Short Reports

Erythrocyte indices for discriminating thalassaemic and non-thalassaemic microcytosis in Indians

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ABSTRACT

Background. Microcytosis is a common red cell change seen in anaemias of varied aetiology. These include iron deficiency, thalassaemia, chronic disease and sideroblastic anaemias. The microcytosis of heterozygous beta-thalassaemia needs to be distinguished from non-thalassaemic microcytosis for its role in thalassaemia control. Red cell indices derived from automated red cell analysers have been used to discriminate between microcytic patients with a high probability of thalassaemia minor from those with a low probability. There is a controversy on the choice of red cell indices to be used and the cut-off values for this distinction, because the prevalence of iron deficiency as a cause of non-thalassaemic microcytosis is variable. Since no Indian study using receiver operator characteristic (ROC) curves was available to determine the above, we conducted this study.

Methods. Red cell Indices (mean corpuscular volume, total red blood cell count, red cell distribution width, linear discriminant function), serum iron, total iron binding capacity and haemoglobin A2 were estimated in 640 adults with microcytosis (mean corpuscular volume 80 fl). The ROC curves were plotted in all.

Results. Total red blood cell count was observed to be the most efficient single test followed by linear discriminant function and Bessman index. Mean corpuscular volume had the least efficacy. The cut-off values obtained for the Indian population were mean corpuscular volume <76 fl, total red blood cell count >4.9 x 10^12/L and red cell distribution width >18% and a positive linear discriminant function. These were different from those observed in the West, possibly because of the high prevalence of iron deficiency in India.

Conclusion. In countries with a high prevalence of iron deficiency, cut-off values for red cell indices should be recalculated using ROC curves.


INTRODUCTION

Anaemias of varied aetiology (such as iron deficiency, thalassaemia, chronic disease and sideroblastic anaemias) can cause microcytosis. Whereas the red cell changes in homozygous beta-thalassaemia are classical, microcytosis due to heterozygous beta-thalassaemia needs to be distinguished from that due to non-thalassaemic causes, for its role in thalassaemia control. Red cell indices have been reported to have a major role in this distinction.1-3 Among these, mean corpuscular volume (MCV) <75 fl,4 MCV <80 fl and MCV <72 fl have been observed to be effective screening tests. Some workers5-10 have reported red cell distribution width (RDW; Bessman index) to be of value, whereas others have observed it to be of no use.11-14 Thus, there is controversy not only on the choice of red cell indices but also on the cut-off values to be used for distinguishing thalassaemic from non-thalassaemic microcytosis. The above cut-off values and interpretations are primarily based on western population studies. In India, iron deficiency is the commonest cause of non-thalassaemic microcytosis and may co-exist with thalassaemia minor;15 thus altering the red cell indices. However, most Indian studies are based on western cut-off values. No Indian study assessing critical red cell indices to discriminate thalassaemic from non-thalassaemic microcytosis using receiver operator characteristic (ROC) curves is available, though one such study has been recently published from the West.6 In the present study, we used ROC curves to calculate the optimum cut-off values for various red cell indices in our population and to determine their utility.

SUBJECTS AND METHODS

Subjects

Adult patients (n=640) presenting to the Department of Haematology, All India Institute of Medical Sciences, between November 1997 and November 1998 with an MCV <80 fl were included in the study. Blood samples were collected every fourth day in order to randomize the day of the week on which collection was done.

Red cell indices were determined on a Sysmex K100 counter (MCV, total RBC count, RDW). The linear discriminant function (DF) [DF=1.89 (RBC)-0.33 (RDW)-3.281] was determined in all cases.16 Serum iron, total iron binding capacity (TIBC) and haemoglobin A2 (HbA2) were estimated in all cases.

Statistical analysis

Receiver operating curves for the above indices were constructed to determine the index with the best sensitivity and specificity profile and also the appropriate cut-off points for the indices.17,18

RESULTS

Of the 640 patients studied, 67 were excluded since a full analysis was not possible in them. Of the remaining 573 patients whose samples were available, 133 had raised HbA2 levels suggesting beta-thalassaemia trait. Forty-five (33.9%) of these had concomitant iron deficiency. Among the 440 patients with non-thalassaemic microcytosis, 356 (80.9%) were detected to have iron deficiency by estimation of serum iron and TIBC. Among these, anaemia (Hb <11.5 g/dl in women and <13.5 g/dl in men) was present in 241 (54.77%) cases. Forty-one of the 84 non-iron deficiency, non-
This is comparable to that reported by Das Gupta West. The total RBC count and the linear DF (a recently described mathematical index which takes into account the RBC count) were observed to be very efficient screening tests. The MCV, RDW, total RBC counts to identify beta thalassaemia carriers. Am J Public Health 1988;78:1476-7.

Among the red cell indices, the cut-offs having the highest sensitivity and specificity by ROC for MCV, RDW, total RBC count and linear DF were ≤76 fl, ≥18%, ≥4.9x10^12/L and ≥0.0, respectively (Fig. 1 and Table I). Based on the area enclosed by the ROC curves, the total RBC count and the linear DF were found to be the most effective screening tests (Fig. 1). However, the Bessman index was also an efficacious test in distinguishing between thalassaemic and non-thalassaemic microcytosis and enclosed more area under its curve as compared to MCV.

DISCUSSION
Iron deficiency has been observed to be the most common cause of non-thalassaemic microcytosis in India. Most Indian studies have used MCV <70 fl and MCV <75 fl for beta-thalassaemia screening. These cut-off values have been arbitrarily selected and are not based on ROC curves. In the present study, the MCV cut-off (≤76 fl) is higher than the ≤72 fl reported by Lafferty et al. in the West. This may be due to co-inheritance of alpha- and beta-thalassaemic microcytosis cases suspected to have anaemia of chronic disease by serum iron studies, showing not only a reduced serum iron but also a reduced TIBC. In 43 patients, however, no conclusive diagnosis for the non-thalassaemic microcytosis could be arrived at.

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<table>
<thead>
<tr>
<th>Index</th>
<th>Cut-off Value</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean corpuscular volume</td>
<td>≤76 fl</td>
<td>93.9</td>
<td>57.4</td>
</tr>
<tr>
<td>Bessman index</td>
<td>≤18%</td>
<td>88.5</td>
<td>75.5</td>
</tr>
<tr>
<td>Linear discriminant function</td>
<td>≥0.0</td>
<td>96.0</td>
<td>79.1</td>
</tr>
<tr>
<td>Total RBC count</td>
<td>≥4.9x10^12/L</td>
<td>94.7</td>
<td>98.8</td>
</tr>
</tbody>
</table>

The total RBC count and the linear DF were observed to be very efficient screening tests. The RDW (≤18) was observed to be an efficient function to distinguish thalassaemia minor from non-thalassaemic microcytosis. This is comparable to that reported by Das Gupta et al. in South India and others, but different from that reported by Lafferty et al. who observed a straight line ROC curve and found it to be a useless test. Our cut-off for RDW is much higher than that reported by Bessman et al. (≥15). This may be because of the higher prevalence of iron deficiency in our population leading to co-existence of iron deficiency with beta-thalassaemia trait (45/133) and thus a higher cut-off for the RDW.

REFERENCES