Automated Differential Leukocyte Counts

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ABSTRACT

Automated differential counts have the advantage of precision, efficiency, safety, and economy. They could potentially serve effectively in 90 percent of patients with normal counts or in 75 percent of patients with anemia only (64 percent of the total in this study). Even patients with increased white blood cell counts and major population shifts (toward granulocytes or lymphocytes) could be followed with automated differential counts. Such a tactic would decrease turnaround time for results, be less expensive, and reduce exposure of technologists to direct contact with patients' blood. However, presently available instruments fail to detect patients' blood samples with small numbers of abnormal cells, e.g., blasts in early relapse of acute leukemia, atypical lymphocytes in viral diseases such as infectious mononucleosis, eosinophils in allergic or parasitic disease, and band forms in early infectious diseases. Clinical judgment should be used in selectively ordering manual differential counts for these patients.

While automated differential counts can be very useful in screening general medical and surgical patients in the ambulatory setting, in referral centers where hematologic abnormalities are more prevalent, the manual differential count and further examination of a smear is particularly necessary at least on initial presentation. Selective manual differential counts may improve efficiency, economy, and safety while not compromising patient care. Further studies of the correlation of clinical disease with automated differential counts are necessary.

Introduction

Automated differential white blood cell counts have become of great interest recently for improvement in blood cell screening capability, efficiency, economy, and safety. Two fundamental techniques are available: (1) electronic impedance counting in which the size of the cell or cell nucleus is the major differentiating characteristic for three cell types and (2) flow cytochemistry in which internal granular characteristics and stain patterns are detected to differentiate six cell types. Digital image processing, which depends upon cell size and nuclear shape characteristics similar to the manual differential count, is no longer readily available.
Use of automated white blood cell differential counters is not without controversy, which ranges from, "In terms of accuracy, none of these (automated differential instruments) can compete with a trained morphologist" to "The assumption that... visual examination remains the 'gold standard' by which alternative techniques must be judged, ... is open to question, and in hematology, as in economics, the days of the gold standard may be numbered." Each of these technologies has advantages and disadvantages which will be reviewed in this paper.

Automated electronic counting is more precise than manual counting and has been adopted almost universally. The only rival at present is counting and characterizing cells by light scattering and absorption. Both these techniques are adaptable to rapid screening of multiple blood samples economically and safely. Both have been modified for closed tube sampling to prevent direct exposure of technologists to liquid blood. This is an important advantage in the face of a growing AIDS epidemic. Digital image processing, like manual differential counting, still requires open tube sampling and preparation of a monolayer blood film for staining. The instruments count only 100 to 200 cells like the manual procedure. Two of the largest and most popular of the image analyzers are no longer being manufactured because the market for this type of specialized differential counter is limited and image analyzers are not adaptable to whole blood cell counting.

Electronic Impedance Counting

Impedance electronic counters determine both the number and the size of cells by volume displacement of electrolyte solution. The small voltage drop accompanying this displacement is amplified many fold. The voltage change denotes a particle in the aperture. The amplitude of the impulse generated indicates the size of the particle. As the technical capabilities have improved, the apertures have become smaller and more exact and the electronic modifications have allowed more channels for pulse discrimination. In the white cell portion of the counter, nuclei of lymphocytes, monocytes, and granulocytes are clearly distinguishable. This three-part differential counting technique could be very useful to screen large numbers of normal preoperative patients which would otherwise waste much technologist time. The flow system counts 10,000 cells which provides considerable precision, but apparently does not, as originally expected, increase the detection of small numbers of abnormal cells. Such cells are often hidden in the overlapping of the major populations of cells present.

Correlation of major populations of cells counted by both automated and manual techniques is very good even in abnormal ranges, and shifts toward lymphocyte or granulocyte populations are easily and accurately detected. Electronic "flags" are built into the computer system to alert the operator to pattern changes. The instrument fails to detect small increases of blasts (large unstained cells), atypical lymphocytes, band forms, and eosinophils that can be found by manual differential count of a stained blood smear.

Flow Cytochemistry Technique

The flow cytochemistry technique depends upon light scattering and light absorption. One automated analyzer provides a complete blood cell count, a six-part differential with absolute counts, and a left-shift flag. In the white cell channel, the red blood cells are lysed and white blood cells are stabilized and stained for peroxidase activity. Cells are characterized by a combination of their
size (light scatter) and peroxidase activity (light absorbance) as they flow singly, past two detectors.

The cell populations are displayed on a cathode ray terminal screen with the vertical axis demonstrating cell size and the horizontal axis corresponding to intensity of peroxidase staining. Lymphocytes and large unstained cells (blasts), show no horizontal displacement on the cathode ray terminal screen and are measured by size only along the left vertical axis. Monocytes have weak peroxidase staining activity, are moderately large cells, and appear in the middle of the screen. Neutrophils are larger and show more intense peroxidase activity and appear on the upper right mid-portion of the screen (figure 1). Eosinophils are smaller than monocytes and neutrophils but show the most intense peroxidase activity and appear on the lower far right of the screen. Basophils are detected as large cells in a separate channel after other leukocytes have been stripped of their cytoplasm (figure 2). The index of the nuclear lobes remaining is used to determine a left-shift in the neutrophil series.

Analysis of these parameters has shown a good correlation with manual and with image analyzer differential counts, but the instrument has the advantage of counting many more cells and having greater precision. Banez and colleagues showed that the flagging of a left-shift based on lobularity index correlated well with actual band counts by automated image analysis (76 percent sensitivity). However, still greater sensitivity was found by simply using absolute leukocytosis (91 percent in biopsy proven cases of appendicitis). Given the documented diagnosis of appendicitis in 33 cases, 30 patients had leukocytosis, 26 patients had left-shift flag, and 23 patients had left-shift by image analyzer. This kind of clinical correlation study needs greater emphasis.

Review of Patient Data

In our own laboratory, over 600 patient samples have recently been reviewed to determine whether or not the automated three-part differential count could be useful in a general university hospital with a substantial number of patients with hematologic conditions in both adult and pediatric services.
Two observations were made. First, given the choice, physicians ordered three-part differential screens for only 10 percent (58/606) of patients and relied on manual differential counts for 90 percent of patient samples (548/606). In spite of education sessions extolling the potential efficiency and the lower cost of the three-part screen, for almost two years physicians continued to order manual differential counts about 90 percent of the time. Following a crisis in the technologist labor market when there were insufficient numbers of technologists to perform all the differential counts ordered, manual differential counts were performed only during the week unless the physician called the laboratory with a specific request. Three-part screen differentials increased from 11 percent (329/2762) to 35 percent (1017/2983) and remained at that level even after the labor situation stabilized (1060/2958).

Second, repeated manual differential counts were rarely helpful in providing any new diagnostic information about the patients involved. The following definition of normal manual differential\(^7\) (table I) was adopted by us. The proportions of patient samples were determined with automated counts that were normal, abnormal with anemia only, abnormal with thrombocytopenia only, and abnormal with leukocytosis or leukopenia, among 606 samples with which both an automated three-part differential screen and a manual differential count had been performed.

Forty percent (248/606) of the samples showed a normal automated count including three-part differential. Twenty-three percent (140/606) showed anemia only. Only one patient had thrombocytopenia alone, and the remaining 36 percent (217/606) had various abnormalities involving leukocytes, sometimes accompanied by other abnormalities.

Manual differential count showed abnormalities in 10 percent (28/248) of the samples with normal automated counts and in 23 percent (32/140) of the samples with anemia only. Examination of patients’ records showed that the specific abnormalities were usually nondiagnostic. The most frequent abnormality was an increase in proportion of polys and bands over 80 percent in some samples with a normal white count (table II). Anemic patients sometimes showed further shift to the left with rare metamyelocytes and myelocytes.

These findings suggest that 90 percent of patient samples with normal automated counts and 77 percent of patient samples showing anemia only will reveal no abnormalities on the manual differential and that many of the remaining samples will have abnormalities which are not diagnostically helpful. Only two of 606 specimens showed significant abnormalities (bands > 20 percent) with normal white counts. Both were elderly females with clinically obvious disease (sacral ulcer, peritonitis).

Subsequent to the labor related shift towards automated differential screens, a process of monitoring five smears a day from such samples was instituted and confirmed that less than 10 percent (20/218) showed any abnormality on smear. The abnormalities found were minor increases in bands (10/20), eosinophils (1/20), atypical lymphs (7/20), or toxic granulation (2/20), some of which had not been detected by previous manual differential counts.

<table>
<thead>
<tr>
<th>Limits of Various Blood Cell Counts Considered as Normal on Manual Differential Cell Counts from Blood Smears</th>
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<tbody>
<tr>
<td>Normal, No Blasts, Metamyelocytes, or Myelocytes</td>
</tr>
<tr>
<td>&lt; 11 percent Band forms</td>
</tr>
<tr>
<td>&lt; 15 percent Monocytes</td>
</tr>
<tr>
<td>&lt; 10 percent Atypical lymphocytes</td>
</tr>
<tr>
<td>&lt; 10 percent Eosinophils</td>
</tr>
<tr>
<td>White Blood Cells &gt; 4500, &lt; 11,000</td>
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</tbody>
</table>

### TABLE I

**Normal, No Blasts, Metamyelocytes, or Myelocytes**

- < 11 percent Band forms
- < 15 percent Monocytes
- < 10 percent Atypical lymphocytes
- < 10 percent Eosinophils
- White Blood Cells > 4500, < 11,000
TABLE II
Abnormalities Found on Manual Differential Counts from Smears Made from Blood Samples

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Normal*</th>
<th>Anemia Only†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band forms &gt; 11</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>Monocytes &gt; 15</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>Atypical lymphocytes &gt; 10</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Eosinophils &gt; 10</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>Polymorphonuclear neutrophils &amp; band forms &gt; 80</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Metastatic &amp; myelocytes &gt; 0</td>
<td>17</td>
<td>10</td>
</tr>
</tbody>
</table>

* Patients (28/248) who had normal automated differential cell counts
† Patients (32/140) who had anemia only

Discussion

Elaborate schemes have been proposed to define when a manual differential count is warranted after review of automated data. Miers et al demonstrated while evaluating the three-part differential count as a screening tool in a tertiary care hospital that there was a good correlation for granulocytes and lymphocytes between manual and automated differentials, but that small increases in blast cells, monocytes, band forms, and eosinophils were not detected in 15/40 samples, 65/113 samples, 425/548 samples and 84/112 samples, respectively. The authors developed an algorithm for determining when a manual differential was required after review of the automated data from the Coulter S Plus IV.

McClure et al, on the other hand, used a microcomputer analysis to triage blood specimens for manual differential called the “Diff If” strategy. In the first strategy, blood sample for all subjects was examined microscopically, then only subsequent specimens that changed by a stipulated degree would be examined manually. The second strategy was that a manual differential count would be performed only if the automated blood count was abnormal to a stipulated degree. This program was more sensitive than the guidelines using the numeric criteria alone. Specificity depended upon the patient population. McClure and co-workers concluded that the choice of triage strategy should be based upon the individual laboratory’s patient population.

Conclusion

It appears that automated differential screening can be useful in a high proportion of patients being tested preoperatively or who have medical conditions unlikely to affect the blood. Abnormalities of cell counts or differential distribution should trigger a manual differential count. Automated follow-up differential counts will usually be adequate for patients having a major shift toward the granulocyte series or toward the lymphocyte series.

Patients having suspected hematologic disease, e.g., acute or chronic leukemia, infectious mononucleosis, myeloproliferative states, or parasitic disease, should have at least one smear examined and a manual differential count performed. Patients with small numbers of abnormal cells (blasts) must be followed with manual differential counts.

Further studies are needed to determine the significance of either automated or manual differential counts in relation to the diagnosis of and the monitoring of nonhematologic disease. Consideration of technologist safety and availability may also play a role in the decision to do a manual differential count in the near future.

References

1. BANEZ, E. I., and BACALING, J. H. D.: An evaluation of the Technicon H-1 automated hematology analyzer in detecting peripheral blood


