Letter to the Editor

Cut-off Values of Diagnostic Indices to Detect Iron Deficiency in Chinese Breast-fed Infants

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Iron deficiency anemia is one of the most prevalent nutritional deficiency worldwide. The commonly used cut-off values for identifying iron deficiency are extrapolated from older children and may not be suitable for infants. Therefore, our study aimed to establish appropriate cut-off values for the evaluation of iron status in Chinese infants. Pregnant women who delivered at ≥37 gestational weeks with normal iron status were recruited. Later, infants with normal birth weight and who were breastfed in the first 4 months were selected. Blood samples were collected to assess hemoglobin, serum ferritin, soluble transferrin receptor, mean corpuscular volume and free erythrocyte protoporphyrin. Cut-offs of all iron indices were determined as the limit of 95% confidence interval.

Iron deficiency anemia (IDA) continues to be one of the most prevalent nutritional deficiency worldwide. Infants are especially susceptible because of high iron requirement for their rapid growth. It has been demonstrated that even the early stage of IDA is associated with impaired neurodevelopment in infants and children.

The diagnosis of iron deficiency (ID) is based mainly on a battery of laboratory tests. Early ID status in infants is difficult to evaluate because iron parameters change with age, critical values overlap at various stages of ID, and infections and inflammations can influence iron status indices.

WHO/CDC1 recommends hemoglobin (Hb), serum ferritin (SF), soluble transferrin receptor (sTfR), mean corpuscular volume (MCV) and free erythrocyte protoporphyrin (FEP) as the best iron variables to assess iron status. The commonly used cut-off values to identify ID at 6-12 months of age are Hb<110 g/L and SF<10-12 µg/L. These values are extrapolated from older children (approximately 7 million toddlers aged 1 to 2 years) and may not be suitable for infants. Moreover, there is lack of data on cut-off values of other measures of iron status, such as MCV and sTfR in infants and need further research. Furthermore, there are fewer studies which have focused on the iron status indices distribution ranges in Chinese breast-fed infants, even though exclusive breast-feeding is generally recommended for the first 6 months of life.

Therefore, our study aimed to establish appropriate cut-offs for the evaluation of iron status or diagnosis of IDA in Chinese infants.

We recruited 800 pregnant women (>18 years old) who delivered at ≥37 gestational weeks with normal iron status (Hb>120 g/L and SF>12 µg/L on the last prenatal examination) and without any symptoms of anemia from July 2010 to May 2012 at the two hospitals in Beijing. Only infants with normal birth weight (2500-4000 g) were included in the analysis. In subsequent follow-up at 4 months, only infants who were exclusively breastfed and had Hb level >145 g/L were considered for the inclusion. Parents were discouraged from feeding the infants complementary foods containing iron between 4 to 6 months. Signed informed consent was obtained from parents. The review and approval of ethics committee was obtained.

Cut-off iron status indices were calculated as the limit (±2 SD) of 95% confidence interval (CI) by setting a ‘healthy’ group sample with normal iron nutrition. Three methods were used to determine the ‘iron-normative group’. First, we enrolled study

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participants not likely to have a high prevalence of ID, but without any further selection. It was assumed that the infants born at full term with normal body weight who are exclusively breastfed by healthy mothers would have a replete iron store at 4 months of age. We called this the ‘unselected’ normative group.

It is known that infants with borderline ID at 4 months would be expected to become gradually more iron deficient with time (such as at 6 months) if they are not supplemented with iron. Thus, we excluded such 6-months-old infants as per the conventional cut-off values for iron status variables. We called this ‘iron-screened’ normative group. To avoid using adult reference values in infants, conventional cut-offs for infants at 6 months were established by calculating the 95% confidence interval of infants at 4 months. We excluded infants with any abnormal iron status variables [Hb, MCV, FEP, SF, or sTfR] as per 2 SD cut-off values at 4 months.

On the other hand, individuals with slight IDA were identified by analyzing the Hb response after a period of iron supplementation. The infants at 4 months were randomly assigned to either control group or ‘iron-supplement’ group. Infants in the control group were considered as the ‘unselected’ group while infants in the iron-supplemented group were given an iron supplement (liquid amino acid chelated iron, Singcom Pharma) at a dose of 1 mg/kg per day (standard treatment doses is 6 mg/kg per day) for 2 months, from the age of 4 months to 6 months. Hb response to iron supplementation was evaluated as an alternative definition of ID, and we called this ‘iron-supplemented’ group. After 2 months, infants with an increase in Hb level of >10 g/L[8] were regarded as possibly iron-deficient and excluded from the ‘iron-supplemented’ normative group at 4 months to further minimize the risk of including ID individuals (Figure 1).

Venous blood samples were collected from infants at birth, 4 months and 6 months. At each stage, iron status indices including Hb, SF, sTfR, MCV, and FEP levels were tested. The presence of inflammation or infection was assessed by measuring the levels of acute-phase proteins: C-reactive protein (CRP) and α-glycoprotein (AGP). Infants with CRP>5 mg/L or AGP>1 g/L were excluded. Changes in Hb level were assessed following iron supplementation.

Automated analyzers (Sysmex XE 2100, Kobe, Japan) were used to assay Hb and MCV while CRP, SF, sTfR, and AGP levels were analyzed on an automatic analyzer (7080i, Hitachi, Japan) using particle enhanced transmission immunoturbidimetric passed assay. FEP level was tested using aflourescence spectrometer (SB-046, PerkinElmer, USA). All reagents were purchased from Roche, USA.

Our preliminary results which included 40 infants at 4 months at the two hospitals showed that Hb level change in infants with ID in the iron supplement group was 8 g/L greater than that in the

![Figure 1. showing the study design flowchart.](image-url)
control group. Hence, permissible error in the estimated standard deviation (σ) was 8 g/L with power (1-β)=80%, significance level α=0.05, Z means Z value, using the formula:

$$N = 2\left(\frac{Z_\alpha + Z_\beta}{d}\right)^2$$


The minimum sample required was 21 infants in each group at 4 months. Considering the prevailing breastfeeding rates of about 50%-60% and the estimated subsequent loss of participants to follow-up, the minimum sample was 130 at the beginning.

The distribution of iron nutritional indices in the three groups were compared. The 2 SD cut-off values were used to diagnose ID in 4- and 6-months-old infants with nearly exclusive breastfeeding (although some water could be fed to the infants). The ratio of sTfR/SF was calculated by dividing sTfR (in µg/L) by SF (in µg/L).

All statistical analyses were performed using SPSS 17.0. Kolmogorov-Smirnov test was used to verify if iron status indices were consistent with normality. Statistical methods used to compare means were t test and analysis of variance (ANOVA) and significance was defined as P<0.05.

We started with 800 pregnant women at the beginning. As the study progressed, some pregnant women or infants were dropped-out for not meeting our inclusion criteria including not exclusively breastfeeding the infants during their first 4 months and feeding the infants with iron-enriched supplementary foods while some were lost due to relocation. Out of 332 infants at 4 months, 23 were further excluded including 10 infants with CRP>5 mg/L or AGP>1 g/L, 5 infants who were fed iron-enriched supplementary foods, and 8 infants who were not exclusively breastfed. Therefore, the number of eligible newborn participants was 309. They had mean birth weight of 3.3±0.3 kg, were born at gestational week of 40±1, and had mean Hb of 175.0±18.0 g/L. The latest test before the delivery showed that Hb of mothers was 125.0±4.0 g/L and SF was 46.5±26.6 µg/L. At 4 months, the average weight of infants was 7.6±0.9 kg, height was 65.5±4.6 cm, and head circumference was 41.3±1.2 cm. During 4 to 6 months, 86 infants were excluded which included 63 infants who were lost, 8 infants who could not tolerate iron supplements and 2 infants had CRP>5 mg/L or AGP>1 g/L. In addition, 13 infants in the iron-supplement group were excluded for having an Hb response level >10 g/L after 2 months of iron supplementation. Thereby, 223 infants remained in the study at 6 months. Figure S1 (see in www.besjournal.com) showed the change in the sample size during the study.

By normality test, Hb, sTfR, MCV, and FEP were consistent with arithmetic normality, so arithmetic means were used. Since SF and sTfR/SF were consistent with a log-normal distribution, geometric mean was used.

To develop the cut-off values to evaluate the iron status of 4- and 6-months-old infants, t test and ANOVA were used to compare means of different iron variables in different groups (Table 1). As iron status did not differ significantly between the unselected group and the iron-supplemented group at 4 months (P>0.05), the mean of the two groups were used to establish the cut-off values at 4 months. A similar method was used to develop the cut-off values of other iron indices such as sTfR, Hb, sTfR/SF ratio, and FEP for 6-month-old infants (Table 1). SF showed significant differences between unselected group, iron-supplemented group, and iron-screened group at 6 months (P<0.01). After pairwise comparisons, the ‘unselected’ group had a significantly lower SF level than the other two groups which was possibly a reflection of the fact that there was a small proportion of iron-deficient infants in the unselected group (P<0.05). So, the mean SF of iron-supplemented group and iron-screened group was used to establish the cut-off values.

Iron status variable distribution of infants in different groups is shown in Table 1 and the ±2 SD cut-off values for each variable in different groups are shown in Table 2. Our study suggested the following 2 SD cut-off values for the diagnosis of IDA in 4- and 6-months-old Chinese infants: SF<16 µg/L, sTfR>6 mg/L, Hb<101 g/L, MCV<72 fl, and FEP>205 µg/dL at 4 months; SF<11 µg/L, sTfR>6 mg/L, Hb<100 g/L, MCV<69 fl, and FEP>209 µg/dL at 6 months (Table 2). Hb is the commonly used index for the diagnosis of ID and we established the cut-off Hb levels for full-term breastfed infants as 101 g/L and 100 g/L at 4 and 6 months, respectively. As per a WHO report, the cut-off Hb levels for 6-12-month-old infants was 110 g/L[9], which is higher than our result. One of the reason for this difference may be that the WHO data was extrapolated from older age groups[7]. A study conducted on 253 healthy Swedish and Honduran infants suggested 105 g/L as the cut-off value of Hb.
for both 4- and 6-month-old infants\(^3\). Another study in Britain suggested 95 g/L as the cut-off value of Hb for 8-month-old infants\(^7\). Considering that Hb concentration shows a physiological descent during the age of 6-9 months, it is reasonable that cut-off values for the 4- and 6-month-old infants in our study are higher than 95 g/L.

Our suggested cut-off value of MCV of 72 fl at 4 months and 70 fl at 6 months are consistent with those in the literature. Cut-off values in a study on Finnish infants who were fed iron-fortified formula were 76 fl at 4 months and 68-70 fl at 6-9 months\(^10\) while a study in Swedish and Honduran infants suggested 73 fl at 4 months and 71 fl at 6 months\(^3\).

Our suggested cut-off SF levels of 16 µg/L at 4 months and 11 µg/L at 6 months were similar to those reported in a research conducted in Sweden and Honduras infants (n=197)\(^3\) in which -2SD cut-off

### Table 1. Comparing the Means of Iron Status Variables Between Infants in the Unselected, Iron-supplemented, and Iron-screened Groups

<table>
<thead>
<tr>
<th>Items</th>
<th>4 Months</th>
<th></th>
<th>6 Months</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unselected</td>
<td>Iron-supplemented</td>
<td>P VALUE(^d)</td>
<td>Unselected</td>
<td>Iron-supplemented</td>
<td>Iron-screened</td>
<td>P VALUE(^e)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>219</td>
<td>90</td>
<td></td>
<td>92</td>
<td>44</td>
<td>53-87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb(^5) (g/L)</td>
<td>114.0±6.0</td>
<td>112.0±6.0</td>
<td>0.25</td>
<td>116.0±8.0</td>
<td>117.0±8.0</td>
<td>115.0±7.0</td>
<td>0.45</td>
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</tr>
<tr>
<td>SF(^bc) (µg/L)</td>
<td>65.5±2.3</td>
<td>81.2±2.4</td>
<td>0.07</td>
<td>28.6±2.6</td>
<td>43.2±1.8</td>
<td>39.5±2.1</td>
<td>0.01(^b)</td>
<td></td>
</tr>
<tr>
<td>sTfR(^g) (mg/L)</td>
<td>3.5±1.3</td>
<td>3.6±1.1</td>
<td>0.96</td>
<td>3.6±1.5</td>
<td>3.4±1.2</td>
<td>3.3±1.2</td>
<td>0.20</td>
<td></td>
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<tr>
<td>sTfR/SF(^bc)</td>
<td>52.4±2.7</td>
<td>41.6±2.7</td>
<td>0.70</td>
<td>111.5±3.4</td>
<td>70.1±2.3</td>
<td>95.9±3.1</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>MCV(^v) (FL)</td>
<td>78.9±3.7</td>
<td>79.3±3.5</td>
<td>0.43</td>
<td>76.6±3.2</td>
<td>76.1±4.0</td>
<td>77.5±2.3</td>
<td>0.09</td>
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</tr>
<tr>
<td>FEP(^x) (µg/dL)</td>
<td>52.5±2.0</td>
<td>60.3±1.6</td>
<td>0.07</td>
<td>60.3±1.6</td>
<td>64.6±1.6</td>
<td>61.3±1.9</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

**Note.** \(^a\): Geometric mean±SD. The arithmetic s is added (subtracted) to the mean, whereas the geometric s is multiplied (divided) by the geometric x. Geometric=x\(^e\), x\(^e\)= ∑\(_{i=0}^\infty\)lnx\(_i\). \(^b\): SF has significant differences between the unselected group, iron-supplemented group and iron-screened group (P<0.01). \(^c\): Hb: hemoglobin, SF: serum ferritin, sTfR: soluble transferrin receptor, MCV: mean corpuscular volume, FEP: free erythrocyte protoporphyrin. \(^d\): t test between unselected and iron-supplemented group. \(^e\): ANOVA test among the unselected group, iron-supplemented group and iron-screened group.

### Table 2. ±2 SD Cut-off Values for Chinese Infants at 4 and 6 Months of Age

<table>
<thead>
<tr>
<th>Items</th>
<th>4 Months</th>
<th></th>
<th>6 Months</th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unselected</td>
<td>Iron-supplemented</td>
<td>Cut-off Values(^d)</td>
<td>Unselected</td>
<td>Iron-supplemented</td>
<td>Iron-screened</td>
<td>Cut-off Values(^e)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>219</td>
<td>90</td>
<td></td>
<td>92</td>
<td>44</td>
<td>53-87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb(^5) (g/L)</td>
<td>102</td>
<td>100</td>
<td>&lt;101</td>
<td>100</td>
<td>101</td>
<td>101</td>
<td>&lt;100</td>
<td></td>
</tr>
<tr>
<td>SF(^bc) (µg/L)</td>
<td>14</td>
<td>17</td>
<td>&lt;16</td>
<td>5</td>
<td>12</td>
<td>9</td>
<td>&lt;11</td>
<td></td>
</tr>
<tr>
<td>sTfR(^g) (mg/L)</td>
<td>6</td>
<td>6</td>
<td>&gt;6</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>&gt;6</td>
<td></td>
</tr>
<tr>
<td>sTfR/SF(^bc)</td>
<td>285</td>
<td>228</td>
<td>&gt;257</td>
<td>758</td>
<td>318</td>
<td>600</td>
<td>&gt;559</td>
<td></td>
</tr>
<tr>
<td>MCV(^v) (FL)</td>
<td>71</td>
<td>72</td>
<td>&lt;72</td>
<td>70</td>
<td>68</td>
<td>73</td>
<td>&lt;69</td>
<td></td>
</tr>
<tr>
<td>FEP(^x) (µg/dL)</td>
<td>210</td>
<td>200</td>
<td>&gt;205</td>
<td>190</td>
<td>204</td>
<td>234</td>
<td>&gt;209</td>
<td></td>
</tr>
</tbody>
</table>

**Note.** \(^a\): Geometric mean±SD. The arithmetic SD is added (subtracted) to the mean, whereas the geometric SD is multiplied (divided) by the geometric mean. \(^b\): Hb: hemoglobin, SF: serum ferritin, sTfR: soluble transferrin receptor, MCV: mean corpuscular volume, FEP: free erythrocyte protoporphyrin. \(^c\): N of screened: When discussing about Hb, infants with abnormal iron status variables of MCV, FEP, SF and sTfR were excluded, 53 infants remained, so n=53. SF: n=87, sTfR: n=76, MCV: n=82, FEP: n=73, sTfR/SF: n=83. \(^d\): The 2 SD cut-off values for the diagnosis of IDA in 4- and 6-month-old Chinese infants: SF<16 µg/L, sTfR>6 mg/L, Hb<101 g/L, MCV<72 fl, and FEP>205 µg/dL at 4 months; SF<11 µg/L, sTfR>6 mg/L, Hb<100 g/L, MCV<69 fl, and FEP>209 µg/dL at 6 months.
values were 20 µg/L and 9 µg/L at 4 and 6 months of age, respectively. Another study of Finnish infants who were fed with iron-fortified formula (n=36-43) suggested -2 SD cut-off values of 37 µg/L and 19 µg/L at 4 and 6 months of age, respectively[11]. The higher SF values in that study could be a result of iron-fortified formula feeding. All the above-mentioned results differ from the 12 µg/L cut-off value at 6-12 months of age proposed by the World Health Organization (WHO) in 2003[12].

Our study suggested a cut-off stTfR level of 6 mg/L both at 4 months and 6 months. These values are consistent with those in two Canadian studies: one study of 389 Canadian ID infants suggested a cut-off stTfR level of 7 mg/L for male infants and 6 mg/L for female infants at 4-6 months of age[13]; and another study of 485 Canadian healthy infants suggested a cut-off value of 6.6 mg/L at 9-15 months[14]. These two studies also used enzyme immunoassay methods to analyze stTfR, but the reagents used were from a different company. One major problem in comparing the results of stTfR from different studies is the wide distribution range among laboratories and different commercial kits; however, it has been reported that even though the values obtained from different stTfR detection kit may differ, they have good correlation and comparable validity to identify ID if the reference values for that particular assay were used[1].

Our study suggested the stTfR/SF cut-off values of 257 at 4 months and 559 at 6 months. In one study of 515 Chilean infants, aged 8-15 months, the suggested stTfR/SF cut-off value was 975[15]. A possible reason for this difference is the higher age of the infants in the Chilean study as SF level drops after 4 months of age, while stTfR level remains steady.

Our study obtained the appropriate cut-off values for the assessment of iron status in Chinese infants aged 4-6 months using a 'healthy-group approach'. Although, the sample size of our study was smaller than in most other studies on reference values for other age groups, the advantage of our dataset is the strict definition of breast-feeding, presence of an iron-supplemented group of infants as well as an un-supplemented group. In addition, CRP and AGP were used as the inflammation markers and the two-hospital design provided the wide range in iron status, all of which is essential for evaluating iron status cut-off values during infancy.

We will continue to coordinate with clinicians to validate the cut-off values determined in this study.

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These two authors contributed equally to this work.

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Figure S1. The change in the sample size during the study.