonstrate an increase in lipid peroxidation with multiple erythrocyte transfusions in premature infants.(32, 33) Although these previous studies reported an association between multiple erythrocyte transfusions and CLD and retinopathy of prematurity in premature infants, the absence of an increase in measures of oxidative stress in these studies suggested that iron mediated oxidative stress was not the contributing factor for these reported associations. (32–35)

It is unclear if the generation of a significant amount of oxidative stress and secondary adverse neurological effects depends on the magnitude and/or duration of iron overload in premature infants. In older children with primary and secondary hemochromatosis, the clinical manifestations of iron overload are variable, may take years to develop, and are not usually seen with modest iron overload (SF concentrations below 1000 ng/ml) that was observed in our study.(29, 36–40) Chelating agents are therefore not used in older children with primary or secondary hemochromatosis unless the iron overload is severe (SF concentration > 1000 ng/ml).(40) We defined iron overload as SF > 400 ng/ml because of the potential for neurotoxicity at a lower magnitude of iron overload in vulnerable premature infants and the fact that the upper limit of normal SF among premature infants is ~ 400 ng/ml.(20, 21) The available literature suggest that premature infants may be more susceptible to brain injury secondary to iron-mediated oxidative stress compared to older children because of immature antioxidant enzyme system in premature infants.(10) Moreover, there is some evidence to suggest that decreased iron binding capacity in premature infants may make them more vulnerable to oxidative stress at a lower magnitude of iron overload than that usually seen in older children.(10).

The major strengths of the study are prospective and standardized evaluation of iron status at a uniform PMA, evaluation of neurodevelopmental outcome measures without knowledge of iron status, high rate of neurodevelopmental follow up, adequate power to test the hypothesis with the use of 1: 3 ratios of controls to cases, and comprehensive analyses controlling for potential confounders. In addition, we minimized misclassification bias by excluding infants with sepsis or elevated CRP within 10 days before assessment of iron status. We chose SF because it has been most commonly used in neonatal outcome studies related to iron status and SF concentrations corresponding to latent iron deficiency are

known among premature infants.(4) Latent iron deficiency as defined based on SF concentrations in premature infants has recently been shown to be associated with abnormal neurological outcomes and, therefore, it was essential to exclude premature infants with latent iron deficiency based on SF concentration. Compared to SF, the normal reference range of newer biochemical measures of iron status such as soluble transferrin receptor and reticulocyte hemoglobin content are not known for premature infants and therefore these newer tests were not considered. Similarly, we did not consider non-protein-bound iron concentration as a marker of iron overload status because although it is absent in healthy adults, non-protein bound iron concentration has been shown to be present and elevated in healthy term newborn infants and infants with increased erythrocyte turnover and hemolytic process that is so common among premature infants.(12, 41) Moreover, the normal reference range of non protein bound iron concentration for premature infants are not known to accurately define iron overload status and avoid any misclassification bias. All but three subjects in our study had modest iron overload (SF concentrations < 1000 ng/ml) and none of the subjects received an additional erythrocyte transfusion after evaluation of their iron status at 34–35 weeks PMA. Therefore, our study cannot exclude the possibility of NDI with severe neonatal iron overload, such as when SF concentrations are 1000 ng/ml. In addition, although neurodevelopmental assessment during infancy has a good negative predictive value for a subsequent evaluation at a later age, we can not exclude the possibility of the influence of modest iron overload on subtle long-term cognitive processes such as attention and reasoning that may not be apparent until school age. (42–45) Although we found no effect modifiers for any association between the iron status and neurodevelopmental outcome, our findings can not exclude the possibility that premature infants may be more susceptible to brain injury from modest iron overload if it develops concurrent to other oxidant-generating co-morbid conditions such as respiratory distress syndrome and sepsis which are common during the first week after birth.

In summary, we found no association between modest neonatal iron overload in premature infants and neurodevelopmental outcomes during infancy in this study. Our findings of the absence of adverse neurodevelopmental effects with modest neonatal iron overload among premature infants provides valuable safety information to the clinicians and may also facilitate future iron supplementation trials in this vulnerable population.(46)

Acknowledgments

The research was partly supported by Grant Number UL1 RR 024160 from the National Center for Research Resources. The sponsors had no role in study design, data collection, interpretation, analysis, writing of the manuscript, and in the decision to submit the manuscript

We are grateful to our certified psychologist, for administering BSID III for neurodevelopmental evaluation of subjects. We are also grateful to research coordinators for data collection.

Abbreviations

GA	gestational age
PMA	post-menstrual age
SF	serum ferritin
SD	standard deviation
OR	odd ratio
CI	confidence interval
NDI	neurodevelopmental impairment

CLD	chronic lung disease
IVH	intraventricular hemorrhage
PVL	periventricular leukomalacia
CRP	C-reactive protein
BSID	Bayley Scale of Infant Development

REFERENCES

1. Lozoff B. Perinatal iron deficiency and the developing brain. *Pediatr Res.* 2000; 48(2):137–139. [PubMed: 10926285]
2. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. *Am J Clin Nutr.* 2007; 85(2):614S–620S. [PubMed: 17284765]
3. Georgieff MK, Innis SM. Controversial nutrients that potentially affect preterm neurodevelopment: essential fatty acids and iron. *Pediatr Res.* 2005; 57(5 Pt 2):99R–103R. [PubMed: 15557113]
4. Amin SB, Orlando M, Eddins A, MacDonald M, Monczynski C, Wang H. In utero iron status and auditory neural maturation in premature infants as evaluated by auditory brainstem response. *J Pediatr.* 156(3):377–381. [PubMed: 19939407]
5. Armony-Sivan R, Eidelman AI, Lanir A, Sredni D, Yehuda S. Iron status and neurobehavioral development of premature infants. *J Perinatol.* 2004; 24(12):757–762. [PubMed: 15318248]
6. Siddappa AM, Georgieff MK, Wewerka S, Worwa C, Nelson CA, Deregnier RA. Iron deficiency alters auditory recognition memory in newborn infants of diabetic mothers. *Pediatr Res.* 2004; 55(6):1034–1041. [PubMed: 15155871]
7. Tamura T, Goldenberg RL, Hou J, Johnston KE, Cliver SP, Ramey SL, et al. Cord serum ferritin concentrations and mental and psychomotor development of children at five years of age. *J Pediatr.* 2002; 140(2):165–170. [PubMed: 11865266]
8. Chiueh CC, Andoh T, Lai AR, Lai E, Krishna G. Neuroprotective strategies in Parkinson's disease: protection against progressive nigral damage induced by free radicals. *Neurotox Res.* 2000; 2(2–3):293–310. [PubMed: 16787846]
9. Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. *Arch Dis Child Fetal Neonatal Ed.* 2008; 93(2):F153–F161. [PubMed: 18296574]
10. Buonocore G, Perrone S, Bracci R. Free radicals and brain damage in the newborn. *Biol Neonate.* 2001; 79(3–4):180–186. [PubMed: 11275648]
11. Berg D, Youdim MB. Role of iron in neurodegenerative disorders. *Top Magn Reson Imaging.* 2006; 17(1):5–17. [PubMed: 17179893]
12. Shouman BO, Mesbah A, Aly H. Iron metabolism and lipid peroxidation products in infants with hypoxic ischemic encephalopathy. *J Perinatol.* 2008; 28(7):487–491. [PubMed: 18322549]
13. Kaur C, Ling EA. Periventricular white matter damage in the hypoxic neonatal brain: role of microglial cells. *Prog Neurobiol.* 2009; 87(4):264–280. [PubMed: 19428957]
14. Adibhatla RM, Hatcher JF. Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal.* 12(1):125–169. [PubMed: 19624272]
15. Ng PC, Lam CW, Lee CH, To KF, Fok TF, Chan IH, et al. Hepatic iron storage in very low birthweight infants after multiple blood transfusions. *Arch Dis Child Fetal Neonatal Ed.* 2001; 84(2):F101–F105. [PubMed: 11207225]
16. Bader D, Kugelman A, Maor-Rogin N, Weinger-Abend M, Hershkowitz S, Tamir A, et al. The role of high-dose oral iron supplementation during erythropoietin therapy for anemia of prematurity. *J Perinatol.* 2001; 21(4):215–220. [PubMed: 11533837]
17. Miller SM, McPherson RJ, Juul SE. Iron sulfate supplementation decreases zinc protoporphyrin to heme ratio in premature infants. *J Pediatr.* 2006; 148(1):44–48. [PubMed: 16423596]

18. Braekke K, Bechensteen AG, Halvorsen BL, Blomhoff R, Haaland K, Staff AC. Oxidative stress markers and antioxidant status after oral iron supplementation to very low birth weight infants. *J Pediatr*. 2007; 151(1):23–28. [PubMed: 17586185]
19. Rao R, Georgieff MK. Iron therapy for preterm infants. *Clin Perinatol*. 2009; 36(1):27–42. [PubMed: 19161863]
20. Murthy JN, Hicks JM, Soldin SJ. Evaluation of the Technicon Immuno I random access immunoassay analyzer and calculation of pediatric reference ranges for endocrine tests, T-uptake, and ferritin. *Clin Biochem*. 1995; 28(2):181–185. [PubMed: 7628078]
21. Obladen M, Diepold K, Maier RF. Venous and arterial hematologic profiles of very low birth weight infants. European Multicenter rhEPO Study Group. *Pediatrics*. 2000; 106(4):707–711. [PubMed: 11015512]
22. Amin SB, Orlando M, Eddins A, Macdonald M, Monczynski C, Wang H. In Utero Iron Status and Auditory Neural Maturation in Premature Infants as Evaluated by Auditory Brainstem Response. *J Pediatr*. 2009
23. Spittle AJ, Doyle LW, Boyd RN. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. *Dev Med Child Neurol*. 2008; 50(4): 254–266. [PubMed: 18190538]
24. Siegel LS. Correction for prematurity and its consequences for the assessment of the very low birth weight infant. *Child Dev*. 1983; 54(5):1176–1188. [PubMed: 6194943]
25. Worwood M. Serum ferritin. *CRC Crit Rev Clin Lab Sci*. 1979; 10(2):171–204. [PubMed: 378539]
26. American Academy of Pediatrics Committee on Nutrition: Nutritional needs of low-birth-weight infants. *Pediatrics*. 1985; 75(5):976–986. [PubMed: 3921937]
27. Bell EF, Strauss RG, Widness JA, Mahoney LT, Mock DM, Seward VJ, et al. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. *Pediatrics*. 2005; 115(6):1685–1691. [PubMed: 15930233]
28. Vohr BR, Wright LL, Poole WK, McDonald SA. Neurodevelopmental outcomes of extremely low birth weight infants <32 weeks' gestation between 1993 and 1998. *Pediatrics*. 2005; 116(3):635–643. [PubMed: 16143580]
29. Ozment CP, Turi JL. Iron overload following red blood cell transfusion and its impact on disease severity. *Biochim Biophys Acta*. 2009; 1790(7):694–701. [PubMed: 18992790]
30. Hirano K, Morinobu T, Kim H, Hiroi M, Ban R, Ogawa S, et al. Blood transfusion increases radical promoting non-transferrin bound iron in preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 2001; 84(3):F188–F193. [PubMed: 11320046]
31. Bolann BJ, Ulvik RJ. Release of iron from ferritin by xanthine oxidase. Role of the superoxide radical. *Biochem J*. 1987; 243(1):55–59. [PubMed: 3038086]
32. Cooke RW, Drury JA, Yoxall CW, James C. Blood transfusion and chronic lung disease in preterm infants. *Eur J Pediatr*. 1997; 156(1):47–50. [PubMed: 9007491]
33. Hesse L, Eberl W, Schlaud M, Poets CF. Blood transfusion. Iron load and retinopathy of prematurity. *Eur J Pediatr*. 1997; 156(6):465–470. [PubMed: 9208245]
34. Inder TE, Clemett RS, Austin NC, Graham P, Darlow BA. High iron status in very low birth weight infants is associated with an increased risk of retinopathy of prematurity. *J Pediatr*. 1997; 131(4):541–544. [PubMed: 9386655]
35. Dani C, Reali MF, Bertini G, Martelli E, Pezzati M, Rubaltelli FF. The role of blood transfusions and iron intake on retinopathy of prematurity. *Early Hum Dev*. 2001; 62(1):57–63. [PubMed: 11245995]
36. Morrison ED, Brandhagen DJ, Phatak PD, Barton JC, Krawitt EL, El-Serag HB, et al. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. *Ann Intern Med*. 2003; 138(8):627–633. [PubMed: 12693884]
37. Wood MJ, Skoien R, Powell LW. The global burden of iron overload. *Hepatol Int*. 2009
38. Beutler E, Hoffbrand AV, Cook JD. Iron deficiency and overload. *Hematology Am Soc Hematol Educ Program*. 2003:40–61. [PubMed: 14633776]
39. Papanikolaou G, Pantopoulos K. Iron metabolism and toxicity. *Toxicol Appl Pharmacol*. 2005; 202(2):199–211. [PubMed: 15629195]

40. Kontoghiorghes GJ, Pattichi K, Hadjigavriel M, Kolnagou A. Transfusional iron overload and chelation therapy with deferoxamine and deferiprone (L1). *Transfus Sci.* 2000; 23(3):211–223. [PubMed: 11099897]
41. Breuer W, Hershko C, Cabantchik ZI. The importance of non-transferrin bound iron in disorders of iron metabolism. *Transfus Sci.* 2000; 23(3):185–192. [PubMed: 11099894]
42. Frisone MF, Mercuri E, Laroche S, Foglia C, Maalouf EF, Haataja L, et al. Prognostic value of the neurologic optimality score at 9 and 18 months in preterm infants born before 31 weeks' gestation. *J Pediatr.* 2002; 140(1):57–60. [PubMed: 11815764]
43. Picciolini O, Gianni ML, Vegni C, Fumagalli M, Mosca F. Usefulness of an early neurofunctional assessment in predicting neurodevelopmental outcome in very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed.* 2006; 91(2):F111–F117. [PubMed: 16492947]
44. Pedersen SJ, Sommerfelt K, Markestad T. Early motor development of premature infants with birthweight less than 2000 grams. *Acta Paediatr.* 2000; 89(12):1456–1461. [PubMed: 11195236]
45. Burns YR, O'Callaghan M, Tudehope DI. Early identification of cerebral palsy in high risk infants. *Aust Paediatr J.* 1989; 25(4):215–219. [PubMed: 2590117]
46. Stoltzfus RJ. Research needed to strengthen science and programs for the control of iron deficiency and its consequences in young children. *J Nutr.* 2008; 138(12):2542–2546. [PubMed: 19022987]

Table 1

Demographic and Perinatal Characteristics of Infants as a Function of Iron Status at 34–35 Wks PMA

	Normal Iron Status (N=70)	Iron Overload (N=25)	P
Gestational Age (weeks) **	28.17 ± 2	25.9 ± 1.6	0.00
Birth Weight (gms) **	1163 ± 368	892 ± 258	0.00
Male (%)	55	44	0.3
White (%)	67	64	0.8
Hispanic (%)	13	4	*0.3
Maternal Marital Status (% Married)	50	52	0.9
Maternal Education (% college)	51	50	0.9
Small for Gestational age (%)	15	4	*0.3
Pregnancy-induced Hypertension (%)	16	0	*0.07
In-Utero Exposure to Illicit Drugs (%)	33	16	*0.08
Chorioamnionitis (%)	11	8	*1
Antenatal Magnesium Sulfate Use (%)	31	16	0.19
Prenatal Betamethasone (%)	82	80	0.7
C-section (%)	68	48	0.09
Asphyxia (%)	3	0	*1.0

* Fisher exact test;

** Mean ± SD

Table 2

Neonatal Characteristics as a Function of Iron Status at 34–35 Weeks PMA

	Normal Iron Status (N=70)	Iron Overload (N=25)	P
Serum Ferritin [†] (ng/ml) ** (Median)	159 ± 71 (148)	619 ± 202 (567)	0.00 ***
Median # of Prior Erythrocyte Transfusions (range)	2 (0–15)	8 (2–16)	0.00 ***
Amount of Erythrocyte Transfusions (ml) **	27 ± 35	109 ± 48	0.00
History of Respiratory Distress Syndrome (%)	86	100	0.06 *
History of Hypotension Requiring Vasopressor (%)	23	68	0.00
Peak Total Serum Bilirubin ^ψ During First 2 Weeks			
After Birth (mg/dL) ** (Median)	8.9 ± 1.8 (8.6)	7.9 ± 1.5 (7.9)	0.03 ***
History of Postnatal Dexamethasone (%)	7	28	0.01 *
History of Sepsis (%)	28	64	0.002
History of Patent Ductus Arteriosus (%)	31	68	0.001
Any Intraventricular Hemorrhage (%)	23	64	0.000
Grade III or IV Intraventricular Hemorrhage (%)	7	16	0.23 *
History of Necrotizing Enterocolitis (%)	3	8	0.3 *
Periventricular Leukomalacia (%)	3	12	0.12 *
Chronic Lung Disease @ 36 weeks PMA (%)	13	60	0.000
Any Breast Milk Feeding (%)	78	96	0.06 *
Median Days on Breast Milk	22 (0–97)	25 (0–99)	0.12 ***

* Fisher exact test;

** Mean ± SD;

*** Wilcoxon-ranked sum test;

[†] Multiply by 2.247 to convert to pmol/L;^ψ Multiply by 17.1 to convert to μmol/L

Table 3

Bivariate Analysis of Neurodevelopmental Outcome During Infancy as a Function of Iron Status at 34–35 Weeks PMA

	Normal Iron Status (N=70)	Iron Overload (N=25)	<i>p</i> *
Physical, Occupational, or Speech Therapy (%)	41	56	0.21
Abnormal Neurological Exam (%)	15	28	0.17
Mental Developmental Index Score (Corrected for Degree of Prematurity)**	95 ± 10	90 ± 12	0.11
Neurodevelopmental Impairment (%)	41	64	0.05

*
t-test or Chi-square test;

**
Mean ± SD