CLIN. CHEM. 39/11, 2271–2275 (1993)

Iron-Deficiency Anemia and Thalassemia Trait Differentiated by Simple Hematological Tests and Serum Iron Concentrations

Carlos V. Jiménez

The diagnostic value of common hematology tests in differentiating between thalassemia trait and iron-deficiency anemia (IDA) was assessed. Serum iron concentration was included in a second stage of the diagnostic model and its predictive contribution was determined. After applying discriminant analysis to the data, minimization of Wilks's lambda (Λ) criterion was used to select the best predictive variables. A training sample of 754 subjects previously classified as either IDA (428) or thalassemia trait (326) was used to determine the classification rule. When serum iron concentrations were included, the model showed a higher predictive capacity than that constructed from hematological variables only (D = 2.342 RBC − 0.079 Hb + 3.627 log[Fe] − 6.459, where D = discriminant score). The sensitivity, specificity, and diagnostic efficiency associated with this model, as assessed on a control sample of 256 patients (137 thalassemia and 119 IDA), were 94.2%, 91.6%, and 93.0%, respectively. This model, when compared with those of other authors, has the highest diagnostic efficiency.

Indexing Terms: *hemoglobin · erythrocytes · discriminant analysis · heritable disorders*

A high incidence of β- and $\delta\beta$-thalassemia minor occurs in the Mediterranean area (*1–8*). The thalassemia trait is characterized by a reduction in or absence of synthesis of one or more globin chains in the hemoglobin (Hb) molecule.[1] The early detection of healthy carriers of thalassemia (heterozygotes) makes it possible to provide genetic counseling, which may lead to a reduced incidence of homozygous status and its fatal outcome. The thalassemia trait requires a differential diagnosis from iron-deficiency anemia (IDA), both being microcytic. Subpopulations previously classified by our laboratory as having a high incidence of thalassemia trait (β and $\delta\beta$) and IDA were the starting point of the present study. The statistical technique used to distinguish between the two microcytic groups according to routine hematological results was discriminant analysis. The aim of this study was to determine which variables of the routine hemogram would best differentiate between the two microcytic groups, and to develop a classification index based on these variables. This index, or discriminant score (D), can be used to calculate an individual's probability of belonging to each of the groups. With this approach, objective criteria that improve both the sensitivity and diagnostic specificity are defined. This statistical approach eliminates the subjective bias when those hemograms are selected that require complementary determinations to arrive at a definitive diagnosis.

Laboratori d'Anàlisis Clíniques, Centre d'Assistència Primaria "Santa Coloma," Major, 48 Santa Coloma de Gramenet, 08921-Barcelona, Spain. Address for correspondence: Avenida de Madrid, 88-90, 2°, 1ª, 08028-Barcelona, Spain. Fax 34-3-4663548.

[1] Nonstandard abbreviations: IDA, iron-deficiency anemia; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; RCDW, red blood cell distribution width; MCV, mean cell volume; MCH, mean cell Hb; and MCHC, mean cell Hb concentration.

Received November 4, 1992; accepted June 23, 1993.

Materials and Methods

Subjects

To develop the predictive model, I randomly selected 326 cases of thalassemia minor (92.2% β and 7.8% $\delta\beta$) and 428 cases of IDA from our database. For validating purposes, I used a control sample of 256 new cases (137 cases of thalassemia minor and 119 cases of IDA). These microcytic (mean cell volume <80 fL) samples were classified according to complementary determinations such as Hb A_2 (>3.5% in β-thalassemic trait) and (or) fetal hemoglobin (Hb F) measurement (>2.0% in $\delta\beta$ or β-thalassemic trait) and Hb electrophoresis (not structural Hb), as well as patient monitoring [normalization of erythrocyte (RBC) indices after appropriate iron therapy in the iron-deficient group]. Patients younger than 3 years and pregnant women were excluded, as were multifactorial cases and individuals with anemia due to chronic disease or with other hemoglobinopathies.

The original data were collected over 34 months (between March 1986 and December 1988). In 1989 the model was validated and applied in this laboratory with satisfactory results.

Laboratory Data and Analytical Methods

For both groups of individuals, i.e., those with IDA or thalassemia trait, the following potential predictor variables within the hematological constituents were measured with a Technicon H-6000 continuous-flow automated analyzer (Miles Technicon, Tarrytown, NY). We used the manufacturer's reagents: erythrocyte count (RBC × 10^{12}/L), Hb (g/L), hematocrit (HCT, %), and red blood cell distribution width (RCDW, 1). Derived variables, such as mean cell volume (MCV), mean cell Hb (MCH), and mean cell Hb concentration (MCHC), being linear combinations of these variables, were not considered. Serum iron (μmol/L), determined on a Hitachi-705 automated analyzer with the manufacturer's commercial kit (iron H-737, cat. no. 791539), was the single biochemical test, and the result was added later to analyze its contribution to the model.

All of the variables except iron were normally distributed. Because the results of serum iron determinations were significantly skewed in the positive direction, log-transformation was used to normalize the distribution.

Radial immunodiffusion was used to measure Hb F and ion-exchange chromatography to measure Hb A_2 (HbF Quickplate Procedure and Beta-Thal Hb A_2 Quick Column Procedure, respectively, both from Helena Labs., Beaumont, TX). Other hemoglobinopathies were ruled out by means of electrophoresis of blood samples on cellulose acetate, pH 8.6, and citrate agar, pH 6.2.

Statistical Method

Stepwise discriminant analysis (*9–11*) was applied to the study material to differentiate between the two groups of patients and to select the "best" variables. The program used to treat the data was SPSS/PC+ (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, 1986). The selection of variables was based on the minimization of overall Wilks's lambda (Λ) criterion. With this method, after the first variable is entered, the value of the criterion is reevaluated for all variables not yet included in the model. Subsequently, the variable with the best criterion value is entered next, and so on, until no other variable improves the model.

The minimization of Wilks's Λ criterion (*12*) was chosen because of its easy comprehension. It is the ratio of the within-group sum of squares to the total sum of squares; thus, it is the proportion of the total variability of the discriminant function not explained by the differences among groups. $\Lambda = 1$ when the group means are equal. Conversely, values are close to zero when the within-group variability is small compared with the total variability, that is, when most of the total variability is attributable to differences between the means of the groups. In other words, a good discriminant function is one that has considerable between-group variability in comparison with within-group variability. In fact, the coefficients of the discriminant function are chosen so that the ratio of both sums of squares is as large as possible. Any other combination of the predictor variables will have a smaller ratio. The value of Λ is, therefore, inversely related to the F statistic. However, in contrast to the F statistic, Wilks's Λ permits a step-by-step evaluation of the contribution of each variable in terms of variation not explained by the model (its global value decreases every time a relevant variable is incorporated into the model) independently of its statistical significance (a significant Λ does not always improve the predictive capacity of the model). Other criteria for variable selection are sometimes used, such as Rao's V (also known as the Lawley–Hotelling trace) (*13, 14*) or Mahalanobis distance D^2 (*15*). These did not present advantages, at least in this study, over the Wilks's Λ.

Posterior Probabilities

Once an individual's discriminant score (D) is known, the probability of his (or her) belonging to each of the groups may be calculated. A case is classified in the group for which the posterior probability is the largest. For this calculation the mean value for D in each group must be known. The distance of the individual from these centers is determined by using the square of the difference between the subjects' discriminant score (D) to both group centers (C_1 and C_2). In our study the mean values for D of the thalassemic and IDA groups were +1.8268 (C_1) and −1.4597 (C_2), respectively. Hence, the posterior probability of belonging to the thalassemic group for a given D score, $P(T|D)$, may be determined by using the following expression:

$$P(T|D) = \frac{p \cdot e^{-D_1^2/2}}{p \cdot e^{-D_1^2/2} + (1-p)e^{-D_2^2/2}}$$

where $D_i = (D - C_i)$ and p is the prior probability (or prevalence) of thalassemia.

Results

Table 1 displays means and SDs of all variables in the study groups, as well as the univariate diagnostic capacity measured by Wilks's Λ criterion and the F statistic. At the univariate level, RBC and iron are the variables with the least residual variation (lowest Wilks's Λ) and are, therefore, the best choices to differentiate between the groups.

Table 2 shows the pooled within-group correlation matrix. Because interdependencies among the variables affect most multivariate analyses, it is worth examining the correlation matrix of the predictor variables. Thus, the variables with the best correlation are Hb and HCT ($r = 0.981$), RBC and HCT ($r = 0.797$), and RBC and Hb ($r = 0.759$).

Applying stepwise discriminant analysis to the study material with and without serum iron, the two linear functions obtained were:

$$D = 2.919\ \text{RBC} - 0.041\ \text{Hb} - 10.138\ \text{HCT} - 3.808\ \text{RCDW} - 5.971$$

$$D = 2.342\ \text{RBC} - 0.079\ \text{Hb} + 3.627\ \log[\text{Fe}] - 6.459$$

Table 1. Variables in the Training Groups

		Thalassemia (n = 326)		IDA (n = 428)			
	Units	Mean	SD	Mean	SD	Wilks's Λ	F
RBC	× 10^{12}/L	5.69	0.53	4.81	0.55	0.606 (2)	480[a]
Hb	g/L	12.0	1.28	11.5	1.76	0.970 (5)	23[b]
HCT	%	37.4	4.06	35.1	5.42	0.948 (4)	41[b]
RCDW	1	22.0	1.86	20.6	2.61	0.937 (3)	50[b]
Log[Fe]	μmol/L	1.20	0.15	0.76	0.21	0.542 (1)	647[a]

Diagnostic power is measured by Wilks's Λ criterion (rank of each variable given in parentheses).
[a] $P < 0.0001$.
[b] $P = 0.0001$.

Table 2. Within-Group Correlation Matrix

	RBC	Hb	HCT	RCDW	Log[Fe]
RBC	1.000				
Hb	0.759	1.000			
HCT	0.797	0.981	1.000		
RCDW	−0.027	−0.336	−0.335	1.000	
Log[Fe]	0.143	0.392	0.371	−0.283	1.000

where D is the discriminant score of the functions.

Table 3 summarizes the point estimation of the sensitivity, specificity, and diagnostic efficiency of the two models (with and without iron measurement) at a confidence interval of 95%. Note that the point estimations of these characteristics are different in each model (their confidence intervals do not overlap).

Table 4 gives the results obtained in classifying the cases of the training and control (validation) samples by means of the equation above including iron. For the control sample the diagnostic efficiency is 93.0% (95% confidence interval: 91.2–94.8%). The sensitivity, specificity, and diagnostic efficiency were not statistically different from those obtained with the training sample.

Table 5 compares the performance of this method with the results published by other authors. The index proposed by Shine and Lal (*16, 17*) is the most sensitive (100%), followed by those of Srivastava (95.0%) (*18*) and those presented here (94.2%); the Green and King (*19*) index is the most specific (98.3%) followed by that presented here (91.1%) and that of England et al. (83.2%) (*20, 21*); the indices presented here show the best diagnostic efficiency (93.0% and 83.7%), followed by the index of Mentzer (81.8%) (*22*).

Table 6 shows the diagnostic performance of the proposed discriminant function when the prevalence of the thalassemia trait in the microcytic population is taken into account. The efficiency of the model is particularly high when the prevalence reaches 10%.

Discussion

On the whole, all the variables considered in this study showed different mean values in the thalassemia and IDA groups (Table 1), which could lead to the conclusion that they are good discriminant factors for these groups. This may be explained by the large size of the groups (high statistical power of the test) or the slight variability of some of the variables, which, although small and clinically insignificant, does reach statistical significance.

However, a consideration of the variables separately produces only a partial analysis. A primary emphasis in discriminant analysis and other multivariate statistical techniques is on analyzing the variables together. It is interesting that, although both the HCT and RCDW had a Wilks's Λ lower than the Hb, they were eliminated from the model when the serum iron variable was introduced, thus demonstrating the magnitude of the interdependence among the variables (Table 2).

Table 3 discloses that the introduction of iron determination improves the classifying capacity and, therefore, increases the percentage of cases correctly classified because of an increase in both sensitivity and diagnostic specificity (the 95% confidence intervals do not intersect for sensitivity, specificity, and efficiency).

For validation purposes, the function should also be applied to a sample different from that which was used to estimate its coefficients. With this in mind, the model was applied to a validation sample of 256 new cases. The sensitivity, specificity, and diagnostic efficiency results obtained in this control sample were almost identical to those for the original sample.

The discriminant function that I obtained summarizes the information from all the relevant variables in the model in a single unobserved variable (D, or discriminant score). Other authors have done the same, although with different approaches. Thus, Mentzer (*22*), and Srivastava (*18*) proposed the ratios MCV/RBC and MCH/RBC, respectively. Shine and Lal (*16*) considered

Table 3. Sensitivity, Specificity, and Diagnostic Efficiency of the Models, with and without Iron Measurement

	Discriminant function (and 95% confidence intervals)	
	Without log[Fe]	With log[Fe]
Sensitivity, %	84.3 (80.5–88.1)	95.1 (92.1–98.1)
Specificity, %	88.3 (85.3–91.3)	94.6 (92.2–97.0)
Efficiency, %	86.5 (84.1–88.9)	94.8 (93.1–96.7)

Table 4. Comparison of Classification Results (Hematological Variables plus Log[Fe]) between Training and Control Samples

	Training sample		Control sample	
Predicted group	Thalassemia	IDA	Thalassemia	IDA
Thalassemia	310 (95.1)[a]	23	129 (94.2)	10
IDA	16	405 (94.6)	8	109 (91.6)
Total	326	428	137	119

[a] Percent of samples is given in parentheses.

Table 5. Comparison of Different Indices to Predict Thalassemia Trait

	Sensitivity	Specificity	Efficiency
		%	
England and Fraser (1973)	74.3	83.2	78.4
Srivastava (1973)	95.0	62.2	79.9
Mentzer (1973)	86.4	76.5	81.8
Shine and Lal (1977)	100.0	11.8	59.5
Green and King (1988)	20.7	98.3	56.4
Jiménez (present study)[a]	86.9	80.1	83.7
Jiménez (present study)	94.2	91.6	93.0

[a] Hematological parameters only.

Table 6. Diagnostic Performance of Discriminant Function According to Prevalence of Thalassemia Trait in the Microcytic Population

Prevalence	Sensitivity	Specificity	Efficiency
	%		
5	80.5	99.3	90.9
10	87.5	98.4	93.5
15	90.1	97.4	94.2
20	91.0	97.0	94.3
25	92.1	96.5	94.5
50	95.1	94.6	94.8

only derived determinations ($MCV^2 \times MCH \times 0.01$), and Green and King (*19*) incorporated a red cell volume distribution ($100 \times MCV^2 \times RCDW/Hb$). England and Fraser (*21*) proposed a function with a constant term (MCV − RBC − 5 Hb − 3.4), whereas Makris (*23*) combined erythrocyte and platelet variables [(MCV/RCDW)RBC/(MPV/PDW)], where MPV is mean platelet volume, and PDW is platelet distribution width.

Fieschi et al. (*24*), England (*25*), Cesana et al. (*26*), and Han and Fung (*27*) developed functions based on discriminant analysis. The Fieschi model ($D = 0.7$ HCT − 2.3 Hb + 14 RBC + 0.4 MCV − 1.05 MCH − 22) discriminates between the thalassemic population and the nonthalassemic population in general with excellent efficiency (98.5%), but does not differentiate the thalassemic microcytic population from that with IDA. England does not validate the model ($D = -3.0 +$ 0.23 MCV − 0.22 RBC − 0.93 Hb) or describe its diagnostic efficiency. The model proposed by Cesana ($D =$ 12.722 + 0.096 MCV + 0.415 RCDW − 0.139 RBC) reaches a diagnostic efficiency of 88.8%, with a sensitivity and diagnostic specificity of 86.7% and 90.9%, respectively. With the Han proposals, two functions with the same three variables are required, one useful for the hospital-patient population ($D =$ 0.82791 RBC + 0.47269 MCHC − 0.15576 RCDW, with standardized coefficients), with a sensitivity, specificity, and diagnostic efficiency of 88.5% (n = 104), 75.6% (n = 26), and 83.2%, respectively, and another for healthy donors (D = 0.95667 RBC − 0.26565 MCHC − 0.10722 RCDW, with standardized coefficients), with a sensitivity, specificity, and diagnostic efficiency of 96.7% (n = 58), 94.1% (n = 17), and 96.1%, respectively. Other authors have inclined toward developing expert systems (*28, 29*) to differentiate thalassemic patients from those with IDA and (or) other hematological illnesses.

When these indices, except those based on discriminant analysis, are processed over the same validation sample, the one I present here shows the greatest diagnostic power; the next most powerful is that of Mentzer: 93.0% and 81.8% of correctly classified cases, respectively. The Shine and Lal index detects 100% of thalassemia cases but gives 88.2% false positives. The Srivastava proposal identifies 95.0% of the patients with thalassemic trait but gives 37.8% false positives. The Green and King index correctly detects 98.3% of the IDA patients but gives 79.3% false negatives. The indices with fewer classification errors within each group are those proposed by England and Fraser, by Mentzer, and by me.

It is important to evaluate the diagnostic performance of this model for the different levels of prevalence that the thalassemia trait can have in the Mediterranean area and especially in the microcytic population. Recent studies have indicated an incidence of 14.3% in Greece (*30*) and as much as 24% in Sardinia (*24*). In Spain the maximum percentage (5.0%) was found on the island of Minorca (*2*). Thus the prevalence of the thalassemia trait is much higher in microcytic subjects than in the general population. This fact improves the performance of the discriminant function. With a prevalence of 10%, the diagnostic efficiency is similar to that obtained in conditions where prevalences are equal (i.e., 50%), although it implies an increase in specificity and a decrease in sensitivity. After 10%, the sensitivity, specificity, and diagnostic efficiency values are harmonized. In some areas of the Mediterranean, the diagnostic performance may be superior to that presented in this study. Thus, I conclude that, of the proposed options, the function described here gives the best overall performance for screening the microcytic population.

References

1. Gimferrer E, Baiget M. Datos hematológicos de 393 casos de talasemia heterocigota beta y delta-beta en el Hospital de la Santa Creu i Sant Pau de Barcelona. Biol Clin Hematol 1979;3:251–62.
2. Baiget M. Los síndromes talasémicos en España. Datos epidemiológicos preliminares. Sangre 1986;31:609–13.
3. Oliva E, López N, Segui M, Hernández JL, Martí F. A campaign for detection of beta-thalassemia minor and prevention of beta-thalassemia major in the island of Minorca. 2 years' experience. Med Clin Barc 1990;94:81–4.
4. Calero F, Villegas A, Porres A, Valverde F, Potro E, Sánchez P, et al. Hematologic data in 825 cases of beta-thalassemia trait in Spain. Nouv Rev Fr Hematol 1990;32:265–70.
5. Orsini A, Vovan L, Lena D, Maarouf I, Merono F, Jaujou M, et al. School screening of hemoglobinopathies in the Marseilles area. An analytic study of 35289 tests. Arch Fr Pediatr 1987;44:455–9.
6. Cao A, Pintus L, Lecca U, Olla G, Cossu P, Rosatelli C, et al. Control of homozygous beta-thalassemia by carrier screening and antenatal diagnosis in Sardinians. Clin Genet 1984;(Jul)26:12–22.
7. Bianco I, Graziani B, Lerone M, Congedo P, Ponzini D, Braconi F, et al. A screening programme for the prospective prevention of Mediterranean anaemia in Latium: results of seven years' work. J Med Genet 1984;21:268–71.
8. Peruttelli P, Scuderi F, Ragazzini G, Arigliani R, Bottini W, Mori PG. Epidemiologic research for thalassemia and hemoglobinopathy traits in the territory of a local health unit in Liguria. Pediatr Med Chir 1988;10:77–9.
9. Tabachnik BG, Fidell LS. Using multivariate statistics, 2nd ed. New York: Harper and Row, 1989:505–96.
10. Bisquerra Alzina R. Introducción conceptual al análisis multivariante, 1st ed. Barcelona: Promociones y Publicaciones Universitarias, 1989:243–86.
11. Cuadras Avellana CM. Métodos de análisis multivariante. Barcelona: Eunibar, 1981:475–500.
12. Wilks SS. Certain generalizations in the analysis of variance. Biometrika 1932;24:471–94.
13. Rao CR. Advanced statistical methods in biometric research. New York: John Wiley, 1952.
14. Rao CR. Linear statistical inference and its applications, 2nd ed. New York: John Wiley, 1973.
15. Mahalanobis PC. On the generalized distance in statistics. Proc Natl Inst Sci India 1936;2:49–55.
16. Shine I, Lal S. A strategy to detect beta thalassemia minor. Lancet 1977;i:692.
17. Shine I. Nonrandom distribution of genotypes among red cell indices. Blood Cells 1989;15:475–8.
18. Srivastava PC. Differentiation of thalassemia minor from iron deficiency. Lancet 1973;i:154–5.
19. Green R, King R. A new red cell discriminant function incorporating RDW to differentiate iron deficiency anemia and thalassemia minor. Am J Clin Pathol 1988;90:507.
20. England JM, Bain BJ, Fraser PM. Differentiation of iron deficiency from thalassemia trait. Lancet 1973;i:1514.
21. England JM, Fraser PM. Differentiation of iron deficiency from thalassemia trait by routine blood-count. Lancet 1973;i:449.
22. Mentzer WC. Differentiation of iron deficiency from thalassaemia trait. Lancet 1973;i:882.
23. Makris PE. Utilization of new index to distinguish heterozygous thalassemic syndromes: comparison of its specificity to five other discriminants. Blood Cells 1989;15:497–506.
24. Fieschi M, Vovan L, Roux M. Le dépistage des thalassémies hétérozygote. Rev Epidém Santé Publ 1981;29:67–73.

25. England JM. Discriminant functions. Blood Cells 1989;15: 465–71.
26. Cesana BM, Maiolo AT, Gidiuli R, Damilano I, Massano P, Polli EE. Relevance of red cell distribution width (RDW) in the differential diagnosis of microcytic anaemias. Clin Lab Haematol 1991;13:141–51.
27. Han P, Fung KP. Discriminant analysis of iron deficiency anaemia and heterozygous thalassaemia traits: a 3-dimensional selection of red cell indices. Clin Lab Haematol 1991;13:351–62.
28. Houwen B. The use of inference strategies in the differential of microcytic anemia. Blood Cells 1989;15:509–32.
29. Bessman JD, McClure S, Bates J. Distinction of microcytic disorder: comparison of expert, numerical-discriminant, and microcomputer analysis. Blood Cells 1989;15:533–40.
30. Nathannil M, Tawsiopoulou A, Loutradi A. Prevention of thalassemia in Greece. Carrier identification [Abstract]. 2nd Int conf on thalassemia and the hemoglobinopathies, Herakleion, Crete, Oct 1987. Abstr book; 36.