HEMATOPATHOLOGY

Original Article

Reticulocyte Hemoglobin
An Integrated Parameter for Evaluation of Erythropoietic Activity

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Traditional reticulocyte counts provide only a partial estimate of the erythropoietic bone marrow activity and do not account for qualitative variations of reticulocyte cellular indexes and hemoglobin content in particular.

We have studied a new integrated parameter, reticulocyte hemoglobin (retHb), that quantifies in grams per liter the hemoglobin contained in the circulating reticulocyte compartment and is obtained by multiplying the absolute reticulocyte count and the reticulocyte cell hemoglobin content. In 50 normal control subjects, retHb values were 1.76 ± 0.59 g/L. The retHb values were lower in patients double heterozygous for HbS and HbC (SC disease) (3.33 ± 1.52 g/L, n = 13) compared with homozygous HbS disease (SS) with concomitant α-thalassemia (5.27 ± 1.51 g/L and 5.48 ± 1.06 g/L for 12 patients with 3 α-genes and 3 patients with 2 α-genes, respectively) and to SS disease with no α-thalassemia (6.47 ± 3.05, n = 20). The hemoglobin contained in the red blood cell pool (rbcHb) also can be calculated by subtracting retHb from the total hemoglobin. The ratio between the two pools (rbcHb/retHb, normal value 76.6 ± 21.9, n = 50) provides a rough estimate of red blood cell survival. It was 9.8 ± 4.1 in SS disease, 16.2 ± 10.1 and 14.7 ± 5.0 in SS disease with 3 and 2 normal α-genes, respectively, and 36.6 ± 17.8 in SC disease with no α-thalassemia. We also studied retHb in patients receiving hydroxyurea therapy for SS disease, intravenous or oral iron for iron deficiency, or recombinant human erythropoietin (r-HuEPO) therapy. All these conditions are characterized by changes in reticulocyte counts and marked variations in reticulocyte cellular hemoglobin contents, which can be integrated into the retHb parameter.

Measurement of retHb and the rbcHb/retHb ratio may provide an estimate of the reduction in red blood cell survival and the severity of hemolysis in various anemias and allow more precise monitoring of the response to hydroxyurea, iron, r-HuEPO, or other therapies. (Key words: Erythrocyte; Hematology; Laboratory hematology; Automated cell counting; Red blood cell survival; Sickle cell disease; Hemolysis; Erythropoietin; Iron deficiency) Am J Clin Pathol 1997;108:133–142.

The reticulocyte count is used to evaluate bone marrow erythropoietic activity. It provides valuable information in the differential diagnosis of anemias and in the monitoring of the response in patients treated with recombinant human erythropoietin (r-HuEPO). The manual reticulocyte count gradually has been replaced by automated counting.1-5 Automated techniques have better precision and reproducibility than the manual count6-8 and provide several new parameters. Reticulocyte analyzers that use fluorescent staining of reticulocytes provide a reticulocyte maturity index, which is used to quantitate the immature reticulocyte fraction and may be helpful in early detection of engraftment after bone marrow transplantation.9 Flow cytometric analysis of red blood cells allows quantification of their volume, hemoglobin concentration, and hemoglobin content.10-13 The oxazine 750 staining method also allows measurements of reticulocyte staining intensity and provides direct measurements of reticulocyte cellular indexes, such as reticulocyte mean cell volume (MCVr), reticulocyte cell hemoglobin concentration mean (CHCMr), and mean reticulocyte cell hemoglobin (CHr), with their respective distribution widths.14 We have described the changes in reticulocyte indexes in healthy subjects receiving r-HuEPO, and how the appearance of reticulocytes with abnormally low CHr indicates at an early stage the appearance of iron-restricted erythropoiesis.15 Because the percentage or absolute reticulocyte count does not account for variations in the cellular characteristics of reticulocytes, it would be desirable to have a global index for bone marrow erythropoietic activity, sensitive to variations in reticulocyte...
number and cellular characteristics. This index would be helpful to quantify precisely a dynamic erythropoietic response in the presence of a specific marrow stimulation as seen with administration of r-HuEPO, intravenous (IV) iron, or hydroxyurea, in which simultaneous variations in reticulocyte count and reticulocyte indexes occur. In addition, this index could allow comparisons of the relative balance of red blood cell survival and erythropoiesis in different kinds of anemias.

We report some examples of the usefulness of reticulocyte hemoglobin (retHb), a direct measurement of the total amount of hemoglobin contained in all reticulocytes obtained from the measured absolute reticulocyte count and the measured CHr.

MATERIALS AND METHODS

Reticulocyte Studies in Patients With Sickle Syndromes and Other Hematologic Disorders

Complete blood cell (CBC) and reticulocyte counts from patients with various hematologic disorders were studied. Data from children were obtained from samples collected during regularly scheduled outpatient visits. Blood samples were obtained from adult patients with sickle cell (SC) disease when they were in their usual state as outpatients. α-Globin genotypes were determined by Southern blot hybridization of genomic DNA using the restriction endonucleases Bam HI and Bgl II and the α-cDNA JW101 plasmid probe.

Hydroxyurea Treatment of Patients With SS Disease

Informed consent for the study was obtained from each patient, consistent with institutional guidelines. Three patients with homozygous HbS (SS) disease were studied; none had α-thalassemia. Each had a history of severe, recurrent, vaso-occlusive pain that required three or more hospitalizations per year. No patient had a history of acute chest syndrome or stroke. No patient had received chronic transfusion therapy or had been previously treated with hydroxyurea or other antisickling agents. Therapy with hydroxyurea was started at a daily dosage of 20 mg/kg. No patient required dosage adjustment during the study, and none required phlebotomy.

r-HuEPO Administration to Healthy Human Subjects

Twenty-four healthy, nonsmoking, men with normal CBC counts, renal function, serum iron levels, total iron binding capacity, serum ferritin levels, vitamin B12 levels, and folate levels were randomly assigned to receive subcutaneous r-HuEPO (Procrit, Ortho Biotech, Raritan, NJ) according to one of three dosage regimens: (1) group A, 300 U/kg on days 1, 4, 7, and 10; (2) group B, 400 U/kg on days 1, 5, and 9; and (3) group C, 600 U/kg on days 1 and 10.

All subjects received iron therapy twice daily with iron-polysaccharide (Niferex-150, Central Pharmaceuticals, Seymour, Ind), a total dose of 300 mg of elemental iron per day, starting on day 1. Subjects were required to have a baseline hematocrit (Hct) less than 48% (0.48). If the Hct rose to more than 55% (0.55) during r-HuEPO administration, the study protocol required phlebotomy of 1 unit (450 mL) of blood. One subject in group C was phlebotomized for 1 unit of red blood cells on day 13 because of a Hct of 55.2% (0.55).

The protocol for this study was reviewed and approved by the human research committee of Brigham and Women’s Hospital (Boston, Mass) and was in accordance with an assurance filed with, and approved by, the Department of Health and Human Services (Washington, DC). The results of this study have been reported in two separate publications.

Iron Replacement Therapy in Severe Iron Deficiency

A patient with polycystic ovaries and menorrhagia was initially examined because of a hemoglobin level of 7.9 g/dL (71 g/L), Hct of 28.5% (0.28), serum iron level of 4 μg/100 mL (0.72 mmol/L), and serum ferritin level of 2 ng/mL (2 mg/L). Treatment with oral contraceptive agents and two oral iron preparations was unsuccessful, and the patient was treated with intravenous (IV) iron dextran.

Ten iron-deficient women were studied at baseline and after 1 or 2 weeks of oral iron replacement therapy (ferrous gluconate, 324 mg twice daily). The response to therapy was monitored with serial CBC and reticulocyte counts. Data on these patients have been reported in a previous publication.

Reticulocyte Analysis With the Bayer H*3 Hematology Analyzer

Reticulocyte indexes were measured on a Bayer H*3 RTX (H*3) Hematology Analyzer (Bayer Diagnostic, Tarrytown, NY). Whole blood, collected in potassium EDTA, was diluted 1:1,000 with Bayer Reticulocyte Reagent (Product No. T03-3392-50, Bayer Diagnostics). The reagent isovolumetrically spheres
the red cells using the zwitterionic detergent, N-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, and selectively stains the reticulocytes using the dye oxazine 750. The stained reticulocytes were discriminated from mature red blood cells by their increased absorption of light.

Laser light scattering was used to quantify cell volume, hemoglobin concentration, and the light absorbance of cells stained with oxazine 750 to detect reticulocytes and distinguish them from mature cells. A total of 20,000 cells were counted for each sample. Light absorbance is measured over 100 discrete channels. The gate for reticulocytes is established based on the distribution of the negative cells. To establish the three levels of staining intensity, the number of channels between the threshold for reticulocytes and channel 80 (8) is divided by 3. Low staining intensity and medium staining intensity reticulocytes constitute all the positive cells between threshold, threshold plus 1/3 8, and threshold plus 2/3 8, respectively. High-staining intensity reticulocytes constitute all the positive cells between threshold plus 2/3 8 and channel 100. The amount of light absorbed by the reticulocyte is directly proportional to its RNA content.

After the volume (V) and hemoglobin concentration (HC) of individual mature red blood cells and reticulocytes is measured, the hemoglobin content (CH) of individual cells is calculated by the formula: $V \times HC = CH$. Histograms are generated, and distribution widths (DWs) are calculated for each of the measured indexes: cell volume (mean cell volume [MCV] and RDW; MCVr and RDW r for reticulocytes), cell hemoglobin concentration (CHCM and HDW; CHCMr and HDW r for reticulocytes), and cell hemoglobin content (mean cell hemoglobin [MCH] and CHDW; Chr and CHDWr for reticulocytes).

From the absolute reticulocyte count and the CHr, the retHb is calculated, which expresses in grams per liter the hemoglobin content of all reticulocytes. By subtracting from the total hemoglobin the retHb, the amount of hemoglobin contained in the mature red blood cells (rbcHb) can be calculated. The ratio of rbcHb to retHb (all values expressed as g/L) defines the ratio between the hemoglobin contained in the mature erythrocytes and that contained in reticulocytes.

Data Analysis

Kinetic data analysis was performed with PKAnalyst software 1.0 (MicroMath Scientific Software, Salt Lake City, Utah).

RESULTS

retHb and rbcHb/retHb in Sickle Syndromes and Other Hematologic Diseases

Table 1 presents data on the values for retHb, rbcHb, and the rbcHb/retHb ratio in healthy adult control subjects and in adult patients with sickle syndromes. Patients with homozygous HbS disease (SS) were grouped according to hemoglobin α-chain status. There is a significant increase in retHb and reduction in the rbcHb/retHb ratio in patients with sickle syndromes compared with normal controls. The data reveal significant differences in retHb between SC and SS disease, as well as significant differences in the rbcHb/retHb ratio among the four groups. In particular, there is an increase in the rbcHb/retHb ratio with α-thalassemia and an additional increase of this ratio in SC disease. These data are in agreement with published differences on the red blood cell survival among these diseases.20 21

Table 2 presents data collected on healthy pediatric controls (n = 212; age, 3.2 ± 2.3 years) and children with hematologic diseases. A marked increase in retHb and a decrease in the rbcHb/retHb ratio are noticeable in the patients with SS disease or hereditary spherocytosis. In the patients with iron-deficiency anemia, there was a marked reduction in CHr.
TABLE 2. RETICULOCYTE INDEXES, retHb, AND rbcHb/retHb RATIO IN HEALTHY PEDIATRIC SUBJECTS AND CHILDREN WITH HEMATOLOGIC DISORDERS

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hemoglobin (g/L)</th>
<th>rbcHb (g/L)</th>
<th>Reticulocytes (x10⁹/mL)</th>
<th>CHr (pg)</th>
<th>retHb (g/L)</th>
<th>rbcHb/retHb Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control subjects</td>
<td>113 ± 9.0</td>
<td>111.3 ± 9.3</td>
<td>61 ± 22.5</td>
<td>26.5 ± 2.1</td>
<td>1.6 ± 0.6</td>
<td>78.5 ± 30.4</td>
</tr>
<tr>
<td>(n = 212)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>73.9 ± 15.3</td>
<td>65.8 ± 14.4</td>
<td>306.2 ± 118.1</td>
<td>26.0 ± 2.4</td>
<td>8.1 ± 3.4</td>
<td>10.6 ± 7.3</td>
</tr>
<tr>
<td>(n = 17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereditary spherocytosis</td>
<td>100.8 ± 29.8</td>
<td>91.4 ± 30.8</td>
<td>336.1 ± 94.4</td>
<td>28.2 ± 2.7</td>
<td>9.4 ± 2.4</td>
<td>10.8 ± 5.5</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>83.0 ± 13.0</td>
<td>81.5 ± 13.2</td>
<td>91.1 ± 35.8</td>
<td>17.2 ± 3.1</td>
<td>1.5 ± 0.4</td>
<td>59.0 ± 21.9</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

rbcHb = mature red blood cell hemoglobin; CHr = reticulocyte cell hemoglobin content; retHb = reticulocyte hemoglobin.

with normal retHb and a normal to slightly reduced rbcHb/retHb ratio, which may indicate a reduced survival of the iron-deficient erythrocytes.

**The Effect of Hydroxyurea Therapy on retHb and rbcHb/retHb**

Figure 1 shows data on three patients with SS disease who were treated with hydroxyurea. Before treatment, retHb was markedly increased and the rbcHb/retHb ratio was markedly diminished compared with healthy control subjects. Hydroxyurea therapy led to an early and substantial decrease in retHb (from 7.3 ± 1.0 to 3.3 ± 0.3 g/L, \( P < .02 \)). In these three patients, macrocytosis induced by hydroxyurea resulted in increased MCV and MCH for erythrocytes and increased MCVr and CHr for reticulocytes. The increase in CHr was more than compensated by the marked reduction in absolute reticulocyte count, which yielded a significant decrease in retHb. There was also a significant increase in the rbcHb/retHb ratio, (from 10.1 ± 1.4 to 23.2 ± 0.4, \( P < .005 \)), suggesting an improved red blood cell survival (see Fig 1).

**The Effect of r-HuEPO on retHb in Healthy Subjects**

Twenty-four healthy men were studied before and during administration of r-HuEPO. They were divided into three groups, each receiving the same total dose of r-HuEPO (1,200 U/kg, subcutaneously), but with three different administration schedules. Figure 2 shows the effect of r-HuEPO administration on reticulocyte parameters and hemoglobin in group B. The increase in the absolute reticulocyte count induced by r-HuEPO is associated with significant changes in CHr, which decreased significantly.
Fig 2. Changes in absolute reticulocyte count, reticulocyte cell hemoglobin (Hb) content (CHr), hemoglobin and reticulocyte hemoglobin (retHb) in a group of eight healthy subjects treated with recombinant human erythropoietin (group B, 400 U/kg on days 1, 5, and 9). rbcHb = mature red blood cell hemoglobin; CHr = reticulocyte cell hemoglobin content; retHb = reticulocyte hemoglobin.

with r-HuEPO administration, indicating the appearance of functional iron deficiency. Changes in these two indicators are combined into the retHb parameter (see Fig 2). The response of retHb to r-HuEPO was analyzed with classic pharmacokinetic analysis, by determining the area under the curve (AUC), and the activation rate (K_a) and time to reach maximum effect (T_max) for marrow stimulation by r-HuEPO and the elimination rate (K_e) and t_{1/2} (half-life) for the return to the basal state (Table 3). No differences could be found among the three groups for the rate of increase in retHb production or its T_{max}. By comparing the corrected retHb AUC to the measured increase in hemoglobin, it is possible to estimate the residence time of reticulocytes in the peripheral circulation: the ratio of retHb AUC vs the increase in total hemoglobin varied between 2.20 and 2.71 (see Table 3). This could indicate that reticulocytes may remain in the peripheral circulation for 2 to 3 days after r-HuEPO administration.

The production of retHb was positively correlated to the log of baseline serum ferritin (r = 0.677, P<.01), suggesting that healthy subjects with higher ferritin levels, and most likely higher iron stores, tend to produce more retHb. In our previous reports on this clinical study, a positive correlation between baseline serum ferritin levels and increase in Hct (but not hemoglobin) also had been found.18

The Effect of Iron Replacement Therapy on retHb in Severe Iron Deficiency

Figure 3 presents changes in reticulocyte counts, indexes, and retHb after IV iron administration in a
**TABLE 3. ANALYSIS OF retHb RESPONSE TO r-HuEPO ADMINISTRATION IN 24 HEALTHY SUBJECTS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>300 U/kg on 4 Days</th>
<th>400 U/kg on 3 Days</th>
<th>600 U/kg on 2 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin increase (g/L)</td>
<td>6 ± 5</td>
<td>6 ± 12</td>
<td>12 ± 7</td>
</tr>
<tr>
<td>Blood loss due to sampling (g/L)</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Corrected hemoglobin increase (g/L)</td>
<td>15</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Corrected retHb AUC (g/L)</td>
<td>36</td>
<td>40.6</td>
<td>46.2</td>
</tr>
<tr>
<td>retHb AUC (corrected)/hemoglobin increase</td>
<td>2.40</td>
<td>2.71</td>
<td>2.20</td>
</tr>
<tr>
<td>retHb increase: $K_a$</td>
<td>0.139</td>
<td>0.154</td>
<td>0.151</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>5.53</td>
<td>5.54</td>
<td>5.56</td>
</tr>
<tr>
<td>retHb decrease: $K_e$</td>
<td>0.231</td>
<td>0.21</td>
<td>0.212</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>3.0</td>
<td>3.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>

retHb = reticulocyte hemoglobin; r-HuEPO = recombinant human erythropoietin; AUC, area under the curve; corrected retHb AUC, AUC minus baseline retHb values; $K_a$, activation rate; $T_{max}$, time to reach maximum effect; $K_e$, elimination rate; $t_{1/2}$, half-life.

1Hemoglobin increase calculated as the difference between baseline and day 24.

Blood loss due to drawing blood was 250 mL (approximately 6% of total blood volume).

DISCUSSION

We have presented several applications of retHb, a new index of bone marrow erythropoietic activity, based on the measurement of CHr and the absolute reticulocyte count.

The retHb (in grams per liter) corresponds with the hemoglobin contained in all the circulating reticulocytes and allows partitioning of the total hemoglobin into its two pathophysiologically relevant components, retHb and rbcHb. Ratios of rbcHb to retHb allow quantification of the relative variations in these two hemoglobin pools.

Results for healthy subjects and patients with sickle syndromes disease, hereditary spherocytosis, and iron deficiency are reported in Tables 1 and 2. The data reveal that, although higher than that of healthy controls, retHb is lower in SC than SS disease (Table 1), and no differences were found among SS disease with various degrees of concomitant α-thalassemia. The rbcHb/retHb ratios indicated a progressive increase from SS disease, to SS with α-thalassemia, to SC disease, in agreement with published data on red blood cell survival for these sickle syndromes.

The ratio of rbcHb to retHb may provide a rapid means of estimating the extent of reduction in red blood cell survival, with lowest ratios obtained in patients with the most reduced survival. The rbcHb/retHb ratio was significantly increased in three patients treated with hydroxyurea (see Fig 1). We previously showed that hydroxyurea therapy is associated with a significant improvement of red blood cell survival for these sickle syndromes. The ratio of rbcHb to retHb may provide a rapid means of estimating the extent of reduction in red blood cell survival, with lowest ratios obtained in patients with the most reduced survival. The rbcHb/retHb ratio was significantly increased in three patients treated with hydroxyurea (see Fig 1). We previously showed that hydroxyurea therapy is associated with a significant improvement of red blood cell survival for these sickle syndromes.
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blood cell survival.\textsuperscript{16,22} This new ratio may allow an estimate of changes in red blood cell survival without performing Chromium-51 survival studies. Hydroxyurea therapy was also associated with a significant reduction in retHb (see Fig 1).

A significant reduction in retHb and increase in the rbcHb/retHb ratio has been observed in patients with SC disease on treatment with hydroxyurea.\textsuperscript{23}

The retHb allows estimation of the erythropoietic response to r-HuEPO and to iron therapy (see Figs 2-4, Table 3). In these settings, absolute reticulocyte counts are less informative because there are significant changes in the cellular characteristics and the cell hemoglobin content of reticulocytes.\textsuperscript{15,19} We used classic pharmacokinetic analysis to assess the characteristics of the stimulated retHb production. The AUC for retHb provides an estimate of erythropoietic activity for the selected time window. The AUC values for retHb reflect at least three major events that affect the movement from the reticulocyte to the mature red blood cell pool: (1) the rate of increase and final maximal stimulation of bone marrow activity that lead to the rise in retHb; (2) the decrease in retHb resulting from the maturation of reticulocytes into the red blood cell pool; and (3) possible intravascular or extravascular elimination of reticulocytes. The rate for the increase and decrease in retHb may provide information on the maximal stimulated activity of the bone marrow. In the three groups of subjects receiving r-HuEPO, there was a twofold to threefold stimulation of retHb production, with a $t_{\text{max}}$ of 5.5 days, while the $t_{1/2}$ for turning off the erythropoietic response varied between 3 and 3.3 days. A similar value for $t_{1/2}$ was found after IV iron therapy in severe iron deficiency (3.3 days).

Because only a variable fraction of nonuremic patients responds to r-HuEPO, a reliable indicator of response is needed to optimize the use of this costly drug. Previous studies have identified absolute reticulocyte counts and increase in total hemoglobin at 4 weeks of treatment as reliable indicators of response in cancer patients.\textsuperscript{24} However, earlier identification of nonresponders would reduce potential wasting of r-HuEPO and allow for the prompt use of a more aggressive iron supplementation when indicated. Future studies will determine whether retHb can be used to monitor the response to r-HuEPO, and the potential advantages of this approach should be compared with other potential indicators of response to r-HuEPO, such as levels of serum ferritin, serum erythropoietin, or serum circulating transferrin receptor levels.\textsuperscript{25,26}

Iron deficiency is characterized by the production of reticulocytes with reduced volume, hemoglobin concentration, and content (see Fig 3).\textsuperscript{27,28} In the presence of megaloblastic anemia, response to therapy is characterized by the production of reticulocytes that have normal red blood cell indexes, but are smaller than the macrocytic red blood cells.\textsuperscript{29} Use of IV iron induced the production of macroreticulocytes, with
normal CHCMr and CHr (see Fig 3). The ratio of retHb AUC to hemoglobin increase (a possible estimate of the time needed for reticulocytes to become mature cells) yielded for the data in Figure 3 a value of 1.67, which is lower than those observed in the healthy control subjects treated with r-HuEPO (see Table 2). This may indicate that the reticulocytes induced by IV iron treatment of iron deficiency have a shorter maturation time than those induced by r-HuEPO in healthy subjects.

A typical reticulocyte matures in 4 days and spends only the last 24 hours in the circulation. Reticulocyte size has been indirectly measured and studied in various anemias. Stress reticulocytes are macroreticulocytes produced in conditions of enhanced erythropoietic activity. They contain more residual RNA than normal reticulocytes, and they stain more intensely. The survival of reticulocytes and red blood cells generated by stress erythropoiesis (including r-HuEPO administration) may be reduced. In the mouse, stress reticulocytes disappear from the circulation in 32 to 36 hours, while macroreticulocytes disappear in 4 to 12 hours. The red blood cells derived from these macroreticulocytes had a reduced survival. In rats, the reticulocytes generated by r-HuEPO have MCV values that are almost double the normal values (100 fL vs 55 fL), and the derived red blood cells have a substantially reduced life span. Stress reticulocytes have been shown to undergo extensive remodeling and substantial intravascular hemolysis. In humans, the most immature reticulocytes are multilobar and motile and demonstrate a dramatic reduction in membrane deformability and mechanical stability. Extensive membrane remodeling of cytoskeletal proteins leads to the formation of the more mature reticulocytes. This maturation process occurs in the bone marrow unless the most immature reticulocytes are released into the circulation by stress erythropoiesis. The spleen also may have a role in the sequestration and maturation of reticulocytes.

A "corrected" % reticulocyte count can be obtained based on the hemoglobin or Hct values. The reticulocyte production index (RPI) also has been used to correct the reticulocyte count percentage for variations in red blood cell count and in reticulocyte maturation time. The RPI is obtained by dividing the percentage of reticulocytes by their maturation time and multiplying by the ratio of measured Hct to ideal Hct. The reticulocyte maturation time is assumed to increase to 1.5 days for Hct values of 35% (0.35), to 2 days for Hct values of
25% (0.25), and to 2.5 days for Hct values of 15% (0.15). A ratio of more than 2.5 is taken as an indication of hemolytic anemia. In a large group of patients with autoimmune hemolysis, the median RPI was 2.8 times the basal value, and differences in indirect bilirubin levels and transfusion and corticosteroid treatment rates were present in the patients with an RPI of 2.0 or less. However, RPI is not widely used and is seldom reported by laboratories. Only 55% of laboratories in the United States report reticulocyte results as absolute counts, whereas 33% still report and calculate a corrected reticulocyte count.

The retHb, and the rbcHb/retHb ratio are new indexes that allow a precise quantification of the relative distribution of hemoglobin into the mature red blood cell and the reticulocyte pool. These new indexes may be useful in monitoring conditions in which changes in reticulocyte counts are accompanied by changes in the reticulocyte cell Hb content, such as response to r-HuEPO, IV iron, or hydroxyurea therapy. In addition, these indexes may help quantify the differences in erythropoietic stimulation and red blood cell survival in hemolytic anemias.

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