The CBC at the Turn of the Millennium: An Overview

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This review offers a birds-eye view of the state of automated hematology at the turn of the millennium. Despite its shortcomings (mainly flaggings and labor-intensive demands for confirmation), instrument-driven hematology provides much accurate and precise data to clinicians. Advances in technology over the next few decades will undoubtedly improve on the categorization of currently ambiguous mononuclear cells and even introduce channels for the detection and subclassification of poikilocytes. Until then, familiarity with the morphology of blood cell variants will be mandatory for technologists attending to the demands of flagging.

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Until the early 1960s, hematologic evaluations were performed manually. Methods were labor-intensive and involved centrifuges, spectrophotometers, counting chambers with etched grids, and stained wedge smears of blood. From these were generated classic red cell indices, white cell counts/differentials, and platelet counts along with assorted morphologic comments often suggesting specific diagnoses.

Since the advent of electronic cell counters, which can generate all of these test results in seconds, labor requirements have been substantially reduced. The presence of most abnormal white cells can be detected and indicated by a series of instrument-specific flags. Virtually all cases of acute leukemia can be detected by the Coulter STKS analyzer via a combination of electronic cell counting, flagging, and review of sentinel blood smears (1). However, relevant comments on red cell morphology cannot be generated by automated technology at the present time. This review will address the status of these and other issues in technology at the turn of the millennium.

Instrument Technology

Most instruments generate two types of data: graphic displays with or without flags for internal laboratory review, and a series of numbers for export to clinicians. Graphic displays take two basic forms: histograms in which relative numbers of red cells, white cells, and platelets are plotted against cell size, and scatter-plots in which white cell subpopulations are displayed. Both forms are generated by most instruments. National guidelines for evaluation of instrument performance, particularly in leukocyte differential counting, have long been established (2).

Technologies involved in earlier instruments still in use have recently been summarized (3). To these have been added some of the principles utilized in newer emerging devices. Basically, all instruments use two or more of the following methods of measuring blood cells. Impedance techniques record the momentary reduction of conductivity engendered by the passage of blood cells through tiny apertures monitored by electron sensors. Tungsten halogen and helium-neon red laser light sources also are used to analyze white cells and red cells, respectively. In the Technicon (Bayer) H-3, peroxidase histochemistry provides a method of dividing white cell populations into families. Lytic agents are used to eliminate white blood cell (WBC) cytoplasm, leaving only bare nuclei for electronic scrutiny. The resistance of basophils to most lytic agents offers a unique approach to counting an individual cell line. The conductivity of high-frequency current is used by some instruments to determine internal physical and chemical composition of white cells. Flow technology, a feature of many instruments, is used for measurement of cell size by low-angle forward light scatter and nuclear complexity by high-angle forward scatter. This technology can be enhanced with fluorescent dyes to quantify

1 Nonstandard abbreviations: WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; IDA, iron deficiency anemia; and ACD, anemia of chronic disease.
reticulocytes and nucleated red cells. Applications of these techniques to the current crop of instruments are provided in Table 1.

Each of the available devices has its advocates from the standpoints of cost and performance. All manage to perform creditably well in counting red cells and platelets. Much of modern-day concern centers on the accuracy of WBC differential counts and on the specter of false negatives, particularly in the matter of blast detection. With improving technology and appropriate flagging, much concern regarding false negativity is evaporating. At worst, the imprecision of electronic differential counting is substantially lower than that of microscopic methods (4).

Most instruments use impedance and optical technologies to measure the total WBC count (5). Predictably, both technologies have their limitations. In impedance counting, both high and lower range accuracy may be impaired (6). Counts may also be inaccurate in the presence of circulating nucleated red cells. Optical methods, however, enjoy substantially better high and low end accuracy than impedance techniques, but they may be impaired in the presence of fragile WBCs and lysed red cells. These discordances have been rectified in the Cell Dyn 3500 analyzer [a precursor to the Cell Dyn 4000 (7)] listed in Table 1] by the introduction of decision analysis based on comparisons of its dual impedance and optical modules (5).

An interesting addition to the diagnostic armamentarium of the new generation of automated analyzers is the slide-making device attached to the Coulter GEN-S. The instrument can be programmed to generate sentinel slides automatically with any red cell, white cell, or platelet flag; with location (blood labeled as originating in an oncology wing); with doctor name (oncologist); or with patient name. Sample printouts of two Coulter instruments are shown in Figs. 1 and 2.

One odd byproduct of automated technology, however, is the detectability of unlysed red cells in printouts of some cell counters (8–11). In the earlier Sysmex NE 8000, these cells were located to the left of the diagonal in the white cell scattergram. Of 13 patients studied with this instrument, 5 had previously undiagnosed hemoglobinopathies, 4 had previously diagnosed hemoglobinopathies, 1 was suspected of hemoglobinopathy, and 3 had liver disease (11). The hemoglobins detected were EE, EA, CA, SA, and SS. Common to blood smears in most of these cases were target cells, logical candidates for lysed red cells. Thus, unexpected fallout from automated technology may have identified the first poikilocyte by serendipity. Flagging indicative of the presence of unlysed red cells continues to be a feature of the newest member of the Sysmex Series (SE-9000; Fig. 3).

With the explosion of new instruments appearing on the market, many representing improvements in technology over earlier models, it is difficult if not impossible to favor a specific instrument over another at purchase time. Technologies have improved radically over the past decade. True, most systems have some limitations. However, such limitations are monitored by flags, minimizing false negatives and false positives. Although initial cost and the expense of maintenance contracts may play important roles at decision time, other factors such as amount of automation, throughput time, and patient population may be equally important. The latter to a significant degree influences frequency of flagging, a major economic consideration indeed.

### Table 1. Methodologic permutations in a group of modern cell analyzers.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Methodology features (comments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coulter STKS</td>
<td>Impedance, conductivity, laser technology</td>
</tr>
<tr>
<td>Coulter GEN-S</td>
<td>Impedance, flow cytometry, conductivity, laser light scatter</td>
</tr>
<tr>
<td>Sysmex SE-9000</td>
<td>Direct current (resistance to cell volume); radio-frequency (cell density, size)</td>
</tr>
<tr>
<td>Cobas Argos 5-diff (Roche)</td>
<td>Impedance, light absorption (halogen light source)</td>
</tr>
<tr>
<td>Technicon H-3 (Bayer)</td>
<td>Cytochemistry, flow technology (reticulocyte analysis)</td>
</tr>
<tr>
<td>Advia 120 (Bayer)</td>
<td>Cytochemistry, flow technology (reticulocyte analysis)</td>
</tr>
<tr>
<td>Cell Dyn 4000 (Abbott)</td>
<td>Impedance, laser light scatter</td>
</tr>
</tbody>
</table>

**Flagging**

Virtually all laboratories are now using automated hematology instruments to generate cell counts, red cell indices, and white cell differential counts. The addition of flags to printouts is designed to reduce the numbers of false positives and false negatives by triggering morphologic review of sentinel slides. Although this ploy has been broadly successful in avoiding interpretative debates (1), substantial numbers of problems remain to be resolved, varying in frequency from one instrument to another. Many can be avoided at the bench by astute technologists who sequester distorted indices generated by samples taken from patients with obvious hypertriglyceridemia or cold agglutinin disease. The recent development of a new Abbott hemoglobin reagent (as used in the Cell Dyn 4000) may herald the disappearance of hypertriglyceridemia as a cause of spuriously increased hemoglobin results (7). Other error-generating aberrations, however, are silent or instrument-invisible. These include spurious macrocytosis generated by hyperglycemia (12, 13) and spurious thrombocytopenia caused by platelet satellitism in EDTA blood (14). A compilation of error-generating aberrations from several sources is provided in Table 2 (12, 15, 16).

Although flagging serves its purpose well by indicating to the technologist or pathologist that an instrument suspects that data generated by one of its channels are marginal, the process tends to introduce its own set of problems. Automation evolved in a logical bid to reduce the labor-intensiveness of manual methods. A plethora of
flags from a given instrument would therefore seem to negate the advantages of automation by precipitating large numbers of reviews of sentinel slides. The need for such reviews is predicated on several factors: (a) the gravity of the consequences of ignoring specific flags and disseminating potentially erroneous or dangerous information; (b) the establishment of tolerable limits for living with certain flags; and (c) the degree of concern of a laboratory director in mandating morphologic review.

At Northwestern Memorial Hospital (Chicago, IL), the review rate resulting from flagging approaches 30%. Such a high rate of review reflects the fact that this is a tertiary hospital, with an active oncology division, a transplant center, and a busy newborn intensive care unit. This rate is virtually identical to that reported earlier in another large university hospital (3). In contrast, the review rate of sentinel slides at St. Mary's Hospital (Duluth, MN) hovers around 5%. This 400-bed hospital, although classified as tertiary, is not a transplant center. Its low rate of sentinel slide review is influenced by two factors: (a) the large number of patients seen in a huge outpatient department; and (b) the conditioning of local physicians to order single hematologic tests rather than full complete blood counts (CBCs).

**Data for Export**

For most purposes, numeric data screened by the laboratory and released for consumption by clinicians appear entirely adequate in precision and accuracy. Flagging aside, the large numbers of red cells, white cells, and platelets counted in modern instruments generate much more accurate data than those deriving from older manual methods. Red cell indices are a case in point. The seven indices routinely proffered are as follows:

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**Fig. 1.** WBC scattergrams from STKS (A) and from GEN-S (B).

(A), blood from a patient with 47,800 WBCs and 69.7% blasts by manual differential. Diagnosis was acute myelogenous leukemia (M4). Note that most blasts are confined to a specific sector. (B), WBC scattergram of the same blood as in A. Note absence of sector lines. PC2 is a flag indicating the possibility of an interfering substance such as hyperlipidemia, a chemical imbalance, or a drug preventing complete lysis of red cells.

**Fig. 2.** WBC scattergrams from STKS (A) and from GEN-S (B).

(A), blood from a patient with 51,800 WBCs, rare blasts, 8% basophils, but 28% nucleated red cells. Diagnosis was chronic myelogenous leukemia with marked normoblastemia. Note the concentration of normoblasts in the lower left sector of the scattergram. (B), WBC scattergram of the same blood as in A. Note absence of sector lines but similar distribution of normoblasts. PC2 is a flag indicating the possibility of an interfering substance such as hyperlipidemia, a chemical imbalance, or a drug preventing complete lysis of red cells.
Red blood cells (RBC), $10^6/\mu$L
Hemoglobin (Hb), g/dL
Hematocrit (Hct), %
Mean corpuscular volume (MCV), fl
Mean corpuscular hemoglobin (MCH), pg
Mean corpuscular hemoglobin concentration (MCHC), g/dL
Red cell distribution width (RDW), %

Of these, Hb (or Hct) and MCV are critical. Fortunately, both are extremely accurate. The MCHC and RDW, although also accurate, are less widely used. The RBC count and MCH are rarely used in decision-making. Without regard for possible morphologic aberrations or their nuances, clinicians by and large order appropriate second-order (follow-up) test batteries when anemias are either macrocytic (MCV $\geq 100$ fl) or microcytic (MCV $<80$ fl) in adults. The diagnostic possibilities in either instance are relatively limited (17). In the absence of clues from abnormal cell size, purists would consider morphologic review mandatory as a prelude to second-order testing. Although admittedly an ideal approach, such review would be less than cost-effective because of excessive labor demands. The need for morphologic review is further blunted by the distribution of anemias in the US population. Iron deficiency anemia (IDA), anemia of chronic disease (ACD), and thalassemia minor together account for $>85\%$ of such anemias and IDA/ACD for $>50\%$ (18). Furthermore, in early IDAs and in $75\%$ of ACDs, MCVs are normal and morphologic discrimination between the two is difficult to say the least. Indices alone,
notably the combination of Hb, MCV, and RDW, serve this purpose better (19). At the present time, it appears that flags rather than clinicians trigger most requests for morphologic reviews. Red cell morphology is addressed in most institutions during such reviews, even when white cell or platelet data are flagged.

### Laboratory Communication with the Clinician

Routine, unflagged WBC counts and differentials are released immediately to the clinician. Flagged cell counts and WBC differentials, however, are checked morphologically by senior technologists before release. At Northwestern Memorial Hospital, WBCs <1000 or >50 000, Hb <6 or >19 (except in newborns), and platelets <50 or ≥1 000 000 are called directly to the clinician by the technologist. The pathologist calls the clinician directly upon encountering (a) a new hematologic malignancy, e.g., circulating blasts; (b) white cells with ingested material (bacteria, fungi); and (c) schistocytes and thrombocytopenia, i.e., stigmata of disseminated intravascular coagulation.

### Morphology and the Technologist

When instrument-generated flags mandate slide review, it is imperative that instrument-attending technologists be well trained in morphologic hematology. Such technologists should be familiar with abnormal red shapes, including those induced artifactually. Technologists should be especially alert to fragmented forms indicating angio-pathic hemolytic anemias—notably those associated with disseminated intravascular coagulation. The nuances of other poikilocytes have been amply received.

### Table 2. Factors known to cause spurious laboratory results in hematology analyzers.

<table>
<thead>
<tr>
<th>Index</th>
<th>Spuriously increased</th>
<th>Spuriously decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs</td>
<td></td>
<td>Clotting</td>
</tr>
<tr>
<td>Hb</td>
<td>In vivo hemolysis, hyperlipidemia, hyperbilirubinemia, WBC &gt;50 000/mm³</td>
<td>Clotting</td>
</tr>
<tr>
<td>MCV</td>
<td>Cold agglutinins, hyperglycemia, WBC &gt;50 000/mm³</td>
<td>Cryoglobulins</td>
</tr>
<tr>
<td>MCHC</td>
<td>Hyperlipidemia, cold agglutinins</td>
<td>WBC &gt;50 000/mm³</td>
</tr>
<tr>
<td>RDW</td>
<td>Status post transfusion</td>
<td>Clotting</td>
</tr>
<tr>
<td>WBCs</td>
<td>Nucleated red cells, platelet clumps, unlysed red cells, cryoglobulins</td>
<td>Clotting</td>
</tr>
<tr>
<td>Platelets</td>
<td>WBC fragmentation, severe microcytosis, cryoglobulins</td>
<td>Satellitism, clumping</td>
</tr>
</tbody>
</table>

### Future of Automated Hematology

Automated hematology 40 years later is still in its infancy. The recognition of left shifts, blasts, circulating lymphoma cells, or atypical lymphocytes remains an exercise in flagging, providing a mandate for sentinel slide review. Tacit acknowledgement of the current state of the art is provided by the Advia™ 120 Hematology System, which reserves a box on its scatter-plot grid for cells not recognized by cytochemistry. It properly labels them "large unstained cells (LUCs)". Undue activity in this box demands prompt morphologic review.

A pandemic problem among most analyzers performing differential white cell counts is an inability to distinguish between small lymphoid blasts, circulating small lymphoma cells, and normal lymphocytes. Thus, flags may not be displayed even in the presence of abnormal lymphoid populations. It has been suggested that automated laboratories set policies for reviewing sentinel slides in patients with unexpected absolute lymphocytosis to avoid missing malignancies in the lymphoid system (24).

Undoubtedly, in the first few decades of the new millennium advancing technology will routinely identify more white cell subtypes and even blast lineage while reducing the need for flags. Recognition of specific poikilocytes will also evolve, providing a major leap forward in the detection of rarer anemias or specific organ diseases. At the present time, automated hematology cannot provide these services.

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References


