Hematopathology / Automated CSF Cell Count Reference Ranges

 Automated Cerebrospinal Fluid Cell Counts Using the Sysmex XE-5000

Is It Time for New Reference Ranges?

Linda M. Sandhaus, MD,1 Pedro Ciarlini, MD,1 Diane Kidric, DO, JD,1 Christine Dillman, MT(ASCP),1 and MaryAnn O’Riordan, MS2

Key Words: Cerebrospinal fluid cell counts; CSF cell counts; Automated body fluid cell counts; Sysmex XE-5000; Reference ranges

Abstract

The main objectives of the study were to compare manual and automated WBC counts on clear cerebrospinal fluid (CSF) samples. Clear CSF samples from 200 adults and children were studied. Cell counts were performed manually using a hemocytometer and then analyzed on the Sysmex XE-5000. Descriptive statistics and Spearman correlation for nonparametric data were used for method comparison. Manual WBC counts ranged from 0 to 702 cells/μL, and Sysmex counts ranged from 0 to 629 cells/μL. The Spearman rank correlation coefficient for the entire range of data was 0.77 (P < .001); however, the correlation was weaker at the low end of the data spectrum. For manual WBC ranges of 0 to 5 cells/μL and 0 to 10 cells/μL, the corresponding Sysmex 0 to 95th percentile ranges were 0 to 23 cells/μL and 0 to 27 cells/μL, respectively. The results suggest that larger studies are necessary to determine new reference ranges for automated CSF WBC counts.

Cerebrospinal fluid (CSF) cell counts have traditionally been performed by manual methods using a hemocytometer counting chamber. Manual methods are labor-intensive and imprecise and have been obsolete for CBCs for more than half a century. In recent years, the capabilities of several automated hematology analyzers have been extended to include automated body fluid cell counting. Several published studies have examined the performance of these automated methods for a range of body fluid types and cell counts.1-10 While overall correlations with manual cell counts have been generally very good, uncertainty about the accuracy of automated methods for extremely low cell counts has been an impediment to their widespread implementation for all body fluid samples, especially clear CSF samples, which typically have extremely low cell counts, often less than 50 cells/μL. The ability to automate cell counting for these samples might improve accuracy of results and improve laboratory efficiency.

The hematology laboratory of University Hospitals Case Medical Center, Cleveland, OH, performs approximately 325 CSF and 150 non-CSF body fluid analyses monthly. Automated body fluid cell counting was initially implemented in 2006 using the Sysmex XE-2100 (Sysmex America, Mundelein, IL). Cell counts on clear CSF samples continued to be performed manually owing to the analyzer’s lower limit for WBC linearity of 50 cells/μL. With this strategy, manual body fluid cell counts were dramatically reduced; however, the majority of CSF samples still required manual counts. With the introduction of the Sysmex XE-5000, the linearity for WBCs and RBCs was extended to 0 cells/μL. Implementation of these analyzers in 2009 presented the opportunity to evaluate the performance of the analyzer on clear CSF samples, many of which would be expected to have cell counts within...
the accepted normal ranges. Previous investigators have suggested that automated technologies might not be appropriate for such samples because a large proportion of normal samples would be misclassified as abnormal using existing reference ranges. However, good laboratory practices require that reference ranges be verified or new reference ranges be established when methods change. Therefore, the main objectives of this study were to compare manual hemocytometer and Sysmex XE-5000 WBC counts on clear CSF samples and determine the ranges of Sysmex XE-5000 WBC counts that correspond to existing manually derived reference ranges. Secondary objectives were to evaluate the automated WBC differential count and the sensitivity and specificity of the WBC abnormal distribution flag for these samples.

Materials and Methods

A convenience sample of 200 clear and colorless CSF samples from adults and children were selected for the study between February 2009 and January 2010. Samples were collected in EDTA or no anticoagulant, and all samples were analyzed within 4 hours of collection. Duplicate RBC and WBC cell counts were performed on a disposable Levy-Neubauer hemocytometer (INCYTO C-Chip, Seoul, Korea) by counting 5 squares on each side of the chamber. According to laboratory procedure, the duplicate counts must agree within 20% of each other. For all samples, the manual cell count was performed before the automated analysis was done. The analyzer aspirates 130 μL and uses a nucleic acid dye and fluorescent light scatter and side-scattered light to determine the total WBC and to perform a limited WBC differential count that consists of neutrophils (PMNs) and mononuclear (MN) cells on a diluted aliquot of this volume. A direct current detection method is used to determine the RBC count. Background counts are performed by the analyzer automatically before all body fluid cell counts. The manufacturer states that the linearity for RBCs and WBCs extends to zero; however, the reportable range for RBCs does not extend this low because of decimal point limitations on the analyzer output. RBC counts less than 0.0005 × 10⁶/μL (500/μL) are rounded downward to 0.000, and values of 0.0005 × 10⁶/μL (500/μL) or more are rounded upward to 0.001 × 10⁶/μL (1,000/μL). The counts proceed in these stepwise increments until counts exceed 0.01 × 10⁶/μL (10,000/μL).

Cytospin samples were prepared for all samples using a Shandon Cytospin 4 (Thermo Electron, Waltham, MA). Manual differential counts were performed by a technologist. The number of cells counted in the WBC differential count is routinely recorded in the report and is often less than 100 on clear CSF samples. Pathologists reviewed all Cytospin smears from study samples. To evaluate the analyzer’s “abnormal WBC scattergram” flag, positive Cytospin findings were defined as the presence of 1 or more of the following: (1) malignant or “suspicious” cells, (2) reactive lymphocytes, (3) bone marrow contamination, (4) fragments of brain tissue or benign CSF lining cells, or (5) microorganisms. Quantitative leukocytoses were not considered positive findings unless 1 or more of these criteria were also present.

Statistical Analysis

Population characteristics are described with frequencies and percentages. Age was categorized as younger than 1 year, 1 to 16 years, and older than 16 years for the purposes of description. Spearman rank correlation coefficients (ρ) were used to estimate the relationship between 2 continuous, nonnormally distributed variables. The experimental unit was the lumbar puncture procedure, and procedures from different dates on the same patient were considered independent observations for the purpose of statistical analysis. Different tube numbers collected from the same procedure were not considered to be independent initially. To demonstrate whether the potential within-procedure correlation biased the results in any way, we carried out the analyses with and without these duplicate samples. Descriptive statistics were used to compare the distributions of values. The 0 to 95th percentiles of Sysmex WBC values are reported for published manually derived reference ranges. All statistical analyses were performed by using SAS software (SAS Institute, Cary, NC). The level of significance was set at .05.

Results

The 200 clear CSF samples were from 135 patients; 28 patients had samples from more than 1 procedure (range, 2–5), 16 patients had 2 samples from the same procedure, and 1 patient had both. The patient population is summarized in Table 1. The patients ranged in age from 14 days to 95 years. The most common indication for lumbar puncture was to rule out infection/inflammation.

Table 1

<table>
<thead>
<tr>
<th>Characteristics of 135 Patients</th>
<th>No. (%) of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Nonhematologic malignancy</td>
<td>15 (11.1)</td>
</tr>
<tr>
<td>Lymphoma/leukemia</td>
<td>30 (22.2)</td>
</tr>
<tr>
<td>Hydrocephalus/shunt</td>
<td>17 (12.6)</td>
</tr>
<tr>
<td>Cerebral hemorrhage</td>
<td>5 (3.7)</td>
</tr>
<tr>
<td>Rule out infection/inflammation</td>
<td>63 (46.7)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (3.7)</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
</tr>
<tr>
<td>&gt;16</td>
<td>93 (68.9)</td>
</tr>
<tr>
<td>1-16</td>
<td>33 (24.4)</td>
</tr>
<tr>
<td>&lt;1</td>
<td>9 (6.7)</td>
</tr>
</tbody>
</table>

Nos. in parentheses indicate percentages.

© American Society for Clinical Pathology

Hematopathology / Original Article

Am J Clin Pathol 2010;134:734-738
DOI: 10.1309/AJCPABGDXSIA4SMT
out infection or other inflammatory condition. A total of 38 samples were from patients with hematologic malignancies, including 28 samples from 20 patients with acute lymphoblastic leukemia. Five samples had malignant or suspicious cells, 2 from 2 different patients with acute lymphoblastic leukemia and 3 from 2 patients with lymphoma.

The results of the WBC count correlation analysis are shown in Figure 1 and are summarized in Table 2. For the entire range of values (0-702 cells/μL), the Spearman rank correlation coefficient (ρ) was 0.77 (P < .001). As shown in Table 2, the correlation was weaker at the low end of the data spectrum. There was no significant difference in the correlation results when duplicate samples from the same procedure were excluded.

Several features are apparent from the scatter plots (Figure 1) that help to explain the correlation analysis. Figure 1A shows the full range of the data. The linear relationship of the data at the upper end of the distribution is clear (with the exception of the single outlier). While the number of data points for manual values of more than 10 cells/μL is small in comparison with those 10 cells/μL or fewer, the range is much larger and, therefore, is likely responsible for the strength of the correlation overall. Figure 1B is an expanded view of the data for manual values of 10 cells/μL or less. There appears to be much less agreement between the 2 methods at the low end, which is attributable to the considerable overlap in ranges of automated values that were obtained for each increment in the manual WBC count. This distribution of the data suggests that the relationship between the 2 methods might not be linear in the low range, and therefore, the correlation coefficient might not be the best statistic for method comparison for this range of the data. The results of a descriptive analysis are shown in Table 3. For each manual WBC range, the corresponding Sysmex 0 to 95th percentile range is given. For example, 0 to 23 cells/μL is the Sysmex 0 to 95th percentile range that corresponds to manual WBC counts of 0 to 5 cells/μL.

There were only 67 samples for which 100 cells or more were obtained on the Cytospin smear. The correlation coefficient was 0.65 (P < .001) for PMN and 0.60 (P < .001) for MN cell counts for these samples. A sensitivity/specificity analysis for the WBC abnormal scattergram flag was done on the second set of 100 samples (101-200) and showed a sensitivity of 60% and a specificity of 96%. There were 12 true-positive samples; 8 were from postoperative patients and had fragments of brain tissue, 2 had reactive lymphocytes, 1 had lymphoma cells, and 1 had bone marrow contamination with nucleated RBCs and immature granulocytes. There were 8 false-negative samples; 4 had lymphocytosis with few reactive lymphocytes, 3 had neural tissue, and 1 had a rare cell suspicious for a leukemic lymphoblast.
Although manual and automated RBC counts could not be directly compared owing to the reporting limitations of the analyzer, a descriptive analysis showed that the rounding algorithm for the automated RBC counts performed as expected Table 4.

### Discussion

Laboratory method comparison studies often rely on parametric statistical methods, such as Pearson correlation and ordinary linear regression. However, biased results may occur from parametric methods when the data do not meet the distributional assumptions. For this reason, nonparametric methods, which make no assumptions about the distribution of the data, are recommended. In this study, the frequency distributions of the manual and automated WBC counts (not shown) indicated that nonparametric statistical methods were required. The correlation was very good over the entire range of data (\( r = 0.77; P < .001 \)). The weaker correlation obtained at the lower end of the data range can, at least in part, be explained by imprecision of both methods when cell counts are low. The coefficient of variation for manual hemocytometer cell counts has been reported to be as high as 45% for a broad range of values. The manufacturer states that the Sysmex XE-5000 has a coefficient of variation of 30% or less for cell counts that range between 15 and 30 cells/\( \mu \)L. A precision study performed in our laboratory confirmed this level of precision for extremely low cell counts (data not shown).

Published age-related reference ranges for CSF WBC counts are widely used by laboratories and accepted in clinical practice. Yet, it is not clear how these ranges were obtained or if they have any clinical value. There is no agreement on reference ranges for infants. The consistently higher range of values for Sysmex XE-5000 cell counts that were obtained for each manual WBC range (Table 3) suggest that manually derived reference ranges are not appropriate for this automated technology. If cell counts on clear CSF samples are to be reported on patient samples using the Sysmex XE-5000, new reference ranges might be necessary. Because CSF samples cannot be obtained from healthy volunteers for reference range studies, patient data must be used for this purpose. By using this indirect sampling method, the 0 to 95th percentile ranges obtained from the new method may be used to establish a new reference range. The results of this study further suggest that the upper limit of normal for Sysmex CSF WBC counts might be in the neighborhood of 20 to 30 cells/\( \mu \)L.

A weakness of this study is that patients with known central nervous system (CNS) disease, such as treated malignancies, were included. Additional larger studies that include a wide range of patient ages and that exclude patients with known CNS disease are needed to validate reference ranges for automated analyzers and to determine what, if any, age-related reference ranges are clinically important.

Correlation of the automated and manual PMN and MN cell counts was of limited value because the study focused on samples with extremely low cell counts. Of perhaps greater interest and diagnostic usefulness is the performance of the abnormal WBC scattergram flag for the detection of pathologically significant findings. However, there were too few samples with pathologic findings in this study population to draw conclusions about the accuracy of the analyzer’s flagging capabilities.

The decimal point limitations for reporting RBC counts less than 10,000/\( \mu \)L on the Sysmex XE-5000 precluded direct comparison of automated and manual RBC counts in this study. However, for CSF and other body fluid samples that have elevated RBC counts, the RBC/WBC ratio generally contributes more to the interpretation of clinical significance than the reference range. High ratios (\( >500:1 \)) favor peripheral blood contamination or hemorrhage, while lower ratios are more consistent with inflammation. Intermediate ratios are ambiguous and can be problematic in certain clinical circumstances, particularly the diagnosis or exclusion of CNS leukemia. More precise automated RBC counts would not only make the RBC/WBC ratio more clinically useful, but would also remove a potential barrier to clinical acceptance of automated CSF cell counts.

From the laboratory perspective, replacement of labor-intensive and imprecise manual methods by automated technology is highly desirable. However, clinical practice poses some barriers for CSF cell counting that must be addressed and overcome for automated cell counts to become widely accepted for all CSF samples. First and foremost, the time-honored, manually derived, age-related reference ranges for CSF cell counts have been around for so long that most physicians have committed them to memory and accept them as “truth.” Their replacement by multiple analyzer-specific reference ranges would be problematic. Ideally, uniformity of reference ranges across multiple automated platforms would facilitate acceptance of automated cell counts in clinical practice. However, this outcome might not be possible at present, given the variety of automated analyzers currently available and the rapidly evolving technology. The issue of uniform

### Table 4

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Sysmex RBC</th>
<th>Maximum Manual RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>0</td>
<td>437</td>
</tr>
<tr>
<td>27</td>
<td>1,000</td>
<td>1,325</td>
</tr>
<tr>
<td>5</td>
<td>2,000</td>
<td>1,750</td>
</tr>
</tbody>
</table>

* Data are given as cells per microliter.
reference ranges is important for several reasons. Many cooperative clinical protocols, particularly those for pediatric acute leukemia, use the existing manually derived reference ranges to define CNS involvement by leukemia. Therefore, multi-institutional studies with well-defined sample inclusion criteria that compare a variety of hematology analyzers are needed to realistically determine if standardized, automated CSF reference ranges can replace existing reference ranges. Clinical collaboration in these studies will facilitate acceptance of new reference ranges and the adaptation of clinical protocols for CNS diseases.

From the Departments of 1Pathology and 2Pediatrics, University Hospitals Case Medical Center, Cleveland, OH 44106.

Address reprint requests to Dr Sandhaus: Dept of Pathology, University Hospitals Case Medical Center, 11100 Euclid Ave, Cleveland, OH 44106.

References


