Diurnal Change of Blood Count Analytes in Normal Subjects

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Short-term, within one 24-hour day (diurnal period) within-person changes of the principal blood count analytes in healthy subjects were studied at three major institutions. The results from each test site were indistinguishable and were therefore combined to make a database of 96 healthy subjects. Analytical imprecision of each analyte was subtracted from the total observed variation to give true diurnal change. Each analyte showed characteristic changes. As would be expected, cellular properties of erythrocytes, such as MCV (mean cell volume) and MCH (mean cell hemoglobin) showed negligible change. The red cell count, hematocrit, and hemoglobin showed changes that were consistent with fluid balance change. Total white cell count and some differential count components showed major changes that raised questions of the confidence limits of clinical decision levels and the validity of commonly used reference intervals. Platelet count changes were typically less than analytic imprecision, suggesting the need for improvement in this aspect of analyzer performance. (Key words: Within-person variation; Diurnal changes; Blood count; Analytical imprecision; Confidence limits) Am J Clin Pathol 1996; 106:723-727.

Plus ça change, plus c’est la même chose.
—French proverb

The National Committee for Clinical Laboratory Standards has proposed in document H26-A a goal for automated blood cell analyzers by which analytical imprecision should not exceed 25% of the within-person variation of the analyte. As reported by Cotlove, Harris and Williams in 1970, and since used as a benchmark by many laboratories, it is not well validated for many analytes. The goal of the present study is to re-examine this empirical standard for analytic precision using automated blood cell analyzers. This study shows that it is appropriate for some blood count analytes and overgenerous for others.

A description of short-term changes in the complete blood count (CBC) in five subjects was included in a definitive paper by Statland and colleagues. Short-term changes in the leukocyte differential count in 21 subjects, measured by 2 different types of automated counters, were analyzed by Winkel and associates. Other work on the variation of within-person blood count analytes has addressed long-term change extending over months or years. Notable contributors are Ross and colleagues and Dot and coworkers. Fraser and associates confirmed the individuality and limited heterogeneity of within-person patterns of blood count analytes in the elderly and cautioned against using only reference limits to set boundaries of normality. Shipkov and Ruseva did not find significant discrepancies among the results of weekly measurements over a period of 3 months in 14 subjects. Rocker and colleagues observed minor seasonal variation in a group of 78 subjects and cautioned against this effect on the measurement of reference intervals.

Costongs and associates, in a comprehensive study of short-term (16 cases) and long-term (274 cases) concluded that within-day, day-to-day and month-to-month blood count variations were remarkably similar.

The possibility of blood count diurnal variation being circadian (ie, cyclical or nonrandom short-term variation), was studied by Swoyer and colleagues in 23 elderly subjects. These variations resembled those seen in a group of 150 young adults. In an earlier communication Haus and coworkers, the same authors stressed the clinical importance of circadian rhythms in relation to cell proliferation.

These cited studies used analyzers from major manufacturers, namely Coulter, Sysmex, Technicon and Ortho. The similarity of the data from this diverse set of independent studies suggests that the short- and long-term biological variation of blood count analytes, be it rhythmical or random, is independent of analytical variation.

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Long-term studies do not provide data that focus on the relation between physiologic change and instrument imprecision. Factors such as instrument drift, recalibration, preventive maintenance, laboratory personnel changes and the like inevitably intrude in the long term. For testing conformance to the proposed goal, we believe that the variables should be limited to both short-term analytical imprecision and short-term (diurnal) within-person physiologic imprecision. This reduces the experimental variables to manageable dimensions, allows the specifications of the study to be exactly defined and helps others to repeat the work more expeditiously. Furthermore, short term studies address the not uncommon problem of clinical interpretation of changes seen in the values of closely repeated specimens.

**MATERIALS AND METHODS**

Ninety-six outwardly healthy volunteer subjects were assayed for routine blood count analytes at three institutions as shown in Table 1. Subject gender was equally divided. The subjects were instructed to not alter their normal daily pattern of work, exercise, and relaxation. However, they were asked to avoid fatty foods that might influence hemoglobin assay results by causing lipemia-induced turbidity. A venous blood specimen was collected from each subject at approximately 9 AM on each of two successive days into an evacuated tube containing K3EDTA (1.5 mg/mL). Prior to assay, the specimens were stored at room temperature (18–23 °C). Specimens were assayed between 30 minutes and 3 hours after phlebotomy, using the hematology analyzers, calibration and quality control procedures routinely used in the investigators' laboratories.

Each specimen \((x_i)\) was assayed in duplicate at VAMC and UMHC, and singly at SFGH. The purpose of duplicate assays was to obtain a real-time estimate of analytical imprecision \((S_{ana})\) by calculating the standard deviation of the differences between the results of pairs of assays for each set of assays using the following conventional equation

\[
s = \sqrt{\frac{\sum (x_i - x_a)^2}{n - 1}}
\]

where \(n\) is the number of pairs contributing the string of differences for VAMC and UMHC.

Analytical imprecision was measured off-line at SFGH by simple replication with \(n = 31\). Inter-institutional comparison of data is nevertheless permissible since replicate and pair difference estimates of imprecision yielded similar results.

Diurnal variation \((S_{diurn})\) was defined as the standard deviation of the differences between assays performed on Day 1 and Day 2, after subtraction of the analytical imprecision \((S_{ana})\), by the formula:

\[
S_{diurn}^2 = \sqrt{(S_{total}^2 - S_{ana}^2)}
\]

To simplify comparison of the diurnal variation of different analytes and to harmonize the dispersion of data among institutions, standard deviations were converted to coefficients of variation (CV) by dividing \(S_{diurn}\) for each analyte by the mean value \((X_a)\) of the of the analyte in that institution.

\[
CV = \left(\frac{S_{diurn}}{X_a}\right) \times 100
\]

**RESULTS**

The columns in Table 2 headed Min and Max describe the limits of the dispersion of values around the mean difference of assay results on Day 1 and Day 2. Some of these differences show isolated limiting values lying beyond the second standard deviation and should not be taken to indicate bias.

The column headed Mean is an indicator of whether values systematically increased or decreased during 24 hours. Inspection of the data in this column indicates that polarity of change is symmetrically distributed and apparently random in character. There was no influence of gender on the magnitude or frequency of diurnal change.

Circadian rhythm was thought to be an unlikely contributor to the re-
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<table>
<thead>
<tr>
<th>Analyte</th>
<th>Units</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Total</th>
<th>Anal</th>
<th>Diurnal</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs</td>
<td>(×10⁷/L)</td>
<td>-3.19</td>
<td>1.9</td>
<td>-0.19</td>
<td>0.98</td>
<td>0.12</td>
<td>0.97</td>
</tr>
<tr>
<td>RBCs</td>
<td>(×10⁶/L)</td>
<td>-0.57</td>
<td>0.58</td>
<td>0</td>
<td>0.18</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>Hgb</td>
<td>(g/dL)</td>
<td>-0.9</td>
<td>1.43</td>
<td>0.02</td>
<td>0.44</td>
<td>0.13</td>
<td>0.42</td>
</tr>
<tr>
<td>Hct</td>
<td>(%)</td>
<td>-4.85</td>
<td>4.84</td>
<td>0.07</td>
<td>1.68</td>
<td>0.62</td>
<td>1.56</td>
</tr>
<tr>
<td>MCH</td>
<td>(pg)</td>
<td>-1.13</td>
<td>1.87</td>
<td>0.01</td>
<td>0.43</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>(fL)</td>
<td>-2.5</td>
<td>2.9</td>
<td>0.1</td>
<td>0.91</td>
<td>0.81</td>
<td>0.42</td>
</tr>
<tr>
<td>RDW</td>
<td>(%)</td>
<td>-1.6</td>
<td>2.1</td>
<td>0.02</td>
<td>0.44</td>
<td>0.24</td>
<td>0.37</td>
</tr>
<tr>
<td>Plt</td>
<td>(×10⁹/L)</td>
<td>-49.58</td>
<td>35</td>
<td>3.57</td>
<td>15.18</td>
<td>7.15</td>
<td>13.39</td>
</tr>
<tr>
<td>MPV</td>
<td>(fL)</td>
<td>-1</td>
<td>0.8</td>
<td>-0.04</td>
<td>0.36</td>
<td>0.15</td>
<td>0.33</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>(×10⁹/L)</td>
<td>-0.85</td>
<td>0.91</td>
<td>-0.05</td>
<td>0.29</td>
<td>0.07</td>
<td>0.28</td>
</tr>
<tr>
<td>Monocytes</td>
<td>(×10⁹/L)</td>
<td>-0.27</td>
<td>0.22</td>
<td>0.01</td>
<td>0.09</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>(×10⁹/L)</td>
<td>-4.15</td>
<td>1.67</td>
<td>-0.13</td>
<td>0.89</td>
<td>0.09</td>
<td>0.89</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>(×10⁹/L)</td>
<td>-0.14</td>
<td>0.14</td>
<td>0</td>
<td>0.05</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Basophils</td>
<td>(×10⁹/L)</td>
<td>-0.14</td>
<td>0.04</td>
<td>-0.01</td>
<td>0.03</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

WBCs = white blood cells; RBCs = red blood cells; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; RDW = red cell distribution width; MPV = mean platelet volume.

Both Tables 2 and 3 express the differential count values in absolute units. Proportional or percentage values are eschewed for reasons discussed by Richardson Jones and colleagues. The International Council for Standardization in Hematology has made a formal recommendation that, for both clinical and publication purposes the differential leukocyte count should always be expressed as the absolute number of each cell type per unit volume of blood. For similar reasons, reticulocyte...
DISCUSSION

Causation of diurnal change is reviewed by Win trovbe. We have made no attempt to enlarge upon that aspect of this phenomenon. We have focused on the interpretative implications rather than causes. It is reasonable to suppose that diurnal changes of blood count analytes have several biologic components. One of these is hematopoietic activity. Others are hemolysis or bleeding. Other factors involve alteration of the subject’s hydration status or transient sequestration of formed elements.

In the absence of active bleeding or transfusion of blood, a change of red cell count, hemoglobin or hematocrit might be presumptive evidence of fluid balance change as in the classical observation of Hedin. In a similar vein, the small changes seen in MCV raise the possibility of their relationship to changes in plasma osmolarity. In our limited database, it was not possible conclusively to associate the degree of diurnal change with gender.

Another question on which within-person variation has a bearing is its effect upon reference intervals. Depending on the time of day the specimen was collected, an individual whose analyte was normally close to either end of the reference range, might extend or diminish the range as a result of this type of change. Although Cotlove and colleagues raise this issue, it has not been incorporated in procedural instructions for reference interval measurement.

The CVs of RBC, HGB, and HCT are similar, in the range of 3% to 3.7%. The physiologic processes that drive normal short-term changes of red cell concentration would necessarily have a simultaneous effect on hemoglobin concentration and hematocrit. The resident cellular properties of the erythrocyte, MCV, MCH, and MCHC show changes that are too small to be measured by a single assay. Alteration of MCH would require short-term production of a large cohort of cells having a major change of hemoglobinization. In normal subjects, this would be an unlikely event.

Reticulocytes (RET) show diurnal CV of 20.0%. This large change might appear to be at variance with the small changes of erythrocyte intracellular properties. It is not inconsistent with the low frequency of this cell type and its short intravascular life span.

The change in platelet count is slightly greater than the erythrocyte count change. This suggests that there may be factors such as variation of rates of proliferation that are additional to fluid balance.

The CVs of the total leukocyte count (WBC) and the major components of the leukocyte differential count show diurnal changes as much as five times greater than those seen in red cells. This relatively high rate of diurnal variation may be associated with the high rate of turnover of this class of cells.

Our results indicate that clinical action limits for the absolute leukocyte differential count can be strongly affected by diurnal variation. The CVs in Table 3 are shown in conventional terms. In other words, they are based on one standard deviation. A better appreciation of the impact of this variable is obtained by multiplying the CVs by two to give an approximation of 95% confidence limits. Expressed in this manner, one may expect up to ±44% diurnal change in the absolute neutrophil count due to normal diurnal variation. Whether, in a patient, a neutrophil count change of this magnitude indicates a response to antibiotic therapy or whether it is the result of inherent within-person variation may thus not be readily decided. Some relaxation of the tolerances of clinical action limits would appear to be in order.

The original purpose of this investigation was to test the proposition that analytical imprecision should be one quarter the within-person variation according to the equation

\[
\frac{S_{\text{anal}}}{S_{\text{diurn}}} < 0.25
\]

The degree to which this goal is realized in practice is shown in the final column of Table 3 which displays the ratios between diurnal variation and analytical variation. To satisfy the goal that analytical imprecision should be equal to or less than one quarter of the within-person imprecision, values in the final column should not exceed 0.25. It is apparent that this is achieved only for WBC and the differential components, NEU and LYM. The reason for this lies in the large diurnal variation of these analytes rather than the high precision with which they are measured.

The simplistic "25%" rule may not be appropriate for all blood count analytes. The value of the ratio for WBC and associated differential components should not be interpreted as inadvertent overkill by the designers of automated blood cell counters but as an opportunity to attach clinical significance to closely spaced assays. The 24-hour rate-of-change of NEU translates into analytically significant change being measurable in 12 hours.

The small changes in RBC cellular parameters simply reflect their normal physiologic rates of change. Assays that seek to detect change of MCV, MCH, and MCHC will be unlikely to yield useful information when spaced closer than 7 days unless there is a high rate of compensatory erythropoiesis for lysis or blood loss.
The most critical analyte is PLT. In almost all cases presented for primary diagnosis or for monitoring, it is unlikely that waiting until the rate of change extrapolated from these data might lead to a useful result, particularly when the count is abnormally low. There is a real need for improvement in analytical precision of PLT counting.

The results of this and other cited studies serve to emphasize the futility of testing an additional specimen solely for the purpose of verifying a blood count result. Results from repeat specimens taken for the reasonable purpose of monitoring change should be interpreted in the light of change possibly being due to diurnal variation rather than clinical response or laboratory error.

Summary

Physiologic, short-term, or diurnal changes in blood count analytes require consideration when evaluating the results of the complete blood count. Changes in RBC in healthy subjects are greater than would be expected from normal erythropoiesis or erythrolysis and may reflect changes in fluid balance. Changes in WBC exceed RBC changes by a wide margin, leading to the speculation that they are caused by normal proliferative activity linked to the brief life of these cells. Therefore, the possibility of diurnal change should be taken into account when monitoring WBC changes in patients. The rule of thumb, which is valuable in clinical chemistry, that analytical imprecision should not exceed 25% of the within person imprecision does not find a comfortable home in CBC analysis. The range of analyzers tested at three different medical centers are in substantial agreement on this point. The analyte for which the 25% goal might have real value is the platelet count (PLT). It is recommended that manufacturers strive for improvement in the precision and specificity of this measurement.

REFERENCES