

Measurement of reticulocyte and red blood cell indices in the evaluation of anemia in inflammatory bowel disease

Pantelis Oustamanolakis^a, Ioannis E. Koutroubakis^a,*, Ippokratis Messaritakis^b, Georgios Kefalogiannis^b, Maria Niniraki^c, Elias A. Kouroumalis^a

^a Department of Gastroenterology, University Hospital Heraklion, Crete, Greece

^b Laboratory of Hematology, University Hospital Heraklion, Crete, Greece

^c Laboratory of Immunology, University Hospital Heraklion, Crete, Greece

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KEYWORDS Anemia;	Abstract
Crohn's disease; Ferritin; Iron deficiency; Ulcerative colitis	<i>Background</i> : The commonest types of anemia in inflammatory bowel disease (IBD) are iron deficiency (IDA) and anemia of chronic disease. The differentiation between these two conditions is important for the management of the patient. The aim of this study was to investigate the usefulness of reticulocyte and red blood cell indices in the evaluation of anemia in IBD. <i>Methods</i> : One hundred IBD patients [49 ulcerative colitis (UC), 51 Crohn's disease (CD)] and 102 healthy controls were enrolled. Measurement of reticulocyte and red blood cell indices was performed using the Coulter LH780 Hematology Analyzer (Beckman Coulter). Additionally, serum levels of ferritin, transferrin saturation (Tsat) and soluble transferrin receptor (sTfR) were analyzed in all patients and controls. <i>Results:</i> The prevalence of anemia was 41.2% for UC and 42.9% for CD, whereas 30 IBD patients (30%) had IDA. Red cell Distribution Width (RDW), Red blood cell Size Factor (RSF), and Reticulocyte Distribution Width-Coefficient of Variation (RDWR-CV) were found significantly correlated with both Tsat and sTfR but not with ferritin levels. Patients with IDA had significantly higher RDW and RDWR-CV and significantly lower RSF levels compared with those without IDA. High values of RDW (sensitivity 93%, specificity 81%) and low values of RSF (sensitivity 83%, specificity 82%) were the best markers for the diagnosis of IDA. Both RDWR-CV and RDWR-SD were
	significantly correlated with disease activity and CRP levels.

Abbreviations: UC, Ulcerative Colitis; CD, Crohn's Disease; HC, Healthy Controls; IDA, iron deficiency anemia; ACD, anemia of chronic disease; MCV, Mean corpuscular volume; RDW, Red cell distribution width; RSF, Red blood cell Size Factor; IRF, Immature Reticulocyte Fraction; RDWR-CV, Reticulocyte Distribution Width-Coefficient of Variation; RDWR-SD, Reticulocyte Distribution Width-Standard Deviation; Tsat, transferrin saturation; sTfR, Soluble transferrin receptor.

* Corresponding author at: Dept. of Gastroenterology, University Hospital Heraklion P.O. Box 1352, 71110 Heraklion, Crete, Greece. Tel.: +30 2810392253; fax: +30 2810542085.

E-mail address: ikoutroub@med.uoc.gr (I.E. Koutroubakis).

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Conclusion: RDW, RSF and RDWR, could be useful markers for the evaluation of anemia and disease activity in IBD.

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1. Introduction

Anemia is one of the commonest extraintestinal manifestations of inflammatory bowel disease (IBD)¹⁻³ with significant impact on the patients quality of life.⁴ The most common types of anemia in IBD are iron deficiency anemia (IDA, up to 90% of patients) and anemia of inflammation or anemia of chronic disease (ACD, prevalence 11–42%).⁵⁻⁸ Other rare causes of anemia are vitamin B12 or folic acid deficiency, hemolysis and drug-induced anemia.^{9,10}

One common and complicated problem which a clinician faces when taking care of IBD patients is to diagnose the type of anemia in an individual and to determine the degree of iron deficiency and the degree of inflammation in each patient, in order to decide, in an evidence-based manner, for the best management of anemia, tailored for each individual.¹¹ The evaluation of anemia requires a determination of body iron status. Serum ferritin is an indicator of the storage iron content, but during inflammatory conditions may not exactly reflect iron stores as its expression is affected by cytokines.¹² Serum iron concentration and transferrin saturation (Tsat) are low in both conditions (IDA and ACD), so they are not useful in differential diagnosis.¹² Soluble transferrin receptor (sTfR) serum levels are increased in conditions of low iron availability for erythropoiesis but in ACD are normal, due to the fact that transferrin receptor expression is negatively affected by inflammatory cytokines.¹³ It seems that conventional markers of iron status cannot safely define IDA in a chronic inflammatory setting.

During the last decade, several new parameters have been introduced to our armamentarium for the diagnosis of ACD or IDA as a result of the development of more sophisticated and technologically advanced automated blood cell counters. COULTER® LH780 Hematology Analyzer (Beckman Coulter, Inc., CA, USA) is a last generation hematology analyzer providing automated measurements of a variety of new red cells and reticulocytes parameters. Red blood cell Size Factor (RSF) is the square root of the product of Mean Corpuscular Volume (MCV) multiplied by the Mean Reticulocyte Volume (MRV) and seems to be a suitable parameter for the study of bone marrow erythropoietic activity, as it provides a very good level of agreement with reticulocyte hemoglobin content (CHr).¹⁴ The Immature Reticulocyte Fraction (IRF) is an early and sensitive index of erythropoiesis and is useful in distinguishing anemias characterized by increased marrow erythropoiesis from anemias due to reduced marrow activity and from situations such as acute infections and myelodysplastic syndromes.¹⁵ Reticulocyte Distribution Width-Standard Deviation (RDWR-SD) is the standard deviation of the retic volume multiplied by the volume factor. Reticulocyte Distribution Width-Coefficient of Variation (RDWR-CV) is the ratio of RDWR-SD and the retic volume mean multiplied by 100. Both RDWR-SD and RDWR-CV are derived from the reticulocyte histogram. RDWR is an indication of the size dispersion within the reticulocyte population.¹⁶

The aim of this study was to investigate the usefulness of reticulocyte and red blood cell indices in the evaluation of anemia in inflammatory bowel disease.

2. Materials and Methods

2.1. Patients

One hundred consecutive IBD patients followed up at the Gastroenterology Department of the University Hospital of Heraklion were included in the study. They were compared with 102 matched for age healthy controls (HC). The main demographic and clinical parameters of the patients and HC are shown in Table 1. Individuals considered HC were all healthy blood donors, with no family history of IBD or any other significant medical history. All patients and controls were of Caucasian origin. Exclusion criteria for the study were hemoglobinopathy or thalassemia trait, history of GI bleeding, malignancy, chemotherapy or radiotherapy, renal insufficiency or hematologic, liver and autoimmune disorders, as well as female patients with history of ongoing pregnancy or recent delivery (<12 months from the study initiation). Moreover, none patient or control had received blood transfusion or treatment with iron or erythropoietin during the last three months, from the study initiation.

Diagnosis of ulcerative colitis (UC) and Crohn's Disease (CD) was based on the European Crohn's and Colitis Organization (ECCO) evidence based consensus on the diagnosis and management of Crohn's disease and Ulcerative Colitis,^{17,18} while disease phenotyping was based on Montreal classification.¹⁹ As far as the disease activity is concerned, it was calculated at the time of blood collection and in CD patients was measured based on the CDAI score²⁰; they were divided in two groups: "Active" with CDAI>150 and "Inactive" with CDAI<150. In UC patients, the Simple Clinical Colitis Activity Index (SCCAI)²¹ was used for the evaluation of disease activity and "Active" disease was considered with a score of 3 or more points. Serum CRP levels were also used as a marker of evaluation of the disease activity. Definition of anemia in our study was based on the established WHO criteria of hemoglobin <13 g/dl and <12 g/dl, for men and women respectively. $^{2\bar{2}}$ None of the healthy controls had anemia.

Informed consent was obtained from all patients. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Ethics Committee of the Medical Faculty of Crete.

2.2. Laboratory Studies

Whole blood samples were collected from patients and HC for a full blood count and erythrocyte sedimentation rate (ESR) measurement at the time of collection. All full blood counts were processed using the COULTER® LH780 Hematology Analyzer (Beckman Coulter, Inc., CA, USA), according to the manufacturer's instructions. Besides full blood count, RSF, IRF, RDWR-SD and RDWR-CV were measured and reported for every patient and HC. Serum samples were separated at the same time by centrifugation at room temperature for measurement of the following laboratory parameters: C-reactive protein (CRP), iron (Fe), ferritin (Fer), transferrin (TRF), soluble transferrin receptor (sTfR) and Transferrin saturation (Tsat). All measurements were processed according to standard laboratory practice.

2.3. Statistical Analysis

All results are expressed as means \pm standard deviation. Comparisons among the three diagnostic groups in terms of continuous measurements were made by the Kruskal–Wallis test (nonparametric ANOVA). Post hoc multiple comparisons tests were made by Dunn's test. Comparisons between two groups were made by either the Student's *t*-test or Mann–Whitney *U* test. Kolmogorov and Smirnov test was used to assess the assumption that data were sampled from populations that follow the Gaussian distributions. Correlations Download English Version:

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