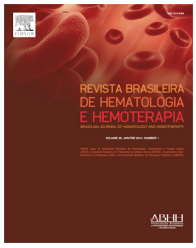
Revista Brasileira de Hematologia e Hemoterapia
Brazilian Journal of Hematology and Hemotherapy

www.rbhh.org



Original article

Evaluation of erythrocyte and reticulocyte parameters as indicative of iron deficiency in patients with anemia of chronic disease

*Ana Beatriz Barbosa Torino, Maria de Fátima Pererira Gilberti, Edvilson da Costa, Gisélia Aparecida Freire de Lima, Helena Zerlotti Wolf Grotto**

Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 25 March 2014
Accepted 24 July 2014
Available online 17 February 2015

Keywords:
Automation
Anemia, iron-deficiency
Erythrocyte indices
Reticulocytes
Erythropoiesis

ABSTRACT

Objective: The aim of this study was to evaluate the effectiveness of mature red cell and reticulocyte parameters to identify three conditions: iron deficiency anemia, anemia of chronic disease, and anemia of chronic disease associated with absolute iron deficiency.
Methods: Peripheral blood cells from 117 adult patients with anemia were classified according to iron status, inflammation, and hemoglobinopathies as: iron deficiency anemia ($n = 42$), anemia of chronic disease ($n = 28$), anemia of chronic disease associated with iron deficiency anemia ($n = 22$), and heterozygous β-thalassemia ($n = 25$). The percentage of microcytic erythrocytes, hypochromic erythrocytes, and the levels of hemoglobin in both reticulocytes and mature red cells were determined. Receiver operating characteristic analysis was used to evaluate the accuracy of the parameters in differentiating anemia.
Results: There was no difference between the groups of iron deficiency and anemia of chronic disease associated with absolute iron deficiency for any of the parameters. The percentage of hypochromic erythrocytes was the best parameter to identify absolute iron deficiency in patients with anemia of chronic disease (area under curve = 0.785; 95% confidence interval: 0.661–0.909 with sensitivity of 72.7%, and specificity of 70.4%; cut-off value 1.8%). The formula microcytic erythrocyte count minus hypochromic erythrocyte count was very accurate to differentiate iron deficiency anemia from heterozygous β-thalassemia (area under curve = 0.977; 95% confidence interval: 0.950–1.005 with a sensitivity of 96.2%, and specificity of 92.7%; cut-off value 13.8).
Conclusion: The erythrocyte and reticulocyte indices are moderately good to identify absolute iron deficiency in patients with anemia of chronic disease.

* *Corresponding author at*: Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), 13083-970 Campinas, SP, Brazil.
E-mail address: lenagrotto@gmail.com (H.Z.W. Grotto).
http://dx.doi.org/10.1016/j.bjhh.2015.02.004

Introduction

New automated blood cell analyzers can provide information about individual cell characteristics, such as hemoglobin content of reticulocytes, hemoglobin content of mature erythrocytes, and percentages of microcytic erythrocytes and hypochromic cells. These parameters have been used in the diagnosis of iron deficiency anemia (IDA), β-thalassemia (β-Thal),[1–3] and anemia of chronic disease (ACD).[4,5] The differentiation between these three conditions is very important as the clinical approach is different in each particular diagnosis.

As reticulocytes have a normal life span of one or two days, information concerning the hemoglobin content of young red cells is a good indication of iron availability and an early marker of iron-deficiency erythropoiesis.[6] Reticulocyte hemoglobin equivalent (Ret-He) reflects real-time information on the synthesis by young erythrocytes in bone marrow. Other available parameters are the percentage of red cells with Hb content equivalent to or less than 17 pg (%HypoHe), and the percentage of red cells with a volume less than 60 fL (% MicroR),[1] which corresponds to a sub-population of mature red cells exhibiting evidence of insufficient iron content.

A mathematical formula using %MicroR and %HypoHe (MHe) proposed by Urrechaga et al.[7] tested discriminant indices in healthy individuals, β-Thal and IDA patients. A sensitivity of 97.4% and specificity of 97.1% was reported in differentiating β-Thal from mild IDA.

Anemia associated with chronic inflammation, infection or malignancy is the most common anemia in hospitalized patients. Although stainable iron is present in bone marrow, elevated levels of inflammatory cytokines interfere in erythropoiesis leading to a hyporegenerative anemia and defective iron incorporation into erythrocyte progenitors. Reduced concentrations of circulating iron and normal or increased iron stores characterize the state of functional iron deficiency.[8]

Anemia of inflammation can be associated with absolute iron deficiency (ACD combi) generally in patients with inflammatory disease and chronic blood loss. Differentiation between ACD and ACD combi is clinically important, but in the clinical practice, differentiation is difficult using conventional biomarkers such as ferritin concentration and transferrin saturation.[9] The soluble transferrin receptor/log ferritin ratio may be useful in distinguishing ACD from ACD combi.[10]

The aim of this study was to analyze the effectiveness of new laboratory parameters related to mature red blood cells and reticulocytes to differentiate three conditions related to iron deficiency: IDA, ACD and ACD combi. Moreover, the performance of these parameters was tested to distinguish IDA from β-Thal, two common causes of microcytic anemia.

Methods

This project was approved by the Ethics Committee of the Faculty of Medical Sciences, Universidade Estadual de Campinas (UNICAMP), São Paulo, Brazil. All samples were selected from routine collections and informed consent was waived.

Peripheral blood samples from 117 adult patients with anemia (Hb < 12.0 g/dL for women and Hb < 14.0 g/dL for men) were selected from routine workload. Blood analysis had been requested by general practitioners, in general to investigate anemia.

Patients were classified according to iron status analysis (commercial kits from Roche Diagnostics Germany): IDA when serum iron (SI) levels were <45 mg/dL for men and <30 mg/dL for women; percentage transferrin saturation (%TS) <15%; serum ferritin (SF) <30 μg/L for men and <13 μg/L for women.

Patients were classified as ACD when SI levels were normal or reduced (40–160 mg/dL and 30–160 mg/dL for men and women, respectively), %TS normal or decreased (30–50%), normal or high SF (30–400 μg/L and 13–150 μg/for men and women, respectively) and C-reactive protein (C-RP) > 5 mg/dL (Tina-Quant C-Reactive Protein, Roche Diagnostics, Germany).

Soluble transferrin receptor (sTfR) levels (Roche Diagnostics, Germany) were measured in all samples, and the sTfR/log ferritin ratio was used to identify iron deficiency in patients with ACD. Patients with ACD having a sTfR/log ferritin ratio > 2.06 or sTfR > 3.71 μg/mL (cut-off values indicative of iron deficiency in our laboratory) were classified as ACD combi.

Twenty-six patients had the diagnosis of β-Thal according to the hemoglobin A_2 level determined by high performance liquid chromatography (HPLC- Variant II – Hemoglobin Testing System, Bio-Rad Laboratories, Inc., CA, USA).

Patients with β-Thal associated with other kinds of anemia, patients with reticulocytosis or pancytopenia, individuals who had received transfusions within the previous three months, and patients on iron replacement therapy were excluded from the study.

A control group (CG) was compounded by healthy individuals with no clinical signs or symptoms of disease, including acute inflammatory/infectious conditions, normal hematologic findings, and C-RP < 5 mg/L. Healthy individuals were students or laboratory staff, all of whom donated blood samples on a voluntary basis.

Determination of erythrocyte and reticulocyte parameters was performed using the Sysmex XE-5000 automated hematological analyzer (Sysmex, Kobe, Japan), which provides the following parameters: Ret-He, %MicroR, hemoglobin content of erythrocytes obtained from the optical counting of red blood cells (RBC-He), and %HypoHe. The MHe index was calculated as: %MIcroR-%HypoHe.[7]

Mann–Whitney test was applied to compare groups. Receiver operator characteristic analysis (ROC) was used to evaluate the accuracy of the parameters to differentiate between types of anemia. The level of significance was set for a p-value <0.05. Data were analyzed using the SPSS statistics program for Windows (version 13.0. SPSS Inc. Chicago, IL, USA).

Results

According to adopted criteria, individuals were classified as IDA (42 patients), β-Thal (25 individuals), ACD combi (22 patients), ACD (28 patients) and CG (54 individuals).

Table 1 describes the demographic characteristics and laboratorial data of the patients and the CG, and Table 2 shows