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Diagnosis of Iron Deficiency in Infants

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Abstract

The assessment of iron deficiency anemia in infants is a clinical challenge because of the high requirements for iron to support expansion of the blood volume during rapid growth and development. Infants are endowed with only adequate storage iron to support this iron requirement for the first 4 to 6 months of life and premature infants even less than that. Hemoglobin is a measure of anemia but is not specific to iron deficiency, which requires the additional measurement of ferritin, soluble transferrin receptor, and protoporphyrin levels to assess iron nutrition. Premature infants that receive transfusion or erythropoietin therapy are special diagnostic challenge. An accurate classification of iron status requires multiple biomarkers of iron storage and transport adequacy and in the absence of standardized assay material for several tests, becomes quite problematic.

The assessment of iron nutrition status in both clinical and health populations of individuals is a difficult task but there is the fortunate circumstance that a number of biomarkers exist that enable the clinician and the public health professionals to evaluate adequacy of iron storage, transport, and utilization in tissue. This review will examine the most recent evaluations of the biomarkers available in the diagnosis of iron status in infants and will hopefully provide some guidance regarding the difficulties in interpretation of commonly utilized indices. In clinical terms, anemia is defined as an insufficient mass of red cells needed to provide adequate oxygen transport to tissue. In public health terms, the definition of anemia is a statistical derivation that represents the lower limit of the 95th percentile range for healthy individuals of a given age and sex. Iron deficiency is defined as the situation when there is insufficient iron to maintain normal cellular and physiological functions in tissues such as blood, immune cells, or muscles. Iron deficiency can exist without the presence of anemia if the iron deficit is short enough or severe enough to cause a downward shift in amount of circulating red cells. The *storage* of iron occurs in tissues in a sequestered form in the protein, ferritin, in order to prevent oxidative damage to cells as well as to provide a ready pool of iron for newly synthesized iron requiring proteins such as myoglobin, cytochromes, and catalase. These *functional* forms of iron can be altered by poor delivery of iron to organs with a resulting tissue iron deficiency due to impaired transport. The clearest examples of this are simple iron deficiency where the storage pool is empty or when there is blockade of transport of iron as in infection and inflammation.

Iron Homeostasis and Iron Status Indicators

Body iron balance is primarily determined by regulation of absorption of iron where the efficiency of absorption is inversely related to the body iron status.[1] This efficiency may vary from as low as 1% to 2% to nearly 50% to 60% depending on the characteristics of the food source of iron and the iron status of the individual. Regulation is exerted at the level of movement both into the cell through receptor mediated process as well as movement across the basolateral membrane into the plasma pool. This former process likely involves both the transferrin receptor and the divalent metal transporter proteins while the latter involves the cellular iron exporter, ferroportin.[2,3] The amount of ferroportin expressed on the basolateral membrane is influenced by a hepatocyte secreted signal peptide named hepcidin. As liver iron stores increase, more hepcidin is released into the plasma pool to decrease ferroportin mediated movement of gastrointestinal cell iron across the basolateral membrane.

Iron in the plasma pool is transported by transferrin, which has 2 identical binding sites for ferric ions. This taxi cab will deliver iron to any cell that expresses the transferrin receptor; the developing red cell mass takes about 80% of this plasma iron turnover on a daily basis. The remaining 20% is distributed to other body iron pools and the excess iron put into storage in the multi-unit protein, ferritin. The amount of transferrin receptor (TfR) located on the cell surface is regulated by cellular iron status through translational regulation involving Iron Response Elements (IREs) on the non-coding regions of the mRNA and rates of recycling of the TfR on and off the membrane.[1] As a cell becomes iron deficient, there is an up-regulation of the translation of TfR-mRNA and more receptor is produced; the inverse is seen when the cells are replete with iron.[4,5] The amount of ferritin made in cells is also regulated by the IRE mediated translation regulation of gene expression. While iron losses through excretion appear slightly higher in some individuals with very high body iron stores, regulation of losses is not thought to be a mechanism of homeostasis.

Biomarkers

A number of biomarkers of iron status have been developed; some are quite common and well established while others have only been recently identified and have not be standardized.[6] The

biomarkers frequently mentioned in this review and their stability as indicators of iron status is as follows:

- **Hemoglobin (Hb):** a measure of circulating hemoglobin and necessary for the transport of oxygen from alveolus to tissue. The units are expressed as a concentration per unit volume of blood. The variable is not specific to iron deficiency and has poor positive predictive value regarding iron deficiency in most situations.
- **Mean cell volume (MCV):** a measure of red cell volume where small microcytic red cells are reflective of insufficient iron delivery to bone marrow. The unit of measure is volume. There are associated indices such as mean corpuscular hemoglobin concentration (MCHC) that can also reflect the relative loading of Hb into cells of varying sizes. This index of cell size can be a characteristic of a type of anemia; microcytic for iron deficiency and macrocytic for folate or B_{12} deficiency. MCV is not specific to iron deficiency per se.
- **Red cell distribution width (RDW):** This measure is a product of automated cell counters which gives a range in the distribution of red cell sizes. It is computed as the standard deviation of the MCV per unit of MCV. A larger RDW is reflective of new cells entering the circulating pool with might be microcytic and thus indicate poor iron delivery to marrow. Again, RDW is not specific to iron deficiency.
- **Protoporphyrin (FEP or ZPP):** The assembled porphyrin ring structure precursor to the heme molecule part of hemoglobin can be detected in circulating red cells and is elevated when iron delivery to marrow is insufficient. The last step in hemoglobin synthesis is the insertion of iron by the enzyme ferrochetalase. Instead trace amounts of zinc are incorporated into protoporphyrin. The normal ratio of iron to zinc in protoporphyrin is about 30,000:1, but a lack of iron available to ferrochetalase during the early stages of iron deficient erythropoiesis results in a measurable increase in the concentration of zinc protoporphyrin. Some clinical assays report this as zinc protoporphyrin (ZPP), while others utilize an assay to detect "free erythrocyte protoporphyrin (FEP). As with MCV, this reflects inadequate delivery of iron specifically to bone marrow but is also quite sensitive to other factors such as lead intoxication.
- **Soluble transferrin receptor (sTfR):** This biomarker in serum or plasma is a cleaved fragment of the transmembrane protein and the plasma pool is derived primarily from reticulocytes. It is a useful marker of tissue iron deficiency and iron deficiency erythropoiesis because the expression of the protein is tightly linked to intracellular iron stores. The concentration of sTfR is strongly affected by erythropoietic drive but does not appear to be affected by inflammation. This measure is very new and there is not yet an external quality control standard available. There is a wide variety of reported values in healthy subjects suggesting assay variability and not biological variability.
- **Serum ferritin:** This is a measure of the storage protein for iron, tissue ferritin. The amount in circulation is proportional to the amount of storage iron in tissue. As cells accumulate iron, there is an increased production of ferritin through the same IRE system described for TfR.[1] This is a poorly described leakage of this tissue ferritin into the plasma pool such that 1 µg/L of serum ferritin represents approximately 8 mg of storage iron in tissue pools (primarily in liver hepatocytes and splenic macrophages). Ferritin is also an acute phase protein and becomes non-reflective of iron status when there is inflammation. There is an external quality control standard available and standardized method available to the clinical community. As with serum iron, there is a large within-subject variability.[7,8]
- **Serum Fe and transferrin saturation:** A measure of the amount of iron being transported in plasma and expressed as a concentration for Fe and a percentage bound for transferrin saturation. These measures have large within subject variability and are sensitive to contamination of samples during analysis.
- **Body iron stores:** A new biomarker that combines sTfR and ferritin measures as a log of the ratio.[9] It offers a full continuous range from iron deficiency to iron sufficiency but the lack of standardization of sTfR measurements limits its application at the moment. The within subject variability has not been examined.
- **Hepcidin:** A new biomarker in plasma or urine that measures the amount of this regulator protein that is in the circulation.[3] The ELISA assay method is very limited in availability at the moment and lacks external quality controls or measurements of variation within or between individuals. The assay approach is still under development but a number of clinical studies are now using hepcidin levels in urine and plasma as an index of iron status.

These biomarkers of iron status are often used in combination when population surveys and clinical studies are conducted. As each marker is sensitive to a different pool of iron (storage, transport, cellular), a number of combinations of markers have been advocated with different cut-off levels employed.[10] The confidence in a correct diagnosis of iron status clearly increases with increasing numbers of abnormal indicators.

Maternal Fetal Iron Transport

The transplacental iron transport to the fetus increases with the duration of gestation and averages 1.35 mg/kg fetal body weight per day during the third trimester. The average iron content of the fetus during the third trimester of gestation is 75 mg/kg of body weight, with 70% to 80% being present in the red blood cells as hemoglobin, 10% in tissues as myoglobin and cytochromes, and the remaining 10% to 15% as storage iron in tissues.[11-13]

Iron is transported against a concentration gradient, especially during the later stages of pregnancy, from the mother to her fetus. Iron transported across the placenta is used for fetal erythropoiesis and development of organ systems, and is stored in iron containing proteins, including myoglobin, cytochromes, ferritin, and hemosiderin. Erythropoiesis begins very early in gestation, with a well-documented rise in hemoglobin from a mean of 11.5 g/dL at 18 to 20 weeks gestation to a mean of 16.8 g/dL at term.[14,15] Fetal erythropoietin is adaptive for the relatively hypoxic in utero environment as fetal hemoglobin has a higher affinity for oxygen than adult hemoglobin. Furthermore, hemoglobin concentrations in the late gestation fetus are higher than in the child or adult, aiding in oxygen delivery to the fetal tissues. In term infants, the higher hemoglobin concentrations seen at birth represent a storage pool of iron[16] that will be available to meet the needs of rapid growth and blood volume expansion the infant during the first 6 months of life.

Iron Status at Birth

The duration of gestation and certain maternal conditions during pregnancy can influence the iron status in the newborn period. When compared with the full term newborn infants, preterm newborn infants have lower cord serum ferritin and serum iron concentrations, lower total iron binding capacity, and higher reticulocyte counts and cord serum transferrin receptor concentrations.[17-22] The lower total body iron endowment combined with postnatal factors make preterm infants vulnerable for early onset iron deficiency during infancy (see below).

Maternal iron deficiency during pregnancy negatively influences fetal iron status. Maternal iron deficiency anemia (hemoglobin ≤87 g/L) is associated with iron deficiency in full-term infants.[23,24] Even the infants of iron-deficient but non-anemic mothers with iron deficiency have lower ferritin concentrations at birth.[25,26]

Besides maternal iron deficiency, gestational conditions, such as intrauterine growth retardation (IUGR), maternal diabetes mellitus, and maternal smoking during pregnancy are the most common risk factors for fetal and neonatal iron deficiency in developed countries.[27-29] These clinical conditions are associated with impaired placental function and increased fetal iron demand in excess of placental transport capacity. Intrauterine growth retardation puts the infants at risk for iron deficiency because of impaired iron transport due to placental vascular insufficiency and increased iron requirement for augmented fetal erythropoiesis secondary to chronic hypoxia.[18,20,27] Compared with the full-term infants, the liver and brain iron concentration of these infants is decreased at birth.[30]

Chronic fetal hypoxia with its attendant augmented fetal iron demand for erythropoiesis is also characteristic of pregnancies complicated by maternal diabetes mellitus.[28] Maternal diabetes mellitus, either preexisting or gestational, produces fetal hyperglycemia and hyperinsulinemia, increased fetal metabolic rate and oxygen consumption and resultant fetal hypoxemia.[27] Increased fetal iron utilization for erythropoiesis combined with suboptimal transplacental iron transport secondary to placental dysfunction and structural abnormalities of placental transferrin receptors results in depletion of tissue iron stores in the liver, brain, and heart in the infants of diabetic mothers (IDM).[28,31-33] Sixty-five percent of the IDM have ferritin concentrations <60 mcg/L with a mean value of 26 mcg/L.[27] Approximately 25% of these infants are at risk for brain iron deficiency, with the brain iron concentration being 40% decreased in the most severe cases.[33]

Maternal smoking during pregnancy is also a risk factor for fetal and neonatal iron deficiency. Fetal hypoxemia due to elevated carboxyhemoglobin levels, decreased uteroplacental blood flow, and increased placental vasoconstriction caused by nicotine and catecholamines induce enhanced erythropoiesis and depletion of tissue iron stores in these infants.[17,34,35]

Methods to Assess Iron Status at Birth

As noted above, a 3-compartment model has been used to characterize the distribution of iron in the fetus and neonate.[12] The compartments are the red cells, typically indexed by the hemoglobin concentration, the storage pool indexed by the serum ferritin concentration, and the non-heme, non-storage tissue pool, which has no readily available serum marker. The amount of iron in red cells can be estimated by multiplying the serum hemoglobin concentration (g/100 mL) by the estimated blood volume of the neonate (85 mL/kg body weight) by 3.46 mg of elemental iron/g of hemoglobin by the infant's weight (kg). A normal serum hemoglobin concentration at birth is between 140 and 200 g/L. The mean serum ferritin concentration at term birth is 134 μg/L with a range from 40 (5th percentile) to 310 (95th percentile). Serum transferrin concentration increases throughout the third trimester reaching a mean of 228 mg/dL at term with a range of 100 to 350 mg/dL.[36] Other indices commonly used in the postnatal period such as serum transferrin receptor and free erythrocyte or zinc protoporphyrin concentrations do not have standards at birth.

Clinical Conditions That Lead to Postnatal Iron Deficiency

The fetal endowment is likely to be an important determinant of the risk of postnatal iron deficiency. Thereafter, postnatal clinical conditions, such as low dietary iron intake, gastrointestinal parasitic infestations, malaria, and chronic gastrointestinal blood loss due to cow's milk consumption are the most important causes of postnatal iron deficiency in developing countries.[37-40] Even in developed countries, such as the United States, approximately 9% to 11% of toddlers between 1 and 3 years of age have iron deficiency anemia.[10,41] The percentage with low iron stores (decreased serum ferritin) at this age varies between 18% and 35%.[41] Low socioeconomic background, recent immigration from a developing country, consumption of low iron formula or cow's milk/evaporated milk, breastfeeding without iron supplementation and occult gastrointestinal hemorrhage are responsible for iron deficiency in such populations.[38,42,43]

Fetal and neonatal iron deficiency also predisposes the infant to postnatal iron deficiency. Full term IDM with low neonatal iron stores have significantly lower ferritin concentrations at 9 months of age, and thus at risk for iron deficiency during the second year of life.[16] Preterm birth puts the infant at greater risk of iron deficiency as discussed below.

Premature, low birth weight infants represent a group particularly at risk for earlier onset of iron deficiency anemia when compared with the full-term controls.[44] Although placental iron transport begins during the first trimester of gestation, it is only during the third trimester that significant accretion occurs. Two-thirds of total body iron present in the term infant is accreted during the third trimester. Infants born prematurely are therefore born with lower iron stores in direct proportion to their degree of low birth weight. Postnatally, preterm infants also may have significant phlebotomy blood (and consequently iron) losses due to the need for frequent laboratory testing.[45] Many preterm infants are critically ill for prolonged periods of time and do not receive supplemental iron parenterally or enterally, further compromising their already low iron stores. Periods of nutritional compromise are often followed by rapid "catch up" growth, requiring adequate iron to maintain erythropoiesis. Unfortunately, preterm infants also have low erythropoietin levels and develop anemia after birth.[46] In the past, preterm infants were liberally transfused, which supported their hemoglobin and provided supplemental iron, but more recently, the use of blood transfusions in preterm infants has been substantially curtailed.[47] Due to all of these factors, preterm infants are vulnerable to iron deficiency and iron deficiency anemia.

Biomarkers in Preterm Infants

Laboratory investigation of iron deficiency and iron deficiency anemia in preterm infants can be complex, as normative values do not exist for the most preterm and smallest of infants. At the same time, the diagnosis of iron deficiency is of special importance in preterm infants as iron deficiency is one of the few treatable conditions affecting development.[48-50]

Evaluation of iron status in preterm infants using other indicators shows evidence of lower iron stores in preterm infants[51,52] likely due to the normal acquisition of iron during the latter half of gestation.[18,53] There is a gradual increase in cord serum ferritin concentration throughout gestation with a median ferritin value of 45 µg/L at 14 to 16 wks gestation and 200 µg/L at 39 weeks gestation.[54]

Serum iron, TIBC levels, sTfR levels, and ZnPP/H ratios in cord blood have also been used to evaluate neonatal iron status at birth.[29,55-57] Serum iron and TIBC levels have been shown to rise with increasing gestational age.[17] Serum transferrin receptors at birth have not shown a correlation with gestational age, though higher levels have been reported in neonates compared to adults.[17,56] ZnPP/H ratios are inversely correlated with gestational age, with levels near adult and child normal values seen near term.[55] ZnPP/H ratios were found to be elevated in extremely preterm patients, infants with intrauterine growth restriction, infants of diabetic mothers, and infants with maternal histories of chorioamnionitis.

Birth Iron Indicators

Table 1 gives a summary of the birth iron indicators.

Birth-to-term: All preterm infants have a progressive decline of hemoglobin, MCV, and ferritin in the first few months postnatally.[14,20,58] This decline is attributable largely to an abrupt decrease in erythropoeisis in response to increased postnatal delivery of oxygen to the tissues coupled with an accelerated growth rate. Extramedullary hematopoiesis comes to a halt during the same period. During this marked depression of erythropoeisis, the concentration of hemoglobin decreases at a rate that is determined primarily by the life span of red blood cells that were produced before birth. The nadir falls to a mean of 11.2 g/dL at 2 months in term infants.[59] In preterm infants, the growth rate is faster; the nadir occurs earlier and is more pronounced than in term infants, often reaching levels as low as 7 to 8 g/dL.[60]

Low birthweight infants have decreased iron stores at birth and periods of nutritional deprivation followed by accelerated rates of "catch up" growth, their iron stores may be exhausted early in life and they may have an earlier dietary dependence on iron than full term infants. Supplementation during this period is effective at increasing serum ferritin in these infants.[58] Recombinant human erythropoietin has been shown to decrease ferritin levels[61] while transfusion use increases the ferritin, though these effects are transient.[62]

Serum iron levels and transferrin saturations gradually fall in preterm infants from 2 weeks with a coexistent rise in total iron-binding capacity and transferrin,[17] while ZPP/heme ratios appear to vary widely with an overall downward trend in all gestational age groups after the first week of life.[55,63,64] During this time period, ZPP/heme ratios do not correlate with reticulocyte count, MCV, MCH, or ferritin. Winzerling and Kling[65] measured ZPP/heme ratio's on infants born at less than 1,500 grams and found a mean ZnPP/H ratio of 95 at discharge. Values in their study correlated with the red cell distribution width.

Neonatal serum TfR levels are complicated by the utilization of recombinant human erythropoietin to treat infants.[55,66] This therapeutic approach, in combination with transfusion causes dramatic changes in iron status measures which reflect the dynamic movement of iron from storage pools to a rapidly increasing red cell mass. Premature infants supplemented with iron required fewer transfusions and decreased ZPP/H ratios.[67] The ZPP/H ratio in premature infants was initially high, fell rapidly over the first 6 months of life and then stabilized.[63]

Birth to 6 months of age: As infants age, erythropoeisis becomes more active by the second month of life and the reticulocyte count and hemoglobin increase preterm infants frequently experience a period of rapid "catch-up" growth with improving growth percentiles and a high requirement for iron.[68] Infants fed iron fortified breast milk had successful gain in iron status as determined by serum ferritin over the next several months. Lower iron intakes and ferritin concentrations early in life predisposed infants to iron deficiency later: by 6 months, a majority of infants had ferritin concentrations less than 10 µg/L.[58] A higher iron formula can reduce the prevalence of iron deficiency by >50% in the first year of life.[69] Low ferritin concentrations were most commonly noted between 4 and 8 months of age.

Krallis and colleagues[70] studied sTfR in preterm infants treated with erythropoietin and iron supplemented control infants over the first year of life. For control infants, the TfR levels were highest at birth, dropped to a nadir at 2 to 4 weeks and then rose to stable levels by the second month. Infants treated with erythropoietin showed a rise in sTfR during therapy, but by the second month, levels were similar to untreated infants. Transferrin receptors have not been studied in other groups of preterm infants, but in term infants, mean plasma TfR levels also were at their lowest levels at month 1 with the peak levels

Table 1_Median and Ranges

Variable	26-28 weeks	29-31 weeks	32-36 weeks	37-41 weeks	Author
Ferritin (µg/L)	75 (44-117)		90 (45-142)	131 (90-238)	Sweet et al 2001
TIBC[a] (µg/dL)	31 (27.0-35.0)		36 (31.5-44.0)	42.0 (36.0-49.5)	Sweet et al 2001
sTfR[b] (mg/dL)	10.3 (7.5-16.5)		8.2 (5.4-12.3)	8.4(6.4-10.6)	Sweet et al 2001
ZPP[c]/heme (µmol/m)	122 (34)	103 (18)	122 (34)		Juul et al 2003

[a] *TIBC refers to Total Iron Binding Capacity*
[b] *sTfR refers to soluble Transferrin receptor (mg/dL)*
[c] *ZPP/heme refers to the molar ratio of zinc protophorphrin/heme.*

occurring at months 3 and 4.[64] In term infants, sTfR levels have been found to be highly variable in the first 7 months. Since sTfR levels are heavily dependent upon the specific assay used, further investigation with this methodology.

6 months to 1 year: During the second half of the first year, hematologic adaptations to birth in the premature infant have been completed and assessment of anemia and iron status is similar to full term infants of the same age. However, preterm infants continue to have high growth rates and dietary dependence on iron continues to be important. Iron supplementation of 2 mg/kg/day prevents iron deficiency anemia in preterm infants out to 1 year in older studies.[71,72] Though these studies imply that supplemental iron of 2 mg/kg/day is effective in preventing iron deficiency anemia in preterm infants for the entire first year of life, they must be interpreted with caution as the populations of smaller preterm infants are surviving today and their iron endowment at birth may be significantly less than infants studied years ago.

Conclusions

This review notes the challenges in the diagnosis of iron status in infants during the first years of life. Active erythropoiesis and rapid growth puts a large demand on available storage pools of iron and diet. Newer analytical tools such as measurement of the sTfR and hepcidin have not yet been calibrated across clinical laboratories. The application of these measures, and others, to an evaluation of infant nutritional and clinical status will continue to be a challenge though standardization of approaches could provide a better diagnostic opportunity. LM

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