Validation of Several Formulas to Differentiate Thalassemia from Iron Deficiency Anemia and Proposal of a Thalassemia–Iron Deficiency Discrimination (TID) Predictive Score

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ABSTRACT

Objective: This study aimed to validate the sensitivity analysis of all the available formulas for their ability to differentiate between IDA and thalassemia and propose a novel formula to improve the sensitivity of all thalassemia subtypes screening.

Materials and Methods: We conducted a 5-year, single-center, Cohort study on 227 microcytic anemia patients diagnosed between June 2015 and September 2020 at Chaophraya Yommarat Hospital, Suphanburi, Thailand to validate the sensitivity of all the available formulas and invent the novel predictive score.

Results: Approximately three-quarters of our cases were all subtypes of thalassemia diseases while 26.9% were IDA. The sensitivity of almost all the previous formulas for thalassemia prediction ranged between 13.9%-44.0%, while the specificity varied between 0%–98.4%. Nevertheless, the sensitivity of the formulas that had favorable sensitivity was quite low. Here, a novel thalassemia–iron deficiency discrimination (TID) predictive score is proposed, which demonstrated a sensitivity of 90.4% the specificity of 78.7%, the positive predictive value of 92.0 %, the negative predictive value of 75.0%, and the accuracy of 87.2%.

Conclusion: The proposed TID predictive score is a novel uncomplicated formulation which offers high sensitivity for all thalassemia subtypes prediction.

Keywords: Iron deficiency anemia; microcytic anemia; predictive score; thalassemia (Siriraj Med J 2022; 74: 256-265)

INTRODUCTION

According to the World Health Organization, iron deficiency anemia (IDA) is the major cause of nutritional anemia worldwide.[1] The incidence of IDA in Thai women of reproductive age was reported to be 28.7%, 30.2%, and 31.8%, in 2013, 2014, and 2015, respectively.[1] Another study reported an anemia rate of 21% in educated young Thai women, with the two most prevalent causes among those cases being thalassemia (28%) and IDA (21%).[2]

Patients with IDA and thalassemia may both present with microcytic anemia (defined as a mean corpuscular volume (MCV) < 80 fL), which should be further investigated to distinguish between these two entities due to their different treatment approaches. Iron supplementation and the correction of occult blood loss remain the standard treatments for IDA. On the other hand, certain types of thalassemia diseases, such as hemoglobin (Hb) E/β-thalassemia and homozygous

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β-thalassemia, require regular blood transfusion and iron chelation to prevent iron deposition in various organs, which could lead to multiple organ dysfunction, i.e., liver cirrhosis, endocrinopathies, and heart failure.[3]

Detailed evaluation to confirm IDA involves an iron study test, consisting of measuring the levels of serum ferritin, serum iron, total iron-binding capacity (TIBC), and transferrin saturation (TSAT). If the serum ferritin value is ≤30 ng/mL or the TSAT value is <16%, IDA diagnosis can be confirmed with high sensitivity and high specificity.[4] Meanwhile, Hb typing is employed for the diagnosis of thalassemia.[5] However, in developing countries, some primary hospitals have limited resources to manage iron studies and Hb typing test. As a result, to make a diagnosis, blood samples have to be transferred to a comprehensive laboratory center, which can be a time-consuming process. As such, other available tools to initially discriminate these conditions could be of value. For thalassemia diagnosis, a range of associated factors can be assessed to develop a thalassemia predictive score.

According to the extensive literature review we performed, several formulas exist for thalassemia prediction among microcytic anemic patients, including the Red Blood Cell Count (RBC), Red Cell Distribution Width (RDW), Red Cell Distribution Width Index (RDWI), Green and King formula, Srivastava formula, Mentzer formula, Ehsani formula, Ricerca formula, England and Fraser formula, Sirdah formula, and Shine and Lal formula.[6-14] The reported sensitivity and specificity of these formulas range from 40% to 100%.[6-18] Another formula, the 11T score is an interesting formula that combines 11 other formulas to calculate its score, providing a higher discrimination ability.[15-18] In a previous study that attempted to validate this score among a Thai population, the 11T score showed a sensitivity of 82.1% and specificity of 91.7% for thalassemia prediction. However, it should be noted that only β-thalassemia subtype was included in previous studies. In addition, IDA in those trials was diagnosed when serum ferritin was <10 ng/mL.[18]

In the present study, we aimed to validate the sensitivity assessment of all the available formulas for their ability to differentiate between IDA and all thalassemia subtypes. In addition, we propose a novel formula to improve the sensitivity of thalassemia screening. In addition, to increase the diagnostic sensitivity in our study, the diagnosis of IDA could be established when ferritin was <30 ng/mL and TSAT was <16%.[4]

MATERIALS AND METHODS

Study design and population

We conducted a 5-year, retrospective, single-center, cohort study on microcytic anemia patients diagnosed between June 1, 2015, and September 30, 2020, at Chaophraya Yommarat Hospital, Suphanburi, Thailand. The inclusion criteria were: (1) patients aged 15 years old or older, and (2) patients with microcytic anemia. (3) patients who had the result of iron study in the IDA group and Hb typing and/or PCR in the thalassemia group. The exclusion criteria were patients receiving erythropoiesis-stimulating agents or receiving iron supplementation before blood testing. We categorized patients into 2 groups by different timeframes; a group for internal validation using patients during June 2015 - August 2017 and a group for calculation score using patients during September 2017 - September 2020. The study was approved for registration in the Thai Clinical Trial Registry with the identification number TCTR20210725003.

Instrument and evaluation parameters

All blood samples were collected by using 3-ml dipotassium ethylenediaminetetraacetic acid tubes (K_2EDTA) for a complete blood count (CBC) test and analyzed within 2 hours after taking the samples by Mindray BC-6200 automated blood counter (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China). This device used impedance technology to count and size RBC and platelet (PLT) together with cyanotic-free colorimetric method for Hb. MCV and % RDW were calculated based on the RBC histogram. In addition, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was also calculated from RBC, Hb, and hematocrit parameters. The patient's demographic data and initial laboratory results were collected. Patients with microcytic anemia were classified into two groups: the IDA group and the thalassemia group. In the thalassemia group, three included thalassemia disease subtypes were as follows: α-thalassemia, β–thalassemia disease, and α- combined β-thalassemia disease.

Study size consideration

At least 200 microcytic anemia cases were required to validate the formulas and develop a novel predictive score. Furthermore, 150 patients (40 patients with IDA and 110 patients with thalassemia) were separately assigned for an internal validation of this score.

Handing of continuous predictors

The proposed predictive score was developed followed by the predictive model study Risk of Bias Assessment Tool (PROBAST). Four red blood cell parameters were incorporated for this predictive score calculation including MCH, RDW, RBC and PLT.

Terminology

Anemia is defined by a hemoglobin (Hb) level < 13/dL in males or Hb < 12 g/dL in females.[19] Anemia with small red blood cells (MCV < 80 fL) is termed microcytic anemia.[4] A diagnosis of IDA is established if a patient has microcytic anemia with serum ferritin < 30 ng/mL and transferrin saturation < 16%.[4] The 11T score is a summary score from 11 formulas, comprising RBC ($\times 10^{12}$/L), RDW, RDWI (RDW × MCV/RBC), Green and King formula (MCV2 × RDW/Hb × 100), Srivastava formula (MCH/RBC), Mentzer formula (MCV/RBC), Ehsani formula [MCV - (10 × RBC)], Ricerca formula (RDW/RBC), England and Fraser formula [MCV - RBC - (5 × Hb) - 3.4], Sirdah formula [MCV - RBC - (3 × Hb)], and Shine and Lal formula (MCV2 × MCH/100).[18]

Statistical analysis

PASW Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA) was applied for the data analyses. The patients' demographic and clinical characteristics were summarized descriptively by causes of microcytic anemia. Continuous variables were reported as the mean±standard deviation for normally distributed continuous variables, and the median with interquartile ranges (Q1, Q3) for nonnormally distributed continuous variables. Categorical variables were reported as the frequency and percentage and were compared using Fisher's exact test or chi-square test. Continuous variables were compared using the Student's t-test or Mann–Whiney U test. The univariate and multivariate predictors of thalassemia were estimated using Cox proportional hazards analysis (backward stepwise method) and presented as an odds ratio (OR) and 95% confidence interval (CI). The receiver operating characteristic (ROC) curve for the cutoff score and for thalassemia diagnosis was presented as the area under the curve (AUC), accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). For all the tests performed, a two-tailed p-value < 0.05 was considered to be statistically significant. The calibration belt model was used for model calibration. The model was attended by the Hosmer –Lemeshow χ^2 goodness-of-fit test.

Ethics approval and consent to participate

This study was approved by the Ethics Committee for Research in Human Subjects at Chaophraya Yommarat Hospital, Suphanburi, Thailand. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was waived due to a retrospective study.

RESULTS

Baseline patient characteristics

In total, 227 microcytic anemic patients were included in this study. Approximately three-quarters (73.1%) were diagnosed with thalassemia disease, including Hb E/β-thalassemia, homozygous β-thalassemia, Hb H disease, Hb H/CS disease, AE Bart's disease, and EF Bart's disease, whereas 61 patients (26.9%) were IDA.

In the thalassemic group, the mean patient age was 42.1±20.5 years old. The mean Hb and MCV were 8±1.7 g/dL and 61.7±9.2 fL, respectively. The median PLT count was 308,000/μL (range, 189,000-413,000/μL). Among the IDA group, the mean patient age was 57.6±18.3 years old. The mean Hb was 6.1±1.7g/dL and the mean MCV was 62.9±8.3 fL. The median PLT count and serum ferritin were 384,000/μL (range, 263,000-478,000/μL) and 16.7 ng/mL (range, 4-22.2 ng/mL), respectively. Several factors were significantly different between the thalassemic and the IDA groups, such as age, body mass index, Hb level, MCH, MCHC, red blood cell distribution width (RDW), red blood cell counts, PLT count, and iron profiles. Table 1 displays the baseline patient features and initial laboratory results of the thalassemic and IDA patients.

Validation of the previous formulas predicting thalassemia

We analyzed the sensitivity, specificity, PPV, NPV, and accuracy of each previous formula to predict thalassemia, including RBC, % RDW, RDWI, Green and King, Srivastava, Mentzer, Ehsani, Ricerca, England, and Fraser, Shine and Lal, and 11T score, by using the included patient's data in this study. The sensitivity of almost all the formulas ranged between 13.9% - 44%. Only the RDW and Shine and Lal formulas yielded high sensitivity (97.6%), but with low specificity results, with figures of 0% and 3.3%, respectively. The specificity of each formula varied between 0%-98.4%. The high specificity of above 90% was found with several formulas, including RDWI, Green and King, England and Fraser, Sirdah, and 11T score; unfortunately, the sensitivity of these formulas was quite low. The PPV of almost all the formulas provided high results, which were above 90%. In contrast, the NPV of all the formulas was as low as approximately 30% (range, 0%-36.3%). The accuracy of each formula varied between 36.1%-72.3%. Table 2 demonstrates the sensitivity, specificity, PPV, NPV, and accuracy of each formula for predicting thalassemia.

Subgroup analysis of the formulas for predicting each type of thalassemia

We performed a subgroup analysis of each thalassemia subtype, including β-thalassemia disease, α-thalassemia

TABLE 1. Baseline patient features and initial laboratory results of the thalassemic and iron deficiency anemia patients.

Parameters	Total (N=227)	Thalassemia (All) (N=166) (73.1%)	β -thalassemia (1) (N=89) (39.2%)	α - thalassemia (2) (N=62) (27.3%)	α-thalassemia combined with β-thalassemia (3) (N=15) (6.6%)	Iron deficiency anemia (0) (N=61) (26.9%)	*P*-value for multiple comparisons			
							1 vs. 0	2 vs. 0	3 vs. 0	All vs. 0
Age(mean±SD) (years)	46.3±21	42.1±20.5	42.8±20.4	41.5±21.5	40.9±17.1	57.6±18.3	<0.001	<0.001	0.002	<0.001
Sex (Male)	74(32.6%)	54(32.5%)	33(37.1%)	18(29%)	3(20%)	20(32.8%)	0.589	0.652	0.531	0.971
BMI	21.4±4.1	20.8±3.6	20.8±3.9	20.3±3.1	22.5±3.3	23.2±4.9	0.001	<0.001	0.602	0.001
Hemoglobin typing	181 (79.7%)	166(100%)	89(100%)	62(100%)	15(100%)	15(24.6)	<0.001	<0.001	<0.001	<0.001
PCR for α-thalassemia	14(6.2%)	14(8.4%)	4(4.5%)	3(4.8%)	7(46.7%)	0(0%)	0.146	0.244	<0.001	0.024
Comorbidities	77(33.9%)	44(26.5%)	26(29.2%)	15(24.2%)	3(20%)	33(54.1%)	0.002	0.001	0.018	<0.001
Hypertension	34(15%)	16(9.6%)	12(13.5%)	3(4.8%)	1(6.7%)	18(29.5%)	0.016	<0.001	0.097	<0.001
Diabetes	22(9.7%)	12(7.2%)	10(11.2%)	1(1.6%)	1(6.7%)	10(16.4%)	0.361	0.004	0.682	0.039
Dyslipidemia	15(6.6%)	6(3.6%)	5(5.6%)	0(0%)	1(6.7%)	9(14.8%)	0.059	0.001	0.676	0.005
CAD	11(4.8%)	10(6%)	4(4.5%)	5(8.1%)	1(6.7%)	1(1.6%)	0.649	2.07	0.358	0.296
CKD	11(4.8%)	9(5.4%)	8(9%)	1(1.6%)	0(0%)	2(3.3%)	0.202	0.619	1.00	0.732
Liver disease	8(3.5%)	7(4.2%)	5(5.6%)	1(1.6%)	1(6.7%)	1(1.6%)	0.402	1.00	0.358	0.686
Arthritis	7(3.1%)	6(3.6%)	4(4.5%)	2(3.2%)	0(0%)	1(1.6%)	0.649	1.00	1.00	0.678
Others	19(8.4%)	7(4.2%)	2(2.2%)	5(8.1%)	0(0%)	12(19.7%)	<0.001	0.062	0.109	<0.001

TABLE 1. Baseline patient features and initial laboratory results of the thalassemic and iron deficiency anemia patients. (Continue)

Parameters	Total (N=227)	Thalassemia (All) (N=166) (73.1%)	β -thalassemia (1) (N=89) (39.2%)	α - thalassemia (2) (N=62) (27.3%)	α-thalassemia combined with β-thalassemia (3) (N=15) (6.6%)	Iron deficiency anemia (0) (N=61) (26.9%)	*P*-value for multiple comparisons			
							1 vs. 0	2 vs. 0	3 vs. 0	All vs. 0
Laboratory										
CBC										
Mean±SD										
Hemoglobin (g/dl)	7.5±1.9	8±1.7	7.9±1.9	8±1.5	8.5±1.7	6.1±1.7	<0.001	<0.001	<0.001	<0.001
Hematocrit (%)	25±14.3	26.4±16.2	26.2±21.7	26.9±4.4	25.9±6.3	21.2±5	0.082	<0.001	0.003	0.015
MCV (fL)	62±8.9	61.7±9.2	61.3±8.9	63.7±9.5	56.1±7.3	62.9±8.3	0.286	0.605	0.005	0.404
MCH (pg)	18.8±3	19.2±2.8	19.9±3	18.6±2.2	17.3±2.4	17.7±3.3	<0.001	0.066	0.674	0.001
MCHC (g/dl)	31.3±14.7	32.5±17	32.3±2.5	33.2±27.8	30.9±2.5	28.0±2.2	<0.001	0.148	<0.001	0.042
RDW (%)	23.6±5.4	24.7±5.7	24.5±6.3	25.2±5	23.8±4.7	20.4±3	<0.001	<0.001	0.016	<0.001
RBC (x10^{12}/L)	4±1	4.2±1	4±1	4.3±0.9	4.9±0.9	3.4±0.8	<0.001	<0.001	<0.001	<0.001
Median±IQR										
WBC (cells/μL)	7,410	7,595	7,860	6,900	7,750	6,320	0.009	0.495	0.096	0.031
Platelet(/μL)	316,000 (224,000-436,000)	308,000 (189,000-413,000)	307,000 (172,000-409,000)	298,000 (202,000-401,000)	353,000 (298,000-470,000)	384,000 (263,000-478,000)	0.015	0.006	0.759	0.006
Iron study										
Median±IQR										
serum ferritin (ng/ml)	249 (8.96-859)	590 (259-1,427)	882.5 (323-2387.5)	383.6 (184-759)	548 (268.7-1499)	16.7 (4-22.2)	<0.001	<0.001	<0.001	<0.001
serum iron (μg/dl)	17 (11-51)	69 (44-104)	66 (41-104)	70.5 (47.0-89)	79 (49.0-105)	12 (10-15)	<0.001	<0.001	0.004	<0.001
Transferrin saturation (%)	4.9 (3.2-24.2)	27.5 (19.1-44)	26.3 (16.6-46.8)	32.9 (23-44)	36 (23-38.6)	3.5 (2.5-4.6)	<0.001	<0.001	<0.001	<0.001
Mean±SD										
TIBC (μg/dl)	322.7±90.2	250.6±80.3	258.1±96.8	241.9±57.7	235.7±31.8	371.3±58.6	<0.001	<0.001	0.027	<0.001

Abbreviations: MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, RDW = Red blood cell distribution width, RBC = Red blood cell, WBC = White blood cell, TIBC = Total iron binding capacity (μg/dL), TSAT = Transferin saturation (%).

TABLE 2. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of each formula to predict thalassemia.

Formula	Cutoff	Thalassemia n (%)	Iron deficiency anemia n (%)	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)
RBC (x10^{12}/L)	≥5	36(97.3)	1(2.7)	21.7%	98.4%	97.3%	31.6%	42.3%
	<5	130(68.4)	60(31.6)	(15.7-28.7)	(91.2-100)	(85.8-99.9)	(25-38.7)	(35.9-48.7)
RDW (%)	≥14	162(72.6)	61(27.4)	97.6%	0%	72.6%	0%	71.4%
	<14	4(100)	0(0)	(93.9-99.3)	(0-5.9)	(66.3-78.4)	(0-60.2)	(65.48-77.25)
RDWI [6]	<220	23(92)	2(8)	13.9%	96.7%	92%	29.2%	36.1%
	≥220	143(70.8)	59(29.2)	(9-20.1)	(88.7-99.6)	(74-99)	(23-36)	(29.9-42.4)
Green and King [7]	<72	27(96.4)	1(3.6)	16.3%	98.4%	96.4%	30.2%	38.3%
	≥72	139(69.8)	60(30.2)	(11-22.8)	(91.2-100)	(81.7-99.9)	(23.9-37)	(32-44.7)
Srivastava [8]	<3.8	56(84.8)	10(15.2)	33.7%	83.6%	84.8%	31.7%	47.1%
	≥3.8	110(68.3)	51(31.7)	(26.6-41.5)	(71.9-91.8)	(73.9-92.5)	(24.6-39.5)	(40.6-53.6)
Mentzer [9]	<13	65(90.3)	7(9.7)	39.2%	88.5%	90.3%	34.8%	52.4%
	≥13	101(65.2)	54(34.8)	(31.7-47)	(77.8-95.3)	(81-96)	(27.4-42.9)	(45.9-58.9)
Ehsani [10]	<15	73(90.1)	8(9.9)	44%	86.9%	90.1%	36.3%	55.5%
	≥15	93(63.7)	53(36.3)	(36.3-51.9)	(75.8-94.2)	(81.5-95.6)	(28.5-44.7)	(49-62)
Ricerca [11]	<4.4	35(81.4)	8(18.6)	21.1%	86.9%	81.4%	28.8%	38.8%
	≥4.4	131(71.2)	53(28.8)	(15.1-28.1)	(75.8-94.2)	(66.6-91.6)	(22.4-35.9)	(32.4-45.1)
England [12]	<0	24(96)	1(4)	14.5%	98.4%	96%	29.7%	37%
and Fraser	≥0	142(70.3)	60(29.7)	(9.5-20.7)	(91.2-100)	(79.6-99.9)	(23.5-36.5)	(30.7-43.3)
Sirdah [13]	< 27	63(94)	4(6)	38%	93.4%	94%	35.6%	52.9%
	≥ 27	103(64.4)	57(35.6)	(30.5-45.8)	(84.1-98.2)	(85.4-98.3)	(28.2-43.6)	(46.4-59.4)
Shine and Lal [14]	<1530	162(73.3)	59(26.7)	97.6%	3.3%	73.3%	33.3%	72.3%
	≥1530	4(66.7)	2(33.3)	(93.9-99.3)	(0.4-11.3)	(67-79)	(4.3-77.7)	(66.4-78.1)
11T score [16]	≥7	40(97.6)	1(2.4)	24.1%	98.4%	97.6%	32.3%	44.1%
	<7	126(67.7)	60(32.3)	(17.8-31.3)	(91.2-100)	(87.1-99.9)	(25.6-39.5)	(37.6-50.5)
11T score (cutoff 5)	≥5	64(88.9)	8(11.1)	38.6	86.9	88.9	34.2	51.5
	<5	102(65.8)	53(34.2)	(31.1-46.4)	(75.8-94.2)	(79.3-95.1)	(26.8-42.2)	(45.0-58.0)
11T score (cutoff 6)	≥6	58(95.1)	3(4.9)	34.9	95.1	95.1	34.9	51.1
	<6	108(65.1)	58(34.9)	(27.7-42.7)	(86.3-99.0)	(86.3-99.0)	(27.7-42.7)	(44.6-57.6)
11T score (cutoff 8)	≥8	30(96.8)	1(3.2)	18.1	98.4	96.8	30.6	36.7
	<8	136(69.4)	60(30.6)	(12.5-24.8)	(91.2-100.0)	(83.3-99.9)	(24.2-37.6)	(33.3-46.0)
11T score (cutoff 9)	≥9	20(95.2)	1(4.8)	12.0	98.4	95.2	29.1	35.2
	<9	146(70.9)	60(29.1)	(7.5-18.0)	(91.2-100.0)	(76.2-99.9)	(23.0-35.8)	(29.0-41.5)

Abbreviations: PPV = Positive predictive value, NPV = Negative predictive value, RBC = Red blood cell count interval, RDW = Red blood cell distribution width, RDWI = Red blood cell distribution width index.

disease, and β-thalassemia combined with α-thalassemia disease. In the subgroup of the β-thalassemia group, the results were not significantly different from in the full analysis. Similarly, the results remained similar to the full analysis in the α-thalassemia disease group. However, when we validated the formulas in the β-thalassemia combined with α-thalassemia disease patients, the NPV and the accuracy of almost all the formulas were better than in the full analysis, with figures ranging between 79.0%-93.0%, while the sensitivity and specificity were not different from in the full analysis.

Proposed novel thalassemia–iron deficiency discrimination (TID) predictive score

Because the validation of each previous formula was imperfect, we attempted to determine the significant factors to differentiate between thalassemic and IDA patients. We found that MCH, % RDW, RBC, and PLT were significant factors related to thalassemia. Therefore, the predictive score is calculated by using these factors as the following:

y = (-4.643) + (2.273 if MCH 17 to 20) + (3.888 if MCH > 20) + (2.025 if RDW 21 to 25) + (4.986 if RDW > 25) + (0.485 if RBC 3.5 to 4.5) + (4.787 if RBC > 4.5) + (0.785 if PLT < 265,000) + (1 if PLT 265,000 to 400,000)

Subsequently the TID predictive score was simplified by multiplying with 2 as the following:

y = (-9) + (5 if MCH 17 to 20) + (8 if MCH > 20) + (4 if RDW 21 to 25) + (10 if RDW > 25) + (1 if RBC 3.5 to 4.5) + (10 if RBC > 4.5) + (2 if PLT < 265,000) + (2 if PLT 265,000 to 400,000) (Fig 1)

We used the ROC analysis for the TID predictive score. The most appropriate cutoff level for predicting thalassemia was ≥2, in which the AUC was 0.93 (95% CI: 0.890 - 0.969; Fig 2). The sensitivity and specificity of the score were 90.4% and 78.7%, respectively (Table 3).

Internal validation of the TID predictive score

The split 150 sample profiles were utilized for the internal validation study of the TID predictive score. The AUC was 0.88 (95% CI: 0.815 - 0.947). The sensitivity to predict thalassemia from the internal validation was 96.4%, with the specificity and the accuracy of 50.0% and 84.0%, respectively. There was a non-statistically significant difference in AUCs between the predictive TID predictive score creation and the internal validation (*P*-value = 0.805).

DISCUSSION

The most common cause of microcytic anemia in developed countries is IDA. However, thalassemia should not be overlooked in patients with microcytic anemia, especially in Asian populations.[20-21] In Southeast Asian subjects, the prevalence of α-thalassemia among anemic patients is 20%–30%, and β-thalassemia is about 3%–9%.[20-21] A CBC is a worthwhile initial investigation that can be performed in every hospital. Although CBC is a simple test, it gives an instant result, but it cannot totally differentiate the cause of microcytic anemia. Hence, confirmation tests, such as iron study, Hb typing, and PCR for α-thalassemia, are still mandatory for a definitive diagnosis. However, such confirmation tests are invariably more sophisticated, quite expensive, and can take several days to several weeks to get the results back. So these are not always practical in developing countries with limited resources.

Several formulas have been developed to predict thalassemia and used as a screening tool for thalassemia diagnosis. For example, predictive formulas, such as Green and King, Srivastava, and Mentzer have shown a sensitivity of 87.7%–93.8% and specificity of 82.5%–95% according to the previous results.[7-9,15] The 11Tscore was developed to improve the sensitivity and specificity for improving thalassemia diagnosis.[15] It is composed of 11 predictive formulas.[15-18] A previous study from France found it had a sensitivity of 85.7% and specificity of 97.5%,[15] while the study from Thailand reported a sensitivity of 82.1% and specificity of 91.7%.[18] However, the 11Tscore has been applied for predicting only the β-thalassemia subtype.[15-18] Another study reported that the Jayabose RDW index, the Green and King formula, and the Janel 11T score are good formulas to differentiate thalassemia trait from IDA among their population.[22] Moreover, the serum ferritin cutoff value from previous studies for IDA diagnosis varied between <10 ng/ml and <16 ng/ml.[16-18] Currently, the definition for IDA diagnosis is serum ferritin < 30 ng/ml and TSAT < 16 %.[4] therefore, we defined IDA according to this recent suggestion in this study.

In our study, we validated all the previous formulas with all subtypes of thalassemia, including α-thalassemia disease, β-thalassemia disease, α-thalassemia combined with β-thalassemia disease, and IDA patients. In contrast to the previous results, the sensitivity and specificity to predict thalassemia disease among these included patients were not high. Therefore, we proposed a novel TID predictive score composed of 4 red blood cell indices, namely % RDW, RBC, and PLT count. The TID predictive score had a sensitivity of 90.4% and specificity of 78.7% to differentiate all thalassemia subtypes from IDA. Although, the specificity from the internal validation was insignificantly lower compared

TID predictive score = (-9) + (MCH points) + (RDW points) + (RBC points) + (Platelet points)

Parameter	Value	Points
MCH (pg)	<17	0
	17-20	5
	>20	8
RDW (%)	<21	0
	21-25	4
	>25	10
RBC ($x10^{12}$/L)	<3.5	0
	3.5-4.5	1
	>4.5	10
Platelet (/µL)	≤400,000	2
	>400,000	0

Fig 1. The thalassemia–iron deficiency discrimination (TID) predictive score and their values.

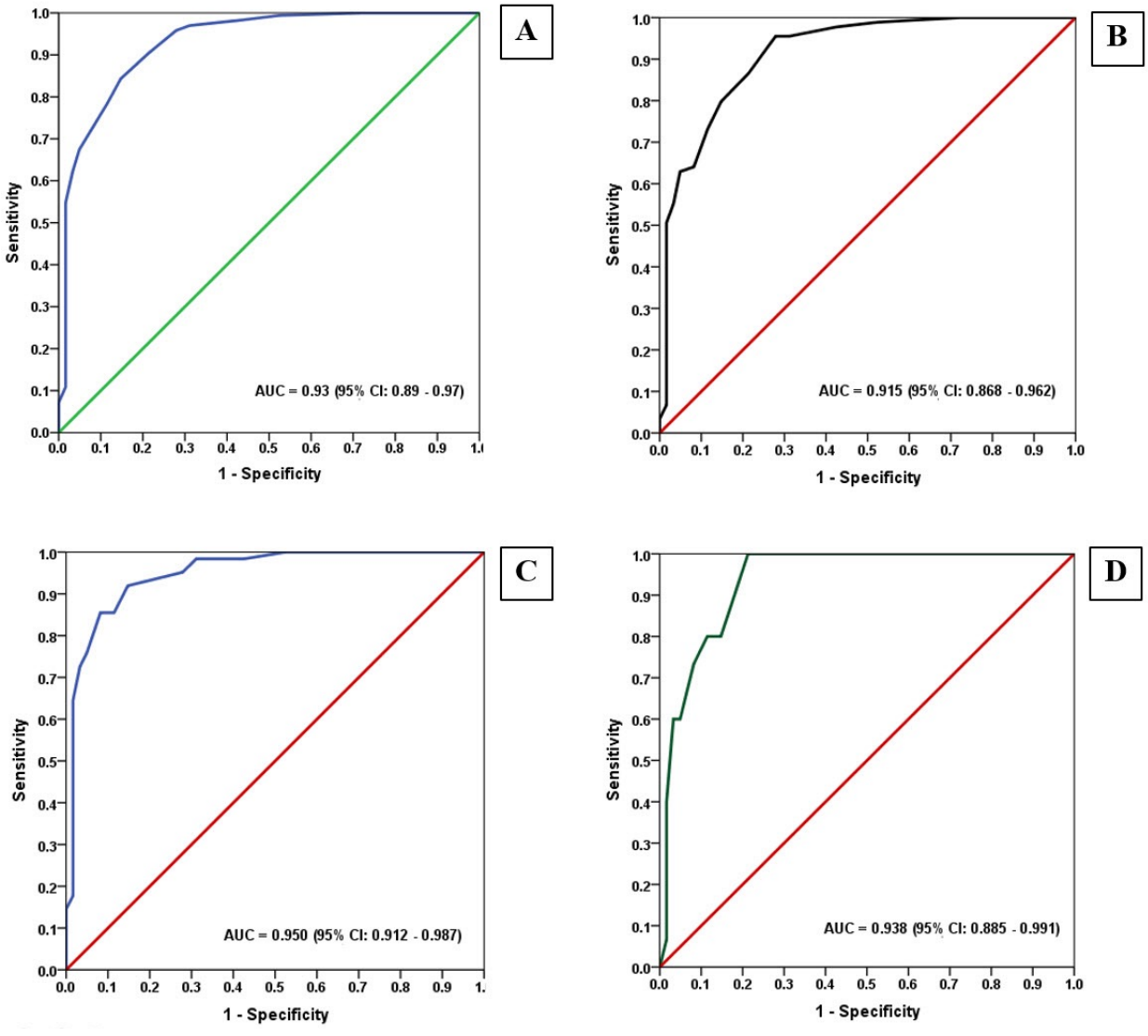

Fig 2. Receiver operating characteristic curve and the area under the curve for obtaining the cut off value for thalassemia prediction using the TID predictive score (A) all thalassemia subtypes (B) β-thalassemia disease (C) α-thalassemia disease (D) α-thalassemia combined with β-thalassemia

TABLE 3. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the TID predictive score.

Logistic model	Cutoff	Thalassemia n (%)	Iron deficiency anemia n (%)	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)
Logistic model score	≥2	150(92)	13(8)	90.4%	78.7%	92.0%	75.0%	87.2%
	<2	16(25)	48(75)	(84.8-88.1)	(66.3-88.1)	(86.7-95.7)	(62.6-85.0)	(82.9-91.6)

Abbreviations: PPV = Positive predictive value, NPV = Negative predictive value.

to the figure from the predictive score generation, the sensitivity remained satisfying. The TID predictive score applies % RDW for calculation because in Thailand CBC report is practically presented with % RDW. However, a recent study showed that absolute RDW is more specific to differentiate thalassemia from IDA in microcytic anemia comparing with relative RDW.[23]

This score might be beneficial for thalassemia screening, whereby patients who have a score ≥2 can be selected for further investigation to confirm thalassemia disease. This could reduce unnecessary expenses from over investigation, which would be especially important in resource-limited countries. In other words, patients who have a lower likelihood of having thalassemia as assessed from the predictive score could be treated as IDA while waiting for their iron study.

There are some limitations of this study to note. First, because this was a retrospective study, some information may have been missing. Second, the TID predictive score demonstrated a specificity of 50.0% from the internal validation, some IDA patients who have high TID predictive scores might experience a treatment delay while awaiting for Hb typing result. Third, IDA patients from this cohort had significantly more severe anemia than the thalassemia subjects. This factor might influence the sensitivity/specificity of discriminant formulas. Lastly, in the subgroup analysis, the size of some subgroups is quite small which leads to imprecise formula validation.

CONCLUSION

Thalassemia and IDA are the most common causes of microcytic anemia. Here, a TID predictive score was proposed that demonstrated higher sensitivity for thalassemia prediction while remaining uncomplicated to be applied due to its few involved parameters.

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