Clinical Significance of The New Beckman-Coulter Parameters in the Diagnosis of Iron Deficiency Anemia

Demir Eksikliği Anemisinin Tanısında Yeni Beckman-Coulter Parametrelerinin Klinik Önemi

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ABSTRACT

Summary: Iron deficiency anemia (IDA) is the most common form of nutritional anemia worldwide. Despite the availability of various biochemical parameters for its diagnosis, their validity is still debatable. Therefore a simple, cheap and useful tool for diagnosis of IDA is under research. The aim of this study was to find the threshold values of the new Beckman-Coulter parameters in Turkish population, show their utility in the diagnosis of IDA and compare them with other conventional iron parameters.

Materials and Methods: A total of 115 female patients with iron deficiency anemia (serum hemoglobin <120 g/L and serum ferritin <20ng/ml), 55 female patients with iron deficiency (serum hemoglobin >120 g/L and serum ferritin <20 ng/ml) and 165 female with healthy individuals were enrolled.

Findings: Red blood cell size factor (RSf) and microcytic anemia factor (MAF) showed a significant positive correlation with hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), serum iron, transferrin saturation and a significant negative correlation with transferrin and total iron binding capacity. Low hemoglobin density (LHD) showed a significant negative correlation with hemoglobin, MCV, MCH, MCHC, serum iron, transferring saturation and a significant positive correlation with transferrin and total iron binding capacity. The receiver-operating characteristic (ROC) curve analysis demonstrated 92.45 fl, 6.71% and 10.14 for RSf, LHD and MAF, respectively.

Discussion: Our data demonstrated that these parameters are useful and can be confidentially used in the diagnosis of IDA.

Keywords: Beckman-coulter, diagnosis, hemoglobin, iron deficiency anemia

Introduction

Iron deficiency anemia (IDA) is the most common form of nutritional anemia worldwide (1). Various biochemical parameters including ferritin, transferrin saturation, serum iron and mean corpuscular volume (MCV) are used to diagnose it. Despite the availability of these parameters, their validity for the diagnosis of IDA is still debatable. Serum ferritin, the most specific indicator of iron deficiency, is an acute phase reactant and its level is affected by inflammation. Transferrin saturation fluctuates due to the diurnal variation of serum iron and serum iron levels decrease with infection, inflammation, and malignancy whereas they increase with liver disease (2-9). Therefore for some patients, clinicians have to order these tests more than once for accurate diagnosis and every test require extra blood sampling.

New Beckman-Coulter device can make some calculations from complete blood count parameters of red blood cell size factor (RSf) = √MRV X MCV, low hemoglobin density (LHD) = 100 X √1-{1/(1 + e 1.8 (30-MCHC))} and microcytic anemia factor (MAF)={(Hb X MCV)/100}. RSf joins together the volumes of mature red cells (MCV) and reticulocytes (MRV) both related to erythropoietic activity and hemoglobinization (10). Reticulocytes are the youngest erythrocytes released from the bone marrow into blood and circulate for 1-2 days before becoming mature erythrocytes. The RSf reflects the amount of iron available for hemoglobin production in the bone marrow. Therefore, RSf has been proposed as an iron status marker (10). Recent studies have indicated that RSf measurements in peripheral blood samples are useful for diagnosis of iron deficiency (10-15). It was shown to be an accurate measure of iron status and reliable iron marker for monitoring iron therapy's effectiveness (10).

LHD derives from the mean cell hemoglobin concentration (MCHC) and is calculated by using a mathematical sigmoid transformation (16). MCHC is an all-inclusive measure of both the availability of iron over the preceding 90-120 days and of the proper introduction of iron into intracellular hemoglobin. In the same way, LHD is related to iron availability and the hemoglobinization of the mature erythrocytes (11). Microcytic anemia factor (MAF) is a parameter for examining abnormal red cell modalities, as its calculation accounts for both size and hemoglobin content (13). These parameters require neither extra blood sampling nor extra cost. Urrechaga et al (10). Have been concentrated on these parameters but there is still need for more studies. Their threshold values should be validated in different populations before they can be routinely used.
In this study, we aimed to evaluate the significance of new three parameters (RSf, LHD and MAF) in the diagnosis of

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IDA, compare them with other conventional iron parameters and find their threshold values in Turkish Patients.

Materials and Methods

Patients

After obtaining the approval of the Ethics Committee and informed consent, 115 female patients with iron deficiency anemia (serum hemoglobin <120 g/L and serum ferritin <20ng/ml), 55 female patients with iron deficiency (serum hemoglobin >120 g/L and serum ferritin <20 ng/ml) and 165 female control (healthy individual) were enrolled. There was no commorbidities without IDA in patients and sensitive C- reactive protein (CRP) was normal. The patients were not receiving iron replacement therapy when the study was conducted. Ethical approval was obtained from Non-Interventional Clinical Research Ethics Committee (12.02.2013-47) at Eskişehir Osmangazi University.

Sample Collection and Laboratory Methods

Samples for complete blood count were collected in K3EDTA tubes and analyzed with an automated hematology analyzer, Beckman-Coulter. Serum iron and total iron binding capacity (TIBC) were measured by LISA 500 Plus automated chemical analyzer (Hycell Diagnostics, Paris, France). Serum ferritin was measured on the Hitachi E170 automated analyzer (Hitachi, Tokyo, Japan). Transferrin saturation was calculated by dividing serum iron by TIBC X 100. Transferrin was measured by the BN II automated chemical analyzer (Siemens, Marburg, Germany). LHD is derived from MCHC and calculated using the mathematical sigmoid transformation formula of LHD= 100 X √1-{1/(1 + e 1.8 (30-MCHC))}. The RSf joins together the volumes of reticulocytes and erythrocytes. RSf= √MRV X MCV. The MAF is calculation account for both size and hemoglobin content: MAF={(Hb X MCV)/100}.

Statistical Analysis

Data were analyzed using IBM SPSS 20. Independent Samples T-test was applied for normally distributed variables and results were given as mean ± standard deviation. Mann-Whitney U Test was applied for abnormally distributed variables and results were given as median (quartiles) values. Receiver-operating characteristic (ROC) curve analysis was performed to identify the optimal cut-off value for predicting iron deficiency anemia. P value less than 0.05 (p<0.05) was accepted significant.

Results

There was no statistically significant difference between each group in terms of age and gender. Hemoglobin, MCV, MCH, MCHC, serum iron and transferrin saturation (TS) were significantly lower in female patients with iron deficiency anemia in respect to iron deficient patients and healthy controls. Transferrin and TIBC were significantly higher in female patients with iron deficiency anemia compared to female patients with iron deficiency (Table 1). Significant differences in RSf values (p<0.001) were detected when groups with IDA (82.00 fl) were compared with iron deficient patients without anemia ID (90.83 fl) and healthy individuals (97.09 fl) (Table 1). A cut off value of RSf was determined as 92.45 fl (98.3 % sensitivity, 93.9 % specificity) by ROC analysis in patients with IDA (Figure 1).

RSf showed a significant positive correlation with hemoglobin (r = 0.777), hematocrit (r=0.750), MCV(r = 0.948), MCH (r = 0.883), MCHC (r = 0.686), MRV (r=0.902), serum iron (r = 0.682) and TS (r = 0.732), ferritin level (r=0.766) and a significant negative correlation with transferrin (r = -0.604) and TIBC (r = -0.495) (Table 3).

Table 1. Hematologic and iron parameters in iron deficiency anemia group, iron deficiency group and control group.

	Iron Deficiency Anemia Group (n=115)	Iron Deficiency Group (n=55)	Control Group (n=165)	P value
Hemoglobin(gr/L)	10.00	13.00	13.80	<0.001
MCV (fL)	70.00	82.00	88.00	<0.001
MCHC (g/L)	31.00	32.00	33.00	<0.001
Htc (%)	32.00	39.00	41.00	<0.001
Iron (µmol/L)	28.00	47.00	84.00	<0.001
Transferin saturation(%)	7.50	13.00	31.00	<0.001
Transferin (mg/dl)	316.00	281.00	249.00	<0.001
Iron bindıng capacity	398.00	351.00	311.00	<0.001
Ferritin (ng/ml)	5.00	10.00	71.00	<0.001

median values (25th/75th percentiles), * p value <0.05

Table 2. New Beckman-Coulter parameters in iron deficiency anemia group, iron deficiency group and control group.

	Iron Deficiency Anemia Group* (n=115)	Iron Deficiency Group* (n=55)	Control Group* (n=165)	Cut-off	P value
RSf (fl)	82.00 (78.38-85.24)	90.83 (89.06-94.43)	97.09 (95.03-99.13)	92.45	<0.001
LHD%	37.66 (16.31-70.71)	16.3 (6.71-16.31)	6.71 (2.73-6.71)	10.14	<0.001
MAF	7.11 (5.72-8.25)	10.71 (10.48-11.09)	12.19 (11.38-12.91)	12.19	<0.001

*median values (25th/75th percentiles), * p value <0.05

Table 3. Parametric correlations

	LHD	RSf	MAF
Hemoglobin	r= -0.710 P=0.000	r=0,777 P=0.000	r= 0,964 P=0.000
Hematocryte	r= -0,605 P=0.000	r= 0,750 P=0.000	r= 0,931 P= 0.000
MCV	r= -0,681 P=0.000	r= 0,948 P=0.000	r= 0,883 P= 0.000
RDW	r= 0,690 P=0.000	r= -0,728 P=0.000	r= -0,769 P= 0.000
MCH	r= -0.741 P=0.000	r= 0.883 P=0.000	r= 0.783 P=0.000
MCHC	r= -1.000 P= 0.000	r= 0,686 P= 0.000	r= 0,724 P= 0.000
MRV	r= -0,606 P= 0.000	r= 0,902 P= 0.000	r= 0,745 P= 0.000
Serum Ferritin	r= -0,621 P= 0.000	r= 0,766 P= 0.000	r= 0,781 P=0.000
Serum İron	r= -0,608 P= 0.000	r= 0,682 P= 0.000	r= 0,741 P= 0.000
TIBC	r= 0.361 P= 0.000	r= -0,495 P= 0.000	r= -0,466 P= 0.000
TSAT	r= -0,619 P= 0.000	r= 0.732 P= 0.000	r= 0.774 P= 0.000
Transferrin	r= 0. 360 P= 0.000	r= -0.604 P= 0.000	r= -0.493 P= 0.000

Significant differences in LHD values (p<0.001) were detected when IDA patients (37.66 %) were compared with ID patients (16.3 %) and controls (6.71%) (Table 1). A cutoff value of LHD was determined as 6.71 % (91.3 % sensitivity, 81.2 % specificity) by ROC analysis in patients with IDA (Figure 2) .

LHD showed a significant negative correlation with hemoglobin (r = -0.710), hematocrit (r= -0.605), MCV (r = -0.681), MCH (r = -0.741), MCHC (r = -1.00), MRV (r= -0.606), serum iron (r = -0.608) and TS (r = -0.619), ferritin level (r= -0.621) and a significant positive correlation with transferrin (r = 0.360) and TIBC (r = 0.361) (Table 3).

Significant differences in MAF values (p<0.001) were detected when IDA patients (7.11) were compared with ID patients (10.71) and controls (12.19) (Table 1). A cutoff value of MAF was determined as 10.14 (96.5 % sensitivity, 97.6 % specificity) by ROC analysis in patient with IDA (Figure 3).

MAF showed a significant positive correlation with hemoglobin (r = 0.964), hematocrit (r=0.931), MCV(r = 0.883), MCH (r = 0.783), MCHC (r = 0.724), MRV (r=0.745), serum iron (r = 0.741) and TS (r = 0.774), ferritin level (r=0.781) and a significant negative correlation with transferrin (r = -0.493) and TIBC (r = -0.466) (Table 3).

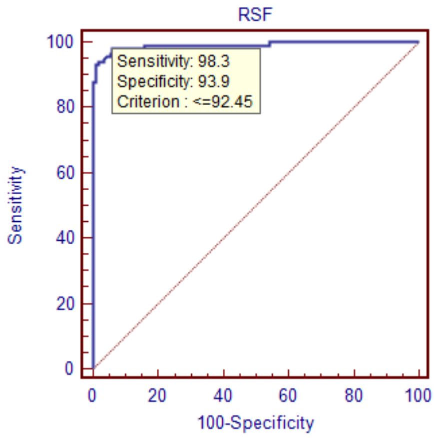

Figure 1. The ROC curve analysis for RSf

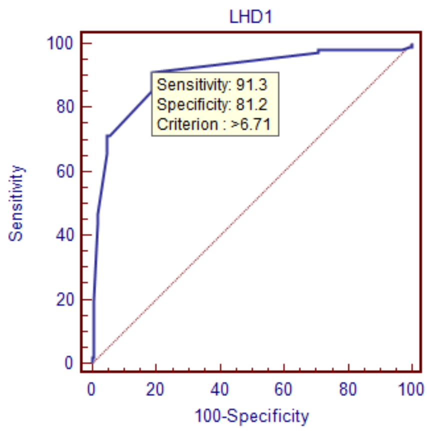

Figure 2. The ROC curve analysis for LHD

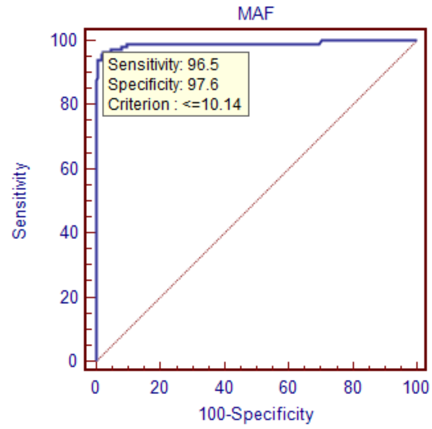

Figure 3. The ROC curve analysis for MAF

Discussion

Various biochemical parameters are being used for the diagnosis of IDA. Technological advances in automated full blood count analyzers enable to show iron status of patients based on some calculations. Beckman-Coulter has proposed LHD%, RSf and MAF as potential markers for iron deficiency.

Reticulocytes have a mean volume (15-20 fl) greater compared to the mature erythrocytes staying in the blood for 1-1.5 days. Therefore, the measurement of reticulocyte number and cellular characteristics provides real-time data regarding certain aspects of erythropoiesis that can influence the dimensions of red cells such as iron status. The examination of both precursors and mature cells provides an opportunity to detect acute and chronic changes in hemoglobin status (10). In a study performed by Urrechaga et al (11). İt was reported that RSf of < 91 fl had an optimal sensitivity (98.8 %) and specifity (89.6%) for diagnosis of IDA. This study showed that RSf could be reliable parameters for the iron status. In our study, in patients with IDA, RSf values were lower than the reference group (97.09 fl) and iron deficient group (90.83 fl) (Table 1). In particular, a cutoff value of 92.45 fl for RSf showed the best diagnostic efficiency, with a sensitivity of 98.3 % and specifity of 93.6 % (Figure 1). Diagnostic sensitivity, specifity and efficiency of RSf was found to bebetter when compared to LHD.

LHD (Low hemoglobin density) derives from the MCHC and calculated using the mathematical sigmoid transformation formulae. MCHC is an all-inclusive measure of both the availability of iron over the preceding 90-120 days and of the proper introduction of iron into intracellular hemoglobin. In the same way, LHD% is related to iron availability and hemoglobinization of the mature red cells (11). In a study by Urrechaga reported LHD% as a potential indicator of available iron for erythropoiesis. In this study, significant differences in LHD% values (p<0.001) were detected when

IDA patients (27.3 %) were compared the control group (2.3%) and iron deficient patient (16.3%). ROC analysis for LHD% in the diagnosis of IDA results are as follow: cutoff 4.0%, sensitivity 95.2%, specifity 93.3 %. This study showed that LHD% suitable parameter for determining iron status and its availability for erythropoiesis, with the same clinical significance (17). In our study, the LHD% values obtained in the IDA group of patients (37.66%) were statistically higher than the control groups (6.71%) and iron deficient (ID) group (16.3%). The optimal cutoff point for LHD% was 6.71% which provided 91.3% sensitivity and 81.2 % specifity.

Microcytic anemia factor (MAF) is parameter for examining abnormal red cell modalities, as its calculation account for both size and hemoglobin content. Dopsaj et al (18). Reported that MAF performs very well discriminating different stages of iron deficiency. Also, in cost/benefit terms, monitoring of MAF is justified as a low cost, effective screening parameter of determining iron status. In this study, ROC curve analysis for MAF in the diagnosis of iron deficient erythropoiesis indicates sensitivity of 61.5% and specifity of 93.0% and cutoff 11.4. In our study, in a patient with IDA (7.11). And ID (10.71), MAF values were lower than the control groups (12.19). In particular, a cut-off value of 10.14 for MAF showed the best diagnostic efficiency, with a sensitivity of 96.5 % and specifity of 97.6 %.

With the present investigation, we identified the value of these parameters in the diagnosis of IDA. RSf and MAF showed significant correlation with hemoglobin, hematocrit, erythrocyte indices, ferritin and transferrin saturation (r>0.7). LHD% was also significantly negative correlated with hemoglobin but correlation with erythrocyte indices and ferritin and transferrin was weak. Diagnostic sensitivity, specifity and efficiency of MAF was better when compared to LHD.

In conclusion, Beckman-Coulter automated complete blood count device provides very useful parameters in diagnosing IDA and ID. These parameters can be obtained in the course of routine blood counts, with no additional cost or need for more blood sampling. In order to use these parameters in daily practice, more studies should be carried out to determine the cut-off values.

Conflict of Interest: The authors declare that they have no conflict of interest

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