Improved method for assessing iron stores in the bone marrow

K S Phiri,1 J C J Calis,1,2 D Kachala,1 E Borgstein,3 J Waluza,3 I Bates,4 B Brabin,4 M Boele van Hensbroek1,2,4

ABSTRACT

Background: Bone marrow iron microscopy has been the “gold standard” method of assessing iron deficiency. However, the commonly used method of grading marrow iron remains highly subjective.

Aim: To improve the bone marrow grading method by developing a detailed protocol that assesses iron in fragments, in macrophages around fragments and in erythroblasts.

Methods: A descriptive study of marrow aspirates of 303 children (aged 6–60 months) with severe anaemia and 22 controls (children undergoing elective surgery) was conducted at hospitals in southern Malawi (2002–04).

Results: Using an intensive marrow iron grading method, 22% and 39% of cases and controls had deficient iron stores, and 40% and 46% had functional iron deficiency, respectively. Further evaluation of the iron status classification by the intensive method showed that functional iron deficiency was associated with significantly increased C-reactive protein concentrations (126.7 (85.6) mg/l), and iron stores deficiency with significantly increased soluble transferrin receptor concentrations (21.7 (12.5) μg/ml).

Conclusions: Iron assessment can be greatly improved by a more intense marrow examination. This provides a useful iron status classification which is of particular importance in areas where there is a high rate of inflammatory conditions.
Fe, Darmstadt, Germany). Positive controls were included in each batch of slides.

**Bone marrow smear iron grading**

Bone marrow smears were graded by the conventional Gale’s method and by a new more intensive grading method.

Marrow smears were first assessed by one of the authors (KP) according to Gale’s histological grading method, which assesses only marrow fragments (table 1). In order to reduce subjectivity, predefined descriptions and sample illustrations of each iron grade were used to grade fragments of all marrow smears. Only iron smears with at least seven fragments were assessed. Deficiency of iron stores was defined as an iron grade of none (grade 0) or very slight (grade 1).

All marrow smears were then systematically assessed using an intensive histological grading method in which iron is assessed in three sites—the fragments (as in Gale’s method), macrophages and erythroblasts. Iron assessed in the fragments and macrophages represented iron stores while iron in the erythroblast represented utilisable iron. Additionally, 20 fields around and behind the fragments were examined at high power (×1000) and all macrophages in these fields were examined for the presence of iron (fig 1). At high power magnification (×1000), 100 erythroblasts were examined and the percentage containing iron granules in their cytoplasm (ie, sideroblasts) were enumerated. Erythroblast iron deficiency was defined when <30% of erythroblasts had visible iron granules.

Results of iron smear assessment using the intensive histological grading method were interpreted as **normal status** (normal iron stores and normal erythroblast iron); **functional iron deficiency** (normal iron stores and deficient erythroblast iron); **iron stores deficiency** (depleted iron stores and normal erythroblast iron); and **combined functional iron and iron stores deficiency** (depleted iron stores and deficient erythroblast iron; table 2).

**Other laboratory tests**

Bone marrow iron status assessment was compared to peripheral blood iron markers from samples taken at the same time as the bone marrow aspirate. Haemoglobin was measured using a Coulter counter machine (Beckman Coulter, Durban, South Africa). Ferritin, a measure of iron stores deficiency, was determined using the electrochemiluminescence immunoassay (Modular Analytics E170, Roche Diagnostics, Switzerland), and soluble transferrin receptor (sTfR) levels, a measure of cellular iron need, using ELISA (Ramco Laboratories, Texas, USA). Immunoturbidimetric assay (Modular P800, Roche Diagnostics, Switzerland) was used to determine C-reactive protein (CRP) levels (measure of inflammation) in blood.

**Table 1** Histological grading for bone marrow iron status according to Gale et al

<table>
<thead>
<tr>
<th>Grade</th>
<th>Classification</th>
<th>Iron detected in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Present Present Present</td>
</tr>
<tr>
<td>1</td>
<td>Very slight</td>
<td>Present Present Present</td>
</tr>
<tr>
<td>2</td>
<td>Slight</td>
<td>Present Present Present</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Present Present Present</td>
</tr>
<tr>
<td>4</td>
<td>Moderate heavy</td>
<td>Present Present Present</td>
</tr>
<tr>
<td>5</td>
<td>Heavy</td>
<td>Present Present Present</td>
</tr>
<tr>
<td>6</td>
<td>Very heavy</td>
<td>Present Present Present</td>
</tr>
</tbody>
</table>

Table 1: Histological grading for bone marrow iron status according to Gale et al

**Table 2** Classification of iron status using the intensive grading method

<table>
<thead>
<tr>
<th>Iron detected in:</th>
<th>Fragment*</th>
<th>Macrophage†</th>
<th>Erythroblast‡</th>
<th>Iron status category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Normal</td>
</tr>
<tr>
<td>Present</td>
<td>–</td>
<td>Present</td>
<td>–</td>
<td>Functional iron deficiency</td>
</tr>
<tr>
<td>Present</td>
<td>Present</td>
<td>–</td>
<td>Present</td>
<td>Iron stores deficiency</td>
</tr>
<tr>
<td>–</td>
<td>Present</td>
<td>Present</td>
<td>–</td>
<td>Functional and iron stores deficiency</td>
</tr>
</tbody>
</table>

*Positive fragment iron: fragment grade >2.
†Positive macrophage iron: iron present in reticular cell.
‡Positive erythroblast iron: iron present in >30% of erythroblasts.

**Figure 1** Bone marrow fragment showing iron deposits at ×400 magnification (top), and ×1000 magnification showing erythroblasts, iron and malaria pigment within macrophages (bottom).
RESULTS

A total of 381 cases and 23 controls were recruited. Cases had an average age of 1.7 years (SD 1.1), and 46.7% (178/381) were male; controls had an average age of 1.8 years (SD 0.9) and 86.4% (19/22) were male. Death in-hospital occurred in 6.3% (24/381) of cases, with no deaths in controls (table 3).

Of a total of 381 cases, 334 bone marrow aspirations were attempted, from which 303 marrow smears were prepared (fig 2). Forty-seven (12%) cases did not have a bone marrow aspiration for the following reasons: refusal by guardian, child being too sick, unsuccessful aspiration. Twenty-two of 23 (96%) controls had a bone marrow aspirate collected and smear prepared. Therefore 303 case smears and 22 control smears were available for assessment.

**Gale’s grading method**

From the total smears considered for assessment, 66 of 303 (22%) cases and 4 of 22 (18%) controls were not assessed because they contained inadequate bone marrow fragments for proper assessment. Assessment of the remaining smears showed that iron deficiency was present among 33.8% (80/237) of cases and 61.1% (11/18) of controls. According to conventional grading, iron stores deficiency was more frequent among the controls than cases, but this difference was not significant (OR = 1.8, 95% CI 0.8 to 4.25).

**Intensive grading method**

Staining quality was adequate to enable assessment of macrophage and erythroblast iron in 79% of cases (187/237) and 72% of controls (13/18). Cases and controls were classified into different iron status categories depending on iron assessment in fragment, macrophage and erythroblast as shown in table 4. Functional iron deficiency was the most common iron status category among both cases (39.6%; 74/187) and controls (46.2%; 6/13). Iron stores deficiency was less frequent among cases (21.9%; 41/187) than controls (38.5%; 5/13; p = 0.2).

Categories of iron status classified by the intensive grading method were compared with values for peripheral blood markers of iron stores (fig 3). Low levels of ferritin and high levels of sTfR signify deficiency of iron stores in the absence of inflammation. Mean ferritin concentration was lower in
children with deficiency of iron stores (1.9 (SD 0.7) µg/l) than in those with no deficiency (2.8 (SD 0.5) µg/l, p = 0.05), or with functional iron deficiency (2.6 (SD 0.6) µg/l, p<0.001). Children with deficiency of iron stores had a higher mean concentration of sTfR (21.7 (SD 12.5) µg/l) than those with normal iron stores (12.5 (SD 16.2) µg/l, p<0.001), or functional iron deficiency (11.4 (SD 6.0) µg/l, p<0.001). Children with functional iron deficiency had increased mean levels of CRP (126.7 (SD 85.6) mg/l) compared to those with iron stores deficiency (71.9 (SD 74.7) mg/l, p = 0.001), or normal iron status (99.8 (SD 70.1) mg/l, p = 0.01).

Ferritin, sTfR and CRP were determined for normal iron and iron deficiency states classified by Gale’s grading method (table 5). Mean log ferritin was higher in those with normal status cases compared to those who were iron deficient. The converse was true for mean sTfR levels. Mean CRP levels were increased in children with normal iron status compared to those classified as iron deficient (p<0.001).

**DISCUSSION**

The intensive histological grading method attempts to distinguish four different iron states compared to the two categories using Gale’s method. The ability to distinguish states in which there is decreased cellular iron delivery to erythroblasts in the presence of adequate iron stores (termed *functional iron deficiency*), compared to states with limited availability due to lack of available iron in the reticular endothelial system, is of particular importance in areas of high malaria transmission and infection. Although some of the results of the biochemical tests are lacking and some of the aspirates were too poor to examine for iron, this study managed to assess a substantive number of bone marrow aspirates. There were no identifiable reasons for selection bias for the missing results.

Functional iron deficiency, classified using the intensive histological grading method, was based on marrow findings alone. Although levels of CRP, a marker of an acute phase response, were mostly increased in all children, the finding of significantly raised levels among children with functional iron deficiency supports the hypothesis that these children have anaemia of inflammation. These children appeared also to have adequate iron stores as they had similar levels of ferritin and sTfR to children with normal iron status.

The use of erythroblast iron to assess iron status has been used in other studies and has certain limitations. Marrow smears were counter-stained with Safranin, giving a uniform

### Table 4: Bone marrow iron status category results using the intensive grading method

<table>
<thead>
<tr>
<th>Iron status category</th>
<th>Cases (n = 187)</th>
<th>Controls (n = 13)</th>
<th>OR* (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional iron deficiency (%)</td>
<td>39.6</td>
<td>46.2</td>
<td>0.7 (0.2 to 2.9)</td>
<td>0.9</td>
</tr>
<tr>
<td>Normal (%)</td>
<td>31.0</td>
<td>7.7</td>
<td>5.4 (0.8 to 33.6)</td>
<td>0.1</td>
</tr>
<tr>
<td>Iron stores deficiency (%)</td>
<td>21.9</td>
<td>38.5</td>
<td>0.5 (0.1 to 1.9)</td>
<td>0.2</td>
</tr>
<tr>
<td>Functional and stores deficiency (%)</td>
<td>7.5</td>
<td>7.7</td>
<td>1.0 (0.1 to 44.4)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*OR = [odds in cases]/[odds in controls].

### Table 5: Mean levels of Log ferritin, sTfR and CRP for iron status categories using Gale’s and Intensive grading methods

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>Gale’s grading method</th>
<th>Intensive grading method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Iron deficiency</td>
</tr>
<tr>
<td>Log ferritin (µg/l)*</td>
<td>Mean (SD) [n] 2.7 (0.5) [89]</td>
<td>1.8 (0.7) [61]</td>
</tr>
<tr>
<td>sTfR (µg/ml)†</td>
<td>Mean (SD) [n] 11.8 (10.7) [156]</td>
<td>22.7 (15.9) [86]</td>
</tr>
<tr>
<td>CRP (mg/ml)‡</td>
<td>Mean (SD) [n] 122.0 (67) [156]</td>
<td>71.9 (8.5) [86]</td>
</tr>
</tbody>
</table>

*Normal >30 µg/l.
†Normal <8.3 µg/ml.
‡Normal <10 mg/ml.

sTfR, soluble transferrin receptor; CRP, C-reactive protein.
Take-home messages

- Differentiation between functional iron deficiency and quantitative deficiency of iron stores is difficult, especially in areas of high infection pressure.
- A new method of grading of iron content of fragments, macrophages and erythroblasts in the bone marrow is able to distinguish between functional and quantitative iron deficiency in anaemic children.
- Severely anaemic Malawian children have less quantitative iron deficiency than controls without severe anaemia.

The use of haematoxylin, or May–Gruenwald–Giemsa, for pink background colour, which makes visualisation of cell types difficult, and hence may affect erythroblast iron assessment. The use of haematoxylin, or May–Gruenwald–Giemsa, for counter-staining smears has been recommended as this provides improved cellular detail.18 Tham and Macon23 demonstrated that use of a silver stain to visualise erythroblast iron was more assessed using Gale’s grading method.

Depending on the amount and morphology of iron granules, which assigned an arbitrary value, ranging from 1 to 4 understood, but may support the hypothesis that iron detailed marrow examination. Additionally this study observes settings that are based on simple, less invasive procedures than Methods to identify these children are required in poor-resource has shown that approximately 30% of severely anaemic children anaemia. In practice, most children are prescribed iron, however whether to give iron in the management of children with severe study.


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