The Red Cell Histogram and The Dimorphic Red Cell Population

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Abstract
The RBC histogram is an integral part of automated hematology analysis and is now routinely available on all automated cell counters. This histogram and other associated complete blood count (CBC) parameters have been found abnormal in various hematological conditions and may provide major clues in the diagnosis and management of significant red cell disorders. In addition, it is frequently used, along with the peripheral blood film, as an aid in monitoring and interpreting abnormal morphological changes, particularly dimorphic red cell populations. This article discusses some morphological features of dimorphism and the ensuing characteristic changes in their RBC histograms.

Keywords: histogram, dimorphic red cells, red blood cell distribution width

After reading this article, readers should be able to correlate a RBC histogram and red blood cell distribution width with microscopical findings.

To paraphrase an adage, 1 histogram graph is worth 1000 numbers. A large collection of data, displayed as a visual image, can convey information with far more impact than the numbers alone. In hematology, these data take on several forms, 1 of which is the RBC histogram. Visual scanning of the histogram gives a good initial sense of the range, size, shape, and other salient features of the red cell morphology.

The RBC histogram, a graphic representation of particle size distribution, is now routinely available on automated cell analyzers as a standard part of automated complete blood count (CBC) analysis. This histogram in association with other CBC parameters, such as RBC distribution width (RDW) and mean corpuscular volume (MCV), has been found abnormal in various hematological conditions and may provide major clues in the diagnosis and management of significant red cell disorders. In addition, it is frequently used, along with the peripheral blood film, as an aid in monitoring and interpreting abnormal morphological changes, particularly dimorphic red cell populations. This article discusses some morphological features of dimorphism and the ensuing characteristic changes in their RBC histograms.

Keywords: histogram, dimorphic red cells, red blood cell distribution width

Table 1. Conditions Associated With Dimorphic Red Cells5-12

<table>
<thead>
<tr>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early iron developing microcytic population</td>
</tr>
<tr>
<td>Folate/vitamin B12 developing macrocytic population</td>
</tr>
<tr>
<td>Post-iron treatment of iron deficiency anemia</td>
</tr>
<tr>
<td>Post-iron treatment of iron deficiency with megaloblastic anemia</td>
</tr>
<tr>
<td>Post-iron treatment of megaloblastic anemia with iron deficiency</td>
</tr>
<tr>
<td>Post-iron transfusion macrocytic anemia</td>
</tr>
<tr>
<td>Post-iron transfusion microcytic anemia</td>
</tr>
<tr>
<td>Iron deficiency anemia with either folate or vitamin B12 deficiency</td>
</tr>
<tr>
<td>Sideroblastic anemia (myelodysplasia)</td>
</tr>
<tr>
<td>Hemolytic anemia (reticulocytosis, spherocytosis, fragmentation, pyropoikilocytosis)</td>
</tr>
<tr>
<td>Cold/warm auto agglutination</td>
</tr>
<tr>
<td>Erythropoietin-induced erythropoiesis</td>
</tr>
<tr>
<td>Delayed transfusion reaction</td>
</tr>
<tr>
<td>Homozygous hemoglobinopathies (admixture of many RBC forms)</td>
</tr>
<tr>
<td>Myelofibrosis (admixture of extramedullary hematopoesis)</td>
</tr>
<tr>
<td>Constitutional chromosomal translocation t(11;22)(p15.5;q11.21)</td>
</tr>
</tbody>
</table>

In a dimorphic picture, the histogram may have 2 or more (multiple) red cell populations, whereas in dual populations the histogram has 2 distinct red cell populations (eg, hypochrom-microcytic and normochrom-normocytic red cells). These 2 distinct populations may be comprised of either a patient’s own red cells (post-iron treatment) or a mixture of patient and donor red cells (post-iron transfusion). Although this is an arbitrary categorization, the term dimorphic is less restrictive and therefore more widely used, as it can be applied to either the dual or the multiple red cell populations. Thus, the dimorphic blood picture will look like a dual population of microcytic and normocytic (Image 1A) or normocytic and macrocytic red cells, or an admixture of small, normal, and large cells of different sizes and forms (Image 1B). Some morphological features of dimorphism and the ensuing characteristic changes in their RBC histograms are discussed in this article.

Abbreviations
CBC, complete blood count; RDW, red blood cell distribution width; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin concentration; IDA, iron deficiency anemia; RDW-CV, red blood cell distribution width coefficient of variation; thal, thalassemia; SD, standard deviation; HPP, hereditary pyropoikilocytosis

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RBC Histogram: An Overview

The well-known Coulter principle of counting and sizing red cells provides the basis for generating the histogram. This method relies on the change in conductance as each cell passes through an aperture. The change in conductance results in an electrical pulse, the amplitude of which is proportional to the cell volume. The 256-channel pulse-height analyzer uses a number of thresholds to sort the cells into several size (volume) channels from which the histogram is formed. Each channel on the X-axis represents a specific size (volume) in femtoliter (24-360 fL), increasing from left to right. The Y-axis represents the number of cells per channel, with each cell being stored in the channel representing its size, so that after data accumulation is completed the relative number of cells (frequency) is provided. This data is further processed by the computer, and the RBC curve is smoothed by a moving average technique and displayed on a data management system. Figure 1A shows a typical normal RBC histogram. For a detailed explanation on the construction of histogram, please see reference 14.

Value of Histogram

A histogram, as shown in Figure 1 and Figure 2, is a graphic representation of a collection of data based on cell size and/or cell number depicting variations in the process. It is sometimes referred to as a frequency of distribution curve. Because graphics can show data in ways that are meaningful and quickly understood, the histogram is a very powerful tool in red cell morphological analysis. It enables one to visualize, analyze, and interpret empirical data that displays morphological changes graphically as points, peaks or valleys, or as a line of frequency curve. It allows the users to intuitively see the visual comparison of the center and spread of data and data containing 2 or more variables: dimorphic red cells, subpopulations, and skewed data, leading to quick and cost-effective decision making.

At times, a histogram can provide invaluable information that may not even be apparent in the automated numerical data. For example, in megaloblastic anemia with developing iron deficiency, some of the hypochromic and microcytic red cells can be identified in the histogram but may not be reflected in the numerical data, and this may well be the first clue to a not uncommon occurrence. Likewise, iron deficiency may develop insidiously during the process of some other disease (e.g., essential polycythemia), with normal cell counts and indices, except the RDW, which is elevated. The RBC histogram is also abnormal, showing a shoulder of microcytic red cells.

Furthermore, a histogram can provide useful information for laboratorians in 1) monitoring the reliability of the results generated by the analyzers; 2) investigating the potential cause(s) of the erroneous automated results; and 3) arriving at the presumptive diagnosis. For example, certain conditions like the presence of fragmented red cells or red cell agglutination that could not have been identified earlier without blood film examination can now be presumably detected on the red cell histogram. Likewise, in patients with iron deficiency anemia (IDA) or megaloblastic anemia in treatment, a sequential histogram can clearly show the progressive appearance of a new erythrocyte population well in advance of other numerical parameters.

The presence of a right-sided shoulder usually corresponds to reticulocytosis (Figure 1J), and a trailer of erythrocyte populations on the far right of the histogram correlates to red cell agglutination (Figure 1F). A leftward shift of the RBC histogram signifies microcytosis (Figure 1B and Figure 1C), and a rightward shift suggests macrocytosis (Figure 1D and Figure 1E). Bimodal red cell histograms (Figure 1F, Figure 1G, Figure 1K, Figure 1L and Figure 2B to Figure 2E) are usually associated with therapeutic transfusion and/or hematonic agent response to microcytic and macrocytic anemia, but they may also indicate other hematological disorders as shown in Table 1.

Although the size ranges for RBC histograms are between 24 fL and 360 fL, the instrument counts only those cells with volume sizes between 36 fL and 360 fL as red cells. Those cells counted in the 24 fL to 36 fL range are rejected and not included in the RBC count. They are enumerated and displayed in the histogram area between the 24 fL and 36 fL.
Figure 1. Red cell histograms in various hematological conditions. (Key hematological features of these conditions are summarized in Table 2.) Figure (A) Normal histogram, (B) Microcytosis, iron deficiency anemia, (C) Microcytosis, beta thal trait, (D) Macrocytosis with normal RDW, (E) Macrocytosis, megaloblastic anemia, (F) Cold agglutination, (G) Sideroblastic anemia, (H) Beta thalassemia major, (I) Pyropoikilocytosis, (J) Reticulocytosis, (K) Post-iron therapy, (L) Post-iron therapy.

Table 2. Key Hematological Features of Various Hematological Conditions in Figure 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>HGB (g/L)</th>
<th>MCV (fL)</th>
<th>MCHC (g/L)</th>
<th>RDW-CV (%)</th>
<th>RBC Histogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A Normal</td>
<td>146</td>
<td>89.6</td>
<td>330</td>
<td>14.4</td>
<td>Normal</td>
</tr>
<tr>
<td>1B Iron deficiency anemia</td>
<td>86</td>
<td>54.7</td>
<td>314</td>
<td>21.6</td>
<td>LS</td>
</tr>
<tr>
<td>1C Beta thalassemia</td>
<td>131</td>
<td>66.0</td>
<td>318</td>
<td>14.5</td>
<td>LS</td>
</tr>
<tr>
<td>1D Macrocytosis</td>
<td>131</td>
<td>102.8</td>
<td>351</td>
<td>14.3</td>
<td>RS</td>
</tr>
<tr>
<td>1E Megaloblastic anemia</td>
<td>65</td>
<td>127.8</td>
<td>324</td>
<td>35.7</td>
<td>DM RS SKL</td>
</tr>
<tr>
<td>1F Cold agglutinin</td>
<td>124</td>
<td>110.6</td>
<td>++++</td>
<td>17.5</td>
<td>DM BM</td>
</tr>
<tr>
<td>1G Sideroblastic anemia</td>
<td>92</td>
<td>90.9</td>
<td>320</td>
<td>39.2</td>
<td>DM BM LS SKR</td>
</tr>
<tr>
<td>1H Beta thal major</td>
<td>86</td>
<td>79.6</td>
<td>285</td>
<td>32.1</td>
<td>DM LS</td>
</tr>
<tr>
<td>1I Pyropoikilocytosis</td>
<td>99</td>
<td>60.2</td>
<td>358</td>
<td>41.5</td>
<td>DM LS SKR</td>
</tr>
<tr>
<td>1J Reticulocytosis</td>
<td>87</td>
<td>72.2</td>
<td>298</td>
<td>31.3</td>
<td>DP LS SKR</td>
</tr>
<tr>
<td>1K Post-iron therapy</td>
<td>132</td>
<td>81.1</td>
<td>317</td>
<td>14.8</td>
<td>DP BM</td>
</tr>
<tr>
<td>1L Post-iron therapy</td>
<td>112</td>
<td>85.8</td>
<td>330</td>
<td>35.0</td>
<td>DP BM</td>
</tr>
</tbody>
</table>

HGB, hemoglobin; MCV, mean corpuscular hemoglobin; RDW-CV, red cell distribution width-coefficient of variation; DM, dimorphic; BM, bimodal; DP, dual population; LS, left-shift; RS, right-shift; SKR, skewed to the right; SKL, skewed to the left; MCHC (++++) overrange.
Figure 2. Histograms of iron deficiency anemia after treatment (key hematological features are summarized in Table 3). Figures B to G are representative examples of dimorphic (dual) red cell population response to hematinic treatment of iron deficiency anemia in 6 patients.

Table 3. Key Hematological Features in Iron Deficiency Anemia After Treatment

<table>
<thead>
<tr>
<th>Condition</th>
<th>HGB(PT)</th>
<th>HGB(POT)</th>
<th>MCV(PT)</th>
<th>MCV(POT)</th>
<th>RDW(PT)</th>
<th>RDW(POT)</th>
<th>SF(PT)</th>
<th>RBC Histogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A Normal</td>
<td>144</td>
<td>84.7</td>
<td></td>
<td></td>
<td>13.9</td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>2B IDA</td>
<td>108</td>
<td>70.3</td>
<td>82.8</td>
<td>22.1</td>
<td>25.5</td>
<td>8</td>
<td>DP SKL</td>
<td></td>
</tr>
<tr>
<td>2C IDA</td>
<td>80</td>
<td>69.2</td>
<td>81.1</td>
<td>18.0</td>
<td>29.2</td>
<td>7</td>
<td>DP BM</td>
<td></td>
</tr>
<tr>
<td>2D IDA</td>
<td>86</td>
<td>61.3</td>
<td>77.4</td>
<td>23.0</td>
<td>36.0</td>
<td>&lt;5</td>
<td>DP BM</td>
<td></td>
</tr>
<tr>
<td>2E IDA</td>
<td>98</td>
<td>70.4</td>
<td>78.9</td>
<td>17.9</td>
<td>28.2</td>
<td>8</td>
<td>DP BM LS</td>
<td></td>
</tr>
<tr>
<td>2F IDA</td>
<td>95</td>
<td>65.6</td>
<td>70.2</td>
<td>19.5</td>
<td>28.8</td>
<td>&lt;5</td>
<td>DP LS SKR</td>
<td></td>
</tr>
<tr>
<td>2G IDA</td>
<td>81</td>
<td>62.9</td>
<td>68.6</td>
<td>19.5</td>
<td>25.9</td>
<td>&lt;5</td>
<td>DM LS SKR</td>
<td></td>
</tr>
</tbody>
</table>

PT, pre-treatment; POT, post-iron treatment; IDA, iron deficiency anemia; DP, dual population; BM, bimodal; SKL, skewed to the left; SKR, skewed to the right; SL, left shift; SF, serum ferritin. SF normal range (female)=13-145 UG/L; SF (male)=27-220 UG/L.
range, however, allowing the lower end of the histogram to be monitored. Normally, the space below 36 fL remains clear, but in certain conditions the histogram may begin above the baseline or show a high takeoff on the far left of the curve (Figure 1G to Figure 1A), which generally indicates the presence of small particles. These particles include red cell fragments, microspherocytes, nucleated RBCs, nonlyzed RBCs, elliptocytosis, macrothrombocytes, platelet clumps, bacteria, parasitic organisms, and other interfering substances such as cryoglobulin, cold agglutinin, and macroglobulinemia.7,16-18

Analysis and Interpretations of Histograms in Figure 1 and Figure 2

The red cell histograms presented for illustration were randomly selected to represent various common hematological conditions and the different stages of post-iron IDA therapy. A histogram may be interpreted by looking at the shape of the data distribution. The distributional shape of the histogram can be classified as reflecting either single or dimorphic (multiple) red cell populations. In a single population, the histogram may have a normal Gaussian or bell-shape curve, may be widened with or without left or right shift, may be skewed to the left or right, or may show some combination of all of these characteristics. In dimorphic populations, the histogram may be symmetric and/or bimodal, or bimodal but skewed to the left or right. The centeredness and width of the histogram also define the extent of RBC variability. Potentially, a number of factors may affect the histogram of the aperture impedance cell distribution analysis. These include the coincident doublet of red cells, red cell agglutination, inclusion of reticulocytes with mature red cells, alteration in red cell shape, and inclusion of leukocytes in certain diseases. These factors, in 1 way or another, influence the histogram’s appearances and accordingly will have a variable effect on any measurements made from the histogram.16 To reduce the effect of these problems, manufacturers design their instruments and reagent systems to specifically prevent and correct for interferences.13 They develop mathematical algorithms for particle counting and produce numeric data, graphic data, scatter plots, and interpretative comments that will assist or alert the users to potential incorrect results.19,20 In addition, to avoid interference in the calculations of RDW, the information below 20% of scale on the red cell histogram are excluded (Figure 3). These misleading data include cell coincidence, aperture artifacts, doublets and triplets, and agglutinates on the right side and platelet clumps, and macrothrombocytes on the left side of the histogram.13,16

Before taking up complex histogram graphs, the beginner will find it helpful to examine the simple outline or overall pattern of a regular histogram as reflected in Figure 1A to Figure 1D. Familiarity with typical RBC histograms will make the task of evaluating other notable deviations easier. In view of this, to maximize the usefulness of the histogram, a dotted line depicting a reference normal curve (eg, symmetric normal curve with MCV of 96 fL and RBC distribution width coefficient of variation [RDW-CV] of 15.0%) should be automatically superimposed on every red cell histogram so any discernible deviation from that curve can be clearly delineated for contrast. Simply put, if the patient’s histogram strays from the reference normal range, it means that the histogram may be abnormal.

The following are important points to consider when reviewing/analyzing histograms.13

- position of individual populations compared to normal/typical positions
- amount of separation between populations compared to normal/typical separation
- relative concentration of each population compared to normal/typical concentrations
- presence of unexpected or non-typical populations

Figure 1A to Figure 1D are typical examples of normal, microcytic, and macrocytic red cell histograms. They are symmetric, single-peaked, and “bell shaped” normal curves. The curve is considered symmetric if the 2 sides of the curve coincide when folded in half or are approximately mirror images. When the distribution is not symmetric, it is referred to as skewed wherein 1 of the 2 sides (left or right) of the curve extends much further than the other. In a normal RBC histogram, the majority of each cell falls between 55 fL and 125 fL. The tail of the distribution consists of coincident doublets and anomalous pulses.16

In IDA and beta thalassemia (thal) trait, the red cell distribution curves are shifted to the left, and the percentage of microcytosis is increased. Although their histograms are similar, the degree of anisocytosis, as measured by the RDW, differentiates them. Iron deficiency anemia is characterized by elevated RDW, reflecting the heterogeneity in the acquired erythrocyte populations. In thal trait however, the RDW is usually within range due to the homogeneity of the inherited population of erythrocytes. Although this difference is useful to some extent in distinguishing them, cases involving severe anemia with homogenous microcytosis may give misleading results. Nonetheless, patients showing severe anemia with hemoglobin <90 g/L and with hypochromic-microcytic red cells are most likely (80%) iron deficient.21

Calculation of RDW. The RDW is calculated from the width of the histogram at 1 SD from the mean divided by MCV. The normal RDW-CV is 11.5% to 14.5%. The RDW-SD is the arithmetic width of the distribution curve measured at the 20% frequency curve. The normal RDW-SD is 39 to 47 fL.14

Figure 3. Calculation of RDW. The RDW is calculated from the width of the histogram at 1 SD from the mean divided by MCV. The normal RDW-CV is 11.5% to 14.5%. The RDW-SD is the arithmetic width of the distribution curve measured at the 20% frequency curve. The normal RDW-SD is 39 to 47 fL.
Anisocytosis: Variation in red cell size.

Anomalous pulses: This is an interference by electrical pulses/noises but at a very low level. These anomalous pulses and coincident doublets are reflected as a small tail to the right of a normal curve (Figure 1A and Figure 2A). In most normals, the frequency distribution of red cells does not exceed the range of 50 fL–200 fL. Despite the instrument’s refinement in aperture design, correction for coincidental passage of cells, and the use of sophisticated edit circuitry to exclude aberrant pulses, the presence of these interferences to some degree cannot be avoided.

Coincident doublets: Occasionally, 2 or more cells pass through an aperture at the same time and are counted as a single cell. The analyzer automatically corrects results for coincidence.

Dimorphic: According to the Webster New World College Dictionary, the word dimorphic means the occurrence of 2 types of individuals in the same species (2 different forms/sizes of cells in a sample). Note, however, that the term dimorphic is broadly defined in this article to signify 2 or more (multiple) red cell populations. This is because there are 2 technical meanings for the same word. When “dimorphic” is used, the context will determine the meaning.

Poikilocytosis: Variation in red cell shape.

Cautionary Note When Visually Observing and Interpreting RDW

Automated counters use 2 methods to measure the value of RDW. The first is referred to as RDW-CV (Figure 3). This reflects the ratio of 1 standard deviation (SD) to the MCV. Since it is a ratio, changes in either the SD or MCV will influence the results. Microcytosis will tend to elevate the RDW-CV simply by decreasing the denominator (MCV) of the ratio. As a result, the RDW is probably a more sensitive parameter in microcytic than in macrocytic red cell disorders. Conversely, macrocytosis, by increasing the denominator, may offset the change in the SD and reduce the RDW-CV. Put simply, a population with a large MCV may appear wider on the histogram but may give a normal RDW-CV. Likewise, a population with a very small MCV may appear smaller but may give a large RDW-CV. The second method of calculating the RDW is called the RDW-SD, which is independent of MCV. It is a direct measurement of the width in fL at the 20% frequency level on the RBC curve (Figure 3).

The RDW-SD is a measure of the dispersion or scatter of values about the mean. The more spread apart the data, the higher the SD and vice versa. An RDW-CV is another measure of the dispersion of data around the mean. It represents the ratio of 1 SD to the mean. Although both methods use SD to measure the degree of anisocytosis, they measure cell variation differently.

Consider Figure 1E - an abnormal histogram of megaloblastic anemia with a RDW-CV of 35.7% and a RDW-SD of 172.8 fL. While both results are high, obviously the RDW-SD is relatively higher than the RDW-CV. This is probably because of the way dispersion is measured by the RDW-CV formula: 1 SD divided by MCV. The 1 SD imposed by the formula restricts its capability to measure variability to ± 1 SD.

As a result, other abnormal cells outside the ± 1 SD are excluded from the estimate hence the comparatively low RDW-CV results. In addition, the MCV (the denominator) is not a direct measure of dispersion, but it can affect the RDW-CV result depending on its average value. In this case, the high MCV value of 127.8 fL counterbalances the effect of high SD, hence the further reduction in the RDW-CV value. Thus, this method is not recommended for assessing highly abnormal histograms. However, it shows a better correlation as an indicator of anisocytosis when the MCV is in the low normal range and anisocytosis is difficult to detect, as seen in developing iron deficiency. It is also a good method to monitor the effect of iron therapy and to differentiate IDA from thal trait.

On the other hand, because the RDW-SD is a direct measure across the RBC histogram, it is the better and more accurate measure of anisocytosis, especially in highly abnormal histograms. It seems to represent the genuine morphological and/or pathological status of the patients, as it encompasses the entire spectrum of MCV values. In other words, unlike in RDW-CV, those small and large abnormal cells in varying degrees of anisocytosis and/or poikilocytosis outside the ± 1 SD are included in the measurement, hence the relatively high RDW-SD results. It appears, however, that the higher the RDWs, the higher the degrees of anisocytosis and/or poikilocytosis.

In view of the above, it seems logical that technologists reading blood films should have ready access to both the RDW-CV and RDW-SD results for comparative review and better correlation of results.

Macrocytosis

Macrocytosis with a MCV higher than 100 fL (and a normal RDW) is found in 3% to 5% of adult patients, a significant proportion of whom may not have anemia (Figure 1D). According to Gulati, macrocytic red cells of <25% with MCV of 95 fL to 110 fL are morphologically graded as (1+). Correlation between the grading level of macrocytosis (or other conditions) and the MCV should be verified microscopically.

Megaloblastic Anemia

Patients with macrocytic anemia due to vitamin B12/folate deficiency may sometimes show a single widely distributed peak of cells from very small cells and red cell fragments on the left to macrocytic cells approaching 250 fL on the right (Figure 1E). Note a large number of small cells, reflected as a high take-off on the left, are above the 20% frequency trim line. As a result, they are included in the determination of the MCV and the RDW, leading to their falsely reduced and greatly elevated values, respectively. In severe megaloblastic anemia, the white cell and platelet histograms as well as the counts and the morphology may give additional information.
Cold Agglutination

Because this is a time-, temperature-, and agglutinin titer-dependent reaction, the frequency curves may vary in shapes (Figure 1F). A U-shape-like appearance of the curve would usually suggest the presence of 2 discrete populations, 1 on either side. However, in this case, the abnormal histogram is the result of a high titer cold agglutinin causing red cells to agglutinate and interfere with their sizing and enumeration. Some analyzers with an Interpretive Program of flags and messages may generate an RBC Agglutination flag. Dashes (−−) replace the data for RDW, and an asterisk (*) appears on most red cell associated parameters. This asterisk indicates that these results are unreliable and should be reviewed carefully as per laboratory protocol.

On the other hand, despite the abnormal histogram, other instruments still measure and calculate the RDW, using only the major population of cells. The minor populations and the middle trough are excluded in order to avoid interference in RDW calculation. The high frequency entry on the left of the histogram could be due to the effect of the high titer antibody interfering in cell separation. Since the doublet or triplet red cells are counted as 1 cell and large clumps of cells are excluded from the count, the changes in RBC counts and volumes affect all the CBC parameters, except the hemoglobin. The varying degrees of agglutination in this condition result in a variety of various histogram curves with the varying amounts of trailer of red cells seen on the far right side of the graph.

Sideroblastic Anemia

This is an example of a bimodal distribution with 2 separated small, distinct high points (Figure 1G). To produce a single distribution, the 2 red cell populations must differ by less than 15% in volume. It appears that the particle size distribution is very wide, from a group of very small cells—reflected as a high take-off on the baseline of the curve, to a group of normal and macrocytic cells that are equally spread on the other side. The groups themselves represent different ranges of values so the entire sets of results are actually wide-spread. In other words, it is an admixture of small cells, normal cells, and large cells in various proportions. The results of the red cell indices, however, are within normal range, and if one were to rely on indices alone, the important finding of mixed populations would be missed. This reinforces the importance of examining the blood cell histograms and blood film in tandem in order to detect the bimodal populations. When viewed microscopically, it is clear the red cell volume histogram accurately reflects the dimorphic appearances.

Reticulocytosis

The histogram is bimodal and is skewed to the right (Figure 1J). Usually, this may be the kind of picture seen in initial post-iron treatment IDA. Three to 8 days after effective iron therapy, a reticulocytosis (polychromasia) of this magnitude may occur, producing a secondary peak representing this new population of cells. Generally, reticulocytes are slightly larger than normal mature red cells. The reticulocyte count of this patient is 5.5%. According to some authors, a reticulocytosis in excess of 15% and/or a volume difference of 2 populations of less than 15% are required to affect histogram analysis. However, others have suggested it is possible to detect the appearance of a new population of cells (eg, reticulocytes) even at just more than 5% of the total red cell population, by skewing the histogram. Thus, depending on the instrument algorithm, a cohort of cells even at 5% may be interpreted as another population, hence, the dimorphic instructive comment.

These 2 histograms (Figure 1K and Figure 1L) seem to be similar in appearance: both have 2 unevenly high peak points with middle trough or valley. What is surprising in these curves is the difference in the RDW results. Despite the bimodal histogram, Figure 1K shows a normal RDW compared to Figure 1L, which has a higher RDW. The reason for the normal RDW is that only the major populations are included and measured for the MCV and RDW calculations, so the MCV and RDW are that of the main population only. The
middle trough, which is below the 20% frequency truncation, and the minor populations, as represented by the low peak, are excluded from the MCV and RDW calculations. The inclusion of minor populations will also require the inclusion of the middle trough, and thus may spuriously elevate the RDW. On the other hand, because the valley in Figure 1L has surpassed the detection level, the minor and the major populations are included in the MCV and RDW calculations; hence, the higher RDW value. Microscopically, both show a dual population of hypochromic-microcytic and normochromic-normocytic red cells.

Figure 2A to Figure 2F are representative histograms of post-iron treatment IDA from 6 patients. The red cell histogram assists in confirming the diagnosis of IDA and in following its treatment. Patients with microcytosis due to IDA usually have a unimodal frequency curve but with appropriate therapy, a second population of normocytic cells appears in the majority of cases. The readout on Figure 2G shows the initial result after 3 days of iron treatment. There is a small subpopulation of reticulocytes that merely contribute to the right skew of the graph. At this stage, the MCV starts to increase and the RDW further increases.

After 2 to 4 weeks therapy, a distinct population of new emerging normocytic cells with some polychromasias is evident on the graph and the blood film, though a secondary peak is just starting to shape up as seen in Figure 2F. Figure 2E shows 2 unequal distinct population peaks of microcytic and normocytic cells, meaning that >15% of the total cell population are normocytic cells and the remainder is microcytic cells. Five to 6 weeks later, Figure 2D shows 2 symmetric peaks representing an almost equal number of hypochromic microcytic and normochromic normocytic cells. Seven, and up to 15 weeks (105 days) forward, normocytic cells become the predominant cells, and the histogram starts to skew to the left as shown in Figure 2G and Figure 2B. After 17 weeks (120 days) of effective therapy, a population of mostly normochromic normocytic cells and a normal MCV and RDW ensue as seen in Figure 2A. Barring any complications or coexistence of vitamin B12/folate deficiency, it appears the histograms and/or MCV in different stages of post-iron treatment may vary; however, the pattern of red cell size changes from initial reticulocytosis to gradual normocytosis appear to be the same.

Conclusion

In assessing a histogram, the overall pattern is observed, and the histogram is described by its shape, center, and spread. This pattern by itself as seen in the red cell distribution curve is meaningless unless it is compared with a reference normal curve and/or confirmed microscopically. Some distributions have simple shapes, such as symmetric and skewed, but others may be more challenging, especially when dimorphic (multiple) populations of red cells are present.

Given that dimorphic and dual populations are synonymous and may cause confusion in the reporting of results, full recognition of dimorphic as more than just 2 cell populations has certain practical and clinical significance. Clinically, it is associated with many conditions as shown in Table 1. Practically, since dimorphic is usually associated with abnormal red cell populations, morphological findings should be correlated with the graphical and numerical data for better interpretation of results.

Although direct inspection of the distribution curve offers a sensitive method for detecting small populations of microcytic or macrocytic red cells, the estimation of the number of cells from the distribution curve should be avoided. Misleading results can occur because the frequency curve shows only the relative and not the actual number of cells in each size range. Even though the MCV can be easily located on any symmetric distribution curve, attempting to do so in a skewed, bimodal, or dimorphic population is discouraged because the MCV is an average value and does not reflect the heterogeneity or the presence of different cell populations.

When combined with the concept of the normal curve and the knowledge of particular CBC parameters, such as RDW and red cell indices, histograms become a practical working tool in the initial stage of morphological analysis. Observation of their outlines and the relations they suggest may be so evident that the presence of fragments, hypochromic microcytic, macrocytic, or dimorphic red cells, as well as different combinations, can be presumed. In addition, the histogram provides a concise idea not only of the different sizes of cells but also of the distribution of cells from the center (MCV) and the spread (RDW), as these 2 CBC parameters are determined from the red cell histogram. Consequently, whatever instrument methods are used to obtain the red cell histogram, electrical impedance or optical flow cytometry, the relationship of the different sizes of cells can be readily delineated and contrasted, especially when a dotted line of reference normal is superimposed on all automated abnormal red cell histogram results.

From reviewing the histograms, one can get a good idea of what to expect when actually evaluating the peripheral blood film. Unfortunately, most technologists have a limited understanding in correlating the graphic displays with the morphological findings. This is probably because graphical representation of results, such as scatter plots or histograms, have been largely ignored in favor of the RDW, hemoglobin distribution width, and reticulocyte hemoglobin content that provide very useful information along with the red cell indices that have been traditionally used. In fact, in some laboratories histograms are not being used when evaluating red cell morphology.

Moreover, there have been very few studies on the utility of red cell histograms in identifying common hematological disorders, with most studies favoring white cell histograms and their use in identifying and characterizing leukemic blast populations. Hopefully, the analysis and interpretations of histograms in this article provide good guidance in correlating histograms with microscopical findings.

The speed and reliability of the modern analyzers allow technologists time to evaluate abnormal blood films, consider diagnostic clues, and correlate clinical findings to histograms and other hematologic parameters with greater confidence and efficiency, all of which produce high returns in terms of patient health care.


