Red cell cytogram in CELL-DYN® Sapphire: a ready-to-use function for recognizing thalassemia trait

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Abstract

Single-cell optical analysis of red blood cells provides information on the cellular hemoglobin concentration and volume of red cells. We evaluated the reliability of the typical profiles of the cytogram hemoglobin concentration/volume (Mie Map), produced by the CELL-DYN® Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA), in the discrimination of iron deficiency anemia (IDA) and thalassemia trait. A total of 380 patients with microcytic anemia were studied: 220 with IDA, 101 β-thalassemia trait, 30 β-thalassemia trait with concomitant iron deficiency, 29 α-thalassemia trait. Three professionals, two experts in laboratory medicine and a trainee, reviewed the Mie maps, with no information regarding the disease of the patient. The observers made a presumptive diagnosis (genetic or acquired anemia) and the percentages of correct classifications were recorded. IDA showed broad shaped shift of the cytogram while carriers presented narrow clustering in the lower microcytic area: 100% IDA were correctly classified and 96-82% of carriers were recognized. Visual inspection of the Mie map reveals different profiles in IDA and thalassemia trait; those patterns are in concordance with the numerical data Mie map helps in the evaluation of large amounts of data.

Introduction

Microcytic anemia is commonly the consequence of iron deficiency anemia (IDA), of thalassemia trait or a combination of these. Differentiating mild IDA from thalassemia trait can be a diagnostic dilemma, as both conditions share many analytical characteristics.

The differentiation between IDA and microcytosis due to a genetic cause has important clinical implications, to avoid unnecessary potentially harmful iron therapy in thalassemia carriers and to improve the overall management of the disease, with an appropriate screening, detection of patients and counsel of couples at risk.

The final diagnosis requires additional laboratory investigations like serum ferritin concentration, hemoglobin-A2 (HbA2) assay and molecular analysis.

Hematology analyzers, based on principles of flow cytometry, can provide information on individual cell characteristics, identifying small subpopulations of erythrocytes within the total red cell population (RBC). The quantification of the percentages of microcytic and hypochromic red blood cells reported by the Advia 120 (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) has proven its clinical usefulness in the differential diagnosis of microcytic anemia. A complementary and simple approach is the visual analysis of the cytograms produced specifically by these analyzers, which show characteristic recognizable patterns in both diseases.

The CELL-DYN® Sapphire analyzer (Abbott Diagnostics, Santa Clara, CA, USA), introduces flow cytometry technology and similar RBC cytograms are reported. Since the correlation between both counters have been reported and the value of the extended RBC parameters assessed, it is reasonable to assume that this first sight detection of thalassemia carriers may be performed with the CELL-DYN® Sapphire analyzer.

Our aim was to establish the utility of RBC cytogram (Mie map) as a tool for recognizing carriers in the routine practice of a middle-size workload Laboratory.

Materials and Methods

Patients

During an 8-week period, we selected samples in the routine workload of the laboratory from all consecutive known and new patients with microcytic anemia (Hb <12.0 g/dL and mean corpuscular volume (MCV) <80 fL).

We excluded patients younger than 18 years of age, patients with inflammatory disease, and patients who had received blood transfusion in the preceding 3 months; in case of multiple samples from a single patient, only the first was used.

After the requested tests had been completed, we used the residual samples for the present study. This practice is in accordance with the guidelines established by the Institutional Ethic Committee at Galdakao-Usansolo Hospital. The diagnosis of each patient was retrieved from his or her laboratory and medical records.

Analytical methods

Venous blood samples were drawn into evacuated tubes containing K2-EDTA, kept at ambient temperature and processed within 6 hours from the time of blood collection. Extended RBC parameters were measured in reticulocytes mode of CELL-DYN® Sapphire (Abbott Diagnostics, Santa Clara, CA, USA) The CELL-DYN Sapphire analyzer was calibrated, controlled and maintained according to the manufacturer’s recommendations. Biochemical assays of iron status (serum iron, transferrin, and ferritin) were performed using standard methods on a Cobas c711 analyzer (Roche Diagnostics, Mannheim, Germany). HbA2 was assayed using high-performance liquid chromatography in a HA 8160 analyzer (Menarini Diagnostics, Firenze, Italy).

Iron deficiency was considered present when serum ferritin was <15 g/L and/or transferrin saturation <20%; β-thalassemia trait was defined as HbA2>3.5%; α-thalassemia was demonstrated by molecular techniques.

Three professionals, two experts in laboratory medicine and a trainee, reviewed the scatterplots (Mie map), with no information regarding the disease of the patient.

The observers made a presumptive diagno...
sis (genetic or acquired anemia) and the percentages of correct classification were recorded. Cohen’s test for inter-rater agreement was calculated to add a quantitative support for the experience statement.

Principle of testing: optical red blood cells analysis

Red cell analysis is done by flow cytometry and laser, applying the Mie theory of light diffraction by spherical objects. The amount of diffracted light is a function of factors such as the wavelength of the light, the size and the external/internal structure of the object. When monochromatic light like laser light is used, the diffraction becomes a function of only the size and the refractive index of the object. Since the content of RBC exists for more than 95% of hemoglobin, the refractive index of a RBC essentially represents its intracellular Hb concentration. Therefore, when the Mie theory is applied to sphere RBC, the scatter signals can be used to derive RBC volume and Hb concentration.5,7

Researchers of the then Technicon Company (later Bayer, now Siemens) developed this optical technology, brought to the market in the late 1980’s in the Technicon H1.5,7 later followed by the Advia analyzers.

In 2010, Abbott Hematology launched extended RBC parameters in the CELL-DYN® Sapphire. When CELL-DYN® Sapphire with suitable software operates in reticulocyte mode, it will combine impedance and optical technology simultaneously for measuring RBC and apart from the traditional Wintrobe indices (MCV, mean cell hemoglobin and mean cell hemoglobin concentration) and red cells distribution width (RDW), the analyzer will report a set of extended RBC parameters, erythrocyte subsets and reticulocyte derived parameters as the Hb content and volume.

When sphere, RBC scatter light homogeneously, depending on their size, internal structure and contents. Four detectors, three for scattered light and one for fluorescence, are used for extended RBC analysis.

CELL-DYN® Sapphire reports the extended RBC parameters as numerical values. In addition, two graphs can be displayed. First there is the histogram of cellular Hb concentration (CHC) from which hemoglobin distribution width is derived, in full analogy with the RDW. The most useful graph however is the scatter-plot of cellular volume vs cellular Hb concentration (V vs CHC). In this graph or cytogram called Mie map, each mature RBC is represented by a red dot whereas a reticulocyte is shown as a green dot. Since reticulocytes are usually larger and contain less Hb than mature RBC, the green reticulocyte cluster is generally located somewhat higher than and to the left of the red RBC cluster. The two vertical lines in the graph represent the CHC cut-off values for calculating hypo- and hyperchromic RBC proportions (28 and 41 g/dL, respectively). Likewise, the horizontal lines are at 60 and 120 fL, the values for defining micro- and macrocytic RBC.

Results

During the study period 380 patients with microcytic anemia were studied: 220 with IDA, 101 β-thalassemia trait, 30 β-thalassemia trait with concomitant iron deficiency, 29 α-thalassemia trait. The analytical data are summarized in Table 1.

In microcytic anemia, the Mie map graphs present characteristic patterns, depending on the underlying disease. As Figure 1 illustrates, a thalassemia carrier shows a narrow clustering in the lower microcytic area on the cytogram as the main key feature, along with low red cell indices and normal or slightly increased RDW. This pattern is more extreme in β carriers than in α carriers (Figure 2). In β thalassemia trait, the red cells though being microcytic hypochromic as in iron deficiency, show minimal anisocytosis and polychromasia. The RBC cytogram consequently shows a narrower spread with close clustering of the cell plots owing to the uniformly shaped microcytic red cells. A characteristic narrow shaped cytogram is seen in β thalassemia trait compared to that seen in iron deficiency, which has a wider triangular fan-shaped spread in the microcytic zone. Other red cell indices are similar as seen in iron deficiency except for the RDW, which is normal or near normal, reflecting the uniform microcytosis and minimal poikilocytosis.

Table 1. Results (mean and standard deviation) of red blood cells parameters in the group of patients: 220 iron deficiency anemia, 101 β-thalassemia trait, 30 β-thalassemia trait with concomitant iron deficiency and 29 α-thalassemia trait.

<table>
<thead>
<tr>
<th></th>
<th>IDA (n=220)</th>
<th>β (n=101)</th>
<th>β+IDA (n=101)</th>
<th>α (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBC (10^12/L)</strong></td>
<td>4.47 (0.6)</td>
<td>5.57 (0.6)</td>
<td>5.56 (0.6)</td>
<td>5.56 (0.6)</td>
</tr>
<tr>
<td>**Hb (g/dL)</td>
<td>10.4 (1.5)</td>
<td>12.3 (1.8)</td>
<td>11.9 (1.4)</td>
<td>12.6 (1.4)</td>
</tr>
<tr>
<td>**MCV (fL)</td>
<td>74 (6.5)</td>
<td>66.0 (4.3)</td>
<td>66.9 (5.5)</td>
<td>73.4 (3.1)</td>
</tr>
<tr>
<td>**MCH (pg)</td>
<td>223 (2.8)</td>
<td>214 (2.1)</td>
<td>215 (1.8)</td>
<td>228 (1.3)</td>
</tr>
<tr>
<td>**MCHC (g/dL)</td>
<td>31.2 (1.8)</td>
<td>32.4 (2.8)</td>
<td>32.1 (1.9)</td>
<td>31.2 (1.4)</td>
</tr>
<tr>
<td>**RDW (%)</td>
<td>17.3 (3.4)</td>
<td>14.7 (1.3)</td>
<td>15.6 (1.5)</td>
<td>14.3 (1.3)</td>
</tr>
<tr>
<td>**HDW (%)</td>
<td>6.6 (2.6)</td>
<td>5.5 (1.4)</td>
<td>5.9 (1.9)</td>
<td>5.5 (1.3)</td>
</tr>
<tr>
<td><strong>MIC%</strong></td>
<td>15.6 (12.8)</td>
<td>29.3 (13.6)</td>
<td>27.8 (15.4)</td>
<td>14.8 (6.3)</td>
</tr>
<tr>
<td><strong>HPO%</strong></td>
<td>5.2 (5.8)</td>
<td>1.6 (1.5)</td>
<td>2.8 (1.4)</td>
<td>1.7 (1.6)</td>
</tr>
</tbody>
</table>

IDA, iron deficiency anemia; RBC, red blood cells; Hb, hemoglobin; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cells distribution width; HDW, hemoglobin distribution width; MIC%, percentage of microcytic red cells (cell volume <80 fL); HPO%, percentage of hypochromic red cells (cellular Hb concentration <28 g/dL).

Table 2. Three professionals, two experts in laboratory medicine and a trainee, reviewed the cytograms, with no information regarding the disease of the patient. The diagnosis of each patient was retrieved from his or her laboratory and medical records. The observers made a presumptive diagnosis (genetic or acquired anemia) and the percentages of correct classification recorded as shown.

<table>
<thead>
<tr>
<th></th>
<th>Expert 1 n (%)</th>
<th>Expert 2 n (%)</th>
<th>No Expert n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total genetic anemia (n=160)</td>
<td>154 (96)</td>
<td>153 (95)</td>
<td>131 (82)</td>
</tr>
<tr>
<td><strong>β</strong></td>
<td>101 (100)</td>
<td>101 (100)</td>
<td>101 (100)</td>
</tr>
<tr>
<td><strong>β+IDA</strong></td>
<td>30 (100)</td>
<td>29 (98)</td>
<td>22 (75)</td>
</tr>
<tr>
<td><strong>α</strong></td>
<td>23 (80)</td>
<td>23 (80)</td>
<td>8 (25)</td>
</tr>
</tbody>
</table>

IDA, iron deficiency anemia.
clear that the typical cytograms in case of IDA and pure thalassemia carriers can be reliably recognized by laboratory professionals of different qualifications, while cases of mixed IDA and thalassemia and α carriers are more difficult to recognize at first sight, and appear to require experience in the field.

When a mixed state of iron deficiency and thalassemia coexist the typical patterns start to overlap. Red cells are microcytic and tend to be more hypochromic as they are in iron deficiency; the anisocytosis and polychromasia are reflected on the more spread signals than the narrow shape characteristic of a genetic anemia (Figure 4).

The hemograms of the α thalassemia carriers in our population tend to be less pathologic than seen in β carriers; the mild anemia is less microcytic, as shown in Table 1 (MCV and % of microcytic red cells in α and IDA groups overlap). At first sight, the relatively high position on the y-axis (volume) may look confusing in view of the microcytosis, but one should bear in mind that the scattergram only provides qualitative information in two dimensions: the quantitative component is not visible (Figure 2). Four of the 6 α carriers incorrectly classified as IDA by the experts were the same patients. All were women, and revision of their clinical histories revealed that 3 of them were puerperae. The other 2 had recovered from iron deficiency anemia that had been treated during the previous months.

Agreement between observers: expert 1/ expert 2 κ=0.878, expert 1/No expert κ=0.665, expert 2/No expert κ=0.705.

Discussion and Conclusions

As hematology analyzers have evolved over the past decades, many additional parameters have become available. Whereas the earlier instruments used electrical impedance as the
sole counting principle for blood cells, modern analyzers, in addition, use conductivity differences, cytochemical staining, light scatter, and flow cytometric principles. While enhancing the speed, accuracy, and precision of test results, this has also added new information to the standard hematology reporting.8

Some of these newer parameters, like hemoglobin distribution width, percentages of erythrocyte subsets and reticulocyte hemoglobin content provide very useful information in addition to the red cell indices that are traditionally used. Not only numerical data, but also graphical results in the form of histograms or scatterplots are now available in modern counters. Most literature on the graphical output of automated hematology analyzers is focused on the white cells, especially in identification and characterization of blasts, atypical lymphocytes and nucleated RBC populations. There have been few studies outlining the utility of red cell histograms or scatterplots in identification of common hematological conditions.3,9

Erythrocyte size and hemoglobin distribution cytograms provide very useful qualitative information, which is an adjunct to the numerical data, and sometimes even are an important clue to certain clinical conditions.13 Interpretation of histograms and scatterplots, in conjunction with the numerical data can also be clinically useful in the diagnosis and follow up of different diseases. Certain conditions like microcytic anemia of different etiologies present significantly different profiles of the RBC subsets, which can be recognized in the VCHC cytogram (Mie map). Changes in the numerical RBC indices, being a mean of many overall measurements, usually lag behind a direct representation of the whole red cell population seen on the cytogram, where the contribution of the different subsets can more readily be detected.3,11

Visual inspection of the Mie map reveals different and characteristic profiles in IDA and in genetic anemias; those patterns are in concordance with the numerical data of RBC subsets and give insights to the pathological conditions in both anemias.

In iron deficiency states, RBCs are continuously produced in the bone marrow, the iron stores progressively decrease, and they tend to be more hypochromic. Because of their long life span, several cohorts of normochromic and hypochromic RBCs coexist in the peripheral blood leading to anisocytosis. On the contrary, the underlying pathogenetic anomaly in thalassemia has no fluctuations: the bone marrow consistently produces a higher than normal amount of RBC, which are uniformly microcytic.12 Thus, the level of anisocytosis is a significant difference between IDA and thalassemia13,14 and for a certain degree of anemia, MCV tends to be lower in thalassemia than in IDA.10 These characteristics are more evident in β carriers than in α thalassemia carriers, in particular when only one α gene is affected.

The quantitative measurement of microcytic and hypochromic red cells also shows different results in patients with uncomplicated β-thalassemia and IDA: the percentages of microcytic and hypochromic cells show opposite trends in both conditions.1,2

Iron-deficient erythropoiesis is characterized by the production of RBC with a decrease in Hb content, so a high percentage of hypochromic cells is present,15 while owing to the impaired globin synthesis, microcytes of thalassemia have smaller volume16 and a high percentage of microcytic cells.

Our study shows that well trained persons can easily recognize a carrier, so the inspection of RBC cytograms constitutes a simple, fast and inexpensive tool for deciding on the most efficient additional diagnostic pathway in patients with mild or moderate microcytic anemia.

It is difficult to talk about thalassemia globally as the social situation and the Health Systems are diverse anywhere in the world. In the developing countries, where these diseases are endemic, they represent a problem of public health; but in the developed countries with the general budgetary reductions, the presumptive identification of hemoglobin disorders must rely on inexpensive methods of detection, to allow an efficient use of the resources: a good system for screening can help, allowing to efficiently select samples for further analysis to confirm the disease.

The Mie map analysis should form part of routine hematology practice because it can often significantly extend the information of a traditional hemogram.

References