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The Efficiencies of Erythrocyte Indices in Differential Diagnosis of Beta Thalassemia Minor and Iron Deficiency Anemia

Zeynep Yildiz[1]*; Abdulkadir Deniz[2]; Asuman Orcun[1]

[1] Department of Biochemistry Laboratory, Dr Lutfi Kirdar City Hospital, İstanbul, Turkey

[2]Istanbul Pendik Omer Hekim Family Health Center, Turkey.

***Corresponding Author:** Zeynep Yildiz*

Email: kuantum2011@gmail.com

Abstract

Background: The most common causes of microcytic anemias are Iron Deficiency Anemia (IDA) and Beta Thalassemia minor (BTm). Correct diagnosis is important to prevent unnecessary iron loading and to manage thalassemia states. In this study, we aimed to evaluate the performances of erythrocyte indices to support the differential diagnosis in a practical, quick and economic way.

Materials and methods: We evaluated the premarital screening data and extracted microcytic anemic patients and classified as IDA and BTm according to Complete Blood Cell counts (CBC), ferritin, C-Reactive Protein (CRP) and HbA2 analysis results. 442 patients with IDA, 205 with BTm were enrolled in the study. Diagnostic performances of 23 indices were evaluated. The sensitivity, specificity, positive and negative predictive values, Youden's index, Likelihood Ratios, Diagnostic Odds Ratios and Area Under Curve values out of Receiver Operating Characteristic curves were calculated. New cut-off values for each index was determined.

Results: None of the indices had a 100 percent sensitivity or specificity. Sirdah Index (SI) had the best rank in overall performances followed by Mentzer Index (MI), England-Fraser (E-F), Ehsani Index (EI) and Green and King index (G&K) while Telmissani MCHD, Bessman, Huber-Herklotz, Sirachainan, and Ricerca Index (RI) had the lowest performance.

Conclusion: Indices are efficient enough for a preliminary differentiation of IDA and BTm before further diagnostic evaluation. Index performances differ among studies because of ethnic and genetic variations among study populations. Study settings as patient selection and cut-offs may also effect the performances of indices. Correct interpretation of performance criteria is also important in assessment of proper index.

Keywords: Erythrocyte indices; Differential diagnosis; Beta thalassemia minor; Iron deficiency anemia.

Introduction

Microcytic anemia is defined as low hemoglobin levels with microcytic and hypochromic erythrocytes. In clinical practice, IDA and BTm are the most common causes of microcytic anemia. IDA occurs mainly as a result of inadequate intake, blood loss by the menstrual cycle and gastrointestinal bleeding. BTm results from impaired globin chain synthesis causing inadequate levels of corresponding hemoglobin types. Thalessemias are common in specific localizations such as Mediterranean area, the Middle East, Africa and Southeast Asia, and as a result of population spread thalassemia genes are over nearly the entire globe [1]. Given in the definitions, routinely used Complete Blood Cell Count (CBC) indices, Mean Corpuscular Volume (MCV) and

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Mean Corpuscular Hemoglobin (MCH) cannot discriminate IDA/BTm; besides their clinical presentations generally overlap, making the correct diagnosis a challenge. IDA diagnosis should be supported by a low serum ferritin <15 ng/mL in the absence of an acute phase state in the patient [2]. Diagnosis of BTm requires a HbA2 level of >3.5% which is provided by hemoglobin electrophoresis, high-performance liquid chromatography and finally DNA identification, all of which require significant expenditures of time, technical skill and financial resources [3]. Instead researchers have proposed formulas based on Red Blood Cell (RBC) indices to discriminate BTm and IDA, (Table 1). It is widely agreed that none of these indices is 100% sensitive or 100%specific. Besides there is considerable differences among the performances of the same indices in different studies. Though not clear, these variabilities may be attributed to regional differences in thalassemia genotypes and the study designs [1].

Hemoglobinopathy is the most common hereditary disorder in our country. According to a survey carried out by the Ministry of Health and National Hemoglobinopathy Council, the prevalence of BTm was stated as 2.1% overall in Turkey with highest prevalence in Western and Southern Anatolia [4]. In 2003, National Hemoglobino-pathy Screening Programme was introduced by Turkish Ministry of health in order to reduce hemoglobinopathy induced morbidity and mortality. Premarital screening is a part of this programme which aims to identify carriers of genetic disordersand guiding them with proper health care. Besides the population taken into premarital screening programme, it would be beneficial to identify any patient presenting with symptoms and signs of microcytic anemia before demanding high cost laboratory techniques.

The aim of this study was to examine the diagnostic accuracies of 23 discrimination indices in differentiating IDA and BTm for an economical, quick, accurate and practical preliminary evaluation.

Materials and methods

Study design

Thisis a retrospective data analysis study carried on premarital variant hemoglobin screening results out of our laboratory information system. Blood samples from couples were obtained in their regional primary care units in the Anatolian Region of Istanbul. Transfer of samples and measurements were done on the same day in Kartal Dr Lutfi Kirdar City Hospital Biochemistry Laboratory and evaluated by laboratory specialists. Data between April and July 2022 was screened retrospectively.Patient written consent was required as a part of screening programme in the primary care setting and any identifying patient information was secured in this study.

Methods

In all primary care units, blood samples were drawn into Beckton Dickinson (BD, Franklin Lakes, NJ, USA) tubes; 5 mL Vacutainer® SST™II tubes for iron and ferritin and 3mL Vacutainer K2EDTA tubes for CBC.

HbA2 analysis was carried on ion exchange high performance liquid chromatography (Trinity Biotech Premier Resolution) analyzer. Routine Complete Blood Count (CBC) analyses were done on Sysmex XN 1000 automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Serum ferritin, C-Reactive Protein (CRP) and iron levels were measured on modular Roche Cobas e801/c701 system.

Classification of data

Screening tests included CBC and variant hemoglobin analyses for both partners. We extracted the data containing ferritin and CRP measurements besides. Patients with a history of acute or chronic disease, bleeding, taking any medication were excluded.Data having Hb values <13 g/dL (for men) and <12 g/dL (for women) with a MCV value <80 fL were diagnosed as microcytic anemia. Of these, individuals with HbA2>3.5% and a normal ferritin (>15 ng/mL) were considered possible carriers of BTm whileindividuals with normal HbA2 results but low ferritin values (<15 ng/mL) were considered as IDA. Patients of both groups had CRP values <5 mg/L to exclude accompanying acute phase states which interfere with ferritin values.

Discriminative indices

23 discrimination indices were evaluated and compared. hese indices, their formulas and proposed cut-off values in distinguishing BTm from IDA are given in Table 1.

Statistical analysis

Descriptive statistics were presented as median and interquartile range (IQR) (25%-75%). Shapiro Wilk test was applied for testing normality. Mann-Whitney U test was used for the comparison of the 2 groups. $p<0.05$ was considered statistically significant. MedCalc version 19.2.1 (MedCalc Software Ltd, Ostend, Belgium) software was used for statistical calculations.

Diagnostic performance criteria [5]

Receiver Operative Characteristics (ROC): ROC curve analysis is a graphical illustration for the goodness of a test with 1-specificity on the x-axis and sensitivity on the y-axis. Given the sensitivity and specificity for each individual cut-off, a graph is formed Area Under Curve (AUC) is calculated. AUC is a global measure of discriminative power of a test and is widely used to compare the discriminative powers of different tests. A perfect diagnostic test has an AUC of 1.0 where a non-discriminative power corresponds to an AUC of 0.5. Relationship between the AUC with the diagnostic accuracy is defined as: 0.5-0.6 (bad), 0.6-0.7 (sufficient), 0.7-0.8 (good), 0.8-0.9 (very good), 0.9-1.0 (excellent).ROC analysis was performed and AUC values and optimum cut-off values for each index was calculated. By use of these cut-off values the following performance criteria were assessed for 23 discrimination indices.

Sensitivity (True Positive Rate) = True Positive / (True Positive + False Negative)

Specificity (True Negative Rate) = True Negative / (True Negative + False Positive)

Positive predictive value (PPV) = True Positive / (True Positive + False Positive)

Negative predictive value (NPV) = True Negative / (True Negative + False Negative)

Youden's Index (YI) = (Sensitivity + Specificity) -1

YI is a global measure of diagnostic accuracy. It compares the discriminative power of a test with others. YI is expressed between 0 -1; a perfect test has a YI =1.

Positive Likelihood Ratio (LR+) = Sensitivity / (1-Specificity)

Negative Likelihood Ratio (LR-) = (1-Sensitivity) / Specificity

Diagnostic Odds Ratio (DOR) = (True positive / False negative) / (False positive / True negative)

Results

442 patients with IDA and 205 patients with BTm were included in the study. CBC parameters Hb, RBC, MCV, MCH, RDW and ferritin values of 2 groups were significantly different from each other (all p's < 0.0001) while patient ages and CRP values were not (p=0.063 and p=0.84, respectively).Baseline characteristics and test results of IDA and BTm patients and statistical significances are given in Table 2.

The best cut-off values for our study group were calculated according to the AUC results and the corresponding sensitivity, specificity, PPV (%), NPV (%), YI, LR+, LR- and DOR (%) of discriminant indices were given in Table 3.

Ten indices had AUC values >90%, corresponding to excellent discrimination power. These are SirI>MI =E-F =G&K>EI>Kerman II = Sehgal > Telmissani-MDHL = Janel Index (11T) = Wong prachum. Sir I as the best with an AUC (%95 CI) of 0.94 (0.92-0.96). YIs of these indices were also superior to the others;SirI again with the highest YI = 0.80. RDW had the lowest AUC value with 0.52 (0.47-0.56), followed by Telmissani-MCHD with 0.67 (0.63-0.71) and Huber-Herklotz (HH) 0.71 (0.67-0.75). Other indices had AUC values ≥0.80. None of the indices had 100% sensitivity/specificity or 100% NPV/PPV. Bordbar index and S&L indices with highest sensitivity of 92.7% and 92.68% respectively, had low corresponding specificity (62.3%, 61.4%). These 2 indices had highest NPV% values (Bordbar 93.4% and S&L 93.3%) with lower corresponding PPV% values (59.6% and 59%, respectively). Ten indices with highest (sensitivity +specificity) values were SirI>RDWI>, E-F > G&K >EI > Sehgal >KermanII > Janel Index >MI > RBC count. These indices had also highest (NPV+PPV) values. SirI had the highest (sensitivity+specificity) and (PPV+NPV) values (88.3+91.2) and (85.8+92.9)%respectively.These indices also had the highest DOR values; SirI again with the highest DOR (77). RDW, Telmissani-MCHD, HH and Sirachainan indices had DOR<10 values showing insufficient performances.In evaluation of LRs, only SirI had a LR+>10 (exact value is 10.07) with LR- value of 0.13. None of the indices had a LR-<0.1.

Ranking of diagnostic performances of indices in discriminatingBTm from IDA in patients with microcytic anemia is shown in Table 4. In the total summary of ranking (Table 4, last column) SirI had the best rank in overall performances (with the smallest sum-off) followed by MI, E-F, EI and G&K while Telmissani-MCHD, Bessman, Huber-Herklotz, Sirachainan, and RI had the lowest performances.

Table 1: Discrimination indices for distinguishing thalassemia from iron deficiency anemia patients with microcytic and/ or hypochromic RBC.

Discrimination Index	History	Formula	Cut-off *	Ref.
RBC count	1973	RBC	>5.0	[26]
Mentzer Index (MI)	1973	MCV/RBC	<13	[27]
Srivastava Index (SI)	1973	MCH/RBC	<3.8	[28]
England-Fraser (E-F)	1973	MCV–RBC–(5 Hb) – 3.4	<0	[26]
Shine and Lal (S&L)	1977	MCV2x MCH/100	<1530	[29]
Bessman	1979	RDW	<15	[30]
Ricerca Index (RI)	1987	RDW/RBC	<4.4	[31]
Green and King (G&K)	1989	MCV2 x RDW/(100Hb)	<65	[32]
Das Gupta	1994	1.89 RBC-0.33 RDW-3.28	>0	[33]
Jayabose RDW Index (RDWI)	1999	MCV x RDW/RBC	<220	[34]
Telmissani-MCHD	1999	MCH/MCV	<0.34	[35]
Telmissani-MDHL	1999	(MCH /MCV) × RBC	>1.75	[35]
Huber-Herklotz (HH)	2004	(MCH × RDW/10RBC) + RDW	<20	[36]
Sirdah Index (SirI)	2008	MCV -RBC-(3xHb)	<27	[37]
Kerman I	2008	MCV × MCH/RBC	<300	[38]
Kerman II	2008	KERMAN I × 10/MCHC	<85	[38]
Ehsani Index (EI)	2009	MCV – (10 RBC)	<15	[39]
Janel (11T) index**	2011	Combination of 11 other indices*	≥8	[40]
Nishad	2012	0.615MCV + 0.518MCH + 0.446 × RDW	<59	[41]
Wongprachum	2012	(MCV × RDW/RBC)-Hb	<104	[42]
Sehgal	2013	MCV2 /RBC	<972	[43]
Sirachainan	2014	1.5Hb-0.05 MCV	>14	[44]
Bordbar	2015	|80 – MCV| × |27 – MCH|	>44.76	[45]

*The cut-off thal. Value favoring thalassemia is as originally published.

**Janel et al. combined 11 existing indices into a single score: RBC, Mentzer, Shine and Lal, England and Frazer, Srivastava, Green and King, RDW, RDWI,Ricarce, Ehsani, and Sirdah. Ref: reference number

Table 2: Baseline characteristics and laboratory test results of IDA and BTm groups.

	IDA	BTm	P value
	Median (25%-75%)	Median (25%-75%)	
Number	442	205	
Age (year)	29 (27-39)	28 (24-36)	0.063
Hb (g/dl)	10.4 (9.3-11.4)	11.8 (10.7-12.7)	<0.0001
RBC (10^6/ µL)	4.69 (4.4-5.01)	5.8 (5.34-6.26)	<0.0001
MCV (fL)	75.2 (70.5-77.8)	66.0 (63.48-69.5)	<0.0001
MCH (pg)	22.1 (20.2-23.6)	20.2 (19.0-21.03)	<0.0001
RDW (%)	18.5 (17.5-19.9)	17.5 (16.0-18.4)	<0.0001
HbA2 (%)	2.0 (18.-2.3)	4.8 (4.3-5.1)	<0.0001
Ferritin (µg/L)	4.32 (2.12-8.42)	92.16 (55.42-180.12)	<0.0001
CRP (mg/L)	1.42 (0.42-4.08)	1.20 (0.18-4.42)	0.84

Hb: Hemoglobin, RBC: Red Blood Cell Count, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, RDW: Red Cell Distribution Width; Hba2: Hemoglobin A2; CRP: C-Reactive Protein.

Table 3: Performance data of discriminant formulas for differentiating thalassemia from iron defiency.

Number	Parameters	AUC	(%95 CI)	Cutt-off*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	YI	LR+	LR-	DOR
1	RBC count	0.897	(0.87-0.92)	>5.3	77.1	89.2	81	86.6	0.66	7.12	0.16	45
2	Mentzer Index (MI)	0.93	(0.90-0.95)	≤13.64	88.3	82.83	86.6	92.5	0.75	6.56	0.14	47
3	Srivastava Index (SI)	0.89	(0.86-0.91)	≤4.06	84.9	79	70.7	89.7	0.64	4.03	0.19	21
4	England-Fraser (E-F)	0.93	(0.90-0.95)	≤6.18	83.4	91.8	85.9	90.2	0.75	10.2	0.18	57
5	Shine and Lal (S&L)	0.80	(0.77-0.84)	≤1157.99	92.68	61.4	59	93.3	0.54	2.4	0.12	20
6	Bessman	0.52	(0.47-0.56)	>16.8	11.2	75.2	21.3	58.5	0.14	0.45	1.18	0.38
7	Ricerca Index (RI)	0.83	(0.80-0.86)	≤3.12	72.7	81.6	70.3	83.3	0.54	3.95	0.33	12
8	Green and King (G&K)	0.93	(0.90-0.95)	≤73.5	84.9	90.1	83.7	90.9	0.75	8.54	0.17	50
9	Das Gupta	0.88	(0.85-0.91)	>0.84	84.4	79.5	71.2	89.5	0.64	4.12	0.2	21
10	Jayabose RDW Index (RDWI)	0.85	(0.81-0.88)	≤230.58	90.2	85.7	79.1	93.6	0.76	6.3	0.11	57
11	Telmissani-MCHD	0.67	(0.63-0.71)	>0.29	81.5	50.9	50	82.1	0.32	1.66	0.36	5
12	Telmissani-MDHL	0.91	(0.88-0.93)	>1.61	78.5	90.4	83	87.5	0.69	8.14	0.24	34
13	Huber-Herklotz (HH)	0.71	(0.67-0.75)	≤24.48	80	58.8	53.8	83.1	0.39	1.94	0.34	6
14	Sirdah Index (SirI)	0.94	(0.92-0.96)	≤32.53	88.3	91.2	85.8	92.9	0.8	10.1	0.13	77
15	Kerman I	0.88	(0.85-0.91)	≤303.02	90.7	72.5	66.4	92.9	0.63	3.3	0.13	25
16	Kerman II	0.92	(0.89-0.94)	≤99.05	90.7	82.1	75.3	93.7	0.73	5.09	0.11	46
17	Ehsani Index (EI)	0.92	(0.90-0.95)	≤19.5	90.2	83.9	77.1	93.5	0.74	5.61	0.12	47
18	Janel (11T) index*	0.91	(0.88-0.93)	>3	87.3	84.8	77.5	91.8	0.72	5.74	0.15	38
19	Nishad	0.85	(0.82-0.88)	≤62.53	90.2	71.6	65.6	92.5	0.62	3.18	0.14	23
20	Wongprachum	0.91	(0.88-0.93)	≤124.49	87.8	80.4	72.9	91.7	0.68	4.48	0.15	30
21	Sehgal	0.92	(0.89-0.94)	≤990.64	90.7	82.5	75.6	93.7	0.73	5.17	0.11	47
22	Sirachainan	0.80	(0.76-0.83)	>12.7	80.5	66.4	58.9	85	0.41	2.39	0.29	8
23	Bordbar	0.81	(0.77-0.84)	>36.96	92.7	62.3	59.6	93.4	0.56	2.46	0.12	21

*The cut-off value favoring thalassemia as found by ROC analysis. AUC: Area Under Curve; PPV: Positive Predictive Value ; NPV: Negative Predictive Value; YI: Youden's Index; LR: Likelihood Ratio; DOR: Diagnostic Odss Ratio.

Table 4: Ranking of diagnostic performance of discrimination indices based on cut-off values obtained out of ROC analysis.

Number	Parameters	AUC	Sensitivity	Specificity	PPV	NPV	YI	DOR	Sum-off
1	RBC count	11	21	5	6	18	12	9	82
2	Mentzer Index (MI)	3	9.5	9	1	9.5	4	6	42
3	Srivastava Index (SI)	12	13.5	15	14	15	13.5	16	99
4	England-Fraser (E-F)	3	16	1	2	14	4	2.5	42.5
5	Shine and Lal (S&L)	19.5	2	21	19	6	18.5	18	104
6	Bessman	23	23	16	23	23	23	23	154
7	Ricerca Index (RI)	17	22	12	15	20	18.5	19	123.5
8	Green and King (G&K)	3	13.5	4	4	13	4	4	45.5
9	Das Gupta	13.5	15	14	13	16	13.5	16	101
10	Jayabose RDW Index (RDWI)	15.5	7	6	7	3	2	2.5	43
11	Telmissani-MCHD	22	17	23	22	22	22	22	150
12	Telmissani-MDHL	9	20	3	5	17	10	11	75
13	Huber-Herklotz (HH)	21	19	22	21	21	21	21	146
14	Sirdah Index (SirI)	1	9.5	2	3	7.5	1	1	25
15	Kerman I	13.5	4	17	16	7.5	15	13	86
16	Kerman II	6.5	4	11	11	1.5	7.5	8	49.5
17	Ehsani Index (EI)	5	7	8	9	4	6	6	45
18	Janel (11T) index*	9	12	7	8	11	9	10	66
19	Nishad	15.5	7	18	17	9.5	16	14	97
20	Wongprachum	9	11	13	12	12	11	12	80
21	Sehgal	6.5	4	10	10	1.5	7.5	6	45.5
22	Sirachainan	19.5	18	19	20	19	20	20	135.5
23	Bordbar	18	1	20	18	5	17	16	95

AUC: Area Under Curve), PPV: Positive Predictive Value, NPV: Negative Predictive Value), YI: Youden's Index, LR+: Positive Likelihood Ratio, LR: Negative Likelihood Ratio, DOR: Diagnostic Odds Ratio.
Sum off coloumn shows the sum of all ranking numbers of an index lower sum indicatingbetter diagnostic performance.

Discussion

IDA and BTm is the most frequent causes of microcytic anemia and differential diagnosis is essential for prevention and appropriate treatment of diseases. Hemoglobin variant analysis by means of high technology methods such as chromatography, electrophoresis and/or DNA analysis is a challenge because of their high economic costs and hard laboratory procedures. The aim of the current study was to compare the discriminative performances of pre-defined 23 RBC indices by use of sensitivity, specificity, PPV, NPV and to calculate LRs, DOR and YI with newly calculated cut-off values out of ROC analysis.

In our study SirI followed by MI, E-F, EI and G&K indices had the best performances in overall ranking while Telmissani-MCHD, Bessman, Huber-Herklotz, Sirachainan, and RI had the lowest performances. First emphasize should be set on performance tests and their correct interpretation. In this study we used an overall ranks scoring because an ideal index should perform well in all of the measures. Different measures represent different characteristics of a test and many aspects should be taken into consideration for a final decision. Sensitivity, specificity, LRs, DOR and YI are not affected by the prevalence of the disease but PPV+ and PPV- are. Within a sole study prevalence dependent measures can be used for comparison but they cannot be used for comparison of different studies with different prevalence backgrounds. YI, on the other hand, is another measure of diagnostic accuracy but is not sensitive for differences in the sensitivity and specificity of the test. Any two indices with the same YI may have different percents of sensitivity and specificity. LR+ and LR- values are good measures for diagnostic accuracy because their value represents the sensitivity and specificity of a test together out of one calculation. In a study of Demir et all. [6] they found highest YIs in RBC and RDWI indices and they concluded that if a patient is proven to have a defined value with these indices, the diagnosis is most likely correct. This is not the case. One has to know about the tests LR+, in order to rule in the diagnosis. When calculated out of given data, RBC index has a LR+ of 22, but RDWI has a LR+ of 5. The later seems to be insufficient to rule in. LR+ is the best indicator for rule-in as LR- is for rule-out the diagnosis. A test with a high sensitivity performs well in ruling out, while a test with high specificity is good for ruling in. In our study S & L had the highest sensitivity (92.68%) with the best LR- of 0.12. Besides E-F Index had the highest specificity (91.8%) corresponding to the highest LR+ of 10.19. If a diagnostic test has a LR+>10 and LR-< 0.1 this test is considered to have a significant contribution to the diagnosis. In our study 10 of 23 indices had excellent discriminative power (AUC>90%) but only SirI had LR+= 10.07 and LR- = 0.13. DOR also, depends significantlyon the sensitivity and specificity of a test. A test with high specificity and sensitivity that is with low rate of false positives and false negatives has high DOR. With the same sensitivity of the test,

DOR increases with the increase of the test specificity.SirI had the highest DOR in our study. Briefly in comparison of studies, prevalence dependence should be taken into consideration and performances should not be matched directly in different study groups. On the other hand, new cut-off values should be calculated and used for each individual study. Genetic expression of beta thalassemias varies considerably and this effects their CBC presentation. Also a significantinverse correlation between MCV values and the severity of thalassemia is stated [7]. Cut-off values change according to the study cohorts' ethnicity, gender and ages and new cut-off values should be calculated for each population analyzed.Some studies used conventional cut-offs and applied them to their study [8,9]. While some found new cut-offs out of their study population and used this new cut-off in evaluating the performance of indices andsaw that 7 of 10 indices improved when using new cut-offs [3]. In our study we calculated new cut-offs out of ROC analysis for each index and evaluated diagnostic performances using these new cut-offs. SirI with a new cut-off of <32.53 had the highest AUC value (0.94) with a 88% sensitivity and 91.2% specificity and taking the first order in overall ranking. In Jahangiri et al. Study [8] that used the conventional cut of <27 for BTm SirIhad a sensitivity of 80.26% and a specificity of 88.65% although had an AUC value of 0.845; one of the highest values in that study. This difference may be attributed to the different cut-off levels used in studies. And finally each performance criteria represents a different aspect of a test and all of them should be evaluated carefully [5].

In a meta-analysisby Hoffman et al [1]. Performances of 12 indices that were investigated five or more times in medical literature were compared by using DOR and ROC analyses. These authors preferred DOR as the accuracy measure because DOR reflects a combination of sensitivity and specificity, independent of disease prevalence, making it very appropriate for comparing different studies [10,11]. They also used summary ROC curves and calculated AUCs, saying AUCs of ROC analysis is quite robust to heterogeneity [12]. In this meta-analysis covering many medical literature results, the ratio of microcytic to hypochromic RBCs (M/H ratio) showed the best performance (DOR = 100.8). In BTm RBC do tend to be more microcytic, whereas iron deficient RBC are often more hypochromic [13]. However, these parameters are not automatically supplied by most of the analyzersand needs blood smear to perform, so difficult to use [14]. The RBC index had the second high DOR value (DOR = 47.0), closely followed by the SirI (DOR = 46.7) and the EI (DOR = 44.7). They presented Bessmanas the index with the lowest performance (DOR = 6.8).In our study these indices had high DOR values as well: SirI as the highest (DOR=77), followed by E-F (DOR= 57) and G&K (DOR=50). EI (DOR=47) and RBC indices (DOR= 45) performed well also. Bessman had the worst performance in our study too (DOR=0.38).

Though different hematology analyzers are used in measurement of RBC indices this did not cause significant variations among studies, except for RDW. This factor is not well standardized and different analyzers show significant variations in RDW measurement [15,16]. So indices applying RDW in calculations fail to perform well in many studies [17-19].

Ethnical and geographical origin of patients included in studies is another factor for different performances across studies. Although RBC indices MCV, MCH and MCHC differ slightly among different populations all around the world [20] indices did not perform similarly in different locations [1]. G&K, RI, RDWI, SirI and EI indices were superior in European studies and in a Mediterranean population the MI, S&L and SirI indices would be preferred. Likewise,in our study held in Turkiye, SirI followed by MI, E-F, EI and G&K indices had the best performances.

Another apparent difference among the studies was classification of IDA group. Microcytic anemic patients with defined Hb and MCV, MCH values are classified as IDA if they have low ferritin levels.Ferritin is the primary iron-storage protein and is critical to iron homeostasis and hematopoiesis [21]. In the absence of inflammation, the concentration of serum ferritin is positively correlated with the size of the total body iron stores [22,23]. Ferritin concentration was confirmed to be a good marker of iron stores and should be used to diagnose iron deficiency. However, because ferritin is also an acute phase reactant to diagnose IDA, WHO suggests alternative cut-offs or using inflammation markers as CRP and/or α-1 acid glycoprotein to exclude acute phase interference. In this guideline WHO proposes ferritin <15 µg/L as the cut-off for IDA diagnosis, and CRP<5 mg/L to exclude acute phase state. In our study, we used both criteria while selecting and classifying our study group.Balcı et all [17] used both of the criteria for their IDA group classification, but they used 12 µg/L for ferritin cut-off. Other studies used 22 µg/L [3] or 28 µg/L [8] and 10 µg/L [9] for ferritin cut-offs. On the other hand, most of the studies classify IDA and BTm in a similar way by means of their CBC and ferritin results. This protocol may cause misclassification of other microcytic anemias such as anemia of chronicle disease (ACD) if exclusion of chronicle diseases could not be applied strictly. In such case ACD patients are more likely to be classified as IDA, not BTm [24]. In our study we evaluated test results of healthy couples of premarital screening programme, regarding their accompanying tests and patient history records. Another specific case is the presence of BTm with IDA. These patients are generally misclassified as IDA by the indices [25] are probably treated with iron. This heterogeneity of IDA and BTm groups may also effect the performance of indices.

Other possible interferants in study settings was patient populations of different ages and different geographical areas. In general,most indices, E-F, MI and G&K evidently perform better in adults [1]. Our study consisted of pre-marital screening adults older than 18 years old and performances of these indices were quite well.

Conclusion

This study demonstrated that some CBC indices perform quite good performance in distinguishing BTm from IDA. Nevertheless, none of them can be used for a final diagnosis in all of the patients. At least these indices identify borderline patients in whom additional laboratory investigations are required for confirming the presence of BTm.

References

1. 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.

2. WHO guideline on use of ferritin concentrations to assess iron status in individuals and popular- tions. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/331505, accessed 7 July 2020).

3. Huang TC, Wu YY, Chen YG, Lai SW, et al. Discrimination index of microcytic anemia in young soldiers: a single institutional analy-

sis. PLoS One. 2015; 10: e0114061.

4. Cavdar AO, Arcasoy A. The incidence of-thalassemia and abnormal hemoglobins in Turkey. Acta Haematol. 1971; 45: 312-8.

5. Šimundić AM. Measures of Diagnostic Accuracy: Basic Definitions. EJIFCC. 2009; 19: 203-11.

6. 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.

7. Janel A, Roszyk L, Rapatel C, Mareynat G, Berger MG, et al. Proposal of a score combining red blood cell indices for early differentiation of betathalassemia minor from iron deficiency anemia. Hematology. 2011; 16: 123-7.

8. Jahangiri M, Rahim F, Malehi AS. Diagnostic performance of hematological discrimination indices to discriminate between βeta thalassemia trait and iron deficiency anemia and using cluster analysis: Introducing two new indices tested in Iranian population. Sci Rep. 2019; 9: 18610.

9. Okan V, Cigiloglu A, Cifci S, Yilmaz M, Pehlivan M. Red cell indices and functions differentiating patients with the beta-thalassaemia trait from those with iron deficiency anaemia. J Int Med Res. 2009; 37: 25-30.

10. Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: A single indicator of test performance. J Clin Epidemiol. 2003; 56: 1129-35.

11. Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, et al. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol. 2005; 58: 982-90.

12. Walter SD. Properties of the Summary Receiver Operating Characteristic (SROC) curve for diagnostic test data. Stat Med. 2002; 21: 1237-56.

13. 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.

14. Urrechaga E. Discriminant value of % microcytic/% hypochromic ratio in the differential diagnosis of microcytic anemia. Clin Chem Lab Med. 2008; 46: 1752-8.

15. 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.

16. 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.

17. Tevfik Balcı, Durmuş Ayan, Cevdet Türkyürek, Ergül Yaylagül. Evaluation of diagnostic accuracy tests of erythrocyte indexes in the differential diagnosis of beta thalassemia minor and iron deficiency anemia: A preliminary report. Cukurova Med J. 2021; 46: 1009-1017.

18. Nagwan I Rashwan, Ahmed El-Abd Ahmed, Mohammed H Hassan, Maha E Mohammed, Ali Helmi Bakri. Hematological indices in differentiation between iron deficiency anemia and beta-thalassemia trait. Int J Pediatr. 2022; 10: 97.

19. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, et al. Hematological indices for differential diagnosis of Beta thalassemia trait and iron deficiency anemia. Anemia. 2014; 2014: 576738.

20. Bull BS, Hay KL. Are red blood cell indexes international? Arch Pathol Lab Med. 1985; 109: 604-6.

21. WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva: World Health Organization. 2020.

22. Nutritional anaemias: Tools for effective prevention and control. Geneva: World Health Organization. 2017.

23. Lynch S, Pfeiffer CM, Georgieff MK, Brittenham G, Fairweather-Tait S, et al. Biomarkers of Nutrition for Development (BOND)-Iron Review. J Nutr. 2018; 148: 1001S-1067S.

24. Burk M, Arenz J, Giagounidis AA, Schneider W. Erythrocyte indices as screening tests for the differentiation of microcytic anemias. Eur J Med Res. 1995; 1: 33-7.

25. 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.

26. England JM, Fraser PM. Differentiation of iron deficiency from thalassaemia trait by routine blood count. Lancet. 1973; 1: 449-52.

27. Mentzer WC Jr. Differentiation of iron deficiency from thalassaemia trait. Lancet. 1973; 1: 882.

28. Srivastava PC. Differentiation of thalassaemia minor from iron deficiency. Lancet. 1973; 2: 154-5.

29. Shine I, Lal S. A strategy to detect beta-thalassaemia minor. Lancet. 1977; 1: 692-4.

30. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiency and thalassemia minor. Blood. 1979; 53: 288-93.

31. Ricerca BM, Storti S, d'Onofrio G, Mancini S, Vittori M, et al. Differentiation of iron deficiency from thalassaemia trait: a new approach. Haematologica. 1987; 72: 409-13.

32. Green R, King R. A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor. Blood Cells 1989; 15: 481-95.

33. Gupta, A. D., Hegde, C. & Mistri, R. Red cell distribution width as a measure of severity of iron deficiency in iron deficiency anemia. Indian J Med Res. 1994; 100: 177-183.

34. Jayabose S. et al. # 262 Differentiating iron deficiency anemia from thalassemia minor by using an RDW-based index. Journal of Pediatric Hematology/Oncology. 1999; 21: 314.

35. Telmissani OA, Khalil S, Roberts GT. Mean density of hemoglobin per liter of blood: A new hematologic parameter with an inherent discriminant function. Laboratory Hematology. 1999; 5: 149-152.

36. Huber AR. et al. In Schweiz Med Forum. 2004; 947-952.

37. 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.

38. Kohan N, Ramzi M. Evaluation of sensitivity and specifcity of Kermanindex I and II in screening beta thalassemia minor. 2008.

39. Ehsani M, Shahgholi E, Rahiminejad M, Seighali F, Rashidi A. A new index for discrimination between iron deficiency anemia and beta-thalassemia minor: results in 284 patients. Pakistan journal of biological sciences: PJBS. 2009; 12, 473-475.

40. Janel A, Roszyk L, Rapatel C, Mareynat G, Berger MG, et al.

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.

41. Nishad AAN, Pathmeswaran A, Wickremasinghe A, Premawardhena A. The Thal-index with the BTT prediction. Exe to discriminate ß-thalassaemia traits from other microcytic anaemias. 2012.

42. Wongprachum K, Sanchaisuriya K, Sanchaisuriya P, Siridamrongvattana S, Manpeun S, et al. Proxy indicators for identifying iron deficiency among anemic vegetarians in an area prevalent for thalassemia and hemoglobinopathies. Acta haematologica. 2012; 127: 250-255.

43. Dharmani P, Sehgal K, Dadu T, Mankeshwar R, Shaikh A, et al. Developing a new index and its comparison with other CBC-based indices for screening of beta thalassemia trait in a tertiary care hospital. International Journal of Laboratory Hematology. 2013; 35: 118.

44. Sirachainan N, Iamsirirak P, Charoenkwan P, Kadegasem P, Wongwerawattanakoon P, et al. New mathematical formula for differentiating thalassemia trait and iron deficiency anemia in thalassemia prevalent area: a study in healthy school-age children. Southeast Asian Journal of Tropical Medicine and Public Health. 2014; 45: 174.

45. Bordbar E, Taghipour M, Zucconi BE. Reliability of Different RBC Indices and Formulas in Discriminating between β-Thalassemia Minor and other Microcytic Hypochromic Cases. Mediterr J Hematol Infect Dis. 2015; 7: e2015022.