Iron and vitamin D deficiency in children living in Western-Europe
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Predictive factors of iron depletion in late preterm infants at the postnatal age of 6 weeks


ABSTRACT

Background
Late preterm infants (born ≥32 weeks of gestation) are at risk for developing iron deficiency and iron deficiency anaemia, and this may lead to impaired neurodevelopment. In The Netherlands, there is no guideline for standardized iron supplementation in these infants. Individualized iron supplementation has been suggested (i.e. treating those infants with the highest risk), but risk factors for deprived iron stores in this specific group of infants are not well documented.

Methods
In this prospective multi-center study we analyzed the iron status at the postnatal age of 6 weeks of 68 infants born between 32 and 35 weeks of gestation in The Netherlands. Serum ferritin (SF) <70µg/L in the absence of infection (CRP <5mg/l) was defined as iron depletion, and, whenever in combination with a hemoglobin level <110g/L, as iron depleted anaemia. Medical charts were reviewed to identify risk factors.

Results
Iron depletion and iron depleted anaemia were present in 38.2% and 30.9% of the infants, respectively. Infants with a birth weight <1830gram and a SF <155µg/l in the first week of life, had a 26.4 times higher risk to develop iron depletion (95% CI 3.1-227.0, p=0.003). Multivariate regression analyses also showed that iron depletion was associated with a higher number of blood draws.

Conclusion
Iron depletion is common in late preterm infants at the age of 6 weeks in a setting without standardized iron supplementation. One should consider early individualized iron supplementation for late preterm infants with a low birth weight (<1830gram), and a low SF in the first week of life (<155µg/l), as they have a high risk to develop iron depletion.
INTRODUCTION

Preterm infants are at risk for developing iron deficiency (ID) and iron deficiency anaemia (IDA) because of insufficient iron storage at birth, phlebotomy during the first weeks of life and high iron requirements due to rapid growth after birth. Since both ID and IDA can have long-lasting detrimental effects on neurodevelopment, prevention, early detection, and treatment are important.

According to international recommendations, all preterm infants should be supplemented with 2-3 mg/kg per day of elemental iron from 2-6 weeks to at least 6 months of age to prevent ID. However, these recommendations are mostly based on studies in very low birth weight infants (birth weight <1500 gram) and/or infants born <32 weeks of gestation. Iron requirements in older preterm infants are poorly defined. One randomized controlled trial, including both preterm and term infants with a birth weight of 2000-2500 gram, showed that iron supplementation at a dose of 1-2 mg/kg per day prevented ID at the age of 6 months. In this study, 35.8% of the infants that received no iron supplementation developed ID. In The Netherlands, as in many countries worldwide, there is no guideline for standardized iron supplementation in late preterm infants. A prospective cohort study among Dutch infants born between 32 and 37 weeks revealed a lower prevalence of ID at the age of 4 and 6 months of 18.9% and 4.9%, respectively. As the majority of infants in the latter study did not develop ID, and because iron supplementation might be harmful in iron sufficient infants, we have suggested that individualized iron supplementation might be justified in late preterm infants.

Individualized iron supplementation (i.e. treating those infants with the highest risk) requires early identification of risk factors that contribute to the development of ID in each infant. In infants born <32 weeks of gestation, several risk factors for ID are described, like conditions altering maternal-fetal iron exchange, multiple gestations, acute and chronic fetal hemorrhage, uncompensated blood losses due to phlebotomy, and inadequate and delayed iron supplementation. However, in late preterm infants (born ≥32 weeks of gestation) risk factors for ID are less clear. The previously mentioned Dutch cohort study showed that low ferritin concentrations in the first week of life are associated with an increased risk of ID at six months of age. Since early detection and subsequent treatment of ID requires early identification of risk factors, we investigated clinical risk factors for a deprived iron status in late preterm infants at the postnatal age of 6 weeks. Around this time, on average, birth weight of late preterm infants has doubled, making them highly susceptible for iron deficiency.
CHAPTER 5

METHODS

Medical charts of all infants born between 32+0 and 35+0 weeks of gestation, and who participated in the IPI (Iron status of Premature Infants) study, were reviewed. The IPI study was a prospective cohort study designed to analyze the prevalence of and risk factors for ID at the age of 4 to 6 months in infants born between 32+0 and 36+6 weeks of gestation in The Netherlands. The infants were born between March 2011 to May 2013 in three large, but non-tertiary hospitals in The Netherlands: Juliana Children’s Hospital/Haga Teaching Hospital (JCH) and the Medical Center Haaglanden (MCH), both in The Hague, and the Medical Center Alkmaar (MCA) in Alkmaar. Exclusion criteria were congenital malformations, chronic or inherited metabolic disease, haemoglobinopathies, active blood loss during delivery/major bleeding, twin-to-twin transfusion syndrome or hemolytic disease (defined as a positive Coombs’ test). In this study, obstetricians performed early cord clamping (<60 sec), which was the standard procedure at time of the study. After hospitalization, infants underwent blood draws while visiting the outpatient clinic for routine follow-up at the postnatal age of 6 weeks, 4 and 6 months. The observational design of the study allowed paediatricians to prescribe iron supplementation in the way they considered appropriate. We excluded the infants that received oral iron supplementation and/or an erythrocyte transfusion. Maternal characteristics were recorded after their own medical records were accessed. The study was approved by the Medical Ethics Committee of South-West Holland. Written informed consent was obtained from all the parents of the infants.

Biochemical analysis & definitions
In the first week of life, during hospitalization, and 6 weeks after birth (timing of regular visit to our outpatient clinic), venous blood was collected and analyzed for serum ferritin (SF), hemoglobin (Hb) and C-reactive protein (CRP). CRP was measured to detect cases of infection, because SF is an acute-phase protein that increases in the presence of infection or inflammation. To prevent false normal or elevated SF levels, infants with elevated CRP levels (CRP >5 mg/l) were excluded from our final analyses.

Iron depletion and iron depleted anaemia at the postnatal age of 6 weeks were defined as SF <70 µg/L in the absence of infection (CRP <5 mg/l) (based on previous analyses of the IPI study population) and the combination of a hemoglobin level <110 g/L and iron depletion, respectively.
Data collection
The following possible risk factors for iron depletion were investigated: gestational age (GA), birth weight, feeding data (type and amount of feeding leading to a mean iron intake, details are below), episodes of illness (sepsis, necrotizing enterocolitis (NEC) or respiratory distress syndrome (RDS)), maternal data (pregnancy induced hypertension (PIH), iron supplementation during pregnancy, gestational diabetes, smoking or maternal anaemia during pregnancy), the number of blood draws (in total and of ≥ 0.2 ml) during hospitalization and ferritin concentrations in the first week of life. Small for gestational age (SGA) was defined as a birth weight <10th percentile of Dutch references. Sepsis was defined as a positive blood culture or in case a neonatologist decided to treat an infant with antibiotics during at least 7 days, because of elevated infection parameters or clinical symptoms of sepsis. The presence of NEC was defined as a modified Bell’s stage 2a or higher. The diagnosis RDS (primarily caused by surfactant deficiency) was defined by the need for oxygen support and the radiological appearance of RDS on a chest X-ray.

The previously mentioned mean iron intake was based on the mean of two calculated iron intakes: 1. on the day total fluid of 150 ml/kg was reached and 2. 7 days after that. The exact amounts of mother’s milk (participating hospitals not routinely used donor milk at the time of the study) and/or formula were obtained from feeding records that were recorded by nurses. To calculate the mean iron intake, we reasoned from the following assumptions: preterm mother’s milk, whether with or without breast milk fortifier (Nutrilon Nenatal Breast Milk Fortifier, Nutricia, Zoetermeer, The Netherlands, commercially available, on prescription), contains no iron. When mother’s milk is insufficiently available, current practice in The Netherlands is to provide the infants with preterm formula before and after discharge until reaching a weight of approximately 3500 gram. The preterm formula that was used (Nutrilon Nenatal Start, Nutricia, Zoetermeer, The Netherlands, commercially available, on prescription) has an iron content of 1.6 mg/100 ml. Parenteral feeding (i.e. total parenteral nutrition), recommended in case of a birth weight <1750 gram, contains no iron.

Statistical analysis
Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS version 21.0; SPSS Inc., Chicago, IL, USA). Before analysis, normality of distribution of all data was assessed using histograms and Kolmogorov-Smirnov tests. To assess the prevalence of and risk factors for iron depletion and iron depleted anaemia, we analyzed complete samples of infants with non-elevated CRP concentrations (CRP <5mg/l).
Univariate analyses of characteristics were performed using a Student’s t-test for normally distributed continuous variables and a Chi-square test for dichotomous variables. In case of a non-normal distribution, a Mann Whitney test was used to compare groups. To analyze risk factors for iron depletion and iron depleted anaemia, we used logistic regression models. Possible risk factors with a p-value <0.100 in the univariate analyses were combined in multiple logistic regression analyses, using a backward Wald method.

Finally, to visualize the efficacy of the risk factors to discriminate infants with iron depletion from infants with no iron depletion, we constructed receiver operating characteristic (ROC) curves. The optimal cut-off for risk factors was chosen by finding the value with a maximized combination of sensitivity and specificity (equal weight of sensitivity and specificity). Subsequently, the calculated cut-off levels of the risk factors were combined to generate a model with an odds ratio (OR) and a 95% confidence interval (CI). A p-value of <0.05 was considered to be statistically significant.

RESULTS

Study population
Of the 200 infants who participated in the IPI study, 99 infants were born between 32+0 and 35+0 weeks of gestation and therefore eligible for our study. Two infants were excluded because they met one of the exclusion criteria (1 infant with a Pierre Robin sequence and 1 infant with deletion of chromosome 12). The blood sample at the age of 6 weeks was incomplete in 19 infants (19.6%) and CRP was elevated in another 4 infants (4.1%). These 23 infants were excluded prior to our final analyses. Furthermore, 4 (5.4%) and 2 (2.7%) infants received oral iron supplementation and an erythrocyte transfusion, respectively. These 6 infants were also excluded prior to our final analyses. In total, 29 infants were excluded and they had similar demographic characteristics compared to the non-excluded infants (data not shown). The final study population consisted of 68 infants: 54 infants from JCH, 11 infants from MCH and 3 infants from MCA. Mean birth weight and the median GA were 2015 gram (± 390 gram) and 34.0 weeks (33.3-34.6 weeks), respectively. Other characteristics of the study population are summarized in Table 1.
Iron status

SF concentrations in the first week and at 6 weeks were analyzed on the average (± standard deviation) age of the infants of 4.2 days (± 1.9) and 47.9 days (± 15.3), respectively. Iron depletion and iron depleted anaemia at 6 weeks were present in 26 (38.2%) and 21 infants (30.9%), respectively.

Risk factors for iron depletion

The infants with iron depletion had a marginally lower GA, and a significant lower birth weight (and were more frequently SGA) than those without iron depletion, and therefore received more frequently parenteral feeding. They also underwent more blood draws (with a volume of ≥0.2 ml), had less frequently an anaemic mother that used iron supplementation, and already had a lower SF in the first week of life, compared to infants without iron depletion at the age of 6 weeks. The mean iron intake during hospitalization was similarly low for both groups (Table 2).
Results of a logistic regression analysis with iron depletion as dependent variable and GA, birth weight, SGA, number of blood draws of ≥0.2 ml, SF in the first week of life, PIH, maternal anaemia and iron supplementation during pregnancy as covariates, showed that a lower birth weight, a lower SF concentration in the first week and a higher number of blood draws were all independently associated with iron depletion at the age of 6 weeks (Table 3).
Of the above mentioned associations, the number of blood draws is unpredictable in the first week. Only birth weight and SF concentrations in the first week can be used in an early stage to identify infants with a high risk for the development of iron depletion. We therefore constructed two ROC curves to investigate the predictive value of these risk factors for the development of iron depletion. The area under the curve-ROC for birth weight and SF in the first week were 0.800 (95% CI 0.684 – 0.916, p<0.001) and 0.760 (95% CI 0.625 – 0.895, p=0.001), respectively. The calculated cut-off values for birth weight and SF in the first week were 1830 gram (sensitivity 65.4% and specificity 88.1%) and 155 µg/l (sensitivity 72.7% and specificity 71.1%), respectively. According to Dutch references, a birth weight <1830 gram in infants born between 32 and 35 weeks of GA does not classify them as SGA. The cut-off values for birth weight and SF were combined in the following model: infants with a birth weight <1830 gram and a SF <155µg/l in the first week had a 26.4 times higher risk to develop iron depletion, compared to infants with a birth weight ≥1830 gram and a SF ≥155µg/l (95% CI 3.1 – 227.0, p=0.003).

**Risk factors for iron depleted anaemia**

The infants with iron depleted anaemia had a significant lower birth weight (and were more frequently SGA) than those without iron depleted anaemia, and therefore received more frequently parenteral feeding. They also underwent more blood draws (with a volume of ≥0.2 ml), and already had a lower SF in the first week of life (Table 4).
Results of a logistic regression analysis with iron depleted anaemia as dependent variable and GA, birth weight, SGA, number of blood draws of ≥0.2 ml, SF concentration in the first week and maternal anaemia as covariates, showed that a lower birth weight and a lower SF concentration in the first week were both independently associated with iron depleted anaemia (Table 5). Furthermore, there was a trend of more blood draws (with a volume ≥ 0.2 ml) in infants with iron depleted anaemia than in those without iron depleted anaemia (Table 5, p=0.062).
Guidelines regarding the prevention and treatment of iron deficiency in late preterm infants are scarce. Late preterm infants usually do well in the NICU or in secondary hospitals, and, sometimes, they masquerade as term infants. However, late preterm infants should be monitored for iron deficiency, because they are at risk. In this study, we found that, in a setting without a guideline for standardized iron supplementation, iron depletion and iron depleted anaemia at the postnatal age of 6 weeks occur frequently in late preterm infants (38.2% and 30.9%, respectively). We showed that a lower birth weight, a lower serum ferritin concentration in the first week of life, and multiple blood draws during admission are all independently associated with iron depletion at the age of 6 weeks. These results suggest that limiting phlebotomy loss may decrease the prevalence of ID, and that birth weight and SF in the first week can both be used as predictors of ID at a later age. In our study, infants with a birth weight <1830 gram and a SF in the first week <155µg/l had a 26.4 times higher risk to develop iron depletion. This information can be used in an algorithm for early individualized iron supplementation to prevent the development of ID in late preterm infants.

A deprived iron status (with and without anaemia) was frequently observed in our study population of late preterm infants at the postnatal age of 6 weeks. To our knowledge, no other studies investigated the prevalence of iron depletion in late preterm infants at this age. Schiza et al. did report iron and erythropoiesis parameters in preterm infants (with a GA of 32-36 weeks) during the first year of life. Their mean ferritin concentrations at six weeks were similar to those in our study population (data not shown). Prevalence rates of a deprived iron status (with or without anaemia) at other postnatal ages vary from 14.4-42.8%, but were based on other definitions than we have used. There is no consensus about how to define low iron stores in late preterm infants at the age of 6 weeks. We

### DISCUSSION

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta</th>
<th>SE of beta</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI of OR</th>
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</thead>
<tbody>
<tr>
<td>Birth weight (grams)</td>
<td>-0.002</td>
<td>0.001</td>
<td>0.047*</td>
<td>0.998</td>
<td>0.996 - 1.000</td>
</tr>
<tr>
<td>Ferritin in the first week (µg/l)</td>
<td>-0.008</td>
<td>0.004</td>
<td>0.046*</td>
<td>0.992</td>
<td>0.985 - 1.000</td>
</tr>
<tr>
<td>Blood draws (n of ≥ 0.2 ml)</td>
<td>0.206</td>
<td>0.110</td>
<td>0.062</td>
<td>1.228</td>
<td>0.990 - 1.524</td>
</tr>
</tbody>
</table>

This table shows the odds ratio’s with 95% confidence intervals for the variables that were associated with iron depleted anaemia at the postnatal age of 6 weeks. Abbreviations: ‘SE’ is standard error; ‘OR’ is odds ratio; ‘CI’ is confidence interval.
used ferritin to define iron depletion since it is an indirect estimate of body iron stores. We chose a cut-off value for SF of 70 mcg/l that is based on previous analyses of the complete IPI study population. These analyses revealed that infants with ID at the age of 4 and 6 months already had significant lower SF concentrations at 1 and 6 weeks postnatal age. The mean SF concentration at 6 weeks was approximately 70 mcg/l for infants with ID at 4 and 6 months of age. Besides the chosen definition of ID, other factors can also attribute to the prevalence of a deprived iron status. For example, the obstetricians in our study performed early cord clamping, although delayed cord clamping has a positive effect on iron stores in infants at birth. This could have led to an overestimation of the prevalence of iron depletion in our study. Currently, delayed cord clamping is internationally recommended, and it would therefore be very interesting to repeat our study. We hypothesize that the prevalence rate of iron depletion would be lower than in the current study. This new setting may also influence our found cut-off levels for birth weight and SF in the first week.

We found that a lower birth weight is an independent risk factor for iron depletion and iron depleted anaemia in late preterm infants at the age of 6 weeks. Low birth weight is a well known risk factor for low iron stores in preterm infants in general, and more specific, in premature infants born after less than 32 weeks of gestation. A low birth weight is mostly due to impaired placental function that could be caused by e.g. PIH, maternal smoking or gestational diabetes. Normally, the placenta serves as the regulating conduit for maternal-fetal iron transport that increases with gestation. In our study, we found no association between the previously described causes of impaired placental function and deprived iron stores after adjusting for birth weight, stating that a lower birth weight is the most important independent risk factor.

Besides birth weight, we also found that SF in the first week is an independent risk factor for iron depletion and iron depleted anaemia at the age of 6 weeks. Other studies have also demonstrated that iron status biomarkers like SF in the first weeks of life are closely associated with future ID. When early individualized iron supplementation is practised, our data suggest that it seems justified to supply iron to those late preterm infants with a birth weight <1830gram and ferritin concentrations <155µg/l in the first week since these infants have a 26.4 times higher risk to develop iron depletion. However, the use of an algorithm following our calculated cut-off values for birth weight and SF should be further investigated in a prospective trial.
To our knowledge, this is the first study that has demonstrated that multiple blood draws (i.e. cumulative number of blood draws with a volume of ≥ 0.2 ml) increased the risk of low iron stores at the age of 6 weeks in late preterm infants. Furthermore, we did not find a significant association between multiple blood draws and iron depleted anaemia, although there was a trend of more blood draws in infants with iron depleted anaemia. It has been found that ferritin and Hb levels of late-preterm infants (≥ 34 weeks) are lower than those of term infants at 2 months of age despite having similar cord blood values. A possible explanation for this difference is that preterm infants more frequently undergo blood draws. Phlebotomy causes iatrogenic blood loss and, hence, iron loss and consequently predisposes a child to ID. In infants born ≤32 weeks, the net erythrocyte balance (cumulative amount of erythrocyte transfusion minus cumulative amount of phlebotomy loss) is negative for those with iron deficiency at 35 weeks post-menstrual age, compared to a positive balance for those without ID.

The mean iron intake (~1.0 mg/kg per day), regardless the type of feeding, was comparable for iron depleted and non-iron depleted infants. In a Dutch study with younger preterm infants, the iron intake varied from 0.82 mg/kg per day (for infants fed with a combination of human milk and iron-fortified milk) to 1.19 mg/kg per day (solely iron-fortified milk fed infants). In contrast to our study, they did find a difference in iron status between the study groups at 3 months. We suggest that iron intake becomes a predictive factor of iron depletion after 6 weeks of age. We believe that the type of feeding (and thereby a certain iron intake) during admission should therefore not be taken into account in an algorithm for early individualized iron supplementation.
CONCLUSION

Iron depletion and iron depleted anaemia at the postnatal age of 6 weeks are common in late preterm infants (born 32-35 weeks of GA), born after early cord clamping, and in a setting without a guideline for standardized iron supplementation. We identified a lower birth weight, a lower SF in the first week of life, and a higher number of blood draws as independent risk factors for iron depletion. The number of blood draws is unpredictable at an early stage. However, early individualized iron supplementation (i.e. treating those infants with the highest risk) should be considered for high risk infants with a birth weight <1830gram and a SF in the first week <155µg/l since these infants have a 26.4 times higher risk to develop iron depletion. The safety and efficacy of an algorithm with these risk factors have to be confirmed, preferably in a randomized controlled trial, while taken in to account the effect of the current practice of delayed cord clamping.
CHAPTER 5

REFERENCE LIST

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