A Review of Cord Blood Concentrations of Iron Status Parameters to Define Reference Ranges for Preterm Infants

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Key Words

Iron deficiency · Premature infant · Cord blood · Hemoglobin · Mean corpuscular volume · Soluble transferrin receptor · Transferrin saturation · Reticulocyte hemoglobin content · Zinc protoporphyrin/heme ratio · Hepcidin

Abstract

Background: Iron plays an essential role in various tissue functions, and hence the reliable assessment of iron nutrition status of preterm infants appears to be mandatory. **Objectives:** To summarize available data on cord blood concentrations of iron status parameters as surrogate reference ranges for preterm infants until term-equivalent age. **Methods:** Review of the literature searching PubMed for cord blood values of hemoglobin, mean corpuscular volume, ferritin, soluble transferrin receptor, ferritin index, transferrin saturation, reticulocyte hemoglobin content, zinc protoporphyrin/heme ratio, and hepcidin and comparison with reference ranges established for adults. **Results:** Gestational age-specific cord blood concentration ranges at term were computed as weighted mean for hemoglobin [15.9 g/dl (13.3–18.4)], mean corpuscular volume [108.1 fl (97.8–118.5)] and transferrin saturation [61.2% (31.5–90.9)] and listed for ferritin, soluble transferrin receptor, ferritin index, zinc pro-

toporphyrin/heme ratio, reticulocyte hemoglobin content and hepcidin. These surrogate reference ranges were markedly different from adult values. **Conclusion:** Reference ranges of iron status parameters established for adults are probably not suitable to define iron status in preterm infants. If iron supplementation in preterm infants should be individually adjusted based on iron status parameters, it may be necessary to aim for cord blood concentration ranges to enable optimal growth and development. © 2013 S. Karger AG, Basel

Introduction

Most fetal iron is transferred from mother to fetus during the third trimester of gestation [reviewed in 1]. This transfer is interrupted by preterm birth, resulting in iron stores at birth being proportional to birthweight [2, 3]. Despite low iron stores at birth, growth velocity of very premature infants is maximal at 28–38 weeks' postmenstrual age reflecting a particularly high iron need during this period [4]. The risk of iron deficiency (ID) in preterm infants is further increased by frequent uncompensated iatrogenic phlebotomy losses [5].

Untreated ID in preterm infants during the fetal and postnatal period may contribute to long-term neurode-

developmental impairments that cannot be corrected by later iron supplementation [reviewed in 6, 7]. For instance, preterm infants with anemia and low ferritin had an increased number of abnormal reflexes evaluated according to the Assessment of Premature Infants Behavior at 37 weeks' postmenstrual age compared to nonanemic preterm infants [8].

Early enteral iron supplementation in very preterm infants reduces the incidence of ID and the need of red blood cell (RBC) transfusion [9, 10] and moreover may be associated with fewer long-term neurological abnormalities compared to those supplemented late [11]. Because enteral iron absorption is highly variable (range: 10–50%) [12, 13], an oral iron dose of 4–6 mg/kg/day may not be sufficient for all preterm infants depending of individual enteral iron absorption and iatrogenic iron loss [10].

In contrast, iron excess may increase the production of oxygen radicals to which preterm infants are especially vulnerable, because of low antioxidant capacity [14]. Fortunately, enteral iron has not been shown to increase markers of oxidative injury [15–17]. Nevertheless, side effects of oral iron supplementation such as lower rate of weight gain [18] and impaired growth in length [19] have been described in older, iron-replete children. However, this has not been observed in very [11] and marginally low birthweight infants [20].

According to both European and American recommendations, all preterm infants should be supplemented with 2–3 mg/kg/day of elemental iron [21, 22]. Because individual needs and enteral absorption are variable and preterm infants are at high risk for both, ID and iron toxicity, measuring iron status to individualize iron supplementation appears to be mandatory. Although many laboratory tests have been developed to assess iron status [reviewed in 23], there is consensus that no single blood test adequately reflects iron status in all circumstances. Beyond parameter-specific limitations, laboratory markers may be confounded by developmental changes occurring in utero. Consequently, this review aims to summarize published data on gestational age-specific cord blood concentrations of iron status parameters that might be suitable to guide iron supplementation in preterm infants.

Materials and Methods

We reviewed the literature searching PubMed for cord blood concentration of iron status parameters, and searched reference lists of retrieved publications for related publications. We attempted to contact the authors of all publications kindly requesting raw data to enable uniform presentation.

Extraction of data on mean, standard deviation (SD), or median and range for three gestational age groups: 23–29, 30–36, and 37–40 weeks was performed. If possible, data were transformed and shown as mean and reference range (defined as 2.5th and 97.5th percentiles = mean $\pm$ 1.96 $\times$ SD), and if normally distributed, a weighted mean was calculated. Not normally distributed data were not transformed and shown as extracted from the original publication. Raw data obtained from contacted authors were included in the tables and shown in a separate line below the original publication.

The majority of the reference ranges of adult iron status parameters were obtained from a standard textbook of clinical chemistry [24], reference ranges of females and males were transformed into a unisex mean.

Results

We identified 30 suitable publications regarding iron status parameters determined in cord blood. Sweet et al. [25, 26] as well as Ervasti et al. [27–29] used the same study population for more than one publication, and just one of each of these publications [25, 27] was listed in the tables. We contacted 22 of 30 corresponding authors of the original publications cited herein. The remaining 8 corresponding authors were not contacted because it was impossible to obtain a current valid email address. Of the 22 contacted corresponding authors, 5 answered without providing additional raw data, and 3 provided additional raw data, which we included in the tables in a separate line below the original publication.

In all publications providing details on cord clamping procedures, cord blood was obtained after immediate cord clamping [30–34].

Hemoglobin

Data on gestational age-specific cord blood concentrations of hemoglobin (Hb) are displayed in table 1. There was a trend towards higher Hb levels with increasing gestational age. Adult reference ranges were lower than term, and higher than very preterm cord blood Hb levels.

Mean Corpuscular Volume

Gestational age-specific mean corpuscular volume (MCV) values in cord blood are displayed in table 2. MCV in cord blood decreased with gestational age and was higher than adult MCV values.

Ferritin

Cord serum ferritin concentrations (table 3) increased with gestational age and were higher compared to adult levels.

Table 1. Hb concentration (g/dl) in cord blood

Study	GA 23–29 weeks			GA 30–36 weeks			GA 37–42 weeks		
	mean	2.5th–97.5th percentiles	n	mean	2.5th–97.5th percentiles	n	mean	2.5th–97.5th percentiles	n
Zaizov and Matoth [35]	19.2	15.3–23.1	25	19.1	15.0–23.2	71	19.2	15.5–22.9	39
Forestier et al. [36] ¹	12.6	10.8–14.3	83						
Burman and Morris [37] ²	14.3	9.8–18.8	14	15.8	11.6–19.9	111	16.3	12.0–20.6	140
Rios et al. [38] ¹							16.0	13.0–18.9	26
Noguera et al. [30] ¹				14.7	11.0–18.4	26	15.5	13.3–17.7	438
Sweet et al. [25] ³	16.2	13.2–19.4	26	15.8	11.7–19.9	50	16.1	12.7–19.4	68
Choi et al. [31] ³							15.8	13.8–17.7	527
Alur et al. [39] ³	14.8	11.6–17.9	100	16.2	12.8–19.5	88			
Kleven et al. [40] ¹							16.0	13.6–18.3	51
Ervasti et al. [27] ⁴							15.9	12.9–18.8	199
Mukhopadhyay et al. [32]				16.6	13.6–19.5	100	15.8	13.0–18.5	50
Weighted mean	14.6	11.8–17.4	248	16.5	12.8–20.2	446	15.9	13.3–18.4	1,538
Hb concentration in adults (95% CI) [24]								12.5–16.5	

SI conversion factor: 0.6206, SI unit: mmol/l.

¹ Coulter counter. ² Standard cyanomethemoglobin method. ³ Sysmex. ⁴ Advia.

Table 2. MCV (fl) in cord blood

Study	GA 23–29 weeks			GA 30–36 weeks			GA 37–42 weeks		
	mean	2.5th–97.5th percentiles	n	mean	2.5th–97.5th percentiles	n	mean	2.5th–97.5th percentiles	n
Zaizov and Matoth [35]	132.5	112.5–152.5	25	124.0	98.9–149.0	71	120.0	98.4–141.5	39
Forestier et al. [36] ¹	124.2	112.2–135.9	83						
Noguera et al. [30] ¹				109.1	103.4–114.8	26	105.1	94.7–115.5	438
Alur et al. [39] ²	114.6	101.2–127.8	100	110.4	97.4–123.3	88			
Choi et al. [31] ²							109.5	99.1–119.9	527
Ervasti et al. [27] ³							109.0	101.1–116.8	199
Weighted mean	120.6	106.9–134.0	208	115.4	98.8–131.9	185	108.1	97.8–118.5	1,203
MCV in adults (95% CI) [24]								80–96	

¹ Coulter counter. ² Sysmex. ³ Advia.

Small for gestational age preterm infants had even lower cord serum ferritin levels (mean: 67 µg/l) compared to appropriate for gestational age preterm infants (mean: 125 µg/l) [2].

Soluble Transferrin Receptor

Data on gestational age-specific cord blood concentrations of sTfR are displayed in table 4. Whereas Kruiper-Kramer et al. [48] did not find an association between gestational age and sTfR concentrations according to a

scatter plot, Sweet et al. [25] showed that increasing gestational age was associated with decreasing sTfR levels, and Hay et al. [43] demonstrated a positive correlation between sTfR and gestational age. In general, cord blood sTfR values tended to be higher than adult levels.

Ferritin Index (sTfR/Ferritin)

Ferritin index in cord blood decreased with gestational age [mean (reference range)]: gestational age at birth (GA) 23–29 weeks: 5.5 (3.3–8.6), n = 26; GA 30–36 weeks:

Table 3. Ferritin concentration (µg/l) in cord blood

Study	GA 23–29 weeks			GA 30–36 weeks			GA 37–42 weeks		
Rios et al. [38] ¹¹							113	80.8–157.9 ¹	n = 26
Haga [2] ¹¹				96	48–207 ²	n = 32	218	115–413 ²	n = 22
Choi et al. [31] ¹¹							183.3	76.1–290.4 ³	n = 527
Sweet et al. [25] ¹¹	75	44–117 ⁴	n = 26	90	45–142 ⁴	n = 50	131	90–238 ⁴	n = 68
Bradley et al. [34] ¹²		90.1±48 ⁶	n = 8		80.2±23.3 ⁶	n = 9		150±68 ⁶	n = 5
Lott et al. [41], detailed raw data ⁹	99.2	47.9–279.3 ⁴	n = 10	166.6	118.8–200.3 ⁴	n = 26	223	158–396.6 ⁴	n = 75
Siddappa et al. [42] ¹²				115	35–267 ⁵	n = 149	134	40–309 ⁵	n = 308
Siddappa et al. [42], detailed raw data ¹⁰	89	40–128 ⁴	n = 12	97	35–136 ⁴	n = 19	157	95–224 ⁴	n = 128
Hay et al. [43] ¹²								196±127 ⁶	n = 363
Kleven et al. [40] ¹³							210.5	58.1–362.4 ¹	n = 51
Ervasti et al. [27] ¹²								198±137 ⁶	n = 199
Verner et al. [33] ¹¹							169	136–202 ⁴	n = 48
Young et al. [44] ¹²								114.1±81 ⁷	n = 82
Rehu et al. [45] ¹³							140.2	119–165.8 ⁸	n = 116
Young et al. [46] ¹³								135±110 ⁷	n = 19
Mukhopadhyay et al. [32] ¹³				128	51.5–140 ⁴	n = 100	141	63–259 ⁴	n = 50
Shao et al. [47] ¹²							170	59–381 ⁵	n = 3,699
Ferritin concentration in adults [24]								13.5–187 ³	

SI conversion factor: 2.247, SI unit: pmol/l.

¹ Geometric mean, limits of 1 SD. ² Not mentioned. ³ Mean and 2.5th and 97.5th percentiles = $1.96 \times \text{SD}$. ⁴ Median and interquartile range. ⁵ 50th and 5th and 95th percentiles. ⁶ Mean ± SD. ⁷ Median ± SD. ⁸ Geometric mean and 95% CI. ⁹ Raw data obtained from Widness [pers. commun., 2013]. ¹⁰ Raw data obtained from Siddappa [pers. commun., 2013]. ¹¹ Radioimmunoassay. ¹² Chemiluminescence immunoassay. ¹³ Enzyme-linked immunosorbent assay.

4.6 (2.8–7.1), n = 50; GA 37–42 weeks: 3.8 (3.0–3.2), n = 68 [25]. Ferritin index in cord blood was higher compared to adult levels (reference range: 0.6–1.8 [49]).

This difference cannot be explained by insufficient assay validation for sTfR, because the same immunoenzymometric assay has been used for both, cord blood and adult values. Cord blood values for ferritin index obtained with an automated immunoturbidometric IdeA TfR-IT assay were lower [GA 37–42 weeks: 0.9 (0.1–1.8), n = 199 [27]].

Transferrin Saturation

Data on gestational age-specific cord blood values of transferrin saturation (TS) are displayed in table 5. TS values increased with gestational age and were higher compared to adult levels.

Reticulocyte Hb Content

Reticulocyte hemoglobin content (CHr) values in cord blood of preterm infants have not yet been reported. CHr values of term infants [mean (reference range): 35.6 pg

(33.0–38.1), n = 199 [27], and geometric mean (95% confidence interval (CI)): 35.6 pg (35.3–36.0), n = 116 [45]] were both higher compared to adult values (reference range: 28–35 pg [24]).

Zinc Protoporphyrin/Heme Ratio

Data on gestational age specific cord blood values of ZnPP/H are displayed in table 6. There is a negative correlation of ZnPP/H with gestational age and adult reference ranges seem to be lower.

Hepcidin

Hepcidin concentrations in cord blood of preterm infants have not been reported, but in term infants [geometric mean (95% CI): 72.3 ng/ml (20.5–231.9), n = 137 [45], and median (SD): 61.7 ng/ml (77.0), n = 19 [46]], values are similar to those in adults [mean (95% CI): 90.7 ng/ml (23.8–267.7) [56]]. All hepcidin concentrations were measured with a competitive enzyme-linked immunosorbent assay.

Table 4. sTfR concentration (mg/l) in cord blood

Study	GA 23–29 weeks			GA 30–36 weeks			GA 37–42 weeks		
Choi et al. [31] ⁵							5.7	4.4–6.9 ¹	n = 527
Sweet et al. [25] ⁵	10.3	7.5–16.5 ²	n = 26	8.2	5.4–12.3 ²	n = 50	8.4	6.4–10.6 ²	n = 68
Hay et al. [43] ⁵							7.1	5.5–9.6 ²	n = 350
Young et al. [44] ⁵								8.0±2.4 ³	n = 83
Young et al. [46] ⁵							8.4	3.1–13.7 ¹	n = 19
Verner et al. [33] ⁵							12.9	8.3–18.7 ²	n = 48
Ervasti et al. [27] ⁶							2.0	0.6–3.3 ¹	n = 199
Rehu [45] ⁶							1.9	1.8–2.1 ⁴	n = 116
sTfR concentration in adults [49] ⁵	1.1–2.7 ¹								

¹ Mean and 2.5th and 97.5th percentiles = $1.96 \times \text{SD}$. ² Median and interquartile range. ³ Median and SD. ⁴ Geometric mean and 95% CI. ⁵ Immunoenzymometric assay. ⁶ Automated immunoturbidometric IdeA TfR-IT assay.

Table 5. TS (%) in cord blood

Study	GA 23–29 weeks			GA 30–36 weeks			GA 37–42 weeks		
	mean	2.5th–97.5th percentiles	n	mean	2.5th–97.5th percentiles	n	mean	2.5th–97.5th percentiles	n
Rios et al. [38]							59.5	33.0–84.9	26
Haga [2]				46.5	6.5–86.5	29	55	27.6–82.4	21
Choi et al. [31] ¹							63.9	36.8–91.0	527
Ervasti et al. [27] ²							55	17.8–92.2	199
Weighted mean				46.5	6.5–86.5	29	61.2	31.5–90.9	773
TS in adults (reference range) [24]	16–45								

¹ Iron + total iron binding capacity: automatic chemical analyzer. ² Iron + transferrin: image analyzer.

Table 6. ZnPP/H ratio (μmol/mol) in cord blood

Study	GA 23–29 weeks			GA 30–36 weeks			GA 37–42 weeks		
Juul et al. [50] ⁷	111.7	60.7–162.6 ¹	n = 26	79.0	49.6–108.4 ¹	n = 26	79.0	49.6–108.4 ¹	n = 26
Lott et al. [41] ⁷	108.0	96.0–120.0 ²	n = 7	84.0	78.0–90.0 ²	n = 12	73.0	66.0–80.0 ²	n = 81
Lott et al. [41], detailed raw data ⁵	96.8	83.4–137.2 ³	n = 10	78.3	57.4–98.5 ³	n = 26	63.5	51.5–76 ³	n = 75
Kling [51] ⁷	125.0	78.0–180.0 ²	n = 12	90.0	42.0–162.0 ²	n = 84	70.0	32.0–180.0 ²	n = 293
Lesser et al. [52] ⁷							66.4	9.6–123.2 ¹	n = 31
Kleven et al. [40] ⁷							68.3	29.2–107.4 ¹	n = 51
Cheng et al. [53] ⁷	114.0	63.0–165.0 ¹	n = 13	84.0	48.7–119.3 ¹	n = 18			
Kleven et al. [40], Kling [51], Lesser et al. [52], Baumann-Blackmore et al. [54], detailed raw data ⁶							83.9	15.1–152.7 ¹	n = 286
ZnPP/H ratio in adults [55]	30–80 ⁴								

¹ Mean and 2.5th and 97.5th percentiles = $1.96 \times \text{SD}$. ² According to the figure. ³ Median and interquartile range. ⁴ Reference range. ⁵ Raw data obtained from Widness [pers. commun., 2013]. ⁶ Raw data obtained from Kling [pers. commun., 2012]. ⁷ Hematofluorometer.

Discussion

This review summarizes gestational age-specific cord blood values of iron status parameters as surrogate reference ranges for preterm infants. It is important to realize that cord blood concentrations of these parameters are markedly different from reference ranges established for adults, and thus cutoff values to define the need for iron supplementation derived from adults or older children may not be appropriate for preterm infants.

Furthermore, all iron status parameters have specific limitations, which may be of particular importance in the preterm population.

Hb, derived from the entire population of RBCs with a mean life span of 60 days in neonates [57] provides a very delayed response to developing ID. Moreover, Hb concentration depends on the blood sampling technique: it may be up to 15% higher when obtained from capillary compared to venous samples [58].

MCV is also a poor indicator of iron status in preterm infants, because of the physiological transition from fetal erythrocytes characterized by high MCV and predominantly containing fetal Hb to adult erythrocytes characterized by lower MCV and predominantly containing adult Hb [35, 39, 59].

High or increased ferritin levels may not only indicate filled iron stores (e.g. following multiple RBC transfusions) or even iron overload conditions (i.e. in neonatal hemochromatosis), but also liver cell injury, or systemic inflammatory response [reviewed in 60], the latter being particularly common in preterm infants.

Increased sTfR concentrations are not specific for iron-deficient anemia but can also be observed in hyperproliferative erythropoiesis, e.g. due to hemolysis or ineffective erythropoiesis [61], and potentially also during erythropoietin administration for prevention and treatment of anemia of prematurity [62]. Furthermore, there are sex-specific differences with higher concentration of sTfR in male compared to female term infants [31, 43]. However the major limitation of sTfR is the lack of assay standardization with a high discrepancy between results from different commercially available tests [63].

TS is a late parameter for diagnosing ID, and it is low only when the iron stores are already exhausted [reviewed in 64].

In contrast, CHr is an early marker of functional ID because of the short lifespan of reticulocytes of just about 24 h. The ZnPP/H fluorometric determination can be influenced by plasma bilirubin, which needs to be washed out before analysis [65]. ZnPP/H and CHr have the ad-

vantage that they do not seem to be affected by chronic inflammatory disease or acute infection [66, 67], whereas hepcidin expression is also upregulated by inflammatory cytokines [68] and systemic inflammation [69] just like ferritin. On the other hand, hepcidin is also downregulated by hypoxia [70]. One of the biggest problems concerning hepcidin measurements both with immunochemical- and mass spectrometric assays is the lack of assay standardization and the availability of routine testing [71].

The more recent parameters to assess iron status like hepcidin and ZnPP/H lack inter-assay standardization, which limits the validity of a reference range based on data obtained with different assays. The more established parameters like ferritin and Hb have been progressively developed over the last decades, potentially limiting the validity of a summary of older and more recent data. It is important to have this limitation in mind when using the reference ranges reported here in clinical practice.

The increase in ferritin, TS (and Hb) and the decrease in ferritin index and ZnPP/H with increasing gestational age may well reflect improved iron availability and growing iron stores during late gestation. In contrast, the observed decrease in MCV values can potentially be explained by the above-mentioned transition from fetal to adult erythrocytes. Controversial data were found for the association of sTfR with gestational age, probably because of a lack of assay standardization. No data have yet been published on the association between both CHr and hepcidin, and gestational age.

For all iron status parameters, higher values were observed in cord blood compared to adult blood. This may be due to high iron transfer from mother to fetus (explaining higher cord blood values for ferritin, Hb, TS) and maximally stimulated erythropoiesis (explaining higher values for sTfR and ferritin index) at the end of gestation.

Moreover, lower ferritin levels indicating lower iron stores were observed not only in preterm infants [26] but also in infants with placental insufficiency [2] or maternal gestational diabetes mellitus [33] leading to a higher vulnerability to ID. In preterm infants, high postnatal growth velocity [4] and uncompensated iatrogenic blood loss [5] further contribute to an even earlier depletion of iron stores.

Iron stores acquired in utero are adequate until approximately the time that birthweight is doubled [72], and this occurs much earlier in premature infant compared to term infants – at least under the rather rare condition that iron losses from phlebotomy are matched by

iron acquired by enteral iron absorption and blood transfusions. Consequently, to prevent anemia of prematurity, preterm infants should receive iron supplementation [21, 22], which can be standardized (i.e. every infant is given a standard dose of 2–4 mg/kg) or individualized (i.e. based on the current iron needs of a given infant). Because iron stores cannot be assessed directly, the individual infant's actual iron status parameter concentrations have to be compared with reference ranges to assess the infant's individual iron need. The latter approach can take into account changes in iron needs based on blood transfusions (taking into account that every milliliter of blood transfused equals a parenteral administration of iron [73]), ongoing enteral iron supplementation, and phlebotomy losses. Because reference ranges derived from postnatal blood samples in preterm infants likely reflect iron supplementation, blood transfusion and phlebotomy practice of the individual unit rather than the physiological range of iron status at this developmental stage, we hypothesize that cord blood reference ranges may better guide individualized supplementation. Similarly, we hypothesize that cord blood reference ranges are more appropriate than the frequently used adult reference ranges [e.g. 74].

Whatever laboratory parameter is currently used to assess iron status, its determination adds to iatrogenic blood and, hence, iron loss and consequently further predisposes the preterm infant to ID. Every milliliter of blood loss represents a loss of 0.35–0.5 mg iron (assuming an Hb concentration of 10–15 g/dl). In very low-birthweight infants, iron losses due to phlebotomy can amount up to 6 mg/kg/week [75]. Therefore, it is desirable to search for a method to measure iron status without causing additional blood loss. Hepcidin, which to date is the only parameter that can also be measured in urine [56, 69], could

serve with some limitations as a parameter to monitor iron status in preterm and term infants without contributing to further blood loss, at least in the absence of systemic inflammation.

Because of the high number of confounding intrauterine as well as postnatal variables that may influence iron status parameters and the fact that measuring iron status parameters leads to further blood loss, standardized iron supplementation is probably still appropriate in preterm infants. However, infants with ID have been described despite standardized iron supplementation, which may not meet the needs of all infants [10].

Conclusion

Reference ranges of iron status parameters established for adults are remarkably different from cord blood values and consequently may not be suitable to guide iron supplementation in preterm infants. Because of inherent limitations of iron status parameters, a standardized iron supplementation to prevent anemia of prematurity still seems to be justified as long as an individualized supplementation, e.g. on the basis of gestational age-specific reference ranges, has not been proven superior.

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