Errors in automated reticulocyte counts due to Heinz bodies

R F Hinchliffe

Abstract
Serial reticulocyte counts on a patient with an unstable haemoglobin haemolytic anaemia whose red cells contained Heinz bodies were obtained by two automated methods. One false low and several false high counts were obtained with standard threshold settings; each was accompanied by activation of the appropriate alarm. With the FACScan a bimodal red cell distribution was found, corresponding to the proportions of Heinz body positive and negative cells. This case illustrates the fact that Heinz bodies can interfere with automated reticulocyte counting methods, and to a degree that could be clinically important.

Automated reticulocyte counting methods represent an improvement over the manual method in terms of speed and precision, and are capable of giving clinically useful data in the low and near-normal ranges of values. One problem, however, is false positive staining of Howell-Jolly bodies, but Heinz bodies are not recognised as causing difficulty.

The opportunity recently arose to perform serial automated reticulocyte counts on samples from a patient many of whose red cells contained Heinz bodies after his spleen was removed.

Case report
A 12 year old boy was found to be anaemic at the age of 6 months. He had an unstable haemoglobin haemolytic anaemia, identified as Hb Bucaresti ($\beta^42$ (CD1) Phe-Leu). As a result of increasing splenomegaly and a falling platelet count his spleen was removed in October 1992.

Manual reticulocyte and Heinz body counts were obtained by standard methods.
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Reticulocyte and Heinz body counts before and after spleen removal

<table>
<thead>
<tr>
<th>Reticulocyte x 10^11 (%)</th>
<th>Preoperative period</th>
<th>Postoperative day 3</th>
<th>Postoperative day 4</th>
<th>Postoperative day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>265 (7.5)</td>
<td>18 (0.45)</td>
<td>64 (1.6)</td>
<td>69 (1.6)</td>
<td></td>
</tr>
<tr>
<td>FACScan</td>
<td>293 (8.3)</td>
<td>49 (1.2)</td>
<td>0 (0)*</td>
<td>17 (0.4)</td>
</tr>
<tr>
<td>(a) reticount software</td>
<td>ND</td>
<td>183 (4.5)</td>
<td>112 (2.8)</td>
<td>80 (1.9)</td>
</tr>
<tr>
<td>(b) consort 30 software</td>
<td>ND</td>
<td>635 (15.7)†</td>
<td>77 (18-9)†</td>
<td>1170 (27-0)†</td>
</tr>
<tr>
<td>R-1000</td>
<td>ND</td>
<td>63</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Heinz bodies (% positive red blood cells)</td>
<td>0</td>
<td>60</td>
<td>60</td>
<td>44</td>
</tr>
</tbody>
</table>

*error alarm given; †staining error alarm given; ND Not Done

Automated reticulocyte counts were from a FACScan (Becton Dickinson) and an R-1000 (Sysmex) using the RNA-binding fluorochromes thiazole orange and auramine-0, respectively.

Results
The preoperative reticulocytosis had disappeared by the third postoperative day and Heinz bodies had appeared in many red cells. The preoperative reticulocytosis was detected by the FACScan (fig 1A); postoperatively two populations of red cells were detected on all samples (fig 1B), including controls run without fluorochrome. A zero count, with an error alarm, was persistently given on one sample. Higher counts were obtained using Consort 30 software, which enables the operator to adjust the thresholds delineating the red cell population(s). Studies with the R-1000 gave much higher reticulocyte counts, with the red cell cloud stretching across the counting threshold (fig 1C). The staining error alarm was activated with each sample. Results are summarised in the table. Only very occasional Howell-Jolly bodies were seen in blood films over the postoperative period.

Discussion
Using the R-1000, Tatsumi et al? found no effect of in vitro generated Heinz bodies on the reticulocyte count. The opposite finding in this study may have been due to the frequently large and multiple nature of the inclusions in our patient, similar to those found in other patients with unstable haemoglobin disorders who had had their spleens removed.4 That his Heinz bodies had increased intrinsic autofluorescence over and above the constituents of normal red cells is supported by a second red cell population, to the right of the original, appearing after spleen removal and in the absence of added fluorochrome. This peak remained stable in the presence of thiazole orange, indicating that the dye was not binding to Heinz bodies. The roughly equal size of the populations correlates closely with the proportions of Heinz body positive and negative red cells.

These findings show that automated reticulocyte counters can give spurious results in the presence of Heinz bodies, and this could occasionally be clinically important. Errors may be correctable by operator adjustment of counting thresholds; alternatively, manual counts may be necessary.

Automated reticulocyte counts were performed by Mrs J Peel and Mr R Kendall of the Departments of Haematology the Royal Hallamshire Hospital, Sheffield, and the Leeds General Infirmary, respectively.

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