Use of mathematical indices based on CBC data to identify patients with β thalassemia minor

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Abstract

The discrimination between thalassemic and non-thalassemic causes for microcytosis has important clinical significance. The incidence of β thalassemia trait in countries surrounding the Mediterranean and in Romania is high. In the absence of definitive DNA and quantification of HbA2 and HbF concentrations, the use of discrimination calculations based on complete blood count data has been proposed to identify patients with β thalassemia trait. Using ROC curves, the sensitivity and specificity of several widely used discrimination calculations are calculated. For patients who are iron replete the MCV, RDWI and Shine and Lal provide the best discrimination between normal and patients and those with β thalassemia.

Introduction

The investigation for thalassemia in a patient may be made for clinical or genetic reasons. Initial investigations usually include the complete blood count (CBC) together with evaluation of iron status by ferritin analysis and the quantification of hemoglobins HbA_2 and HbF. In patients who are iron replete, the presence of a raised HbA_2 with thalassemic indices in the CBC is diagnostic of β thalassemia[7]. Although confirmation by DNA studies may be performed, this is unnecessary in the majority of cases. The diagnosis of α thalassemia is made by exclusion (patient is iron replete with thalassemic indices and a normal HbA_2 level) or by a positive hemoglobin H preparation. Confirmation of α thalassemia is by DNA analysis.

Quantification of hemoglobins A_2 and F should be made by a method with good sensitivity and precision as the demarcation between a normal and a clinically significant value is narrow. The College of American Pathologists (CAP) strongly discourages the use of quantification of HbA_2 on alkaline electrophoresis as this method shows very poor precision. High Performance Liquid Chromatography (HPLC) has become the method of choice for the quan-

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tification of HbA_2 and HbF as this method shows excellent precision even at the low concentrations of these hemoglobins in patients without thalassemia or with δβ thalassemia.

The definition of what constitutes thalassemic indices in the CBC is a source of discussion. An increase in the red blood cell count (RBC) or a value in the upper half of the reference interval is considered a necessary parameter. As for a second factor, some authors prefer the use of the mean cell volume (MCV) while others prefer the mean hemoglobin concentration value (MCH). A MCV value below 72 to 74 fL or MCH below 26 pg is associated with thalassemia. Iron deficiency can mimic many of the features of thalassemia and it is important to distinguish between these two microcytic conditions. In situations where the quantification of HbA_2 and HbF is not available, discrimination indices based on CBC parameters have been proposed.

Lafferty[8] investigated the use of several discrimination indices used to differentiate thalassemia from iron deficiency but found that none were better than the use of the MCV cut-off of 72 fl. On this basis, Kiss described[6] an algorithm to aid in the investigation of thalassemia trait in multicultural populations. Demir et al[2] investigated the use of eight discrimination indices and found, using Youden's plots, that the RBC (Red Cell Count) and RDWI (Red Cell Distribution Width Index) had the best sensitivity and specificity. Bernstein et al[1] describe the use of the Mentzer Index in discriminating iron deficiency from thalassemia and, using odds ratios and probabilities, found that a Mentzer Index less than 14.7 provided the best probability of β thalassemia diagnosis. Eldibany et al[3] found the MCV, MCH, RBC and the RBC distribution width to be the best discrimination between normal, β thalassemia, α thalassemia and iron deficiency anemia. Liu et al[9] found that the cell hemoglobin distribution width (CHDW) could segregate thalassemic from non-thalassemic patients independent of whether they were iron deficient and iron replete.

Since Lafferty's paper, many new calculations have been described. Demir et al worked only with a pediatric population and Bernstein et al used only pregnant females in their study. It was felt that a study using all the calculations currently available and based on a general population would be useful.

Methods and Materials

The CBC was performed on a Coulter GenS blood analyzer. The HbA_2 and HbF measurements were performed on a Bio Rad VARIANT II using the β thalassemia reagent kit. Ferritin analysis was performed on a Siemens Medical Solutions Diagnostics Centaur immunoassay system. All methods were performed according to the manufacturer's instructions.

EDTA anti coagulated blood and serum samples were submitted to the laboratory for thalassemia/hemoglobinopathy investigation. Initial investigation included a CBC, HPLC analysis and ferritin quantification. Based on these results, patients were assigned to either the non-thalassemic or thalassemic groups by two of the three study investigators (two clinical chemists and one general pathology resident. The normal group consisted of 161 patient results and for inclusion in this group the MCV was between 80 and 100 fL, the RBC between 3.8 and 6.0 $X10^{12}$, the RDW <15.6 and the ferritin was >12 µg/l. The β thalassemic group had 166 patient results all with elevated HbA_2 (>4.0%) concentrations. Each patient in a group was assigned a patient number for tracking purposes. This data was entered into a Microsoft Excel spreadsheet for the calculation of the indices. The indices calculated were:

- Mentzer Index (MI): MCV/RBC; [10]
- Shine and Lal index (S&L): (MCV^2) x (MCH/100); [11]
- Srivastava Index (S): MCH/RBC; [12]

- Discriminant Factor (DF): $(MCV^2 \times RDW)/100 \times Hb$; [4]
- RDW index (RDWI): $MCV \times RDW/RBC$; [5]

ROC (Receiver Operator characteristic) curves and other statistical data were obtained using Analyze It software.

Results

Figure 1 shows the index value plotted against the patient number for the normal (in blue) and β thalassemia (in red) groups for the six indices evaluated. *Figure 2* shows the ROC curves generated for the six indices evaluated and *Table 1* summarizes the area under the ROC curve (AUC), the sensitivity and specificity, true positive and true negative and false positive and false negative for the six indices evaluated. For some indices only a single optimal value was used to calculate ROC curves. However when a different index value produced significant variation in the previously mentioned parameters a number of index values are shown.

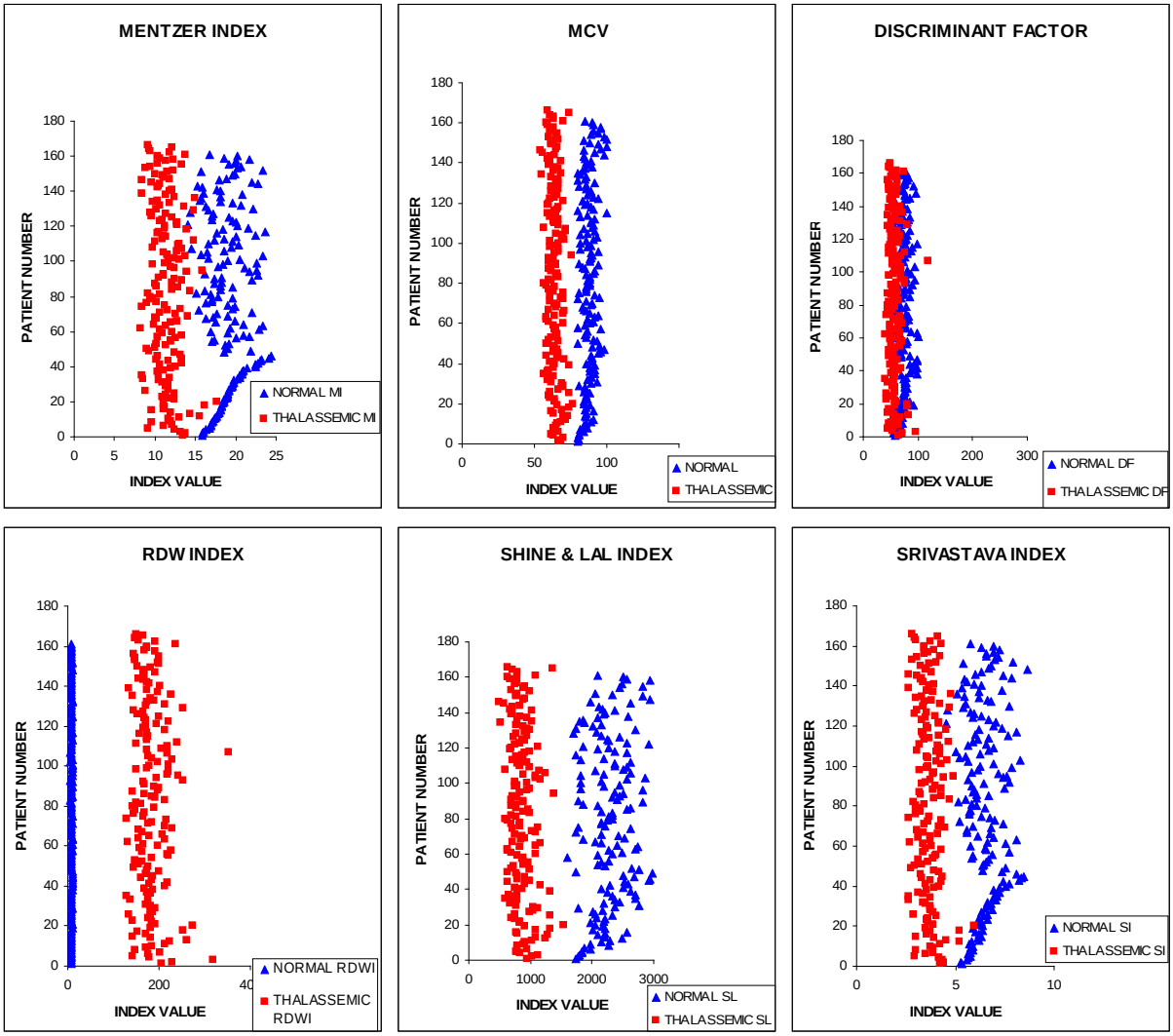

Figure 1. Graphical plots of patient identity number against index for the six indices studied.

Discussion

For patients who are iron replete the MCV, RDW Index and Shine and Lal indices provide the best discrimination between the normal and β thalassemic groups with 100% specificity and sensitivity at the optimal value. However it must be stated that this data is generated with individuals who are iron replete and for inclusion in the normal group the MCV had to be greater than 80. In patients who are iron deficient the MCV will be lower and may not be the best index to discriminate between normal, iron deficient and β thalassemic groups. The Mentzer Index is the next best index with a sensitivity of 94% and a specificity of 100% at the optimal value of <14.0. This is slightly different than Bernstein[1] who found that a value of 14.9 was the best discriminating value. The sensitivity and specificity of the Srivastava Index varies with the value chosen. If a value of 4.6 is chosen the specificity is 100% but the specifici-

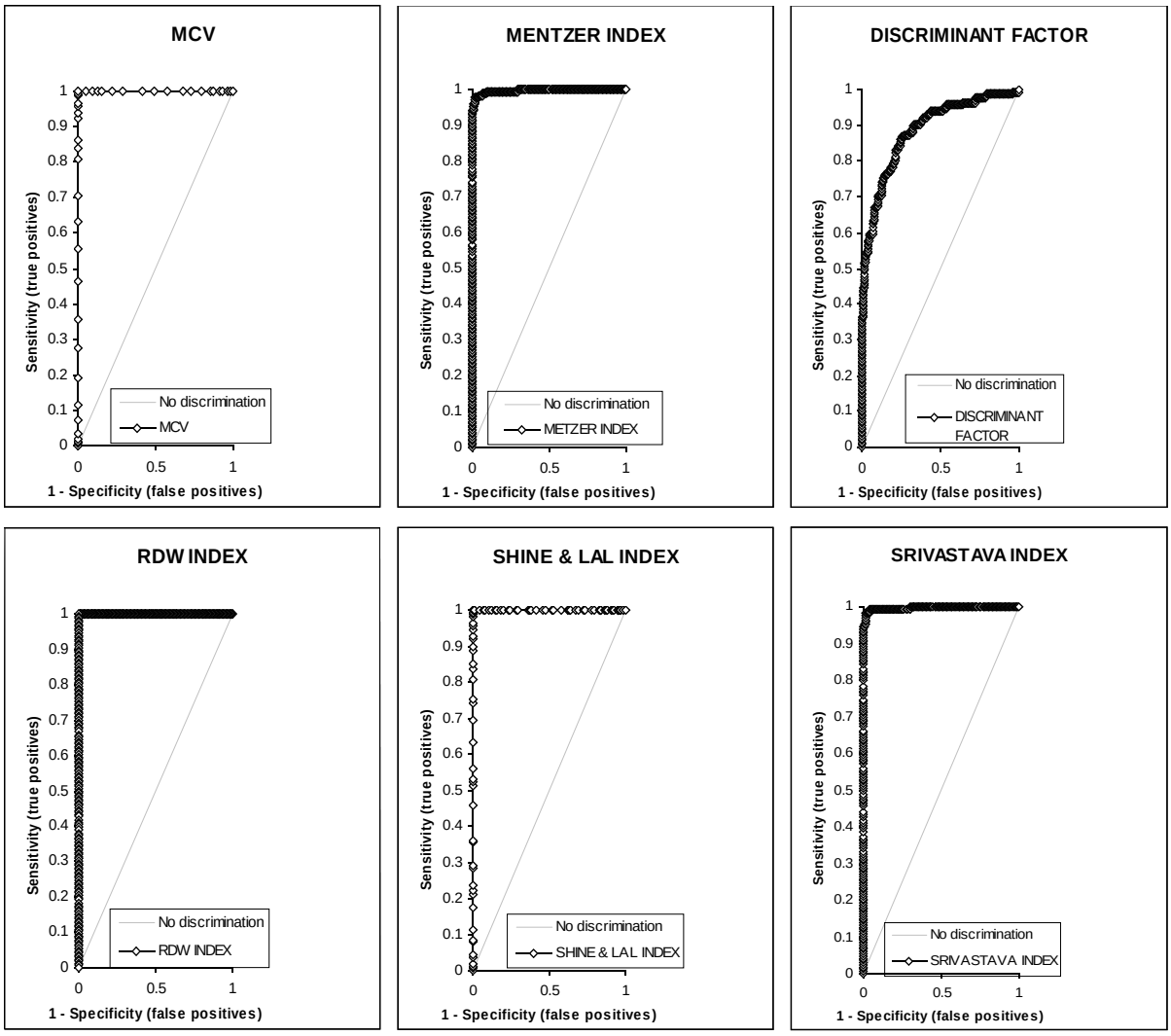

Figure 2. ROC (receiver operator curves) for the six indices studied. The MCV, RDW index and Shrine and Lal indices show excellent discrimination between the normal and β thalassemic groups.

Table 1. ROC statistics for the six studied indices. N=327. Normal patients =161. β thalassic patients =161. TP =True positive, TP=True negative. FP = False Positive, FN=False Negative. n= number of patients.

INDICES	AUC	SENSITIVITY (%)	SPECIFICITY (%)	TP,n	TN,n	FP,n	FN, n
MCV	1.000 (1.00 – 1.00)						
80		100.0	100.0	166	161	0	0
77		99.4	100.0	165	161	0	1
76		98.8	100.0	164	161	0	2
74		96.4	100.0	160	161	0	6
73		95.8	100.0	159	161	0	7
72		94.0	100.0	156	161	0	10
RBC	0.91 (0.88 – 0.94)						
5.91		33.7	100.0	56	161	0	110
5.64		50.6	96.1	84	158	3	82
MENTZER INDEX	0.996 (0.99 – 1.00)						
≤14.0		94.0	100.0	156	161	0	10
DISCRIMINANT FACTOR	0.89 (0.85 – 0.92)						
54		36.1	100.0	60	161	0	106
60		66.9	90.7	111	146	15	55
66		80.1	79.5	133	128	33	33
RDW INDEX	1.000 (1.00 – 1.00)						
≥11		100.0	100.0	166	161	0	0
SHINE & LAL INDEX	1.000 (1.00 – 1.00)						
≥1600		100.0	100.0	166	161	0	0
SRIVASTAVA INDEX	0.997 (0.99 – 1.00)						
4.6		94.6	100	157	161	0	9
5.2		98.2	95.7	163	154	7	3
5.8		99.4	80.7	165	130	31	1
6.0		100	69.6	166	112	49	0

ty is decreased. At a value of 6.0 the sensitivity is 100% but the specificity is markedly decreased. The Discriminant factor shows poor sensitivity at all index values although the specificity is 100% when a value of 54 is chosen.

Conclusion

The RDWI, Shine and Lal, and MCV provide the best discrimination between the normal and patients with β thalassemia. Further studies to determine which are the better indices to discriminate between normal, iron deficient and patients with either α or β thalassemia are planned.

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