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Erythrocytes

Clinical Applications of Automated Reticulocyte Indices

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Automated analysis of reticulocytes provides pathologists and clinicians with several new parameters, which need to be evaluated for their role in the diagnosis and management of diseases. We review here the current knowledge on reticulocyte cell volume, hemoglobin concentration and content. Several studies have provided reference values for reticulocyte cell volume (MCVr), cell hemoglobin concentration (CHCMr) and cell hemoglobin content (CHr). Data are available on the changes of these indices in iron deficiency and megaloblastic anemias and their response to therapy. CHr has been shown to be an early indicator of functional iron-deficiency in subjects treated with recombinant human erythropoietin (r-HuEPO). Reticulocyte changes have also been described in the early phases of hydroxyurea therapy for sickle cell disease and in bone marrow transplantation. The real-time information provided by reticulocyte indices on the functional state of the erythroid marrow is an important tool in the diagnosis and management of several hematological disorders and in the use of r-HuEPO.

Keywords: Reticulocyte, automated hematology, immature reticulocyte fraction

CLINICAL APPLICATIONS OF AUTOMATED RETICULOCYTE COUNTING INTRODUCTION

Early 19th century studies of reticulocytes focused on morphological descriptions of the appearance of the reticulum and hypotheses about the reticulum's nature, biological significance and physiology. Manual techniques for the examination and counting of reticulocytes were developed, which allowed identification of stages of maturation according to the amount of reticulum in the blood. Theories were formulated of the diagnostic value of the amount of reticulum present at a sign of blood regeneration [1].

More in-depth studies on the physical properties of reticulocytes, including shape, size, density and osmotic resistance, were carried out in the first decades of this century, but the

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results were not translated into clinically useful parameters. Only in the last decade has it been possible to obtain reliable automated counts of reticulocytes and just recently the measurement of reticulocyte volume and hemoglobin concentration has been obtained automatically with laser-based technology [2, 3].

This review attempts to correlate the old, forgotten knowledge concerning reticulocyte physical properties with the new measurement of reticulocyte indices. We will also discuss the diagnostic and clinical role of the parameters obtained with automated reticulocyte counting.

1) Physical Properties of Reticulocytes

A) Reticulocyte Size (Cell Diameter, Cell Surface Area, Cell Volume)

Reticulocytes are larger than mature erythrocytes and they gradually diminish in size during maturation [1, 4]. Reticulocyte diameter was initially determined using micrometric eyepieces on dry blood smears after staining the reticulum with a supravital dye. The diameter of normal reticulocytes was found to be about 8,5 μ, about 1–1,5 μ greater than that of their mature counterpart. The diameter of reticulocytes and mature erythrocytes increased in pathological conditions, such as megaloblastic and microcytic anemias, but the ratio between them remained constant.

These findings fit the theory of a progressive decrease in size as a general phenomenon of erythropoietic development. Mean reticulocyte diameter in bone marrow also appeared larger than that of peripheral blood, suggesting that maturation was completed in the circulation. Reticulocyte diameter also showed a progressive decrease through the different stages of the morphological classification proposed by Heilmeyer, especially from stage 0 (orthochromatic erythroblasts) to stage I, because of the loss of the nucleus, and from stage IV to mature erythrocyte [5].

These early studies of reticulocyte size lacked precision and accuracy because of the flattening, distortion, and shape changes induced by absorption of supravital dyes and subsequent drying on glass slides.

In the 1960 s photographic methods allowed studies of reticulocyte size on wet preparations. Photographs were obtained from fresh oxalated blood mixed with brilliant cresyl blue in isotonic saline which was then placed between a slide and coverslip [6]. In each sample 30–35 fields containing reticulocytes were photographed. Prints on glossy paper were enlarged with a final magnification of approximately 4000 x. The area of reticulocytes and adjacent red cells was determined planimetrically using an instrument called a Polarplanimeter. The coefficient of variation of the method ranged from 0.41% to 0.98%. These studies showed that the area of normal reticulocytes was on average 13.0% larger than that of mature erythrocytes (range 7.7–22.0%) the calculated reticulocyte volume was 20% larger. The mean reticulocyte/erythrocyte area ratio was 1.13 and the calculated mean volume ratio was 1.2. These measurements were expressed in arbitrary units and not in actual metric units, so that only relative measurements were possible.

In 1976 Clarkson and Moore [7] used an adapted planimetric method in which reticulocytes identified in brilliant cresyl blue wet preparations were photographed and the resultant Kodachromes were projected onto butcher paper. Pairs of reticulocytes and adjacent red blood cells were circled and traced with a planimeter. The reticulocyte area/erythrocyte area ratio obtained with this method in 17 normal subjects was 1.125, indicating that the normal reticulocyte area was 12.5% greater than the area of mature erythrocytes, a value almost identical to that of Killman. Mean reticulocyte volume was thus calculated as 20% greater than the average volume of mature red cells. The actual mean reticulocyte volume, calculated from an average MCV of 88 fl measured with a

Coulter S, was 106 fl in normal subjects (88×1.20). The mean reticulocyte volume was 79 fl in iron deficiency (mean MCV 73 fl) and 139 in megaloblastic anemia (mean MCV 103), confirming the existence of a consistent difference between reticulocyte and erythrocyte size. The presence of microreticulocytes or macroreticulocytes in the peripheral blood was pointed out as a very early finding during the development of iron deficiency or vitamin B_{12}/folate deficiency, respectively.

A few European authors have tried to determine the mean volume of reticulocytes from the ratio of packed cell volume to cell number in samples with very high reticulocyte concentration. An enrichment in reticulocytes up to 100% was obtained in experimental animals treated with phlebotomies and/or hemolytic agents. These rough methods provided reticulocyte volumes from 1,2–1,5 to 2–3 times larger than those of mature red cells [8, 9]. In addition to the poor reproducibility of manual methods of centrifugation and cell counting, these methods did not measure the volume of normal reticulocytes, but that of the much bigger "stress" reticulocytes produced under very intense erythropoietic stimulation. Severe hemolytic anemia induced by phenylhydrazine in rats was a popular experimental model in the '60 s [10]. The MCV of reticulocytes produced under such an extreme erythropoietic stimulation was nearly twice that of normal mature red cells. The larger size of these "stress" reticulocytes was attributed to the skipping of cell divisions in the bone marrow, due to the accelerated erythropoiesis and shortening of the interval between differentiation of stem cells and emergence of reticulocytes. These early, oversized, reticulocytes had much lower MCHC than normal erythrocytes.

Changes in reticulocyte volume can be indirectly monitored through changes in the red cell size distribution curves obtained with an automated hematologic analyzer [11]. The production of macrocytic reticulocytes was also observed following bleeding, exposure to simulated altitude, injection of erythropoietin [12], and also after successful treatment of iron deficiency anemia [13].

A different experimental model of erythropoietic stimulation uses temporary suppression of erythropoiesis with thiamphenicol and phlebotomy [14]. Immature reticulocytes produced under these stress conditions are larger than normal reticulocytes (mean diameter $9,66 \pm 1,10\,\mu$, compared with $7,04 \pm 1,10\,\mu$ for normal reticulocytes and $6,66 \pm 0,34\,\mu$ for mature red cells) and have a high content in ribosomes, mitochondria and other cellular organelles [15].

On a Romanowsky-stained peripheral blood film, stress macroreticulocytes are seen as polychromatophilic erythrocytes: on the average the diameters of these bluish red cells are 27% larger than those of a adjacent normally stained erythrocytes [16]. Polichromatophilia is abolished by treatment with ribonuclease. As stated by Adolfo Ferrata in 1912, all polychromatophilic red cells correspond to reticulocytes in which preliminary alcoholic fixation has not permitted the precipitation of ribosomes with formation of the reticulum. However, not all reticulocytes appear as polychromatophilic red cells on panoptical stains; this happens only with the most immature, Heilmeyer class I and II reticulocytes.

The count of polichromatophilic red cells corresponds to a "shift reticulocyte count" it was proposed as an indicator of bone marrow response to anemia [17], like the immature reticulocyte fraction which is obtained with flow cytometers (see below).

A typical reticulocyte matures in 4 days, and spends only the last 24 hours in the circulation [18]. Reticulocyte size has been indirectly measured and studied in various anemias [7]. 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 cells generated

by stress erythropoiesis (including r-HuEPO administration) may be reduced. In the mouse, stress reticulocytes disappear from the circulation in 32–36 hours, while macroreticulocytes disappear in 4–12 hours [19]. The red cells derived from these macroreticulocytes had a reduced survival [20]. In rats, the reticulocytes generated by r-HuEPO have MCV values which are almost double the normal values (100 fl *vs.* 55 fL) and the derived red cells have a substantially reduced life span [21]. Stress reticulocytes have been shown to undergo extensive remodeling [22] and substantial intravascular hemolysis [23]. In man, the most immature reticulocytes are multilobar, motile [24], and demonstrate a dramatic reduction in membrane deformability and mechanical stability [25]. Extensive membrane remodeling of cytoskeletal proteins leads to the formation of the more mature reticulocytes. This maturation process takes place in the bone marrow unless the most immature reticulocytes are released into the circulation by stress erythropoiesis. The spleen may also play a role in the sequestration and maturation of reticulocytes [26].

Macroreticulocytes produced during abrupt erythroid expansion are also characterized by the co-expression of adult and fetal hemoglobin (so-called F reticulocytes) [27, 28]. In states of rapid marrow expansion, accelerated maturation of early erythroid precursors yields a progeny that maintains the capacity for primitive globin expression.

B) Reticulocyte Density and Hemoglobin Concentration

Reticulocytes are less dense than mature red cells. When a sample of blood is centrifuged or allowed to stand, reticulocytes tend to remain in the top layer of cells. According to an old observation, when erythrocyte sedimentation rate is measured in samples with a high reticulocyte count there is a trail of more slowly sedimenting reticulocytes which gives a characteristic shading at the red cell/plasma interface. Reticulocytes from anemic rabbits were estimated to have a density of 1.105 g/ml cells, compared to a density of 1.122 g/ml cells for the mature anemic red cells [1, 29]. These early studies demonstrated that such a difference in density largely depended on the lower hemoglobin concentration and higher water content of reticulocytes compared with mature red cells. More than 50 years after these studies, it has been shown that the percentage of reticulocytes correlates with the percentage of hypochromic macrocytes [30].

The lower density of reticulocytes permits the recovery of enriched fractions of these cells from mature erythrocytes using centrifugation and density separation on Percoll columns [29].

2) Automated Measurements of Reticulocytes

The introduction of flow cytometric methods has greatly improved the precision and accuracy of reticulocyte counting. The manual reticulocyte count has been gradually replaced by automated counting [31–35]. Automated techniques have better precision and reproducibility than the manual count [36–38], and provide several additional parameters. These methods are either based on fluorescence (thiazole orange) or absorbance (ozaxine 750, methylene blue) of dye which interact with reticulocyte RNA.

A "corrected" % reticulocyte count can be obtained based on the Hb or Hct values [39]. The reticulocyte production index (RPI) has also been used to correct the % reticulocyte count for variations in RBC count and in reticulocyte maturation time. The RPI is obtained dividing the % 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 d for Hct values of 35%, to 2 d for Hct values of 25%, and to 2.5 d for Hct values of 15% [40]. A ratio > 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, transfusion and steroid treatment rates were present in the patients with RPI $\leq$ 2.0 [41]. However, RPI is not widely used and is seldom reported by laboratories. Only 55% of the laboratories in the United States report reticulocyte results as absolute counts, whereas 33% still report and calculate a "corrected" reticulocyte count [42].

Flow cytometers-based methods for reticulocyte counting use fluorescent staining of reticulocytes. In addition to enumerating reticulocytes, these methods can provide information regarding the distribution of fluorescence intensity among the reticulocyte population. Fluorescence intensity is proportional to the RNA content of the reticulocytes. Thus, young, immature, or stress reticulocytes will have higher fluorescence than mature reticulocytes. Initial studies of this parameter used arbitrary fluorescence units to determine a reticulocyte maturity index [32, 43]. The term immature reticulocyte fraction (IRF) has been proposed to define the fraction of reticulocytes with the highest fluorescence intensity [44]. Several studies and reviews have been published on IRF, discussing its use in the diagnosis of anemias [45, 46], early detection of engraftment after bone marrow transplantation, [47, 48] and technical issues of reproducibility [49].

3) Automated Measurements of Reticulocyte Cellular Indices

Reticulocyte indices are the latest achievement in the field of automated reticulocyte measurements. With laser based technology, simultaneous measurement of volume and hemoglobin concentration can be carried out on both red blood cells [50–53] and reticulocytes [54].

Flow cytometric analysis of red cells allows quantification of their volume, hemoglobin concentration, and hemoglobin content. The oxazine-750 staining method also allows measurements of reticulocyte staining intensity and provides direct measurements of reticulocyte cellular indices, such as reticulocyte MCV (MCVr) and MCHC (CHCMr) with their respective distribution widths. Mean hemoglobin content of reticulocytes (CHr) is calculated from the product of the volume times hemoglobin concentration of single cells.

The measurement of reticulocyte indices has shown excellent precision both in normal subjects and in patients with reticulocytosis. In one reported study coefficients of variations for measurements of MCVr, CHCMr and CHr were 0.8–1.6% after 21 repeated determinations [54]. The MCVr remains stable after up to 72 hours of storage at 4°C, while CHr and CHCMr show a small statistically significant decrease.

4) Reference Values for Reticulocyte Indices

After the preliminary contribution by Colella *et al.* [55], who published in abstract form some results obtained with a H*3 early prototype, three studies have reported information concerning the reference intervals of reticulocyte indices in healthy subjects (Tab. I).

TABLE I Normal range values for reticulocyte cellular indices

	[54]	[56]	[3]
MCvr (fL)	88.2–107	103.2–126.3	92.4–120.2
RDWr (%)	10.3–18.3	–	13.7–20.1
CHCMr (g/dL)	25.4–31.0	23.5–28.7	26.7–33.0
HDWr (g/dL)	1.9–4.7	–	2.8–4.0
CHr (pg)	23.5–29.9	25.9–30.6	27.1–33.9
CHDWr (pg)	2.5–4.1	–	3.0–4.7

In Ref. [54] data were collected in 110 children, (51 males, 59 females) age 1 to 10 years. In Ref. [56], data were collected in 64 healthy adults (32 males and 32 females). In Ref. [3], data were collected in 133 healthy adults.

In the collaborative Italian study carried out in Rome and Treviso with the Bayer H*3 [56], reference values have been calculated as the 95 central percentiles of the distributions obtained from 32 male and 32 female healthy adult subjects with no clinical symptoms and normal results at a screening blood test including complete blood count, differential count and serum ferritin level. In these subjects all reticulocyte parameters showed a normal distribution and no statistically significant difference between sexes. The average value of MCVr was 111.7 fl, ranging from 103.2 fl and 126.3 fl. This value was 24% higher than the corresponding MCV of mature erythrocytes. Consequently, the ratio MCVr/MCV was consistently higher than one, with a mean value of 1.24. The behavior of H*3 CHCM and CHCMr was almost specular to that of volume. The mean CHCMr was 26.3 g/dl (reference range 30.1 – 33.2), compared with a CHCM of 31.6 g/dl, with a mean negative difference of −16.7%. The mean values of the ratio CHCMr/CHCM were consistently lower than one. The mean reticulocyte hemoglobin content (CHr) was on average 28, 5 pg (reference range 25.9 – 30.6 pg), very similar to the CH of mature red cells, so that the CHr/CH ratio was close to one (average value: 1, 03).

Brugnara *et al.* [54] have measured reticulocyte indices in 110 pediatric outpatients (51 males and 59 females, age 1 – 10 years), who showed normal hematologic parameters according to the age-adjusted reference range. Their results (Tab. I) show a good general agreement with those obtained in adult subjects: although the average volumes are lower, the average MCVr/MCV ratio is again higher than one (1.24), the average CHCMr/CHCM ratio is lower than one (0.81) and the CHr/CH ratio is very close to one (0.96). Thus, in children as in adults, the H*3 measurements indicate that reticulocytes have a larger volume, a reduced cell hemoglobin concentration and a similar hemoglobin content compared with mature red cells. Distribution widths for the reticulocyte indices have also reported: the RDW of reticulocytes, expressed as the coefficient of variation of volumes, is on average very close to that of mature erythrocytes (14.3% *versus* 14.0%), while the variability of hemoglobin concentration (HDW), expressed as the standard deviation, is greater in reticulocytes than in red cells (3.3 g/dl *versus* 2.6 g/dl).

Another study has reported reference intervals for reticulocyte indices in 133 adult normal subjects [3]. Table I presents the normal range for reticulocyte indices based on these 3 published reports.

In the collaborative Rome-Treviso study reticulocyte and red blood cell indices have also been measured in anemic patients with abnormalities of red cell size [56]. The microcytic anemia group included 58 patients with overt iron deficiency anemia before iron treatment and 40 with heterozygous b-thalassemia and no iron deficiency. The macrocytic anemia group included 28 patients with anemia of different etiology and MCV above 100 fl. In these subjects the average values of reticulocyte indices have shown a general behaviour which was very similar to that of healthy subjects (Tab. II). Thus regardless of the final red blood cell size, reticulocytes appear consistently larger than the mature erythrocytes that originate from them, whereas their hemoglobin concentration is consistently lower: as a consequence of these changes, hemoglobin content is almost the same. These results are in surprisingly good agreement with the former data obtained by the authors that used planimetric methods [6, 7]. Similar data for normal subjects and for patients with iron efficiency have been reported by another group [3].

Finally, it must be noted that, although reticulocytes were found capable of hemoglobin synthesis while they are still in the bone marrow [57], the H*3 measurements indicate that hemoglobin content does not change significantly after the delivery of these cells into the peripheral blood.

TABLE II Reticulocyte cellular indices in iron deficiency

d'Onofrio *et al.* [56] mean (range)	Buttarello *et al.* [3]	mean ± SD
MCVr (fL)	100.1 ± 4.9	84.7 (67.6 – 93.4)
RDWr (%)	–	22.3 (20.1 – 28.8)
CHCMr (g/dL)	20.4 ± 2.1	24.6 (22.2 – 26.5)
HDWr (g/dL)	–	4.6 (4.1 – 5.1)
CHr (pg)	19.6 ± 2.5	20.9 (20.1 – 28.8)
CHDWr (pg)	–	4.1 (2.9 – 5.0)

In Ref. [56], data were collected in 58 patients. In Ref. [3], data were collected in 9 patients.

5) Clinical Usefulness of Reticulocyte Indices

The average survival of red blood cells in the circulating blood is about four months in healthy subjects and remains unchanged in most patients with anemia, with the exception of hemolytic anemias. During steady-state erythropoiesis, 20 ml of the total erythrocyte mass is renewed daily, (1 of the circulating red cells). Such relative stability of the erythrocyte population inherently limits the clinical sensitivity of red cell indices MCV, MCHC and MCH as early indicators of erythropoietic changes. Classical studies on both experimental animals and humans have shown that the first modifications of the MCV do become manifest after at least 6 to 12 weeks during experimentally induced deficiencies of iron [58] or folic acid [59]. Clarkson and Moore (1976) [7], have observed with their planimetric method that experimental induction of iron deficiency by phlebotomy is associated with a sequence of events which begins with an increased production of macroreticulocytes, which represents the first response to increased erythropoietin production. Two days after the fall of the transferrin saturation, the mean reticulocyte volume also abruptly diminishes, while the percentage and absolute reticulocyte count decreases after one more week. The MCV does not change for several weeks, then it begins a progressive decline down to values lower than the normal range, which are reached after more that two months. With iron supplementation the mean reticulocyte volume increased rapidly, followed by reticulocyte count and MCV. Similarly, the administration of methotrexate, a potent inhibitor of dihydrofolate reductase, causes an abrupt and sustained increase in reticulocyte volume (up to 142 fl), while the specific replacement therapy given to patients with macrocytosis and folate or vitamin B_{12} deficiency induces a rapid normalization of reticulocyte size, without any early change in the MCV.

These results show that twenty years ago the measurement of reticulocyte size, although still inaccurate and imprecise, could provide very sensitive and early clues to the diagnosis and treatment of patients with abnormal erythropoiesis. Reticulocytes released in the peripheral blood during the last 24 hours represent a very timely indicator of marrow erythropoietic activity. The measurement of their size and hemoglobin content using the new cytometric techniques, which are much more precise and rapid than the tedious planimetric methods, thus possesses a great potential for hematological diagnosis. The following paragraphs report some examples of the clinical values of reticulocyte indices.

Recombinant Erythropoietin Administration and Functional Iron Deficiency

It is known that treatment with recombinant human erythropoietin (*r*-HuEpo) may cause functional iron deficiency in patients with chronic renal failure receiving long term dialysis [60]. Functional iron deficiency occurs because *r*-

HuEpo stimulates erythropoiesis to such an extent that the demand for iron exceeds the body's ability to release it from stores. Functional iron deficiency is one of the main causes of resistance to *r*-HuEpo and can be detected by monitoring transferrin saturation or the percentage of hypochromic red blood cells with CHCM lower than 28 g/dl [61]. Functional iron deficiency is also observed when *r*-HuEpo is administered to non-anemic subjects, such as normal subjects undergoing multiple blood donations for autotransfusion schedules [62]. In healthy volunteers the administration of subcutaneous *r*-HuEpo is associated with a significant increase in the production of reticulocytes, which have an increased MCVr and a decreased CHCMr [63]. In this setting the measurement of the CHr in pg seems to represent a sensitive flag of functional iron deficiency: in particular, the percentage of reticulocytes with CHr less than 23 pg is an effective indicator or iron-deficient erythropoiesis and is inversely correlated with the log value of baseline serum ferritin [64]. These subjects should be treated with intravenous iron supplementation, because oral iron is ineffective in preventing iron-deficient erythropoiesis. When intravenous iron saccharate is administrated in association with *r*-HuEpo, no hypochromic reticulocytes are produced and the CHr remains within the normal range [63].

The effect of IV iron on reticulocyte indices has been studied in normal subjects receiving a single dose of *r*-HuEpo [65]. These data suggest that IV iron potentiates the hematopoietic response to *r*-HuEPO in normal subjects, which may otherwise be rate-limited. IV iron significantly increased the *r*-HuEpo-induced production of Hb and prevented the marked decrease in serum ferritin which is usually associated with *r*-HuEPO administration. While the total number of reticulocytes was not affected by IV iron administration, CHr was increased in the IV iron group compared with the control group. This effect was confirmed by measurements of total reticulocyte Hb, an integrated index which is derived from the absolute reticulocyte count and the CHr [66]. retHb was significantly increased in the group receiving IV iron, indicating that there was a greater production of Hb when IV iron was used.

Functional iron deficiency may also develop as a consequence of increased endogenous erythropoietin production, as in patients with immuno-hemolytic anemia: in these cases a sudden and unexpected fall of reticulocyte counts associated with the production of microreticulocytes and with an inversion of the MCVr/MCV ratio can be the first manifestation of iron-deficient erythropoiesis.

CHr has been shown to be a valuable indicator of the iron status in patients undergoing chronic hemodialysis [67]. The group of patients with CHr values lower than red cell MCH was shown to have lower transferrin saturation and hematocrit. In addition, all these patient had a recent increase in *r*-HuEPO dose. These data suggest that CHr could be an extremely useful index in the management of *r*-HuEPO therapy for patients on chronic dialysis.

Diagnosis and Treatment of Iron Deficiency and Megaloblastic Anemia

There are limited data on the use of reticulocyte indices and CHr in the diagnosis of iron deficiency. We have shown in a groups of pediatric patients undergoing routine office visits, that the estimated probability for iron deficiency is > 90% when CHr is less than 20 pg. When CHr is close to 23 – 24 pg, the probability of iron deficiency is approximately 50% and this probability decreases smoothly for larger values of CHr. Children with CHr above 29 pg have virtually a zero probability of having iron deficiency. Ferritin is not predictive of iron deficiency in this group of patients [68]. CHr was a much stronger predictor of iron deficiency than either the traditional red cell indices (MCV, MCHC, MCH) or erythrocyte zinc protoporphyrin (ZPP).

In patients undergoing hemodialysis, a value of CHr < 26 pg predicts iron deficiency with a sensitivity of 100% and specificity of 80% in dialysis patients [67]. Much lower diagnostic accuracy is achieved with the use of serum ferritin, transferrin saturation, or %hypochromic red cell [67].

The monitoring of erythropoietic response to iron administration in iron-deficient anemia is also improved by the availability of reticulocyte indices [69]. Changes in MCV and RDW become apparent after weeks or months of iron deficiency and change slowly during its treatment as well. However, reticulocyte indices may allow a real-time evaluation of iron deficient erythropoiesis and of the effectiveness of iron replacement therapy, since the lag time for their appearance from the marro is only two days. As shown in Figure 1, oral iron therapy in iron-deficient patients produces a very early increase in both the absolute reticulocyte count and the reticulocyte hemoglobin content (CHr) well in advance of any other change in red indices or total hemoglobin [2]. CHr may also be useful to identify patients who are not responding to oral iron: when abnormally low CHr persists despite oral iron, treatment with intravenous iron is indicated.

The observation that in particular cases reticulocytes may be smaller than the circulating mature red cells was reported for the first time in 1962: according to Brecher and Stohlman [12], in fact, "...reticulocytes produced in a megaloblastic anemia in response to B12 are markedly smaller than the predominant megaloblasts circulating at the time". A similar inversion of the MCVr/MCV ratio, with reticulocytes smaller than mature reticulocytes, has been observed with the H*3 method in a patient with megaloblastic anemia during hematologic recovery following vitamin B12 administration. In this case the circulating reticulocytes produced after 17 days of treatment had an MCVr of 108.8 fl, while the MCV was still 109.8, because most of the circulating mature erythrocytes had been formed before the vitamin administration [56].

Sickle Cell Anemia

The automated measurement of reticulocyte indices can be used to obtain information on the presence and the proportion of dehydrated hyperdense reticulocytes which have CHCMr higher than 38 g/dl [70]. The induction of fetal hemoglobin synthesis by hydroxyurea results in increased cell hydration of sickled reticulocytes, so that the disappearance of dense reticulocytes can be used as an early indicator of the effectiveness of the treatment. Recent studies on the use of oral Mg supplements in patients with sickle cell disease have shown that the reduction in dense erythrocytes induced by Mg is associated with a reduction in the absolute number of high staining intensity reticulocytes [71], and with a reduction in the distribution widths for reticulocyte volume (RDWr) and cell hemoglobin concentration (HDWr) [72].

Bone Marrow Transplantation

During the follow-up of patients undergoing transplantation of bone marrow or peripheral blood progenitor cells, the succession of suppression and regeneration of erythropoiesis is associated with important changes in MCVr and MCVr/MCV ratio [56]. After conditioning chemotherapy a severe decrease in reticulocyte percentage and absolute number occurs, which can be associated with progressive decrease in MCVr and transitory inversion of the MCVr/MCV ratio. Erythroid regeneration, on the other hand, is heralded by an abrupt increase of MCVr, up to values above normal [56].

In conclusion, the availability of automated measurements of reticulocyte indices allows a real-time evaluation of the bone marrow erythropoietic activity. Future studies will deter-

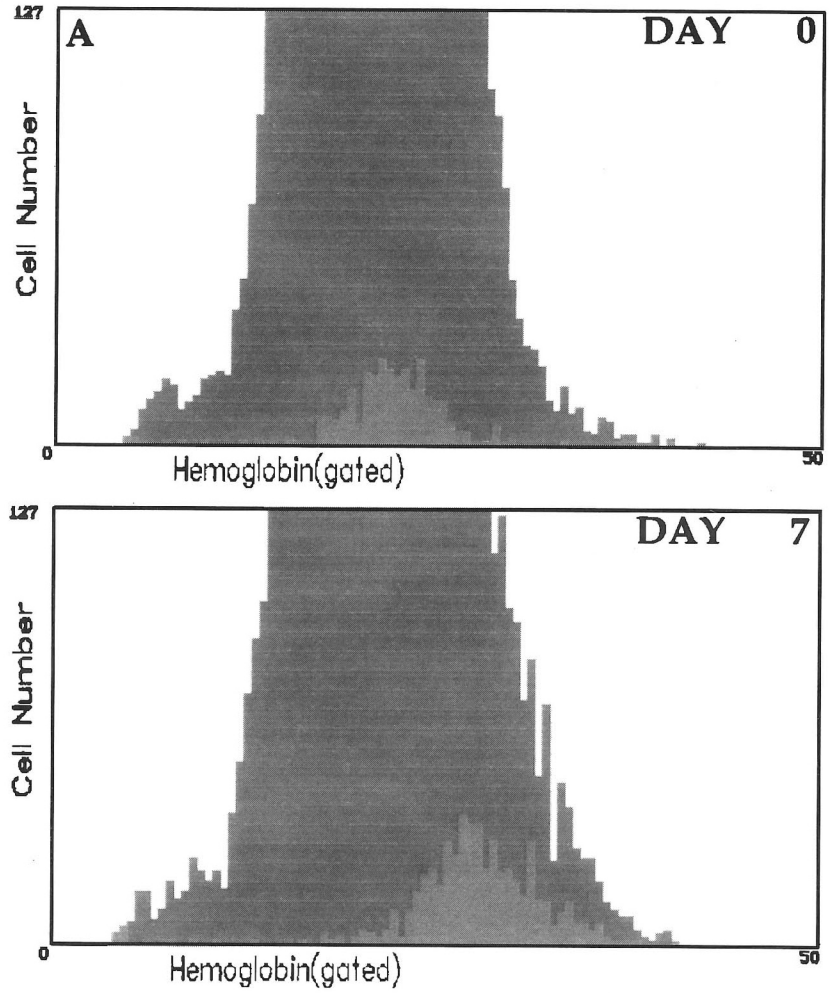

FIGURE 1 Frequency distribution histograms of reticulocyte cell hemoglobin content (CHr, green) and erythrocyte cell hemoglobin content (CH, blue) in a patient with iron deficient anemia before and after oral iron supplementation [2]. Response after one week of oral iron supplementation is demonstrated by an increase in CHr from 22.1 to 28 pg, while MCH changed only from 21.4 to 22.3 pg. (See Color Plate XV).

mine how this information can be translated into clinically and cost effective decisions.

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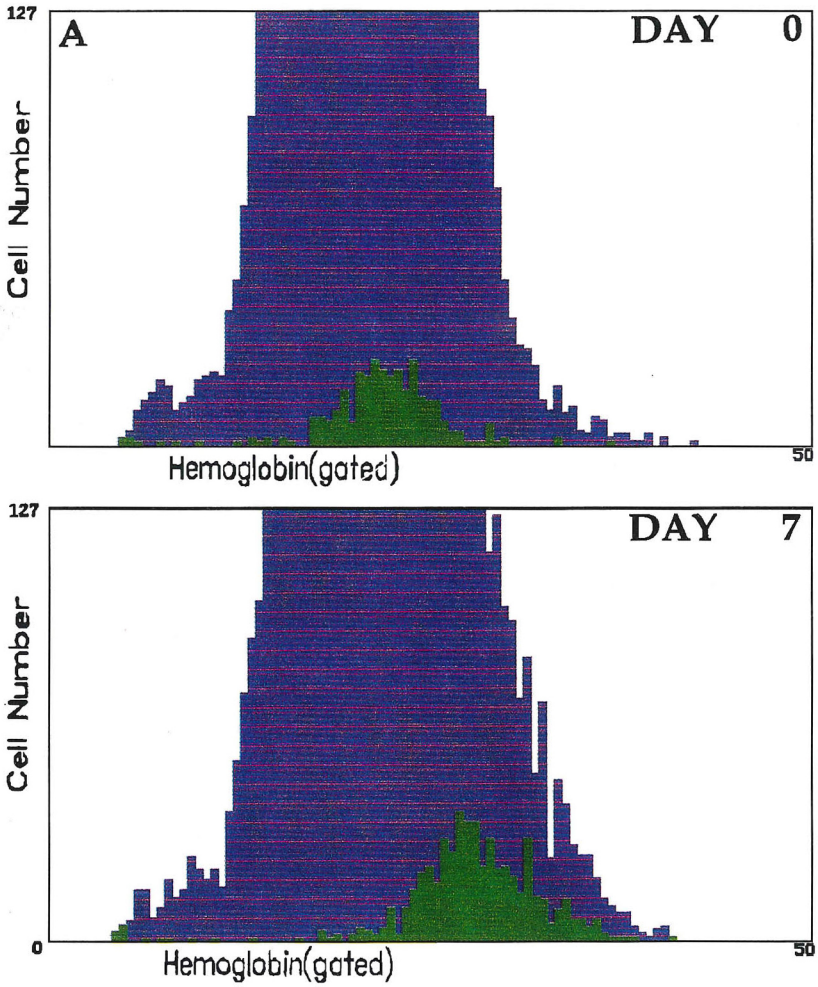

Color Plate XV (See page 174, Figure 1) Frequency distribution histograms of reticulocyte cell hemoglobin content (CHr, green) and erythrocyte cell hemoglobin content (CH, blue) in a patient with iron deficient anemia before and after oral iron supplementation [2]. Response after one week of oral iron supplementation is demonstrated by an increase in CHr from 22.1 to 28 pg, while MCH changed only from 21.4 to 22.3 pg.