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Clinical relevance of erythrocyte ferritin in microcytic anemias

Jose M. Vagace [a,*], Antonio Peças [b], Jorge Groiss [a], Celeste Bento [b], Maria Leticia Ribeiro [b], Guillermo Gervasini [c]

[a] Service of Hematology, Infanta Cristina Hospital, Badajoz, Spain
[b] Service of Hematology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[c] Department of Surgical and Medical Therapeutics, University of Extremadura, Badajoz, Spain

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ABSTRACT

Background: Erythrocyte ferritin (EF) reflects the balance between iron supply and its utilization for hemoglobin synthesis. This balance is altered in microcytosis. We aimed to evaluate the diagnostic value of both EF and the ratio (FRR) plasma ferritin (PF)/EF in these disorders.
Methods: A total of 231 subjects participated in the study. Samples from 93 adult patients with different causes of microcytosis, 57 healthy subjects and 81 full-term newborns were analyzed to determine EF and PF concentrations and other hematological parameters.
Results: In patients with iron deficiency, and in contrast to PF, EF decreased only in the presence of anemia and in direct correlation with the degree of microcytosis (Pearson's $p < 0.001$). EF values for thalassemia patients were higher than those observed in controls ($p < 10e-5$), while PF concentrations were similar between these groups. This EF increase was more marked in the delta-beta thalassemia group ($p < 0.05$). Finally, FRR was much higher in patients with anemia of inflammation than in those with thalassemia ($p < 10e-5$), thus helping to discriminate between these disorders.
Conclusions: EF and FRR are tools that may be useful in the diagnosis of the main causes of microcytosis.

1. Introduction

Microcytic anemias are mainly caused by two mechanisms, namely, iron deficiency and subsequent decrease in the synthesis of the heme group or decreased synthesis of globin chains (e.g., thalassemias). Iron deficiency and its most severe manifestation, iron deficiency anemia, are public health problems worldwide. However, iron deficiency constitutes a broad concept, both in terms of the number of affected subjects and of the deleterious effects that this deficiency has on the patient's health [1,2]. On the other hand, thalassemias are a group of genetic disorders of hemoglobin (Hb) synthesis characterized by a reduced or absent production of one or more globin chains. Thalassemia syndromes constitute one of the most frequent genetic defects in humans. Heterozygous forms may be silent or present with microcytosis, while homozygous forms cause progressively serious conditions, from intermediate to major syndromes [3]. The anemia resulting from either acute or chronic inflammatory processes has been traditionally, but incorrectly, named anemias of chronic disease (ACD). This ACD, anemia of inflammation would be the correct term, are coupled with abnormalities in iron metabolism which result in microcytosis in 25–30% of cases [4].

After the evaluation of the hemogram, the study of microcytosis should begin with the assessment of iron deposits. For this, one of the most valuable parameters currently available is the determination of plasma ferritin (PF) concentrations. However, while PF levels below 20 ng/mL is a sign of iron deficiency [5], normal or even elevated concentrations cannot completely rule out the disorder. This is because PF is a positive acute-phase protein, i.e., numerous circumstances such as inflammation, hepatopathies or malignancies, may result in increased ferritin plasma levels with no relation to iron deposits [6–8]. In contrast, erythrocyte ferritin (EF), which also correlates with iron stores [9], is not an acute-phase reactant, which makes it an advantageous parameter to evaluate iron functional availability in the aforementioned circumstances [2,9,10].

In the present work, we have aimed to evaluate the utility of both EF and the ratio PF/EF for the diagnosis of microcytosis in the hospital setting and to discuss the significance of these parameters as an approach to understanding iron metabolism in several pathologies that present with microcytosis. A secondary goal was to analyze a control population of adults and newborns to investigate putative differences in EF levels that may reflect a different iron metabolism in these two groups.

2. Patients and methods

The study included a total of 231 subjects divided into three groups. A first group consisted of 93 consecutive adult patients (36 males) with

* Corresponding author at: Infanta Cristina Hospital, Av.Elvas s/n 06071, Badajoz, Spain. Tel.: +34 924 215 335.
E-mail address: jvagacev@gmail.com (J.M. Vagace).

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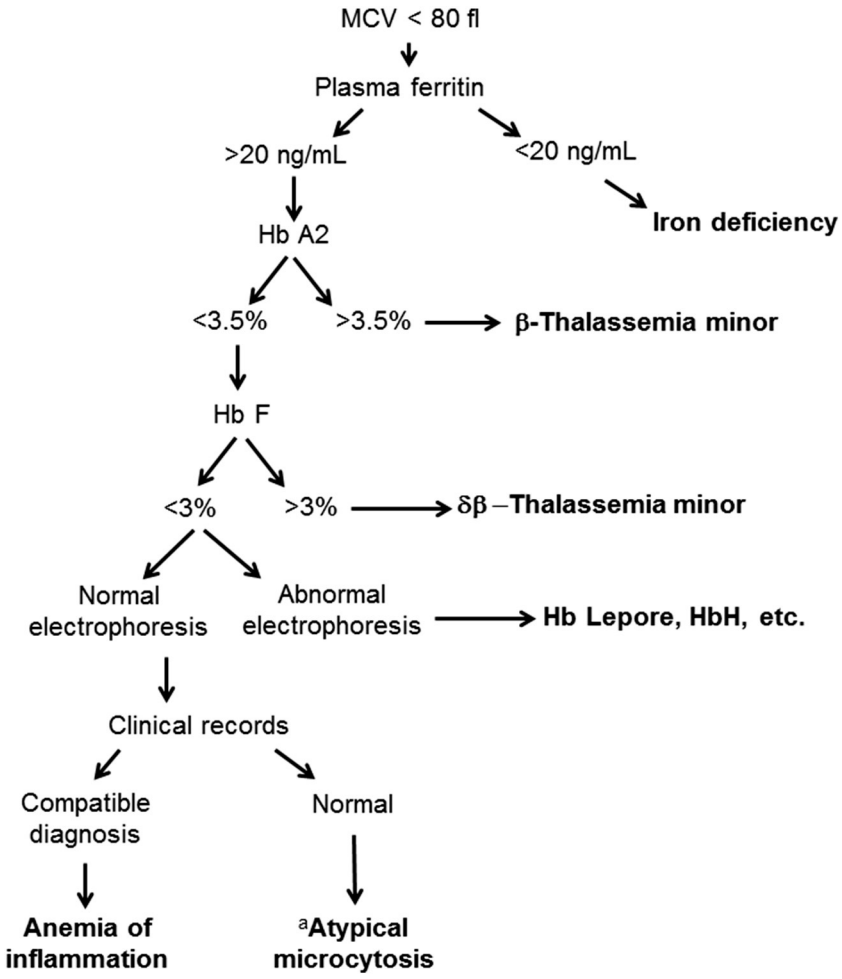

Fig. 1. Nomogram for the diagnosis of the different microcytosis considered. MCV, mean corpuscular volume; Hb, hemoglobin. [a]The term *atypical microcytosis* includes those cases with putative alpha-thalassemia minor.

microcytosis who were diagnosed at the Infanta Cristina University Hospital (Badajoz, Spain) according to the nomogram depicted in Fig. 1. The number of patients with the different pathologies is shown in Table 1. The second study group included 57 control subjects (28 males), recruited among the hospital staff, who showed a normal hemogram with no iron deficiency, thalassemia or hemoglobinopathies. Finally, umbilical cord blood samples were collected from 81 full-term newborns at the Materno Infantil Hospital (Badajoz, Spain) in EDTA tubes immediately after birth by puncturing of the umbilical vein.

Plasma was separated from the cells in all the samples and the erythrocytes were concentrated by centrifugation at 1400 g for 10 min followed by resuspension in saline. An aliquot of this erythrocyte suspension was subjected to cell count in an automated counter (Beckman Coulter® Hmx, Beckman Coulter Inc., Galway, Ireland). Ferritin levels were quantified in plasma or erythrocyte concentrates by a solid-phase chemiluminiscence enzyme immunoassay (Immulite 2000, Siemens Healthcare Diagnostics, Malvern, PA, USA), which uses basic anti-ferritin antibodies. Knowing the concentration of the erythrocyte concentrate, EF concentration was calculated and expressed in attograms (ag) per cell (1 ag = 10e-18 g).

Patients with low plasma ferritin concentrations (<20 ng/mL) with no presence of anemia were classified as latent iron deficiency, regardless of corpuscular parameters. In addition, if the patient had a family history of thalassemia, Hb-A_2 was determined regardless of ferritin levels (eight cases of thalassemia associated with iron deficiency were thus diagnosed). Atypical microcytoses were not included in this

Table 1
Clinical characteristics of patients and control subjects. Plasma and erythrocyte ferritin values are expressed as median (interquartile range).

	Controls	Ferropenia	Thalassemia	Anemia of inflammation	Newborns
Males	28	15	15	6	41
Females	29	31	21	5	40
Hb	14.9 ± 1.1	10.9 ± 1.9	12.0 ± 1.3	10.8 ± 1.6	15.8 ± 1.6
MCV	88 ± 4	73 ± 9	65 ± 4	77 ± 3	109 ± 6
RDW[a]	13 ± 1	18 ± 5	17 ± 3	17 ± 2	-
PF (ng/ml)	64.6 (61.2)	5.9 (11.8)***	50.7 (103.2)	141.0 (183.9)*	179.0 (127.0)***
EF (ag/cell)	9.8 (6.9)	3.6 (16.6)**	74.8 (98.8)***	9.5 (7.2)	509.1 (356.6)***

*$p < 0.001$; **$p < 0.0001$; ***$p < 10e-5$ (all comparisons vs. control group).
Hb, hemoglobin; MCV, mean corpuscular volume; RDW, red cell distribution width; PF, plasma ferritin; EF, erythrocyte ferritin.
[a] RDW was not measured in the newborns.