Quantitative Anisocytosis as a Discriminant Between Iron Deficiency and Thalassemia Minor

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The coefficient of variation (CV) of red cell size, as measured by electronic red cell sizing (erythrography), was less than 14.0% in 20 normal subjects. In 22 of 25 patients with β-thalassemia minor and microcytosis (mean corpuscular volume [MCV] <70 fl), CV was less than 14.0%; in the other 3, CV was 14.0%-14.9%. In 53 patients with iron deficiency anemia and MCV <70 fl, CV always was >14.0%. In 7 patients with α-thalassemia minor and MCV <70 fl, CV was less than 14.0% in all 7. Among patients with microcytosis, erythrography appears to be an excellent technique for rapidly distinguishing between iron deficiency and α or β thalassemia minor.

VARIABILITY of erythrocyte size is assessed routinely as the degree of anisocytosis by examination of the peripheral blood films. However, variation in shape may be confused with variation in size, and artifacts in smear preparation may hamper optimal analysis. Price-Jones first quantified anisocytosis as the coefficient of variation of red cells in a Price-Jones curve. The curves of red cell diameter distribution were technically difficult, measured only 500 or 1000 cells, and still were subject to the artifacts of the peripheral smear. More recently, anisocytosis has been quantified using red cell size-distribution histograms.

We quantified anisocytosis in a large number of patients with microcytosis and demonstrated a marked difference between iron deficiency anemia and thalassemia minor.

MATERIALS AND METHODS

Patients (n = 85) with a mean corpuscular volume (MCV) less than 70 fl, as determined by Coulter Counter Model S in the routine hematology laboratory, were studied. Their blood, anticoagulated with EDTA, was suspended in Isoton to produce a final concentration of 0.5-1.0 × 10^7 RBC/liter. Red cell size-distribution histograms (erythrograms) were generated on a Coulter Counter model ZB1 with Channelyzer C-1000 attachment (Coulter, Hialeah, Fla.). The base channel threshold was 5, aperture current 1 mA, and aperture width 70 μm. The ZB1 was calibrated with Coulter standard solution 4C and also against the MCV given by the routine laboratory’s Coulter Counter Model S. Approximately 100,000 red cells were counted in each erythrogram.

Erythrograms were analyzed for mean cell volume (μ) and SD (σ). The natural logarithm of cell size was plotted against probability. The median cell volume was the size at which probability was 50%; median ± 1 SD was the size at which probability was 16% and 84%; and the median ± 2 SD was the size at which probability was 2.5% and 97.5%. The following formulas then were applied:

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QUANTITATIVE ANISOCYTOSIS

\[ \mu = \text{antilog} \ln(\text{median cell volume}) + (\ln a)^2, \]  

(1)

\[ \sigma = \frac{(\text{median cell volume} + 2 \text{ SD}) - (\text{median cell volume} - 2 \text{ SD})}{4}, \]  

(2)

\[ CV = \frac{\sigma}{\mu}. \]  

(3)

CV thus derived from the erythrogram is the statistical quantitation of variation of red cell size; an increase above normal is anisocytosis. Erythrograms require about 90 sec to perform, including the written record. Derivation of the coefficient of variation requires about 2 min using the Channelizer while the erythrogram is performed.*

Serum iron and iron binding capacity were performed by standard methods. Hemoglobin \( A_2 \) was quantitated by microcolumn chromatography according to the method of Huisman et al. Globin chain synthetic ratios were determined in the laboratory of Dr. Haig H. Kazazian. No patient had hemoglobin \( S, C, \) or \( F \) greater than 7.0%. No patient had received blood transfusions or iron during the 4 mo before being seen in this study.

Normal studies in our laboratory are: red cells \((\pm 2 \text{ SD})\), males 5.1 \((\pm 0.8) \times 10^{12}/\text{liter}, females 4.5 \((\pm 0.7) \times 10^{12}/\text{liter}; \text{MCV} \ 90 \ (\pm 10) \ \mu; \text{hemoglobin} \ A_2 \ 2.2\%-3.7\% \text{of total}; \text{hemoglobin} \ F \text{less than} \ 2.0\% \text{of total.}

From the MCV, hemoglobin concentration (Hb), and red blood cell count (RBC) generated by the Coulter S in the routine laboratory, the differential function (DF') was determined as

\[ \text{MCV} - 5(\text{Hb}) - \text{RBC} \div 3.4 \]

and the ratio of MCV/RBC found. For each patient, the Wright-stained peripheral blood smear was examined by one of us, without knowledge of hematologic values or diagnosis, for anisocytosis and microcytosis.

RESULTS

Twenty male medical students and house officers, all with normal complete blood counts and no known hematologic disorder, were control normal subjects. All had a CV \(< 14.0\%\).

We studied 85 patients, all with MCV \(< 70 \ \mu\). Of the 85, 53 patients had iron deficiency defined by serum iron saturation \(< 10\%\), a return of normal MCV and an increase in hemoglobin level after iron administration, and normal hemoglobin electrophoresis including hemoglobin \( A_2 \) \(< 3.7\% \) of total. In these patients hemoglobin was 4.6\%-13.7 g/dl (mean \( \pm 2 \text{ SD}, 9.2 \pm 2.1 \)); red cell count was 2.68\%-5.73 \( \times 10^{12}/\text{liter} \) (3.78 \( \pm 0.61 \)); MCV was 59\%-69 \( \mu \) (64 \( \pm 4 \)). There were 25 patients with \( \beta \)-thalassemia minor defined by hemoglobin \( A_2 \) level \( > 4.0\% \) (hemoglobin \( F \), 0.9\%-6.3\% in these patients) and serum iron saturation \( > 20\% \). In these patients hemoglobin was 9.8\%-15.6 g/dl (12.4 \( \pm 1.5 \)); red cell count 3.90\%-6.31 \( \times 10^{12}/\text{liter} \) (5.39 \( \pm 0.77 \)); MCV 61\%-69 \( \mu \) (65 \( \pm 3 \)). There were 7 patients with \( \alpha \)-thalassemia minor defined by \( \alpha/\beta \) globin chain synthetic ratio ranging from 0.63 to 0.82 and normal hemoglobin electrophoresis and iron saturation. In these patients hemoglobin was 11.9\%-14.8 g/dl (13.3 \( \pm 1.3 \)); red cell count 4.94\%-6.27 \( \times 10^{12}/\text{liter} \) (5.56 \( \pm 0.52 \)); MCV 60\%-69 \( \mu \) (64 \( \pm 4 \)).

Of 53 patients with iron deficiency, all had CV \( > 14.0\% \) (14.6\%-21.0\%). Of 25 patients with \( \beta \)-thalassemia minor, 22 had CV \( < 14.0\% \) and 3 had CV \( \geq 14.0\% \) (14.0\%, 14.2\%, 14.7\%). Of 7 patients with \( \alpha \)-thalassemia minor, all had CV

*Several companies are adding the same circuitry to standard instruments for complete blood counts, so that erythrograms and CV are automatically generated. The reliability of histograms and values so produced currently is under study.
Fig. 1. Erythrograms of two patients with microcytosis. (A) β-Thalassemia minor: MCV 65 fl, red cells 5.67 × 10^12/liter, CV 13.7%. (B) Iron deficiency: MCV 65 fl, red cells 4.93 × 10^12/liter, CV 17.8%.

<14.0%. Erythrograms of a patient with normal CV and β-thalassemia minor, and of a patient with increased CV and iron deficiency, are shown in Fig. 1.

The discriminant function, DF', is described as >0 in iron deficiency and <0 in thalassemia minor. Of 53 patients in our study with iron deficiency, 49 had DF' >0 and four had DF' <0. Of 25 patients with β-thalassemia minor and no iron
deficiency, 7 had $\text{DF}^+ > 0$ and 18 had $\text{DF}^- < 0$. Of 7 patients with $\alpha$-thalassemia minor and no iron deficiency, 2 had $\text{DF}^+ > 0$ and 5 had $\text{DF}^- < 0$.

MCV/RBC is described as $> 130$ in iron deficiency and $< 130$ in thalassemia minor. Of 53 patients with iron deficiency, 42 had a value $> 130$ and 11 had a value $< 130$. Of 25 patients with $\beta$-thalassemia minor and no iron deficiency, 6 had values $> 130$ and 19 had values $< 130$. Of 7 patients with $\alpha$-thalassemia minor and no iron deficiency, 2 had values $> 130$ and 5 had values $< 130$.

Of the 53 patients with iron deficiency, 18 had a hemoglobin level $\geq 10$ g/dl, comparable to the range in subjects with thalassemia minor, while 35 had hemoglobin $< 10$ g/dl. The two groups were comparable in CV. However, the 18 with less anemia included 7 of the 11 with MCV/RBC atypical of iron deficiency and all 4 with $\text{DF}^+$ atypical of iron deficiency. The distinction of iron deficiency from $\alpha$- and $\beta$-thalassemia minor by the above three methods is summarized in Fig. 2.

On the peripheral blood smear, anisocytosis was described in 38 of 53 patients with iron deficiency, 3 of 7 patients with $\alpha$-thalassemia minor, and 11 of 25 with $\beta$-thalassemia minor. Microcytosis was not described in nine of the smears (two, $\alpha$-thalassemia; four, $\beta$-thalassemia; three, iron deficiency).

**DISCUSSION**

The peripheral blood smear in iron deficiency anemia is well known to show anisocytosis; the degree of anisocytosis is said to correlate with the degree of iron deficiency. In this study, all 53 patients with iron deficiency anemia had increased CV. We suggest two reasons for this anisocytosis. First, iron deficiency per se results in abnormal erythropoiesis: hypochromia, microcytosis, and increased variation in shape and size—poikilocytosis and anisocytosis. Second, without iron replacement, iron deficiency frequently is progressive rather than stable. For example, a patient with chronic blood loss and iron deficiency may be producing red cells with an MCV of 75 fl; 3 mo later, with continued blood loss and more profound iron deficiency, he may produce cells with an MCV of 65 fl. The peripheral blood, reflecting all erythropoiesis during the past 4 mo, will contain an aggregate of cohorts of cells of each intermediate size, with an MCV of 70 fl for the aggregate. This conglomerate of red cell populations should not obscure the fact that in any progressive iron deficiency cells most recently produced are likely to be the smallest. Thus the more rapid the iron depletion, the less accurately the MCV will reflect red cell production at the time of sampling. This second cause of anisocytosis may explain why the increase in CV was independent of severity of anemia.

Patients with uncomplicated $\alpha$- or $\beta$-thalassemia minor are exempt from this second cause of anisocytosis, since the red cell abnormality in thalassemia minor is not progressive. Interestingly, 22 of 25 patients with $\beta$-thalassemia minor and no iron deficiency had a normal CV (no anisocytosis), and the other 3 had minimal anisocytosis; all 7 patients with $\alpha$-thalassemia minor had a normal CV. This suggests that in thalassemia minor, the morphologic abnormalities usually described as anisocytosis on peripheral blood film actually reflect leptocytosis or poikilocytosis. Thus normal CV should be a useful clue to the diagnosis of $\alpha$- or $\beta$-thalassemia minor. Two earlier studies of erythrocyte size distribution in
β-thalassemia minor and in iron deficiency were limited by counter technique\textsuperscript{19} and only single cases.\textsuperscript{3}

No patient in this study had β-thalassemia minor and iron deficiency. In the patients with iron deficiency, α-thalassemia could not be excluded; however, 36 of the 53 patients had erythrograms after a reticulocyte response to iron therapy, and all 36 had normal- or large-sized new red cells.\textsuperscript{20}

Several formulas derived from routine Coulter S determinations have been proposed as discriminants between iron deficiency and thalassemia minor.\textsuperscript{1,6,21,22} Since erythrography is more complicated than such formulas, to be useful for this purpose it must be a better predictor. Among the 85 cases with iron deficiency, or with no iron deficiency and either α- or β-thalassemia minor, the wrong diagnosis (iron deficiency versus thalassemia minor) would have been made in 27 by MCV/RBC, in 18 by DF, and in 3 by erythrography (Fig. 2). Other series show similar rates of error for the formulas.\textsuperscript{1,6,21} These formulas add no new information to the Coulter S values and depend on a disproportionate fall in MCV versus RBC. Cases of iron deficiency with primary or secondary polycythemia\textsuperscript{23,24} or thalassemia minor and chronic disease particularly will be misdiagnosed. The formulas, valid for comparing groups, are questionable in the same cases for which the unmanipulated Coulter S values also are poor discriminants. Erythrography is more accurate and distinguishes by a variable independent of red cell count or indices. Distinction from the peripheral smear also is not very reliable.\textsuperscript{14} Determination of CV by erythrography appears to be a rapid and reliable way to distinguish thalassemia minor, both α and β, from iron deficiency among patients with microcytosis, although making this distinction does not lessen the obligation for an appropriate thorough clinical evaluation. Whether or not in cases with less pronounced microcytosis the same distinction is possible remains to be determined.

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