Instrument-dependent interference of Howell-Jolly bodies in reticulocyte enumeration

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Letter to the Editor

Miranda van Berkel*, Eveline Besselaar, Philip Kuijper and Volkher Scharnhorst

Instrument-dependent interference of Howell-Jolly bodies in reticulocyte enumeration

Keywords: Howell-Jolly bodies; red blood cells; reticulocytes; state-of-the-art hematology analyzer.

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To the Editor,

Reticulocyte enumeration in peripheral blood is an important diagnostic procedure in the evaluation of erythropoiesis in bone marrow. Traditionally, reticulocytes are counted after staining with basic dyes such as Brilliant Cresyl Blue (BCB) or new methylene blue (NMB), which stain the RNA containing reticulofilamentous material in vitally stained unfixed preparation of cells on a glass slide [1]. In subsequent microscopic evaluation, reticulocytes can be discriminated from mature red blood cells by an intracellular deep blue precipitate. Nowadays, state-of-the-art hematology automatic counters replace the labor intensive microscopic evaluation of reticulocytes with quick and reliable enumeration of reticulocytes in whole blood samples [2]. The technique is based on specific dyes, which mostly are polynucleotide-specific, thus binding DNA as well as RNA [2]. Here, we describe a recently observed case where two out of three state-of-the-art hematology analyzers displayed a strong discrepancy with manual counting in reticulocyte enumeration due to the presence of Howell Jolly bodies.

A 38-year-old male visited the outpatient clinic of internal medicine with general symptoms of decreased well-being, suffering from fatigue, loss of concentration and paraestesia after gastric bypass surgery 8 years ago. This surgery had been complicated by stomach bleeding and was followed by splenectomy. The laboratory tests showed strong reticulocytosis (440×10^9/L; ref. value 30–120×10^9/L) with normal number of erythrocytes (4.8×10^12/L, ref. value 4.5–5.5×10^12/L) and hemoglobin (Hb) content (148 g/L, ref. value 136–177 g/L) (analyzed with a CELL-DYN Sapphire analyzer) and a vitamin B12 deficiency (136 pg/mL, ref. value 189–948 pg/mL). Differential leukocyte count showed a minimal leukocytosis (11.4×10^9/L) caused by lymphocytosis. There were no laboratory findings indicating hemolysis (bilirubin and lactate dehydrogenase within reference ranges and normal concentration of haptoglobin). The reticulocytosis in combination with normal erythrocyte- and Hb concentrations was an indication for further microscopic examination. The blood smear showed the presence of a striking number of Howell-Jolly bodies in red blood cells (8% of all erythrocytes, Figure 1). This prompted us to re-evaluate the number of reticulocytes in a subsequent blood sample drawn from this patient. As a reference method, reticulocytes were evaluated microscopically after supravital BCB staining, resulting in a percentage of reticulocytes of 1.2% of total erythrocytes. Automated counting with three systems using different methods for reticulocyte enumeration showed a strong discrepancy between the methods used (Table 1). While Beckman Coulter LH 750 automated counter produced a reticulocyte percentage resembling the BCB staining and manual counting (1.1%, absolute 49×10^9/L, reference 30–120×10^9/L) both Sysmex XE-5000 and especially CELL-DYN Sapphire counted falsely elevated reticulocytes (94×10^9/L and 377×10^9/L, respectively, reference 30–120×10^9/L).

The interference of nucleic acid containing structures present in erythrocytes in the correct determination of reticulocytes has been described for automatic hematological cell counters. For example, the presence of Pappenheimer bodies, basophilic stippling and Heinz bodies were reported to result in falsely elevated reticulocyte numbers [3]. Malaria parasites in erythrocytes, although they are usually faintly stained, caused spurious elevated reticulocyte counts using CELL-DYN 4000 automatic systems [4]. Also, the presence of Howell-Jolly bodies interferes with the automated reticulocyte...
Counting using Coulter Epics Profile II flow cytometer, resulting in falsely elevated levels [5]. In our evaluation, counting with Beckman Coulter LH 750 generated reticulocyte numbers comparable to microscopic examination after supravital BCB staining, while CELL-DYN Sapphire and – to a lesser extent – Sysmex XE-5000 counted falsely elevated numbers of reticulocytes. This discrepancy might be explained by the analyzer-specific staining, detection and gating strategies used. Beckman Coulter LH 750 stains blood cells with new methylene blue, a colorimetric dye comparable to BCB, and discriminates reticulocytes from other cells by volume, conductivity, and laser light scatter; the latter signal is proportional to the residual RNA within the red cell. This staining is specific for RNA remnants in reticulocytes [2]. In contrast, Sysmex XE-5000 and CELL-DYN Sapphire instruments use fluorescent dyes, staining polynucleotides within cells. CELL-DYN Sapphire combines a cyanine dye (Sybr II) and narrow angle light scattering to separate reticulocytes from platelets, RBC and nucleated cells [2, 6]. Sysmex XE-5000 uses similar techniques, with polymethylene dye, and forward light scattering differentiating platelets and RBC/reticulocytes based on cell volume. The variation between these two analyzers in reticulocyte concentration possibly results from a combination of different dyes with different subgating strategies.

Overall, the use of polynucleotide-specific fluorescent dye in state-of-the-art hematological analyzers such as Sysmex XE-5000 and CELL-DYN Sapphire can cause pseudoreticulocytosis in the presence of Howell-Jolly bodies or other nucleic acid remnants. This is not observed using an RNA-specific dye in Beckman Coulter LH750 instruments. Therefore, the reticulocyte numbers obtained with automatic hematological counters can be spuriously elevated and need to be interpreted with caution in the presence of nucleic acid containing inclusions. This case underlines that improvement of some automatic hematology analyzers with respect to the detection of reticulocytes is necessary and achievable, thereby contributing to a better standardization of automated reticulocyte enumeration.

Conflict of interest statement

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Table 1 Reticulocyte numbers evaluated using different techniques.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Abbott CELL-DYN Sapphire</th>
<th>Sysmex XE-5000</th>
<th>Beckman Coulter LH 750</th>
<th>BCB staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulocytes, ×10^9/L</td>
<td>377</td>
<td>94</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes, %</td>
<td>7.9</td>
<td>2.0</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>RBC, ×10^12/L</td>
<td>4.7</td>
<td>4.6</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Reticulocyte numbers evaluated using different techniques. Blood was collected into K3 EDTA containing tubes (Vacutainer ref. 3688610, Becton Dickinson BV, Breda, The Netherlands) and blood counts were measured within 6 h on a Beckman Coulter LH 750, a Sysmex XE-5000 and a CELL-DYN Sapphire (Abbott Diagnostics) automatic hematology analyzer. A blood film was prepared and stained with either May-Grünwald-Giemsa or Brilliant Cresyl Blue (BCB) staining. For the automatic counter, reticulocytes are expressed as a percentage of total erythrocytes as measured by the particular analyzer. After supravital BCB staining 1000 reticulocytes were manually counted (1000 erythrocytes were evaluated).

References


2. Piva E, Brugnara C, Chiandetti L, Plebani M. Automated reticulocyte counting: state of the art and clinical applications

Figure 1 Howell-Jolly bodies included in erythrocytes. A blood film was stained with May-Grünwald-Giemsa (original magnification 630×). Howell-Jolly bodies are indicated with arrows.