The differentiation between thalassemic and non-thalassemic microcytosis has important clinical implications in hematology and medicine. A simplified index, based on red cell parameters derived from automated blood cell analyzers, which could be used to discriminate between microcytic patients with a high probability of thalassemia minor and those with a low probability, would be an extremely useful tool. Five mathematical indices have been proposed as useful for this purpose. These are the: Bessman index, Shine and Lai index, England index, Mentzler index, and mean cell volume (MCV) alone. This study was designed to prospectively evaluate the efficacy of these indices. Patient samples were chosen every fourth day from all patient samples referred to the hematology laboratory at St. Joseph's Hospital over a 6-month period. All patient samples with an MCV < 80 fL and age ≥ 18 years were considered eligible for the study. After enrollment and laboratory analysis were complete sensitivities and specificities were calculated for each of the indices using a variety of cut-off values and receiver operator characteristic (ROC) curves were constructed. Based on statistical analysis of the area under these curves, the authors conclude that MCV alone is as effective as the Mentzler and Shine and Lai indices in selecting microcytic patient samples with a high probability of thalassemia minor for thalassemia testing. They also conclude that the Bessman index and the England index are ineffective indices for this purpose. (Key words: Microcytic anemia; β thalassemia trait; α thalassemia trait; Iron deficiency anemia; Mean cell volume (MCV); Red cell distribution width (RDW)) Am J Clin Pathol 1996; 106:201-205.

Microcytosis is a common presenting manifestation of anemia. It is an important clinical clue as it narrows the differential diagnosis of the anemia to four well-defined conditions. These are iron deficiency, thalassemia minor, the anemias of chronic disease, and the sideroblastic anemias. The differentiation between thalassemic and non-thalassemic microcytosis has important clinical implications in hematology and medicine.

The accepted protocol for the diagnosis of thalassemia minor in routine hematology laboratories is the performance of hemoglobin (Hb) electrophoresis, Hb A2 quantification and investigation for the presence of Hb H inclusion bodies. However, it has been proposed that the routine electronic red blood cell (RBC) counts and indices derived from modern blood cell analyzers can provide valuable clues as to whether thalassemia minor may be present. Specifically, for a given mean cell volume (MCV), the Hb level and RBC count tend to be higher in thalassemia minor than in iron deficiency. A simplified index, based on the complete blood count (CBC), with a high level of specificity and sensitivity for thalassemia minor would be an extremely useful tool in the investigation of microcytic anemia.

Various indices have been developed to differentiate between thalassemia minor and iron deficiency. Indices derived by England and colleagues and Mentzler exploit the above mentioned discrepancies between Hb, RBC count, and MCV. The usefulness of the England and Mentzler indices was evaluated by other researchers. Shine and Lai developed an index based on the MCV and MCH (mean cell hemoglobin), whereas Ghosh and associates found that an MCV of <75 fL provided superior differentiation of β-thalassemia trait from iron deficiency, in pregnant women of Chinese ancestry, than did the England and Mentzler indices. Bessman and

From the Regional Hemoglobinopathy Reference Laboratory, St. Joseph's Hospital, Hamilton, Ontario, Canada; McMaster University Department of Medicine, Hamilton, Ontario, Canada; and Father Sean O'Sullivan Research Centre, St. Joseph's Hospital, Hamilton, Ontario, Canada.

Manuscript received September 18, 1995; revision accepted February 28, 1996.

Address reprint requests to John LaFerry: Regional Hemoglobinopathy Laboratory, St. Joseph's Hospital, 50 Charlton Avenue, E, Hamilton, Ontario L8N 4A6, Canada.
Feinstein relied on the observation that patients with iron deficiency had a greater range of red cell size than did patients with thalassemia minor. Their investigations led to the development of the red cell distribution width (RDW), which was widely publicized and added to the output of new cell analyzers. Bessman and colleagues subsequently proposed that a normal RDW (ie, <15.0), in combination with a decreased MCV, was a strong indicator of thalassemia minor. There is significant controversy in the literature over the effectiveness of the RDW as an indicator of thalassemia minor with supporters and detractors on both sides of the issue.

To date, there has been no report prospectively assessing the efficacy of all the various formulas proposed and their ability to discriminate between patients with thalassemia minor and patients with various non-thalassemic causes of microcytosis.

An effective way to critically assess the various formulas would be to perform a prospective study in which receiver operator characteristic (ROC) curves are constructed for each index. This would evaluate which formula has the best sensitivity and specificity profile and would be the most effective index for predicting the likelihood of thalassemia minor.

The purpose of this communication is to report such a study performed at the Regional Hemoglobinopathy Reference Laboratory at St. Joseph’s Hospital in Hamilton, Ontario, Canada.

MATERIALS AND METHODS

Entrance Criteria

Samples were collected from the hematology laboratory at St. Joseph’s Hospital in Hamilton, Ontario, Canada for a 6-month period. Samples were collected every fourth day to randomize the day of the week on which collection occurred. It is routine practice in this laboratory to screen all adult patients with an MCV of <75 fl for thalassemia syndromes. For the purpose of this study, all patients 18 years of age and older with an MCV < 80 fl were entered. All patients were tested on their first entrance to the study and if encountered on subsequent days were not re-entered.

Laboratory Tests

All samples entered into the study were collected into potassium (K3) EDTA Vacutainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ) and were delivered the same day to the hematology laboratory. Study testing was conducted on the following business day. Each sample had the following laboratory tests performed using standard published techniques:

- CBC (Coulter STKS, Coulter Electronics, Hialeah, FL.)
- Hb H inclusion body investigation
- Hb A2 level
- Hb F level
- Starch Gel Electrophoresis pH 9.0
- Free erythrocyte protoporphyrin (FEP)
- Serum ferritin
- High Performance Liquid Chromatography (HPLC) was performed, when required, to confirm the identity of Hb variants detected on electrophoresis.

Indices Used:

- Mentzler index: MCV/RBC
- Bessman index: RDW
- Shine and Lai index: MCV X (MCH/100)
- England index: MCV - RBC - (0.5 x Hb) - 3.4

Statistical Analysis

Data were collected and sorted using a database in Lotus Version 2.1 (Lotus Development, Cambridge, MA). Data were later copied to Excel Version 5 (Microsoft, Toronto, ON).

Receiver operator characteristic curves for these indices and the MCV were constructed in Excel using sensitivity and specificity calculations. Cut-off values for the various indices were as follows: For the Mentzler index, ≤11, 12, 13, 14, 15, and >17, for the Bessman index, ≤13, 14, 15, 16, 17, 18, 19, and >19, for the Shine index, ≤150, 200, 300, 400, 500, 600, 700, and >800, and for the England index, ≤0, 1, 2, 3, 4, 5, 6, 8, and >10. In addition, the estimate of area under each curve as well as comparisons of the areas under the curves were calculated.

RESULTS

Seven hundred eighty-nine patient samples were eligible for the study. Seventy-seven were excluded because of insufficient sample volume for full analysis. Of the 712 patient samples available, 40 were found to have thalassemia minor. Thirty-one of these were diagnosed as β thalassemia trait on the basis of a Hb A2 level greater than 0.036. Eight were diagnosed as α thalassemia trait based on the detection of the occasional RBC containing Hb H inclusion bodies (most likely α thalassemia 1 cis deletion). One was diagnosed as Hb E trait by detection of Hb E on starch gel electrophoresis, confirmed by HPLC. Six hundred seventy-two patients had no hemo-
globinopathy detected. Of this, 133 were categorized as nonanemic, but iron deplete (defined as ferritin < 20 μg/L and Hb ≥ 115 g/L in females or ≥135 g/L in males), 191 were categorized as iron deficiency anemia (defined as ferritin < 20 μg/L and Hb < 115 g/L in females or <135 g/L in males).34 Three hundred forty-eight patients did not fall into any of these categories and were not classifiable. This group would include patients with iron deficiency (with or without anemia and ferritin levels > 20 μg/L), anemia of chronic disease, sideroblastic anemia as well as cases of α thalassemia trait, which did not yield positive Hb H inclusion body results (most likely α thalassemia 2 or α thalassemia 1 trans deletion).33

Receiver operator characteristic curves generated on all 712 patients for each of the indices as well as for MCV are displayed in Figure 1. Figure 2 displays the ROC curve for MCV alone with the various cut-off values.

Table 1 shows the sensitivity of an MCV ≤ 72 as a predictor of the different thalassemia syndromes detected in the study using all patients.

**DISCUSSION**

Receiver operator characteristic curves readily demonstrate the utility of a test in the detection of a particular disease state. The greater the area under the curve the more useful, in general, the test will be. A straight diagonal line from the origin of the XY intercept to the upper right corner of the graph represents a useless test with no discriminating power.

<p>| TABLE 1. SENSITIVITY OF A MEAN CORPUSCULAR VOLUME ≤ 72 FL AS A PREDICTOR OF THE DIFFERENT THALASSEMIA SYNDROMES DETECTED IN THE STUDY |
|---|---|</p>
<table>
<thead>
<tr>
<th>n</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin E trait</td>
<td>1</td>
</tr>
<tr>
<td>α Thalassemia trait</td>
<td>8</td>
</tr>
<tr>
<td>β Thalassemia trait</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
</tr>
</tbody>
</table>
Based on the data from the study, had this laboratory used an MCV of ≤72.0 fL as an indication for thalassemia testing, 108 of the 672 non-thalassemic patients and 35 of the 40 thalassemic patients would have been tested, yielding a positivity rate of 25%.

Table 1 shows the sensitivity of an MCV ≤ 72 fL in the detection of the different types of thalassemia trait encountered in the study. The sensitivity is essentially identical for α thalassemia trait and β thalassemia trait at 0.88 and 0.90, respectively. Insufficient numbers of Hb E trait were detected to yield significant data. The hemo-globinopathies detected in the study reflect the population of the Hamilton region where β thalassemia trait is the predominant thalassemia minor syndrome followed by α thalassemia trait. This reflects the multicultural nature of the region’s population that contains large Italian and Southeast Asian populations.

Conclusion

The England and Bessman indices are poor discriminators between thalassemic and non-thalassemic microcytosis and should not be used for this purpose. The Bessman index has a further disadvantage in that the RDW is known to be increased in δβ thalassemia trait and Hb H disease. The Bessman index would therefore fail to detect these conditions. The most advantageous index appears to be an MCV of ≤72 fL. However, it is not 100% accurate and its effectiveness in the detection of other forms of thalassemia minor (eg, α thalassemia 2, α thalassemia 1 trans deletion, Hb Lepore trait, and Hb E trait) was not determined in this study. Thalassemia testing should be done on all microcytic patients who are not demonstrably iron deficient, who do not respond to iron therapy or are not suffering from chronic disease.

Using an MCV ≤ 72.0 fL as a laboratory-based criteria for the selection of samples for thalassemia testing should provide similar sensitivities and specificities regardless of the population. However, in certain centers, the population of individuals presenting with thalassemia trait may be so small that this would not be worthwhile. In many multicultural centres, similar results to the 25% positivity rate predicted by this study should be achievable.

Acknowledgments. The authors thank the staff of the Regional Hemoglobinopathy Laboratory at St. Joseph’s Hospital, Hamilton and the staff of the Radio-Immunassay Laboratory at McMaster University Medical Centre for their contributions to this study. The authors also thank Mrs. Millic Danek, Mr. Andrew McFarlane, and Ms. Karen Langan for their assistance with this manuscript.

REFERENCES


