Validation and Implementation of an Algorithm for Reporting the Automated Absolute Neutrophil Count From Selected Flagged Specimens

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Key Words: Cell counters; Automated neutrophil count; Hematology analyzers; Sysmex XE/XT; Flags

Upon completion of this activity you will be able to:

- describe the issues faced by clinical laboratories providing neutrophil counts to outpatient hematology/oncology clinics, and the advantages and disadvantages of various approaches to address these issues.
- identify a set of flags that indicate the potential presence of qualitative or quantitative abnormalities that may call into question specific parameters of the automated differential.
- describe how ordinary linear regression and outlier examination can be used to assess the effect “absolute neutrophil count (ANC) affecting flags” have on the correlation between manual and automated ANCs overall and around specific, clinically relevant threshold values.
- define a set of metrics to quantitatively assess the utility and safety of a newly implemented algorithm to report automated ANC results on samples triggering a selected group of flags.

Abstract

Samples that are flagged by automated cell counters and, therefore, require a time-consuming microscopic review cause unacceptable wait times for patients in hematology/oncology clinics. We used a set of 518 samples to validate that 5 flags on Sysmex XE/XT instruments (Sysmex, Kobe, Japan) could safely be ignored when the absolute neutrophil count (ANC) was the primary clinical question. The R² between automated and manual ANCs was 96.9% for samples triggering non-ANC flags, with 1 clinically significant discrepant sample out of 296 (0.3%). A new test code allowing clinicians to specify “ANC-only” was implemented, and these non-ANC specific flags were disregarded. The new order set was used in 16.3% of patients. Automated reporting of the ANC in selected flagged samples reduced the review rate by 60% and decreased the turnaround time by 100 minutes. This approach to automatically report the ANC in selected flagged specimens in which the ANC is the primary clinical interest safely decreases the turnaround time for many ANC samples.

The absolute neutrophil count (ANC) is the absolute number of segmented neutrophils and band forms in a patient’s blood (ie, [WBC count per microliter] × [percentage of neutrophils + band forms]). Cancer treatment protocols often require the ANC to be above a certain threshold (usually 1,000/μL) before the administration of chemotherapy. Therefore, ANC results must be provided as quickly as possible to minimize wait times for patients and medical staff. Many outpatient hematology/oncology clinics maintain laboratories dedicated mainly or exclusively to obtaining ANC results to avoid delays associated with transport and processing in busy centralized laboratories.

The ANC can be determined using automated or manual methods. Automated cell counters provide a CBC, including the WBC count and a 5-part differential, and the latter includes the percentage of neutrophils. This allows reporting of an “automated ANC” within minutes of loading a sample on the analyzer. The automated ANC provides fast turnaround time (TAT), lack of interobserver variation, and high statistical validity.1 The instruments use “flags” to indicate whether a sample contains qualitative or quantitative abnormalities that may call some or all of the reported results into question. “Flagged” samples then require a labor-intensive manual microscopic review and, frequently, a full manual microscopic differential in order to report a “manual ANC.”

Traditionally, clinicians order a “CBC with differential” for all samples; this necessitates determining the relative...
percentages of WBCs in a patient’s blood, even when the ANC is the sole clinical question. Laboratories prepare slides and perform manual scans and/or manual differentials on all samples for which a predetermined set of flags is triggered, even if the automated neutrophil percentage is unaffected. These manual differentials lead to significant delays in reporting results, increasing wait times for patients.

Laboratories have used 3 approaches to solve this problem as it applies to reporting of the ANC. In the first approach, a “preliminary” ANC is reported from the automated cell counter, even if the sample is flagged. A “final” manual ANC is then reported later. The disadvantage of this approach is that clinicians make treatment decisions based on the preliminary automated ANC; in some cases, the final manual ANC would have led to a different course of action. In the second approach, all flags are ignored for samples when the primary interest is the ANC. This approach is based on studies suggesting that the correlation between automated and manual ANCs is robust. The disadvantage of this approach is that it ignores the recommendations of most manufacturers of automated cell counters.

Finally, the third approach is to perform manual review of samples when flags that could affect the neutrophil count are present but to ignore flags that do not affect neutrophils. This approach is supported by a study that found that one flag, the “WBC ABN Scattergram” flag on the Sysmex XE-2100 (Sysmex, Kobe, Japan), predicts a poor correlation between the manual and automated ANC. In addition, another report recommended that microscopic validation should be performed on samples when Gen-S or HmX analyzer (Beckman Coulter, Miami, FL) flags indicate the presence of blasts, immature myeloid cells, monocytes, nucleated RBCs (NRBCs), or platelet clumps. Advantages of this approach are that no preliminary results are reported and manufacturers’ recommendations regarding flags are respected.

We aimed to improve TAT for ANC results while adhering to the manufacturer’s recommendations regarding flags. Therefore, we first determined which flags indicated possible issues with the WBC count or the neutrophil percentage (which could not be ignored) and which flags indicated potential issues with results other than the WBC and the neutrophil counts (which could safely be ignored, if only the ANC was reported). After validating this approach by comparing manual and automated ANC results from 518 flagged samples, the algorithm was introduced into clinical practice. This communication describes the procedure, its validation, and its effects on TAT and ordering patterns in our outpatient hematology/oncology clinic.

Materials and Methods

Study Site

The study was performed at the Herbert Irving Comprehensive Cancer Center (HICCC) on the Columbia University campus of New York-Presbyterian Hospital (New York, NY), a tertiary care academic medical center. HICCC is designated as a comprehensive cancer center by the National Cancer Institute and serves adult and pediatric patients. More than 3,500 new cancer patients are seen every year. A satellite laboratory dedicated exclusively to HICCC patients is located in the clinic area and staffed by 3 technologists during clinic hours. Technologists rotate through the satellite laboratory and the central laboratory. The satellite laboratory provides CBC, WBC differential, and coagulation results.

The Sysmex XE/XT Lines of Automated Cell Counters

The Sysmex XE/XT automated cell counters (Sysmex) provide a 5-part differential using an optical technique including forward scatter, side scatter, side fluorescence, and an electrical impedance method. These instruments use flags to indicate potential issues in RBC, WBC, and platelet parameters. We presently use 10 flags specific for the WBC differential as triggers for manual review, and these were the focus of this study. Flags indicating possible RBC or platelet issues were not studied.

Manual Differentials

Blood films were prepared using the SP-1000i slidemaker-stainer (Sysmex) (adult samples) or with the push-pull method with a spreader slide (pediatric samples) and stained with a Wright-Giemsa stain. Manual differentials using standard microscopic technique were performed using the laboratory’s standard operating procedure, which is based on Clinical and Laboratory Standards Institute guidelines. For the study, 100 WBCs were counted for each manual differential.

Turnaround Time

TAT was defined as the time from sample receipt in the satellite laboratory to verification of the results in the laboratory information system (Sunquest, Tucson, AZ) and release into downstream hospital information systems. Reports were prospectively compiled using the laboratory information system’s reporting function or with a Crystal Report (SAP, Waldorf, Germany). A Crystal Report uses an automated database query to create a customized report.

Statistical Analysis

Statistical analysis and graphing were performed using Microsoft Excel 2007 (Microsoft, Redmond, WA).

Results

Identification of Flags Not Specific for WBC Count or Neutrophil Percentage

Sysmex XE/XT automated cell counter flags are generated by patterns in the scattergram that are typical for certain
abnormalities. A literature review, discussions with the manufacturer, and our experience with this cell counter identified 5 flags that could affect the reliability of the automated ANC. The “WBC ABN Scattergram” flag indicates that the instrument cannot separate 1 or more classes of cells in the WBC differential and has been identified as invalidating the automated ANC. The possible presence of blasts, monocytosis, and NRBCs were identified as reasons for validating an automated ANC by slide review; in our experience, these findings also indicate a compromised neutrophil count. Therefore, we decided to perform a slide review for cases with the “Monocytosis,” “Blasts,” and “Nucleated Red Blood Cell” flags. The “Eosinophilia” flag was also reviewed because large populations of eosinophils are often not well separated from neutrophils by the cell counter. These 5 flags were defined as the “ANC-affecting flags.”

In contrast, 5 WBC-specific flags (“Immature Granulocytes,” “Left Shift,” “Atypical Lymphocytes,” “Abnormal lymphocyte/Lymphoblast,” and “Basophilia”) indicate possible abnormalities that do not affect the accuracy of the automated WBC differential or neutrophil percentage. Therefore, we hypothesized that these flags can be ignored if the purpose for ordering the test is the ANC (ie, “non-ANC flags”).

Correlation of Manual and Automated ANCs

To prove that flags not specific to the WBC count and neutrophil percentage could be safely ignored, a validation study was performed. The study used 518 samples flagged by the automated cell counters on which a microscopic differential had also been performed. Many samples were identified by more than 1 flag. The average WBC count in this data set was 12,113/μL (SD = 7,298/μL). The average manual ANC (7,780/μL; SD = 6,100/μL) was similar to the average automated ANC (7,749/μL; SD = 6,180/μL). Using standard linear regression, the overall correlation coefficient between the automated ANC and manual ANC was $R^2 = 94.42\%$.

Most (ie, 292) of the 518 samples (56.4%) were only identified by flags not likely to affect the ANC; the ANC correlation for these samples was improved ($R^2 = 96.9\%$). The correlation coefficient for samples flagged by ANC-affecting flags was lower ($R^2 = 93.0\%$).

Examination of Outliers and Discrepant Samples at an ANC of About 1,000/μL

Outliers were defined as samples for which the percentage difference between the 2 ANC methods was more than 50%; 23 (4.4%) were identified. The ANC-affecting flags detected 65% of the outliers (15/23); 8 samples were flagged only by ANC-affecting flags.

By focusing on samples with ANC results near the commonly used clinical threshold of 1,000/μL, we found 8 samples with discrepant results, defined as a manual ANC and an automated ANC on different sides of this cutoff. These discrepancies are most likely to lead to incorrect treatment decisions. Only 4 of these 8 samples had a difference of more than 50% (range, 50%-144%). Of these 4, 3 were detected by the ANC-affecting flags, with 1 significantly discrepant sample unflagged by this method. The performance of this new flagging system is shown in Table 2.
Unique Ordering and Reporting Options

Having established that certain WBC-related flags can be safely ignored when reporting the ANC, the laboratory added a new item to its test menu: “CBC with ANC.” This new test is available only for samples sent to the hematology/oncology clinic satellite laboratory, ensuring its use only by hematologists and oncologists who had been educated about this new option. Samples for which a CBC with ANC had been ordered were analyzed on the automated cell counters in CBC/differential mode. Depending on the flagging status, there were 3 possibilities: (1) If no flags were encountered, all CBC and differential results were directly released from the instrument. (2) When only non-ANC flags were present, the laboratory information system calculated the ANC from the automated differential and WBC count and released the result with the following appended comment: “This ANC (absolute neutrophil count) is based on a white cell differential from an automated cell counter which has not been microscopically reviewed for the presence of abnormal cells. Clinical correlation is required. Please call the laboratory if a manual microscopic differential is clinically indicated.” No other differential parameters were released. (3) If 1 or more ANC-affecting flags were present, a full manual microscopic differential was performed and reported.

The new ordering option was explained to the clinical staff in meetings and written communications. Clinicians were specifically advised to call the laboratory to request a manual differential for all category 2 samples, if a full differential was needed.

Utilization Pattern for the New Test Code

One year after introduction of the new test code, clinicians in the hematology/oncology clinic ordered 333 CBCs with ANC and 1,712 CBCs with differential during a representative 3-month period; thus, 16.3% of orders that would have previously requested a CBC with differential now requested a CBC with ANC.

TAT for the New Test Code

The average TATs for the new test code are provided in Table 3 by result type: CBC with automated differential, CBC with automated ANC with comment, or CBC with full manual differential. Samples without flags constituted 71.5% of the total; the CBC with full automated differential was autoverified by the laboratory information system and reported in an average of 8.9 minutes of sample receipt. For the 17.2% of samples flagged with non-ANC flags, the CBC and automated ANC with comment had an average TAT of 15.4 minutes; these represent manual differentials saved by implementation of the new system. For the 11.4% of samples identified with ANC-affecting flags, a full microscopic differential was required, resulting in an average TAT of 115 minutes.

Comparison of TAT for All Manual Differentials Before and After Implementation

Before implementing the new ANC test code, the results for 62% of all manual differentials were reported in less than 2 hours. After the new order option was put into place, the average number of manual differentials reported in less than 2 hours rose to 78.4%. Results were obtained by averaging percentages from 3-month periods before and after implementation.

Discussion

This study describes the development, validation, and implementation of a new laboratory order set, the CBC with ANC, in a large academic hematology/oncology outpatient

**Table 2** Summary of Discrepant Results at the ANC Threshold of 1,000/μL and Their Detection by ANC-Affecting Flags

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Manual ANC (μL)</th>
<th>Automated ANC (μL)</th>
<th>Difference (%)</th>
<th>Detected by ANC-Affecting Flags</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,074</td>
<td>910</td>
<td>15.27</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>1,093.3</td>
<td>930</td>
<td>14.94</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>1,140</td>
<td>920</td>
<td>19.30</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>1,177.5</td>
<td>960</td>
<td>18.47</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>523.2</td>
<td>1,280</td>
<td>144.66</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>679</td>
<td>1,520</td>
<td>123.86</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>1,128.6</td>
<td>660</td>
<td>50.38</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>1,761.6</td>
<td>730</td>
<td>58.56</td>
<td>Yes</td>
</tr>
</tbody>
</table>

ANC, absolute neutrophil count.
This order set allows immediate reporting of automated ANC results from 57% of flagged specimens in which there is a minimal probability of a compromised result; this focuses valuable technologist time on samples in which the determination of a manual ANC is truly necessary.

This approach is based on previous studies showing that automated ANC results can safely replace manual results, even in certain flagged specimens. For example, Parham et al\(^2\) reported good correlation of manual ANC results with automated ANC results from Cell-Dyn 3500 hematology analyzers (Abbott Diagnostics, Santa Clara, CA) (\(R^2 = 0.97\)). By reporting automated ANC results from flagged specimens, they decreased the TAT for ANC results from 45 minutes to 7 minutes. They recommend that the final report should still include a microscopic evaluation. They do not discuss a clinical approach to cases with discrepant automated and manual ANC results.

In addition, Hijiya et al\(^3\) reported a correlation coefficient of \(R^2 = 0.81\) between manual and automated ANC results obtained with Beckman Coulter Gen-S or HmX analyzers. They suggested that microscopic validation should be performed when the analyzer flags indicate the presence of blasts, immature myeloid cells, monocytes, NRBCs, or platelet clumps.

Finally, Friis-Hansen et al\(^6\) provide the only study examining the correlation of manual and automated ANC results obtained with Sysmex XE-2100 cell counters. Samples flagged by the “WBC ABN Scattergram” alert often had an invalid automated ANC. Exclusion of such flagged samples resulted in an \(R^2\) of 0.992 with very few outliers. They did not discuss the effect of their algorithm on TAT.

The present study considered 10 flags on the Sysmex XE/XT that trigger manual review. We hypothesized that only the abnormalities indicated by the flags for an abnormal WBC scattergram, monocytosis, eosinophilia, blasts, and NRBCs would sufficiently disturb the automated differential so that the neutrophil percentage would not be accurate. The decision to include 4 more flags than Friis-Hansen et al\(^6\) as potentially ANC-related was based on the recommendations of Hijiya et al\(^3\) and on our 7 years of experience with Sysmex automated cell counters, during which we determined that these 5 flags were the only indication of a seriously compromised neutrophil count.

Exclusion of samples flagged by these 5 alerts improved the correlation coefficient from \(R^2 = 94.42\%\) to \(R^2 = 96.9\%\) and reduced the number of discrepant samples at the ANC threshold of 1,000/μL to 5. Of the 5 discrepant samples, 4 were different by less than 50% and were most likely due to the low reproducibility of the manual ANC because of the relatively low number of WBCs counted during slide review. Only 1 sample of 296 (0.3%) showed a clinically significant discrepancy. Review of the manual differential from this sample revealed elevated eosinophil and monocyte percentages (22% and 20%, respectively) that could call the neutrophil percentage estimate into question. These values were below the cutoffs of the quantitative “Eosinophilia” and “Monocytosis” flags that would have caused a blood film to be reviewed in our new system. The sample was from a 2-month-old girl with neonatal sepsis and necrotizing enterocolitis.

Of note, exclusion of samples flagged by these 5 alerts resulted in a 57% reduction in manual reviews in our test set. After introduction of the algorithm in clinical practice, 60% of all flagged samples could be released without microscopic review.

For the entire range of ANCs, 23 samples (4.4%) showed a greater than 50% discrepancy of manual vs automated ANC; 15 of these were flagged by ANC-related flags. The remaining 8 samples had an average ANC and WBC count that were significantly lower than the average ANC of the sample set. The dependence of the correlation between automated and manual ANC on the values of the WBC count and the ANC was noted previously and may actually be due to greater variation in the manual 100-cell differential in leukopenic samples.\(^3\) Review of the manual differentials from these samples revealed an average monocytosis (monocyte count, mean, 10%; SD, 4%; range, 5%-20%) that, although lower than the quantitative threshold for the monocytosis flag, may have negative effects on the automated cell counter’s ability to accurately estimate the neutrophil percentage. One study that examined how the sensitivity of WBC flags on the Sysmex XE-2100 changes with decreasing WBC counts found that the “Blasts?,” “Immature Granulocyte?,” and “Left Shift?” flags

### Table 3

<table>
<thead>
<tr>
<th>TATs for ANC Orders (n = 501)</th>
<th>Frequency (%)</th>
<th>Average TAT (SD, min)</th>
<th>Median TAT, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC with automated differential (samples without flags)</td>
<td>358 (71.5)</td>
<td>8.9 (11.4)</td>
<td>7</td>
</tr>
<tr>
<td>CBC with automated ANC with comment and no differential (samples with non-ANC flags only)</td>
<td>86 (17.2)</td>
<td>15.4 (8.3)</td>
<td>14</td>
</tr>
<tr>
<td>CBC with manual differential, including manual ANC (samples with ANC-related flags)</td>
<td>57 (11.4)</td>
<td>115 (227.6)</td>
<td>57</td>
</tr>
</tbody>
</table>

ANC, absolute neutrophil count; TAT, turnaround time.
maintain their sensitivities at even very low WBC counts. However, there is no information given on the “Monocytosis” or “Eosinophilia” flags, for which the sensitivity may decline with lower WBC.\textsuperscript{10}

One year after implementing the new order set, clinicians in the hematology/oncology clinic replaced 16.3\% of their CBC with differential orders with CBC with ANC orders. This shows a definite interest in this new option, but also indicates that clinicians in our clinic remain interested in a full differential for the vast majority of cases. This is in contrast with the findings of Jatoi et al.,\textsuperscript{4} who reported a 75\% drop in differential orders after introducing an “ANC-only” order. Therefore, laboratories may not be able to replace all differential orders from hematology/oncology clinics with ANC reports. Close cooperation with the clinical staff is necessary before making any significant changes in the available order menu.

Of the CBC with ANC orders, 17.2\% were affected only by non-ANC flags, and results could be reported in an average of 15.4 minutes. This compares with an average turnaround time of 115 minutes for CBC with ANC samples requiring a full manual differential. Therefore, implementing this algorithm decreased the TAT for 17.2\% of the ANC samples by an average of almost 100 minutes, allowing physicians to make much faster treatment decisions and allowing patients to spend much less time waiting for laboratory results. In our laboratory, a well-trained technologist requires about 10 minutes to complete a manual differential (including slide preparation and reporting). The new reporting system reduced the number of microscopic differentials per month by approximately 60, thereby allowing the technologists to finish the remaining microscopic differentials in less time. This explains the increase from 62\% to 78\% in the percentage of all manual differentials completed in less than 2 hours.

We developed and implemented an algorithm for reporting automated ANC results from specimens flagged by Sysmex XE/XT analyzers. Implementing this algorithm safely improved the TAT for 60\% of flagged ANC results and the overall TAT for manual differentials.

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References