Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2021;46(3):1009-1017
DOI: 10.17826/cumj.905007

ARAŞTIRMA / RESEARCH

Beta talasemi minör ve demir eksikliği anemisinin ayırıcı tanısında eritrosit indekslerinin tanısal doğruluk testlerinin değerlendirilmesi: ön çalışma raporu

Evaluation of diagnostic accuracy tests of erythrocyte indexes in the differential diagnosis of beta thalassemia minor and iron deficiency anemia: A preliminary report

Tevfik Balcı[1], Durmuş Ayan[1], Cevdet Türkyürek[1], Ergül Yaylagül[1]

[1]Niğde Training and Research Hospital, Department of Medical Biochemistry, Niğde, Turkey

Cukurova Medical Journal 2021;46(3):1009-1017.

Abstract

Purpose: The most common anemias are iron deficiency anemia (IDA) and beta-thalassemia minor (BTm). A correct differantiation of them is important in terms of preventing unnecessary iron treatment and also preventing thalassemia disease. Considering the financial limitations specifically in countries with high prevalence of thalassemia, mathematical indices, which are simpler solutions, have been used to achieve a differential diagnosis.

Materials and Methods: Total of 71 cases eligible for inclusion criteria were included in the study and 11 indices were examined. 46 of the cases were in the BTm-group and 25 of the cases were in the IDA-group. The BTm-group were accepted as positive-patient-group and the IDA-group were accepted as control-group. Diagnostic criteria were evaluated with erythrocyte indices.

Results: The highest Youden Index was seen in the Sirdah index (89.48%), followed by two identical performances of the Ehsani and the Mentzer indices (84.%), indicating that these three indices showed the best diagnostic performance. There were two indices with a low performance (DOR<10): The Bessmann and Ricerca indices.

Conclusion: Adding appropriate indices to complete blood count (CBC) results before further examinations will be beneficial in terms of efficient use of resources.

Keywords: Erythrocyte indices, differential, diagnosis, beta thalassemia minor, iron deficiency anemia

Öz

Amaç: En sık görülen anemiler demir eksikliği anemisi (DEA) ve beta-talasemi minördür (BTm). Bunların doğru bir şekilde ayırt edilmesi, gereksiz demir tedavisinin önlenmesi ve talasemi hastalığının önlenmesi açısından önemlidir. Özellikle talasemi prevalansının yüksek olduğu ülkelerdeki finansal kısıtlılıklar göz önüne alındığında, ayırıcı tanıya ulaşmak için daha basit çözümler olan matematiksel indeksler kullanılmıştır.

Gereç ve Yöntem: Dahil edilme kriterlerine uygun toplam 71 vaka çalışmaya dahil edildi ve 11 indeks incelendi. Vakaların 46'sı BTm grubunda, 25'i ise IDA grubundaydı. BTm grubu pozitif hasta grubu, DEA grubu kontrol grubu olarak kabul edildi. Tanı kriterleri eritrosit indeksleri ile değerlendirildi.

Bulgular: En yüksek Youden İndeksi Sirdah indeksinde (%89,48) görüldü, ardından Ehsani ve Mentzer indekslerinin iki özdeş performansı (%84,00) bu üç indeksin en iyi tanısal performansı gösterdiğini gösteriyor. Düşük performanslı (DOR <10) iki endeks vardı: Bessmann ve Ricerca endeksleri.

Sonuç: İleri tetkiklerden önce tam kan sayımı (TKS) sonuçlarına uygun indekslerin eklenmesi kaynakların verimli kullanılması açısından faydalı olacaktır.

Anahtar kelimeler: Eritrosit indeksleri, ayırıcı, tanı, beta talasemi minör, demir eksikliği anemisi

Yazışma Adresi/Address for Correspondence: Dr. Tevfik Balcı, Niğde Training and Research Hospital, Department of Medical Biochemistry, Niğde, Turkey E-mail: balcitevfik86@gmail.com
Geliş tarihi/Received: 12.04.2021 Kabul tarihi/Accepted: 24.06.2021 Çevrimiçi yayın/Published online: 23.07.2021

INTRODUCTION

It has been reported that the most common forms of anemia are iron deficiency anemia (IDA) and beta-thalassemia minor (BTm). In addition, globin chain synthesis disorders and heme synthesis disorders in these anemias have been reported to cause microcytic and hypochromic red blood cells (RBCs), respectively[1]. It is known that these two anemias, which can be easily confused, require laboratory tests for the accurate diagnosis.

A hemoglobinopathy control program is implemented for all premarital couples in Turkey. By adding simple mathematical erythrocyte indices to complete blood count (CBC) tests, it will be possible to use hemoglobinopathy screening more beneficially and efficiently in all pediatric and non-marital periods with the high-performance liquid chromatography (HPLC) method.

To diagnose BTm, a CBC should be performed first, followed by a hemoglobin and/or genetic analysis to confirm the diagnosis. Similarly, an analysis of iron status parameters after CBC is required to confirm DEA. However, these confirmatory assays are expensive and the process is complex. Preliminary evaluation with CBC and erythrocyte indices can be done to increase the accuracy of these advanced diagnostic tests[34,35].

It has been reported that IDA can cause a decrease in cognitive, behavioral and motor functions in children. Therefore, it is important to make a preliminary evaluation of the differential diagnosis of IDA and BTm with simple erythrocyte indices, especially in periods outside the scope of the hemoglobinopathy control program[36]. On the other hand, physiological changes during pregnancy in cases of BTm have been reported to worsen the severity of anemia and are significantly associated with fetal growth restriction, preterm delivery, and risk of low birth weight. Therefore, early and accurate diagnosis in pregnant women has important implications for genetic counseling and appropriate treatment[34,37].

In our study, it was aimed to prevent unnecessary iron treatment in patients with erythrocyte index results in favor of thalassemia carriers and to refer these patients to hemoglobinopathy screening with HPLC. On the other hand, it is aimed to prevent cases with erythrocyte index favoring IDA from being referred to unnecessary and expensive HPLC hemoglobinopathy screening. In this way, it is aimed to use resources correctly and efficiently.

Table 1. Discrimination indices for distinguishing BTm from IDA in patients with microcytic and / or hypochromic RBC

			Cut-off value		
	Discrimination Index	**Formula**	**IDA**	**BTm**	**Ref**
1	England-Fraser (E-F)	MCV–RBC–(5 Hb) – 3.4	>0	<0	6
2	RBC	RBC	<5	>5	6
3	Mentzer (Mnt)	MCV/RBC	>13	<13	7
4	Srivastata (Srv)	MCH/RBC	>3,8	<3,8	8
5	Shine-Lal (SH-L)	(MCV^2×MCH)/100	>1530	<1530	9
6	Bessman (Bsm)	RDW	>15	<15	10
7	Ricerca (Ric)	RDW/RBC	>4,4	<4,4	11
8	Green-King (G-K)	MCV^2×RDW/ 100Hb	>65	<65	12
9	Jayabose (Jyb)	MCV/(RBC ×RDW)	>220	<220	13
10	Sirdah (Srd)	MCV–RBC–(3 Hb)	>27	<27	14
11	Ehsani (Ehs)	MCV–(10RBC)	>15	<15	15

Cut-off values transformed into generally used units: Hb in g/dL; RBC in 10^{12}/L; MCV in fL; MCH in pg; RDW in %)
BTm: Beta-thalassemia minor. Hb: Hemoglobin. IDA: Iron deficiency anemia. MCH: Mean corpuscular hemoglobin. MCV: Mean corpuscular volume. RBC: Red blood cell. RDW: Red cell distribution width. Ref: References.

Since erythrocyte indices are calculated from CBC results, it has been reported that similar results are expected in terms of index performances in different parts of the world, but unexpectedly this is not the case. For example; It has been reported that Mentzer, Sirdah and Shine-Lal indices may be preferred in the Mediterranean region societies where our country is located[14,22,31]. Erythrocyte indices were reported to

perform best overall in European countries, but notable differences with other regions were reported. For example, it was reported that Mentzer and Shine and Lal indices performed weaker than other regions, while Green and King, Ricerca, Jayabose, Sirdah and Ehsani indices performed stronger[22,32,33]. Therefore, we believe that each region should choose the appropriate index method

Given the financial constraints in countries with high prevalence of thalassemia, we planned to evaluate the performance of mathematical RBC indices, which are simple and less complex solutions for differential diagnosis.

The aim of this retrospective study was to examine the diagnostic accuracy of 11 discrimination indices in a series of 71 patients (25 IDA and 46 BTm) for assessment of differential diagnosis of IDA and BTm. The eleven commonly used discrimination indices calculated using the CBC results are defined in Table 1.

MATERIALS AND METHODS

This study was carried out in Niğde Ömer Halisdemir University Training and Research Hospital Biochemistry laboratory. Patient results approved by biochemists were evaluated retrospectively according to test results between 01.01.2020 and 10.30.2020. In addition to routine biochemical tests, hemoglobinopathy screening is also performed by HPLC method in our laboratory. We interpret the HPLC test results together with the patient's clinical and other laboratory findings as a whole and assist the clinician in differential diagnosis.

This study was approved by the Niğde Ömer Halisdemir University Clinical Research Ethics Committee with the date of 11.02.2021 and the decision number 2021/17. Since the study was conducted with retrospectively approved results, participant approval was not requested by the ethics committee.

Differential diagnosis with laboratory parameters

It has been reported that low serum ferritin is a sensitive feature of absolute iron deficiency, which indicates iron stores as the gold standard method. In the presence of anemia, ferritin levels have been reported to be low (< 10-12 ug / L). In patients with iron refractory IDA (IRIDA), transferrin saturation (Tsat) was low, hepcidin levels were normal or high, and ferritin levels were normal or high. Therefore, it reflects an increase in iron in macrophages. Apart from this, tests other than ferritin were found unnecessary for the diagnosis of IDA. Measuring serum hepcidin levels may be the diagnosis of this atypical iron deficiency (ID) but it has been known that hepcidin testing is difficult to access widely today. Although ferritin values of higher than or equally 100 mg / L in combination with low (<20%) Tsat are frequently used to diagnose iron deficiency in the presence of inflammation, these discretionary cut-off values have been found to over estimate ID[2].

We used low serum ferritin levels (<12 ug/L) which has high sensitivity in the diagnosis of ID and together with we used hemoglobin (Hb) levels below lower limit of for each age groups in the diagnosis of IDA[2,3]. It has been reported that the normal range for Hb varies between different populations. It is therefore reasonable for laboratories to use the lower limit of the Hb normal range to define anemia[3].

High performance liquid chromatography (HPLC) is known to give an accurate estimate of Hemoglobin A2 (HbA2) levels and is therefore the preferred method. Conditions with decreased RBC indices and with HbA2 levels of > 4.0% were found to be indicative of BTm. It has been reported that normal RBC indices and HbA2 levels > 4.0% can be seen in different conditions such as vitamin B12 deficiency, liver disease and human immuno deficiency virus (HIV) infection. Borderline HbA2 levels (3.3-3.9%) have been reported to be difficult to diagnose with routine methods, as it may require verification with DNA methods for the diagnosis[4]. In the evaluation of hypochromic and microcytic RBCs, we selected cases with mean corpuscular hemoglobin (MCH) <27 pg and / or mean corpuscular volume (MCV) <80 fL for adults and cases with MCV and / or MCH values below the reference values in the pediatric population[4,5].

For the definition of anemia in our study; we used Hb reference ranges that recommended by the hematology analyzer manufacturer insert. Since cases in the gray-zone (i.e IDA + BTm and /or IDA + alpha thalassemia) may need to be confirmed with DNA testing, only the cases with an HbA2 level of 1.5% to 3.3% were selected in the IDA group of in our study. These cases may also include BTm with normal HbA2 levels and /or alpha thalassemia trait situations that cannot be identified by the HPLC screening methods. Fortunately, in these cases, both

premarital screening of the spouse-to-be with HPLC and, if necessary, genetic counseling reduces the risk of having a child with thalassemia. In addition, only the cases with HbA2 levels between 4% and 8%, HbF levels lower than 10% and, with hypochromia and / or microcytosis, normal ferritin levels and, normal C-reactive protein (CRP) levels were selected for BTm diagnosis[4,5,16,17].

Sample

When all the cases admitted to our hospital in 10 months from the beginning of 2020 were evaluated regardless of the HbA2 levels and the inflammation status, the total number of cases (IDA and other unknown conditions) was 1932 in women, 380 in men and therefore approximately 5 times more in women than in men. Regardless of ferritin levels and inflammation, the number of cases with HbA2 levels greater than 3.5% (suggestive of BTm) alone was 52; 27 of whom were men and 25 of whom were women. A total of 71 cases with hypochromic, microcytic anemia and eligible for inclusion criteria were included in the study. 46 of the cases were in the BTm group (24F, 22M) and the median age of BTm group was found to be 26 (min-max: 2-77). 25 of the cases were in the IDA group (20F, 5M) and the median age of IDA group was found to be 28 (min-max: 1-45). The BTm cases group were accepted as positive patient group and the IDA cases group were accepted as negative patient group (control group). Results can be inconsistent and misleading if the definitive diagnosis is not correct, such as selecting subjects from a population of BTm patients who also have concurrent IDA[34]. Power analysis of the study was performed according to Clinical and Laboratory Standards Institute (CLSI) guideline EP24-A2. Inclusion/exclusion criteria were applied in our study to evaluate index performances realistically and accurately. Our study was conducted in Niğde, Turkey, and we believe that this study will be useful for preliminary evaluation. It is seen that there is a need for long-term studies with more participants in different centers. Exclusion criteria of groups and number of eligible participants are summarized in Table 2.

Table 2. Exclusion criteria of groups and number of eligible participants

Group1 (IDA)	**Women**	**Men**	**Total**
Potentially eligible participants	n: 1932	n: 380	n: 2312
Eligible participants	n: 20	n: 5	**n: 25**
Exclusion criteria	Reason 1. (CRP levels)	Cases with unknown and/or high CRP levels (n: 2014)	
	Reason 2. (HbA2 levels)	Cases with unknown and/or outside the range of 1.3% to 3.3% HbA2 levels (n: 273)	
Group 2 (BTm)	**Women**	**Men**	**Total**
Potentially eligible participants	n: 25	n: 27	n: 52
Eligible participants	n: 24	n: 22	**n: 46**
Exclusion criteria	Reason 1. (Ferritin levels)	Cases with unknown and/or low ferritin levels (n: 2)	
	Reason 2. (HbA2 levels)	Cases with outside the range of 4% to 8% HbA2 levels (n:4)	
	Reason 3. (HbF levels)	Cases with above the range of 10% HbF levels (n:0)	

A total of 71 participants were eligible for the study according to the exclusion criteria.
BTm: Beta-thalassemia minor. CRP: C-reactive protein. IDA: Iron deficiency anemia. HbA2: Hemoglobin A2. HbF: Hemoglobin F. n: number.

Diagnostic performance criteria

Sensitivity % (Sens; TP/TP+FN), Specificity % (Spcf; TN/TN+FP), Accuracy % (TP+TN/TP+TN+FP+FN), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR) and Diagnostic Odds Ratio (DOR) were calculated. Finally we used Receiver Operating Characteristic (ROC) curve to summarize overall test performance[18] with Youden Index. A value of 1,00 indicates that there are no false positives or false negatives, i.e. the test is perfect. The index gives equal weight to false positive and false negative values. Youden Index is

often used in conjunction with ROC analysis[19]. The index is defined for all points of an ROC curve, and the maximum value of the index may be used as a criterion for selecting the optimum point when diagnostic tests give numeric results.

Statistical analysis

Data were entered into an Excel (Microsoft, Redmond, WA, USA) spreadsheet which calculated automatically these 11 indices and diagnostic criteria. *SPSS 15.0 software* (SPSS, Inc., *Chicago*, IL, USA) were used to compare CBC and other biochemical parameters results (serum ferritin, CRP and HbA2 levels) for the groups. Descriptive statistics were presented as numbers and percentages for categorical variables, and as the interquartile range (IQR) and median for the numerical variables. Normal distribution was determined by skewness, kurtosis, Kolmogorov-Smirnov (Lilliefors Significance Correction), Shapiro-Wilk tests, and examination of the distribution of histogram graphics. After the Kruskal-Wallis analysis was performed for comparing multiple independent groups without normal distribution, the non-parametric Mann-Whitney U test was used as a verification test for the comparison of the groups. Since the parametric test condition could not be achieved, the relationships between the numerical variables were analyzed using the Spearman Correlation test. The statistical alpha significance level was accepted as $p<0.05$.

Table 3. The CBC and biochemical parameters of two groups

	IDA Median (25%-75%)	BTm Median (25%-75%)	Significance
Hb	10.33 ± 1.88	12.05 ± 3.30	p < 0.001
RBC	4.86 ± 0.71	6.15 ± 1.04	p < 0.001
MCV	75.10 ± 10.2	63.95 ± 5.65	p < 0.001
MCH	22.80 ± 5.65	19.45 ± 1.95	p < 0.001
RDW	15.40 ± 2.35	16.70 ± 2.60	p < 0.05
Ferritin	5.86 ± 3.87	100 ± 136	p < 0.001
CRP	0.85 ± 3.38	1.30 ± 1.50	p = 0.494
HbA2	2.20 ± 0.35	5.35 ± 0.7	p < 0.001

Units: Hb in g/dL; RBC in 1012/L; MCV in fL; MCH in pg; RDW in %; Ferritin in ug/L; CRP in mg/L and HbA2 in %.
Statistical significances of differences between groups were determined using a Mann-Whitney U.
CBC: Complete blood count. CRP: C-reactive protein. HbA2: Hemoglobin A2.

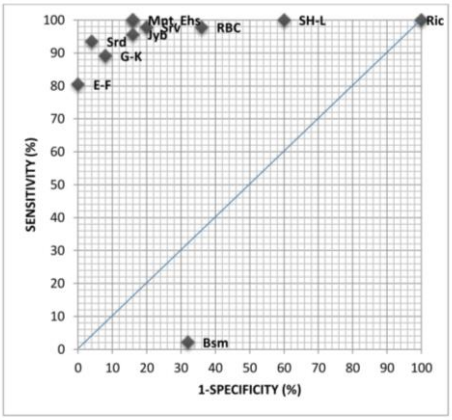

Figure 1. The performance of each discriminant index is graphically illustrated in the ROC plot

Bsm: Bessman. Ehs: Ehsani. E-F: England-Fraser. G-K: Green-King. Jyb: Jayabose. Mnt: Mentzer. Ric: Ricerca. SH-L: Shine-Lal. Srd: Sirdah. Srv: Srivastata.

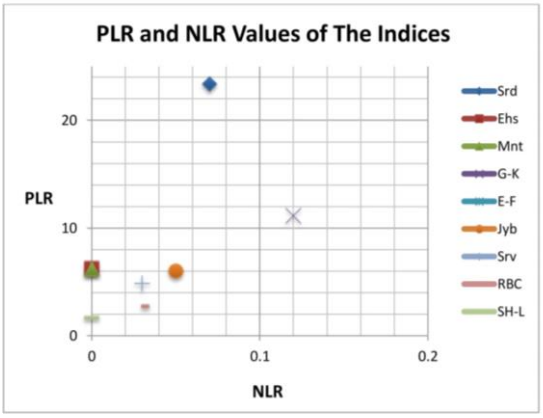

Figure 2. The PLR and NLR values of the indices with Youden Index greater than zero shown below

The diagnostic accuracy performance of the indices is considered to be high when the PLR value is greater than 10 and the NLR value is less than 0.1.
Ehs: Ehsani. E-F: England-Fraser. G-K: Green-King. Jyb: Jayabose. Mnt: Mentzer. SH-L: Shine-Lal. Srd: Sirdah. Srv: Srivastata.

RESULTS

The CBC and biochemical parameters of the two groups of the anemia to use in differential diagnosis study are shown in Table 3.

Sens, Spcf, PLR, NLR, DOR, Youden Index (%) and Accuracy % are presented in Table 4. For each discriminant index, the DOR was calculated using the data from all applicable studies (Table 4). Ehsani, Mentzer, England-Fraser and Shine-Lal indices were found to have the highest DOR. These performance were higher than the DOR performance of all other discriminant indices. There were two indices with a low performance (DOR <10): the Bessmann (RDW) and Ricerca indices (Table 4). The performance of each discriminant index is graphically illustrated in the ROC plot Figure 1 and summarized the overall test performance with Youden Index (%) (Table 4).

The highest Youden Index was seen in the Sirdah index (89,48), followed by two identical performances of the Ehsani and Mentzer indices (84,00), indicating that these three indices showed the best diagnostic performance. Among these three indices, the sensitivity of Ehsani and Mentzer indices was found to be the highest (100%), so false negative rates were found to be the lowest in these two indices. Similarly, the percentage accuracy of all three indices was found to be the highest (94%) (Table 4). PLR and NLR values of indices with Youden Index greater than zero according to Table 4 are summarized in Figure 2. According to (Figure 2), it was seen that the only index with PLR value greater than 10 and NLR value less than 0.1 was the Sirdah (Srd) index.

Table 4. The differential values of each discrimination index and the correctly identified number of the patients using these indices in each group

	Sens (%)	Spcf (%)	PLR	NLR	DOR	Youden (%)	% Acc
Srd	93,48	96,00	23,37	0,07	344,00	89,48	94,00
Ehs	100,00	84,00	6,25	0,00	*inf*	84,00	94,00
Mnt	100,00	84,00	6,25	0,00	*inf*	84,00	94,00
G-K	89,13	92,00	11,14	0,12	94,30	81,13	90,00
E-F	80,43	100,00	*inf*	0,19	*inf*	80,43	87,00
Jyb	95,65	84,00	5,98	0,05	115,50	79,65	92,00
Srv	97,83	80,00	4,89	0,03	180,00	77,83	92,00
RBC	97,83	64,00	2,72	0,03	80,00	61,83	86,00
SH-L	100,00	40,00	1,67	0,00	*inf*	40,00	79,00
Ric	100,00	0,00	1,00	*UD*	*UD*	0,00	65,00
Bsm	2,17	68,00	0,07	1,44	0,05	-29,83	25,00

Acc: accuracy. Bsm: Bessman. DOR: Diagnostic odds ratio. Ehs: Ehsani. E-F: England-Fraser. G-K: Green-King. Inf: infinity. Jyb: Jayabose. Mnt: Mentzer. NLR: Negative likelihood ratio. PLR: Positive likelihood ratio. Ric: Ricerca. Sens: Sensitivity. SH-L: Shine-Lal. Spcf: Specificity. Srd: Sirdah. Srv: Srivastata. UD: undefined.

Since HbA2 level testing from all cases with low erythrocyte indices is neither practical and nor cost effective in the routine practice, we could not precisely detect our local BTm frequency. It is known that the prevalence of BTm is relatively high in our country. Moreover, the treatment of beta thalassemia is very difficult and despite the treatment, it can lead to disastrous results such as heart failure and sudden death. For all these reasons, the % sensitivity of the index method to be selected should be high enough[20,21]. The Bessman index was found to have a negative Youden Index (%) score (-29.83), and therefore the cut-off value of this index should be reversed (Table 4)

DISCUSSION

The prevalence of BTm in our country is 2%, and it has been reported to reach up to 10%, especially in the southern regions of the Anatolia[21]. It has been reported that there are many RBC indices to be used in the distinction between IDA and BTm. None of these RBC indices were found to be perfect (%100 Sens and %100 Spcf) and also the performances of the indices were not found to be consistent across

studies. It was reported that RBC indices shown good perfomance in one study may show unacceptable performance in another study. Although the reasons for this problem are not clearly stated; it has been reported that possible differences in genetic and analytical factors between races and regions may be important.

Correct diagnosis is necessary to ensure that IDA patients are treated with iron and on the other hand, patients with BTm are not given unnecessary iron treatment. In addition, preventing serious consequences of thalassemia syndromes with pre-marital screenings and genetic counseling services is extremely important in regions where BTm is commonly seen[22].

In previous studies, it was reported that microcytosis in cases with BTm and hypochromia in cases with ID were more dominant[23,24]. Since the MCV parameter is used in the diagnosis of both IDA and BTm patients for the presence of microcytic anemia, it is very important that the blood sample to be analyzed is fresh because it has been reported that the MCV parameter may increase up to 5 fL due to waiting for more than 24 hours[25]. In our study, it was determined by Sysmex XN1000 CBC autoanalyzer (Sysmex Corporation, Hyogo, Kobe, Japan). that RBCs were more likely to be microcytic and more likely to be hypochromic in the BTm group than in the IDA group (Table 3).

Considering the studies examining the genetic differences of beta thalassemia cases, it has been reported that there is a link between CBC results and hemoglobin genetics. In addition, it has been reported that there is a significant inverse correlation between MCV result and the severity of thalassemia[26]. These data could explain the differences observed in our study and the necessity to define new cut-off values for each population analyzed.

It has been reported that discrimination performance of BTm with RBC indices is evaluated in almost all studies. Fewer studies evaluated the RBC index performance of alpha thalassemia plus BTm or alpha and/or delta-beta thalassemia only. In all these studies, it was clearly seen that all indices performed better than alpha thalassemias in BTm[24].

Since BTm and alpha thalassemia are common in our country, pre-marital thalassemia screening is mandatory in all cities. For this reason, it becomes important that the indices to be selected has high sensitivity for thalassemia. Since the gray zone cases (IDA + BTm, IDA + alpha thalassemia, ACD, etc.) are more likely to result in favor of IDA in previous RBC index studies, it means that these indexes should be used carefully in the Turkish population in order not to miss BTm cases.

In a meta-analysis, the evaluation of Microcytic RBC % / Hypochromic RBC % ratio was found to have the strongest performance, but this method is impractical and requires peripheral smear assessment[27]. Studies reported in the 21st century where a high level of analytical standardization of RBC parameters has been achieved has shown a high degree of compatibility, and therefore the influence of the analyzer type on the performance of RBC indexes is negligible. When the index formulas are evaluated, RDW stands out as the only parameter that shows significant differences between different analyzers. Therefore, it is reported that the performances of RBC indexes containing this parameter are not good enough[28]. This factor may explain the low diagnostic performance of especially RDW-containing indices (Bessman, Ricerca) in our study.

In previous studies, age group suitability was found to be different for almost every index, and no consensus was stated as a result of these studies, so no age limitation was made in our study[6-8,12-15,22]. Many authors have not used the original cut-off values of the discriminant indices, but applied an alternative cutoff. For example, Mentzer originally published his index with 13 as the cut-off value[3]. Other authors, however, used values even as high as 20, without proper validation[29].

Demir et al. calculated eight discrimination indices in Turkey and concluded that none of the discrimination indices showed 100% Sens and Spcf. It was reported that RBCs and RDWI indices had the highest Youden's Index value (82 and 80% respectively) and could distinguish BTm from IDA in 90% of the patients with RBC and 92% with RDWI[30]. These results were not found to be consistent with the results of our study. Hoffman et al. stated in their meta-analysis study that Mentzer, Shine-Lal and Sirdah indices are preferred indices in a Mediterranean population[22]. In our study, the Sirdah, Mentzer, and Ehsani indices were the best performing indices, and our results were relatively consistent with this study.

Since there is no verification method with DNA

testing in our laboratory; the cases in the gray-zone were not included in our study. In our study, gray-zone cases were excluded in order to evaluate RBC index performances more realistically. Therefore, when we consider previous studies, the number of cases included in our study seems to be relatively insufficient for the cut-off change and this may be a limitation of our study. In order to obtain more realistic performances of the indices, it will be beneficial to verify all cases with DNA testing in terms of thalassemia and also to include cases with microcytic anemia other than IDA and thalassemia in new studies.

To summarize, it can be said that simple mathematical indices are helpful in differential diagnosis in populations where BTm prevalence is high. In the definitive diagnosis, it is seen that no index alone is sufficient. We think that adding appropriate indices to CBC results in pre-evaluation of the patients before further examinations will be beneficial in terms of efficient use of resources and preventing unnecessary test requests.

Yazar Katkıları: Çalışma konsepti/Tasarımı: TB, DA, CT, EY; Veri toplama: TB, DA; Veri analizi ve yorumlama: TB; Yazı taslağı: TB; İçeriğin eleştirel incelenmesi: TB, DA; Son onay ve sorumluluk: TB, DA, CT, EY; Teknik ve malzeme desteği: TB, DA, CT, EY; Süpervizyon: TB, DA, CT, EY; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Niğde Ömer Halisdemir Üniversitesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulundan 11.02.2021 tarih ve 2021/17 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.

Author Contributions: Concept/Design : TB, DA, CT, EY; Data acquisition: TB, DA; Data analysis and interpretation: TB; Drafting manuscript: TB; Critical revision of manuscript: TB, DA; Final approval and accountability: TB, DA, CT, EY; Technical or material support: TB, DA, CT, EY; Supervision: TB, DA, CT, EY; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained for this study from the Niğde Ömer Halisdemir University Non-Interventional Clinical Research Ethics Committee with the decision dated 11.02.2021 and numbered 2021/17.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: Authors declared no financial support

REFERENCES

1. DeLoughery TG. Microcytic anemia. N Engl J Med. 2014;371:1324-31.
2. Camaschella C. Iron deficiency. Blood. 2019;133:30-9.
3. Goddard AF, James MW, McIntyre AS, Scott BB; British Society of Gastroenterology. Guidelines for the management of iron deficiency anaemia. Gut. 2011;60:1309-16.
4. Ghosh K, Colah R, Manglani M, Choudhry VP, Verma I, Madan N et al. Guidelines for screening, diagnosis and management of hemoglobinopathies. Indian J Hum Genet. 2014;20:101-19.
5. Passarello C, Giambona A, Cannata M, Vinciguerra M, Renda D, Maggio A. Iron deficiency does not compromise the diagnosis of high HbA(2) β thalassemia trait. Haematologica. 2012;97:472-3.
6. England JM, Fraser PM. Differentiation of iron deficiency from thalassaemia trait by routine blood-count. Lancet. 1973;1:449-52.
7. Mentzer WC Jr. Differentiation of iron deficiency from thalassaemia trait. Lancet. 1973;1:882.
8. Srivastava PC. Differentiation of thalassaemia minor from iron deficiency. Lancet. 1973;2:154-5.
9. Shine I, Lal S. A strategy to detect beta-thalassaemia minor. Lancet. 1977;1:692-4.
10. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiency and thalassemia minor. Blood. 1979;53:288-93.
11. Ricerca BM, Storti S, d'Onofrio G, Mancini S, Vittori M, Campisi S et al. Differentiation of iron deficiency from thalassaemia trait: a new approach. Haematologica. 1987;72:409-13.
12. Green R, King R. A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor. Blood Cells. 1989;15:481-91; discussion 492-5.
13. Jayabose S, Mahmoud M, Levendoglu-Tugal O, Sandoval C, Ozkaynak F, Giamelli J Jr et al. Corticosteroid prophylaxis for neurologic complications of intravenous immunoglobulin G therapy in childhood immune thrombocytopenic purpura. J Pediatr Hematol Oncol. 1999;21:514-7.
14. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol. 2008;30:324-30.
15. Ehsani MA, Shahgholi E, Rahiminejad MS, Seighali F, Rashidi A. A new index for discrimination between iron deficiency anemia and beta-thalassemia minor: results in 284 patients. Pak J Biol Sci. 2009;12:473-5.
16. Thein SL. Molecular basis of β thalassemia and potential therapeutic targets. Blood Cells Mol Dis. 2018;70:54-65.
17. Giambona A, Passarello C, Renda D, Maggio A. The significance of the hemoglobin A(2) value in screening for hemoglobinopathies. Clin Biochem. 2009;42:1786-96.
18. Walter SD. Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. Stat Med. 2002;21:1237-56.
19. Schisterman EF, Perkins NJ, Liu A, Bondell H. Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology. 2005;16:73-81.
20. Russo V, Melillo E, Papa AA, Rago A, Chamberland C, Nigro G. Arrhythmias and sudden cardiac death in

beta-thalassemia major patients: noninvasive diagnostic tools and early markers. Cardiol Res Pract. 2019;2019:9319832.
21. Bolaman Z, Enli Y, Köseoğlu M, Koyuncu H, Aslan D. Prevalence of beta thalassemia trait in Denizli. Turk J Haematol. 2001;18:85-8.
22. Hoffmann JJ, Urrechaga E, Aguirre U. Discriminant indices for distinguishing thalassemia and iron deficiency in patients with microcytic anemia: a meta-analysis. Clin Chem Lab Med. 2015;53:1883-94.
23. Lafferty JD, Crowther MA, Ali MA, Levine M. The evaluation of various mathematical RBC indices and their efficacy in discriminating between thalassemic and non-thalassemic microcytosis. Am J Clin Pathol. 1996;106:201-5.
24. d'Onofrio G, Zini G, Ricerca BM, Mancini S, Mango G. Automated measurement of red blood cell microcytosis and hypochromia in iron deficiency and beta-thalassemia trait. Arch Pathol Lab Med. 1992;116:84-9.
25. Cappellini MD. Hemoglobin, iron, bilirubin. In Tietz Textbook of Clinical Chemistry and Molecular Diagnostics 6th Edition - E-Book (Nader Rifai):736. St. Louis, Missouri, USA, 2018.
26. Janel A, Roszyk L, Rapatel C, Mareynat G, Berger MG, Serre-Sapin AF. Proposal of a score combining red blood cell indices for early differentiation of beta-thalassemia minor from iron deficiency anemia. Hematology. 2011;16:123-7.
27. Jahangiri M, Rahim F, Malehi AS, Pezeshki SMS, Ebrahimi M. Differential diagnosis of microcytic anemia, thalassemia or iron deficiency anemia: a diagnostic test accuracy meta-analysis. Mod Med Lab J. 2019;3:1-14.
28. Lippi G, Pavesi F, Bardi M, Pipitone S. Lack of harmonization of red blood cell distribution width (RDW). Evaluation of four hematological analyzers. Clin Biochem. 2014;47:1100-3.
29. Kneifati-Hayek J, Fleischman W, Bernstein LH, Riccioli A, Bellevue R. A model for automated screening of thalassemia in hematology (math study). Lab Hematol. 2007;13:119-23.
30. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatr Int. 2002;44:612-6.
31. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S et al. Hematological indices for differential diagnosis of beta thalassemia trait and iron deficiency anemia. Anemia. 2014;2014:576738.
32. Urrechaga E. Discriminant value of % microcytic/% hypochromic ratio in the differential diagnosis of microcytic anemia. Clin Chem Lab Med. 2008;46:1752–8.
33. Janel A, Roszyk L, Rapatel C, Mareynat G, Berger MG, SerreSapin AF. Proposal of a score combining red blood cell indices for early differentiation of beta-thalassemia minor from iron deficiency anemia. Hematology. 2011;16:123–7.
34. Xiao H, Wang Y, Ye Y, Yang C, Wu X, Wu X et al. Differential diagnosis of thalassemia and iron deficiency anemia in pregnant women using new formulas from multidimensional analysis of red blood cells. Ann Transl Med. 2021;9:141.
35. Urrechaga E, Hoffmann JJML. Critical appraisal of discriminant formulas for distinguishing thalassemia from iron deficiency in patients with microcytic anemia. Clin Chem Lab Med. 2017;55:1582-91.
36. Düzenli Kar Y, Özdemir ZC, Emir B, Bör Ö. Erythrocyte indices as differential diagnostic biomarkers of iron deficiency anemia and thalassemia. J Pediatr Hematol Oncol. 2020;42:208-13.
37. Hanprasertpong T, Kor-anantakul O, Leetanaporn R, et al. Pregnancy outcomes amongst thalassemia traits. Arch Gynecol Obstet. 2013;288:1051-4.