Erythroid Marrow Function in Anemic Patients

By Mario Cazzola, Pensri Pootrakul, Helmut A. Huebers, Mary Eng, Joseph Eschbach, and Clement A. Finch

Erythropoietic activity is known to be closely associated with marrow iron uptake. A modification of the standard measure of plasma iron turnover has been developed in which erythron transferrin uptake (ETU) rather than iron uptake has been calculated. The ETU has the advantage of providing a parameter of erythroid marrow activity independent of change produced by plasma iron and transferrin saturation. Measurements in 80 patients with anemia were compared to the normal value of 60 ± 12 μmol/L whole blood/d. The mean ETU for ten patients with severe aplastic anemia and for six patients with pure red-cell aplasia were 12 ± 8 and 12 ± 11 μmol/L whole blood/d, respectively. In ten transfusion-dependent patients with renal failure under dialysis therapy, the mean value was 35 ± 11, while ten other diazoyed patients who were transfusion independent had a mean ETU of 73 ± 21 μmol/L whole blood/d. Sixteen patients with hemolytic anemia had an average ETU of 400 ± 130, while 28 patients with ineffective erythropoiesis had a mean value of 474 ± 147 μmol/L whole blood/d. While patients with hypoproliferative anemia showed no relation between the severity of anemia and ETU, those with hyperproliferative erythroid marrow showed increasing values as the anemia became more severe. Sequential measurements in patients with aplastic anemia under treatment and in thalassemic patients under transfusion therapy showed the value of this measurement in monitoring the effects of treatment on erythroid marrow activity. It is concluded that the measurement of ETU provides a more direct ferrokinetic evaluation of erythroid activity in anemic states.

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DISORDERS of erythroid marrow function may be characterized as affecting marrow proliferation and/or red cell maturation. For clinical purposes the former is evaluated by marrow aspiration or biopsy, and the latter by the relationship between the reticulocyte index and marrow proliferation as judged by marrow examination. Limitations to the quantitation of erythrocyte marrow activity from a minute sample have led to a continued search for a more adequate means of estimating erythroid marrow activity. Beginning with the original measurements of Huff and associates, the measurement of plasma iron turnover has been continually refined to reflect more quantitatively erythron iron uptake.

In the last few years, attention has focused on the reaction between transferrin iron and its membrane receptors. The assumption of a single plasma iron pool, so essential to previous kinetic measurements, has been shown to be erroneous. There are actually two plasma iron pools, one composed of monoferric transferrin and the other of diferric transferrin. The diferric molecule has a greater capacity to deliver iron to tissue receptors than the monoferric species. Since the in vivo loading of iron on transferrin is a random phenomenon, the quantitative relationship between these two plasma iron pools and their respective iron clearance is predictable. The critical reaction is the uptake of the transferrin-iron-receptor complex into the cell, and this occurs at the same rate, regardless of whether there are one or two molecules of iron on the transferrin molecule. In a recent study dealing with normal subjects, a simple approach to the measurement of transferrin-receptor interaction in normal man was presented, and the transferrin uptake was shown to be independent of plasma iron concentration and transferrin saturation. This approach has now been extended to the measurement of the erythron transferrin uptake in anemic patients.

MATERIALS AND METHODS

Patients. Ferrokinetic studies were performed in a total of 80 patients selected to provide extremes in erythropoiesis. A first group included ten individuals with severe aplastic anemia (AA) and six with pure red-cell aplasia (PRCA). Criteria for the diagnosis of severe aplastic anemia included a hypocellular bone marrow on bone marrow biopsy (less than 30% cellularity) and two of the following three factors: reticulocyte index <1, granulocytes <0.5 x 10^9/L, and platelets <20 x 10^9/L. Criteria for the diagnosis of PRCA included the virtual absence of erythroid cells in the marrow but abundant marrow precursors for other cell species, a transfusion-dependent anemia associated with a decrease in reticulocyte index below 1, the presence of circulating granulocytes >2 x 10^9/L, and platelets >100 x 10^9/L. In addition to the above criteria and in order to select the most severely affected individuals, only those with less than or equal to 10% of injected radioiron in circulating red cells at two weeks were included in this first group. A second group was composed of 20 patients with severe renal disease with azotemia, all of whom were on renal dialysis. Within this group ten patients needed repeated transfusions to maintain their hematocrit over 20%, and ten other patients did not require transfusion.

The remaining 44 patients had anemia associated with erythroid marrow hyperplasia. There were 16 patients with hemolytic anemia, eight of whom had sickle cell disease, four hereditary spherocytosis, two red cell enzyme defects (pyruvate kinase deficiency, glucose 6-phosphate isomerase deficiency), and one autoimmune hemolytic ane-
mia, and one fragmentation hemolysis. Another group of 28 patients demonstrated ineffective erythropoiesis with erythroid marrow hyperplasia. Ten of these patients had β-thalassemia intermedia with hemoglobin concentrations between 6 and 9 g/dL, while 16 patients had β-thalassemia/Hemoglobin E disease, the criteria for which have been described elsewhere.17

**Ferrokinetic measurements.** Details of the measurement of plasma iron turnover have been previously summarized.18 In these studies it was standard practice to inject 59FeSO4 (0.2 µg containing 2 µCi 59Fe at pH 2) intravenously (IV) over a period of five minutes. However, in individuals with transferrin saturation >70%, radioiron was bound to normal plasma in vitro, and then the tracer saturation was adjusted to that of the patient’s own plasma using cold ferrous ammonium sulfate.19

Plasma iron turnover was routinely calculated employing the formula:20

\[
PIT (\text{mg/dL whole blood/d}) = \frac{\text{ PI (µg/dL) } \times (100 - \text{Hct} \times 0.9)}{\text{T1/2 (min) } \times 100}
\]

This formula is suitable for turnover measurements as long as the blood volume (BV) is not significantly different from that predicted (±10%). A comparison was made of the determined BV based on the dilution of the injected isotope, as compared to the predicted BV.20 Where there was a significant increase (>10%), the observed PIT was corrected by multiplying it by the ratio between determined and predicted plasma volume for the patient’s plasmacrit.19

In some instances the radioiron red-cell utilization (RCU) was calculated from the red cell activity at 14 days and the amount of radioactivity injected as described by Cook and Finch.19 Additional calculations, discussed more in detail elsewhere, were made to convert the plasma iron turnover to the transferrin-iron complex uptake by the erythron. This involved (a) the subtraction of the extravascular plasma flux (EVF), (b) a correction designed to convert tissue iron uptake (IU) to tissue transferrin uptake (TU), and (c) the subtraction of nonerythroid transferrin uptake to leave erythron transferrin uptake (ETU).21

\[
\text{EVF (mg/dL wb/d)} = \frac{\text{ PI (µg/dL)} \times (100 - \text{Hct} \times 0.9)}{100} \times 0.0015
\]

\[
\text{IU (mg/dL wb/d)} = \text{PIT - EVF}
\]

\[
\text{TU(µmol/L wb/d)} = \frac{\text{IU(µg/dL wb/d)} \times 10,000}{56} \times \frac{200 + 2.25}{200 + 6.45}
\]

where S is the percent transferrin saturation, and wb/d stands for whole blood/day.

The final correction to allow for transferrin-iron going to non-erythroid receptors is made by subtracting the mean volume value of 11 µmol/L whole blood/day21 from the calculated transferrin uptake:

\[
\text{ETU (µmol/L wb/d)} = \text{TU (µmol/L wb/d)} - 11
\]

Plasma iron and transferrin saturation were measured as described elsewhere.15,22 Hematocrit was determined by the micro-method. 59Fe activity was determined by gamma counting. Plasma volume was calculated from the 59Fe-transferrin dilution.19

**Statistical analysis.** Statistical analysis was performed using the CLINFO computer system (University of Washington, Seattle, WA). All results are given as mean ± 1 SD. The significance of the differences between means was tested by the Student’s t test.

**RESULTS**

Data obtained in 80 subjects are summarized in Table 1. Ten patients with aplastic anemia had an average transferrin saturation of 90% ± 9%, a mean plasma iron turnover of 0.57 ± 0.10 mg/dL whole blood/d, and a mean ETU of 12 ± 8 µmol/L whole blood/d. Radioactivity in the blood at two weeks amounted to an average of 4% ± 4% (range 0% to 10%). Six additional patients with PRCA had a mean transferrin saturation of 94% ± 5%, a mean plasma iron turnover of 0.57 ± 0.15 mg/dL whole blood/d, an ETU of 12 ± 11 µmol/L whole blood/d, and a mean red-cell utilization at two weeks of 3% ± 3% (range 0% to 6%). Thus, in these subjects the mean values for PIT were 80% of normal whereas ETU values averaged 20% of normal.

Ten patients with transfusion-dependent renal failure under continuous dialysis treatment had a mean transferrin saturation of 80% ± 15%, a mean plasma iron turnover of 0.73 ± 0.16 mg/dL whole blood/d, and an average ETU of 35 ± 11 µmol/L whole blood/d. Red cell utilization in the ten patients averaged 26% ± 10% (range 12% to 47%). By contrast, a second group of dialyzed patients who were able to sustain their hematocrit without transfusion had a mean transferrin saturation of 28% ± 6%, a mean PIT of 0.77% ± 18% mg/dL whole blood/d, and a mean ETU of 73 ± 21 mmol/L whole blood/d. Their red cell utilization averaged 71% ± 13% (range 55% to 89%). Whereas there was no difference between the mean PITs of the two groups of renal patients (t = 0.55, P > 0.05), the mean ETU of those patients who did not need blood transfusions was significantly higher than that of transfusion-dependent patients (t = 5.17, P < 0.0001).

Sixteen patients with hemolytic anemia were heterogeneous in respect to the cause of their anemia, to its degree (hematocrit varied from 18% to 35.5%), plasma iron concentration (46 to 279 µg/dL), and transferrin saturation (11% to 91%). Plasma iron turnover averaged 3.86 ± 1.45 mg/dL whole blood/d but ranged from 1.26 to 6.64 mg/dL whole blood/d. ETU averaged 400 ± 130 µmol/L whole blood/d but ranged from 168 to 612 µmol/L whole blood/d.

A similar heterogeneity was observed in 28 patients with ineffective erythropoiesis. Hematocrits varied from 15.1% to 34.5%, plasma iron from 52 to 308 µg/dL, and transferrin saturation from 16% to 98%. Mean PIT was 5.11 ± 1.85 mg/dL whole blood/d with a range from 1.40 to 9.23, and ETU values averaged 474 ± 147 µmol/L whole blood/d with a range from 176 to 809.

All patients with aplastic anemia, pure red-cell aplasia, and renal failure associated with anemia had erythroid marrow activity as judged by ETU between 0 and 1.6 × basal. On the other hand, patients with hemolytic anemia and ineffective erythropoiesis showed values between 2.8 and 13.5 times basal (all values but two being greater than 3). When ETU was plotted against the hematocrit (Fig 1), there was no apparent relationship among hyperproliferative patients between the severity of the anemia and ETU (r = 0.32, P = 0.06), whereas there was an inverse correlation among hyperproliferative patients (r = −0.71, P < 0.001).

Sequential studies were carried out in five patients with pure red-cell aplasia under treatment after an appropriate interval of time (Fig 2). These measurements showed no change in marrow activity in two patients and improvement in three by both improvement in hematocrit and clearly...
Table 1. Ferrokinetic Studies

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hct (%)</th>
<th>Retic Index (x basal)</th>
<th>Plasma Iron (μg/dL)</th>
<th>Tf Sat. (%)</th>
<th>T-1/2 (min.)</th>
<th>RCU (%)</th>
<th>PIT (mg/dL)</th>
<th>ETU (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Subjects (53)</td>
<td></td>
<td>0.7 ± 0.2</td>
<td>274 ± 9</td>
<td>90 ± 9</td>
<td>376 ± 88</td>
<td>4 ± 4</td>
<td>0.57 ± 0.10</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>Aplastic Anemia (10)</td>
<td></td>
<td>0.5 ± 0.2</td>
<td>22 ± 7</td>
<td>55 ± 5</td>
<td>285 ± 85</td>
<td>5 ± 5</td>
<td>0.71 ± 0.17</td>
<td>60 ± 12</td>
</tr>
<tr>
<td>Hemolytic Anemia (16)</td>
<td></td>
<td>0.7 ± 0.2</td>
<td>274 ± 9</td>
<td>90 ± 9</td>
<td>376 ± 88</td>
<td>4 ± 4</td>
<td>0.57 ± 0.10</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>Renal Failure A (10)</td>
<td></td>
<td>0.7 ± 0.2</td>
<td>274 ± 9</td>
<td>90 ± 9</td>
<td>376 ± 88</td>
<td>4 ± 4</td>
<td>0.57 ± 0.10</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>Renal Failure B (10)</td>
<td></td>
<td>0.7 ± 0.2</td>
<td>274 ± 9</td>
<td>90 ± 9</td>
<td>376 ± 88</td>
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</tr>
</tbody>
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Average values are given.
demonstrable increases in marrow erythroid activity. Sequential measurements in patients with thalassemia or sickle cell anemia undergoing transfusion therapy showed the suppressive effect of higher hematocrit on erythroid activity but erythropoietic activity was still two to three times basal for hematocrit values around 40% (Fig 3). In these latter studies there was a close correlation ($r = 0.86, P < 0.01$) between ETU and erythroid: myeloid ratio of the marrow.

**DISCUSSION**

Within the circulating blood there is a constant flow of iron from donating cells via transferrin to membrane transferrin receptors of tissues requiring iron. The marrow normally receives about four fifths of all iron passing through
known to be due to thyroid iron uptake, to

tion." Cook et al thought this was due with increasing plasma iron

have previously shown that the plasma iron between transferrin iron and its membrane receptors. We
cular on based

between plasma iron and extravascular flux.4 Transferrin uptake so derived was found to be independent of plasma iron and transferrin saturation, and this suggested that the critical measurement of erythroid marrow function could be best described by erythroid transferrin uptake once allowance had been made for nonerythroid transferrin uptake. The nonerythroid tissues of the body have a limited capacity to assimilate iron. After injection of radioiron, normal subjects have only about 15% of the activity outside of the red cell mass at two weeks. Furthermore, there is little evidence to date that these nonerythroid receptors, most of which reside in the liver, can change greatly in number except in the presence of iron deficiency (Tanin et al, unpublished data). This means that the number of iron-bearing transferrin molecules reacting with membrane receptors on nonerythroid tissues can be considered relatively constant, and the mean normal value of 11 μmol transfernin/L whole blood/d has been assumed in the present study.13 According to these calculations, erythron transferrin uptake in normal subjects averages 60 ± 13 μmol/L whole blood/d.

A first opportunity to examine the appropriateness of the various corrections made to obtain the ETU was provided by patients with severe aplastic anemia and pure red-cell aplasia. Among the 16 patients studied, whose plasma iron turnover ranged from 0.38 to 0.77 mg/dL whole blood/d, values for ETU ranged from 0 to 28 μmol/L whole blood/d. No patient had a value below 0, indicating that estimates of nonerythroid iron turnover were not overcorrected. The red cell utilization of 3% to 4% when compared to the mean ETU of 12% the basal value suggested that a major portion of the residual erythroid activity in these patients did not result in the production of viable red cells. Evidence of dyspoiesis in aplastic anemia has been reported.13,24

Studies in renal patients provided another opportunity to compare the clinical status with the ferrokinetic evaluation of erythropoiesis. Renal patients have a combination of impaired marrow stimulation due primarily to inadequate erythropoietin production and also increased red cell hemolysis, but erythropoiesis is effective.14 Studies of two groups of dialyzed patients, one self-sufficient and the other transfusion dependent, showed no difference in plasma iron turnover (0.77 ± 0.18 v 0.73 ± 0.16 mg/dL whole blood/d) but a conspicuous difference in erythroid activity in the two groups, ie, 73 ± 21 v 35 ± 11 μmol/L whole blood/d. Assuming that both had the same degree of shortening of red cell life span to about one-half normal, the difference in hemoglobin concentration was adequately explained by the greater impairment in production in the transfused group.

Patients with hemolytic anemia had an average ETU of 400 ± 130, and patients with ineffective erythropoiesis 474 ±
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147 μmol/L whole blood/d. These can be translated into mean erythropoietic rates of 6.7 and 7.9 times basal, respectively. In some of these patients the reduction of erythropoiesis after transfusion was measured (Fig 3). Quantitative aspects of the relationship between blood hemoglobin concentration and hematocrit or erythropoiesis were not well defined. There is ample clinical evidence that suppression of erythropoiesis by transfusion is required for thalassemic patients if distortion of the marrow and distortion of the skeleton are to be prevented. Previous measurements on patients with thalassemia were made by Cavill et al25 at hemoglobin levels from 9 to 17 g/dL. According to their detailed analysis of the plasma iron disappearance curve, erythropoiesis as judged by marrow iron turnover became normal or subnormal at a blood hemoglobin concentration of 11 to 12 g/dL. They comment, however, that the marrow still showed normoblastic hyperplasia. It might be anticipated from the type of analysis performed by them that ineffective erythropoiesis could not be completely separated from iron exchange with nonerythroid tissiu..., and this could cause total erythropoiesis to be underestimated.18,26 In studies reported here, erythropoiesis did decrease as the hemoglobin concentration was increased by transfusion but remained two to three times basal at a hemoglobin concentration of 11 to 12 g/dL, and parallel changes in erythroid: myeloid ratio validated the ferrokinetic measurement. Evaluation of erythroid activity can be of value in determining the degree of erythroid suppression after transfusion in individual patients with thalassemia major and also in deciding whether patients with thalassemia intermedia should be transfused.

Taken as a group, individuals with an impaired marrow response to anemia, ie, less than two basal times, showed no relationship between the ETU and hematocrit, the slope of the regression line being not significantly different from 0. This suggested that the impairment in production was the dominant feature in causing the anemia and that the erythroid marrow could not respond appropriately to the erythropoietin stimulus produced by anemia. In contrast, patients with an appropriate proliferation response to anemia showed an inverse relationship between hemoglobin concentration and erythropoietic response. This would be consistent with an increased marrow stimulation by erythropoietin as the hemoglobin fell.1 A similar relationship has been observed employing a more complex ferrokinetic approach.23

REFERENCES


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