Clin Chem Lab Med 2005;43(2):195–197 © 2005 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2005.033

Short Communication

Measurement of reticulocyte and red blood cell indices in patients with iron deficiency anemia and β-thalassemia minor

José F. A. Noronha and Helena Z. W. Grotto*

Department of Clinical Pathology, School of Medical Sciences, State University of Campinas, Brazil

Abstract

New parameters correlated with the hemoglobin content in reticulocytes (RET-Y) and in red blood cells (RBC-Y) have been suggested as helpful in diagnosing iron deficiency anemia. We have studied RET-Y and RBC-Y indices in two groups of patients with microcytosis to verify if these parameters could be used to differentiate iron deficiency anemia from β-thalassemia minor. Blood samples from 33 iron-deficient patients, 25 β-thalassemic minor patients and 50 normal individuals were analyzed on a Sysmex XE-2100 instrument. A significant difference was observed in reticulocyte counting and immature reticulocyte fraction between iron deficiency anemia and β-thalassemia minor groups, but not in RBC-X and RET-Y parameters. Reticulocyte counting was higher in β-thalassemia minor and the immature reticulocyte fraction was higher in severe iron deficiency anemia. The ratio RET-Y/mean cell volume was tested and was significantly different when β-thalassemia minor was compared with mild and severe iron deficiency anemia, and showed better performance than the Mentzer ratio and the Green and King function. A great overlap of RET-Y and RBC-Y individual values was observed in both groups of microcytic anemias; we conclude that these new indices may be used with caution as indicative of iron deficiency, mainly in populations where β-thalassemia minor is frequent.

Keywords: automated cell counting; β-thalassemia minor; iron deficiency anemia; microcytosis; red blood cell; reticulocyte.

Measurement of reticulocyte cellular characteristics may provide useful information about marrow erythropoietic activity in a variety of anemias.

The Sysmex XE-2100 instrument (Sysmex, Kobe, Japan) measures two parameters in the reticulocyte channel according to the mean value of the forward-scatter light histogram of the red blood cells (RBC-Y) and reticulocytes (RET-Y). RBC-Y and RET-Y are measured in arbitrary units (channel numbers), corresponding to the signal proportional to the size of red blood cells and reticulocytes, respectively. Despite the fact that RET-Y is a measurement of reticulocyte size, good correlation was observed between RET-Y and the average reticulocyte hemoglobin content (CHr; provided by Advia 120, Bayer), suggesting that RET-Y may be used for monitoring functional iron deficiency (1). In addition, RBC-Y is correlated with the percentage of hypochromic red cells (%HYPO provided by Advia 120, Bayer Corporation, Tarrytown, NY, USA) (2).

As several different indices have been proposed for differentiating iron deficiency anemia (IDA) from β-thalassemia minor (3, 4), we have compared the new parameters, RET-Y and RBC-Y, in both microcytic anemias. We evaluated RET-Y and RBC-Y in 33 adult patients presenting overt IDA before iron treatment (mild IDA group, Hb $\geq$ 10 g/dL, n = 14; and severe IDA group, Hb $<$ 10 g/dL, n = 19; serum ferritin level below 13 μg/dL for females and 30 μg/dL for males), and 25 patients with β-thalassemia minor (hemoglobin level 7.5–16.8 g/dL; hemoglobin A_2 (HbA_2) level $>$ 4.0%; normal serum ferritin level). A total of 50 healthy individuals with normal results for total blood count, serum ferritin and HbA_2 levels were considered as the control group.

The variance analysis test with RANK transformation was used to explain the variability of parameters. The Tukey test was applied to identify differences. A p-value $\leq$0.05 was considered significant. The capacity of the tests to discriminate between the groups was studied by means of receiver operating characteristic (ROC) curves.

Reticulocyte count was significantly higher in β-thalassemia minor patients, although the immature reticulocyte fraction (IRF) was only higher in the group with severe IDA, suggesting enhanced erythropoietic activity in individuals with severe IDA (Table 1). These data are in concordance with those reported by Tassiopoulos et al. (5), who demonstrated that erythropoietin levels in β-thalassemia minor are significantly lower than in IDA with the same degree of anemia. A possible explanation could be that adjustment of capillary circulation due to life-long anemia or increased oxygen unloading of Hb would lead to diminished hypoxic stimulus and, consequently, to the production and secretion of erythropoietin concentrations lower than theoretically expected.

*Corresponding author: Helena Z. W. Grotto, Department of Clinical Pathology, Faculty of Medical Sciences, State University of Campinas – UNICAMP, PO 6111, 13084-971 – Campinas, São Paulo, Brazil
Phone: +55-19-3788-7064, Fax: +55-19-3788-9434,
E-mail: grotto@fcm.unicamp.br

Table 1 Hematological and reticulocyte parameters (median, minimum–maximum) in controls (n = 50) and patients with mild IDA (n = 14), severe IDA (n = 19) and β-thalassemia minor (n = 25).

	Hb, g/dL	RET, cells × 10^9/L	IRF	RBC-Y	RET-Y
Normal	14.6 (12.1–17.3)	47.6 (20.5–122.8)	8.8 (1.4–17.8)	162.0 (149.0–167.4)	172.7 (161.2–180.7)
Mild IDA	10.7* (10.0–12.3)	47.1** (23.3–63.7)	17.1* (5.2–26.7)	130.65* (112.7–140.1)	135.7* (119.5–144.0)
Severe IDA	7.9*/** (5.9–9.8)	48.5** (23.6–113.3)	22.1*/** (9.8–36.5)	106.7* (82.9–142.0)	118.2* (83.4–152.2)
β-Thalassemia minor	11.2* (7.5–16.8)	67.2* (24.1–202.9)	13.2* (4.2–24.1)	129.4* (111.2–150.0)	136.2* (119.5–163.0)

RET, reticulocyte count. *Significantly different from normal ($p < 0.05$). **Significantly different from β-thalassemia minor ($p < 0.05$).

Table 2 p-Values of red cell discriminant functions.

Group	Red cell discriminant function				
	RBC-Y	RET-Y	Mentzer ratio	Green-King function	RET-Y/MCV
N × β-thal	<0.0001	<0.0001	<0.0001	0.3563	0.0034
N × mild IDA	<0.0001	<0.0001	0.0001	0.0147	0.6370
N × severe IDA	<0.0001	<0.0001	0.0140	<0.0001	0.0023
β-Thal × mild IDA	0.6359	0.6976	0.7400	0.1205	0.010
β-Thal × severe IDA	0.0875	0.0734	0.0490	0.0001	<0.0001

N, normal group; β-thal, β-talassemia minor. Mentzer ratio, MCV/RBC (4); Green and King function, $MCV^2 \times RDW/Hb \times 100$ (3); RBC, red blood cells; MCV, mean cell volume; RDW, red cell distribution width.

There was no significant difference in RBC-Y and RET-Y values between IDA and β-thalassemia minor groups (Table 1).

It has been suggested that values <162.4 for RET-Y are indicative of iron deficiency, with 99% sensitivity and 98.7% specificity as determined by ROC curves (6). Our results showed that values <155.6 for RET-Y presented 100% sensitivity and specificity in distinguishing individuals with iron deficiency from the normal group. However, all of the β-thalassemia minor patients showed lower RET-Y values, showing an important overlap of results. When patients were separated by degree of iron deficiency according to Hb level, we observed that RET-Y > 134.5 showed 61.5% sensitivity and 50% specificity in discriminating β-thalassemia minor from mild IDA. RET-Y values >130.0 showed 80.8% sensitivity and 73.7% specificity in distinguishing β-thalassemia minor from severe IDA.

Thus, in populations where β-thalassemia minor is frequent, it is necessary to eliminate this cause of microcytosis for which RET-Y measurements present an evident overlap of individual values. The same difficulty was observed in assessing iron status in β-thalassemia minor and in α-thalassemias using CHr, because these conditions already exhibit an abnormally low CHr (7). On the other hand, CHr was useful in evaluating the severity of different genotypes in heterozygous β-thalassemia with the degree of hemoglobinization, represented by negative correlation between HbA_2 levels and CHr values (8).

Good correlation was observed between RET-Y and mean cell volume (MCV) in patients with β-thalassemia minor (Spearman coefficient 0.84125, $p = 0.0001$) and then we tested the possibility whether Hb content of reticulocytes, represented by RET-Y (1), although slightly different between both anemias, could be incorporated with MCV using the ratio RET-Y/MCV. We evaluated if this was a sensitive and specific method in the initial screening of patients with microcytic anemias and better than the Green and King function ($MCV^2 \times RDW/Hb \times 100$) (3) and Mentzer ratio (MCV/RBC) (4). The results are summarized in Table 2 and show that the RET-Y/MCV ratio was the only parameter that was significantly different when β-thalassemia minor was compared with mild and severe IDA. The sensitivity of the ratio was 73.1% and specificity was 78.6% in separating β-thalassemia minor from mild IDA, and was better in distinguishing β-thalassemia minor from severe IDA (84.5% sensitivity and 78.6% specificity).

According to our results, RET-Y and RBC-Y are not sufficiently sensitive and specific to differentiate β-thalassemia minor from IDA. Although RBC-Y and RET-Y may be useful in quantifying different degrees of erythropoietic stimulation and in understanding the physiopathology involved in anemias of various origin, these new indices should be used with caution as indicative of iron deficiency, mainly in populations where β-thalassemia minor is frequent.

References

1. Franck S, Linssen J, Messinger M, Thomas L. Potential utility of Ret-Y in the diagnosis of iron-restricted erythropoiesis. Clin Chem 2004;50:1240–2.
2. Briggs C, Rogers R, Thompson B, Machin SJ. New red cell parameters on the Sysmex XE-2100 as potential markers

of functional iron deficiency. Infus Ther Transfus Med 2001;28:256–62.
3. Green R, King R. A new red cell discriminant incorporating volume for differentiating iron deficiency from thalassemia minor. Blood Cells 1989;15:481–95.
4. Mentzer Jr WC. Differentiation of iron deficiency from thalassemia trait. Lancet 1983;1:882.
5. Tassiopoulos T, Konstantopoulos K, Tassiopolulos S, Rombos Y, Alevizou-Terzaki V, Kyriaki P, et al. Erythropoietin levels and microcytosis in heterozygous beta-thalassaemia. Acta Haematol 1997;98:147–9.
6. Buttarello M, Temporin V, Ceravolo R, Farina G, Bulian P. The new reticulocyte parameter (RET-Y) of the Sysmex XE 2100. Am J Clin Pathol 2004;121:489–95.
7. Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. Clin Lab Sci 2000;37:93–130.
8. Skarmoutsou C, Papassotiriou I, Traeger-Synodinos J, Stamou H, Ladis V, Metaxotou-Mavrommati A, et al. Erythroid bone marrow activity and red cell hemoglobinization in iron-sufficient β-thalassemia heterozygous as reflected by soluble transferrin receptor and reticulocyte hemoglobin content. Correlation with genotypes and HbA_2 levels. Haematologica 2003;88:631–6.

Received August 9, 2004, accepted December 7, 2004