EDUCATIONAL COMMENTARY- THE USE OF ERYTHROCYTE INDICES IN THE DIFFERENTIAL DIAGNOSIS OF ANEMIAS

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Learning Outcomes
Upon completion of this exercise, the participant will be able to:

- calculate the erythrocyte indices.
- classify an anemia based on erythrocyte indices.
- correlate morphologic classification with a potential etiology.

Erythrocyte indices are mathematical values obtained from a patient’s erythrocyte count, hemoglobin concentration, and hematocrit results. These indices define the size and hemoglobin content of the erythrocyte. Erythrocyte indices are an important evaluation tool because they provide a basis for the diagnosis of anemia. This initial step in the investigation of anemia is critical because it allows clinicians to rule out a number of etiologies and direct their attention to fewer probable causes, allowing a faster diagnosis. It also lowers healthcare costs by using fewer confirmatory tests. Before discussing each of the indices, a brief discussion of erythrocyte morphology and production as it relates to pathophysiology is helpful to understand the purpose of these indices in classifying and evaluating anemias and subsequently in determining appropriate treatment.

Erythrocyte Production
The erythrocyte requires a variety of nutrients for normal development, but iron is necessary for the production of the actual hemoglobin molecule. Therefore, any condition that interferes with iron absorption, metabolism, or utilization will result in impaired production of hemoglobin. As the erythrocyte is formed in the bone marrow, the lack of iron produces an erythrocyte that contains less hemoglobin than a normal erythrocyte. Ultimately, the mature erythrocyte that enters the peripheral bloodstream is also smaller than normal. These cells are morphologically described or classified as microcytic and hypochromic. Case A in Table I depicts the kind of erythroid values commonly associated with this blood picture. In this case, the number of cells is normal but with a lower hemoglobin concentration and hematocrit value. This occurs because the bone marrow continues to produce the normal number of cells, but the cell size is decreased and the hemoglobin content is low.
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TABLE I. Erythroid values associated with different types of anemia.

<table>
<thead>
<tr>
<th>Value</th>
<th>Case A</th>
<th>Case B</th>
<th>Case C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count, ×10^{12}/L (=RBC ×10^6/µL)</td>
<td>4.5</td>
<td>2.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>9.5</td>
<td>7.6</td>
<td>9.0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>32.0</td>
<td>23.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV), fL</td>
<td>71, microcytic</td>
<td>105, macrocytic</td>
<td>93, normocytic</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH), pg</td>
<td>21</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>MCH concentration, (MCHC) g/dL</td>
<td>30, hypochromic</td>
<td>33, normochromic</td>
<td>33, normochromic</td>
</tr>
</tbody>
</table>

**Image 1** is from a patient with iron deficiency anemia. These cells are smaller than normal and have a larger central pale area. In contrast, normal cells have a central pallor that is <30% of the cell diameter.

Other conditions may also interfere with hemoglobin production. For example, thalassemia is a hereditary condition that results in less hemoglobin in each cell. In this disorder, the patient cannot produce enough of the appropriate amino acid chains needed for the hemoglobin molecule. Therefore, a microcytic-hypochromic blood picture does not always indicate iron deficiency, but could be due to other etiologies. **Image 2** is from a patient with thalassemia and shows hypochromic-microcytic RBCs along with poikilocytes (abnormally shaped cells).

In each condition, different follow-up tests are needed to identify the specific etiology and proper patient treatment.
Another morphologic classification of anemia is macrocytic-normochromic. In this instance, the developing erythrocyte has inadequate vitamin B₁₂ or folic acid needed for proper nuclear maturation. Due to this deficiency, the erythrocyte matures at a slower pace than normal; however, the cell continues to produce hemoglobin at a normal rate. By the time the erythrocyte is ready to enter circulation, it is larger than normal and full of hemoglobin, hence the description macrocytic-normochromic. Case B in Table I depicts the kind of erythroid values commonly associated with this blood picture. In Case B, the number of cells is low in relation to the hemoglobin concentration and hematocrit value.

Finally, erythrocytes that are normal in size and color, described as normocytic-normochromic, may also be seen in anemias. These can be seen in cases where the patient has experienced blood loss, possibly due to trauma. They are also seen in disorders that result in a premature destruction of erythrocytes, thus decreasing the normal life span of the cell. These disorders are referred to as hemolytic anemias. In these cases, there is a decrease in erythrocyte number, but the size and hemoglobin concentration of the cells are not altered. Case C in Table I depicts the kind of erythroid values commonly associated with this blood picture. Image 4 is from a patient with an acquired hemolytic anemia.

**Deriving the Indices**

Table II, on the next page, provides the basis for the following discussion.
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TABLE II. Erythrocyte Indices.

<table>
<thead>
<tr>
<th>Index</th>
<th>Definition</th>
<th>Calculation</th>
<th>Reference Range</th>
<th>Morphologic Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td>The volume of the average erythrocyte in cubic micra (µ³) or femtoliter (fL)</td>
<td>( \frac{Hct (\text{vol} %)}{RBC (\text{millions} / \mu L)} \times 10 = MCV )</td>
<td>80-94 fL (male) 77-97 fL (female)</td>
<td>Microcytic Normocytic Macrocytic</td>
</tr>
<tr>
<td>MCH</td>
<td>The hemoglobin content (weight) of the average erythrocyte in micromicrograms (µµg) or picograms (pg)</td>
<td>( \frac{Hgb (\text{vol} %)}{RBC (\text{millions} / \mu L)} \times 10 = MCH )</td>
<td>27-31 pg</td>
<td>Not applicable</td>
</tr>
<tr>
<td>MCHC</td>
<td>The average hemoglobin concentration per cell reported out as a percent or g/dL (g/L)</td>
<td>( \frac{Hgb (g / dL)}{Hct (\text{vol} %)} \times 100 = MCHC ) or ( \frac{MCH}{MCV} \times 100 = MCHC )</td>
<td>32-36 g/dL (320-360 g/L)</td>
<td>Hypochromic Normochromic</td>
</tr>
</tbody>
</table>

MCV indicates mean corpuscular volume; Hct, hematocrit; MCH, mean corpuscular hemoglobin; Hgb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

The mean corpuscular volume (MCV) is the average volume of the erythrocytes in cubic micra or femtoliter. By relating the number of erythrocytes (RBC count) to the volume they occupy (hematocrit), the average size of the individual erythrocyte can be determined. Depending on the volume or size of the cell, erythrocytes may be classified as normocytic, microcytic, or macrocytic. However, this is a mean measurement, so if both microcytes and macrocytes are present, mathematically a normal MCV would be obtained. For this reason, a stained peripheral blood smear is examined to ensure an accurate evaluation of erythrocyte morphology.

The mean corpuscular hemoglobin (MCH) is the average hemoglobin content of the erythrocytes measured in micromicrograms or picograms. This measurement expresses weight. The MCH value by itself has limited usefulness. One could relate the MCH to the following situation: Fifty people are in a theater but the seating capacity of the theater is not known. Using this information, you do not know if the theater is full, half full, or almost empty. The MCH indicates the amount of hemoglobin in a cell, but does not relate it to the size of the cell.

The mean corpuscular hemoglobin concentration (MCHC) is more useful. It provides information regarding the appearance of a cell by measuring the concentration of hemoglobin in each erythrocyte. It is calculated as the ratio of the weight of hemoglobin to the volume in which it is contained. The result is
expressed either as a percent or as grams per deciliter. If the MCHC falls below the reference range, the cell is referred to as hypochromic. MCHC results that fall in the normal range indicate that the cell is normal in color or normochromic. However, the MCHC can also be normal when both the MCV and MCH are either low or high.

It is less common to have an MCHC that is greater than normal because cells have a limited capacity and can only hold a certain amount of hemoglobin. The weight of hemoglobin in a cell may be increased, but this increase is only proportional to the increase in the size of the cell. Therefore, an MCHC of >36% is not typically found because there is no such thing as a cell supersaturated with hemoglobin. However, in conditions such as hereditary spherocytosis, where there is a loss of cellular membrane, the erythrocyte may be damaged in such a way that the cell volume is decreased while the hemoglobin concentration remains constant, resulting in a MCHC >36%. **Image 5** is from a patient with hereditary spherocytosis. In this blood smear, the cells, referred to as spherocytes, appear smaller than normal in diameter and have no central pallor. These microcytic cells actually look darker due to their change in shape from a biconcave disc to a sphere.

**Summary**
Cost-effectiveness is critical to the delivery of health care. Patients who present with anemia must be properly evaluated to determine the specific etiology. Using erythrocyte indices to determine potential causes leads to appropriate confirmatory tests and appropriate treatment.