Performance of an Automated Immature Granulocyte Count as a Predictor of Neonatal Sepsis

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Abstract

Neonatologists use immature granulocytes (IG) in manual differential counts as an indicator of sepsis. This study was designed to compare the predictive ability of automated vs manual IG counts for neonatal sepsis. Infants undergoing sepsis evaluation were identified prospectively for study if a CBC count was obtained in temporal proximity to the blood culture. Automated IG counts were obtained from the research software of the Sysmex XE-2100 (Sysmex, Kobe, Japan). Manual average IG counts were obtained from two 100-cell manual differential counts independently performed by a technologist and a hematopathology resident. A comparative analysis of manual and automated IG counts showed considerable overlap of ranges. The highest positive blood culture rate occurred in the nonneutropenic preterm subset of infants older than 7 days (21/55 [38%]). For these infants, elevated IG counts by manual and automated methods were associated significantly with positive blood culture results (odds ratio, manual, 3.74; odds ratio, automated, 3.63), albeit with low sensitivity.

The detection of a granulocytic left shift in manual differential counts often is used as an indicator of sepsis in infants. A left shift traditionally has been defined as an elevated neutrophil band count or an elevated immature/total granulocyte (I/T) ratio.1-3 However, in the pertinent published literature, the validity of these granulocyte parameters as predictors of neonatal sepsis continues to be controversial owing to the use of inconsistent criteria for defining left shift and sepsis. The statistical imprecision of 100-cell manual differential counts combined with subjective morphologic criteria and interobserver variation make band counts unreliable.1,4,5 In addition, it is extremely difficult to determine reference ranges for immature granulocytes (IGs) in infants because these cells undergo rapid fluctuation during the first 5 days of postnatal life,2 and it is difficult to obtain samples from infants. Many earlier studies of diagnostic tests in neonatal sepsis have had flaws in study design and statistical analysis.1,4 In a systematic review of the literature on diagnostic testing for neonatal sepsis, Fowlie and Schmidt4 concluded that “[e]ven in rigorous studies, the reported accuracy of the tests varies enormously and they are of limited value in the diagnosis of infection in this population.”

Despite controversy about the predictive value of band counts and I/T ratios, their use persists in clinical practice. A more reliable method for determining the presence of a granulocytic left shift would be highly desirable. Recently, the ability to count IGs by automated flow-through hematology analyzers has been developed. Automated IG counts offer the potential advantages of improved accuracy, precision, and turnaround time compared with manual differential counts. The purpose of the present study was to compare the predictive ability of the manual IG count and the automated IG count.
produced by the Sysmex XE-2100 hematology analyzer (Sysmex, Kobe, Japan) for the diagnosis of neonatal sepsis.

Materials and Methods

The study was approved by the institutional review board of the University Hospitals of Cleveland, Cleveland, OH. From August 1 through December 1, 2003, infants from the neonatal intensive care unit (NICU), NICU step-down unit, or newborn nursery were identified prospectively for inclusion in the study if they had 1 or more CBC counts obtained within 48 hours of a blood culture. In addition, CBC counts from infants who had urine or cerebrospinal fluid (CSF) cultures during this period were included in a comparative analysis of manual and automated IG counts. The CBC samples were obtained by heel stick, collected in microtainer tubes, and analyzed on a Sysmex XE-2100 automated hematology analyzer in the manual mode within 4 hours of collection. The Sysmex absolute neutrophil count (ANC) includes segmented neutrophils, band neutrophils, and all IGs. The Sysmex IG count includes promyelocytes, myelocytes, and metamyelocytes and is available on the Sysmex XE-2100 research screen for research purposes only.6,7 The IG count is performed in the leukocyte differential channel. A surfactant increases permeability of leukocyte cell membranes, which allows a poly-methine dye with high affinity for nucleic acid to enter the cells. When excited by a 633-nm laser beam, the stained cells emit fluorescence proportional to their content of nucleic acid. IGs show an intense fluorescence that permits their separation from mature neutrophils.7,8

Blood smears were made by the automated slide maker/stainer (SP100, Sysmex), if any CBC or differential parameters were flagged by the analyzer or if results were outside acceptable ranges, according to usual laboratory procedures. Samples that did not have a blood smear or manual differential count performed were not included in the study. A 100-cell manual differential count was performed on each blood smear by various technologists in the University Hospitals of Cleveland Core Laboratory. An independent 100-cell manual differential count was done on each blood smear by a hematopathology resident (K.G.N.), who was blinded to the Sysmex IG count and the results obtained by the technologists. Blood smears for which there were discrepant manual vs Sysmex IG counts were reviewed by a hematopathologist (L.M.S.) for verification of manual results. The average IG percentage and absolute IG number were calculated for each CBC count based on the 2 manual differential counts. The manual IG count included promyelocytes, myelocytes, and metamyelocytes but not bands.

Blood culture samples were obtained by venipuncture or through indwelling catheters using sterile technique, collecting approximately 1 mL of blood into PEDS PLUS/F vials (Becton Dickinson Diagnostic Systems, Sparks, MD). Vials were incubated in the BACTEC 9240 (BD Diagnostic Systems), and samples flagged as positive had aliquots removed for Gram staining and subculture.

Statistical Methods

The primary outcome measure was the blood culture result. The primary independent variable was the IG count, measured by manual and automated methods. Secondary variables included the ANC and the calculated IG/ANC, using values taken directly from the Sysmex XE-2100 output. Characteristics of interest for the infants included gestational age (term vs preterm), neutropenic status, and day of life. For purposes of analysis, the experimental unit was the blood culture.

The manual IG values represent true “count” data and, therefore, can be described as frequencies and percentages. The automated data represent continuous data and are described as medians, ranges, and selected percentiles. The data were compared by describing the median and range of the automated IG values for each frequency category of manual IG percentages. All available data, including the CBC counts from infants who had urine and CSF cultures, were used for these analyses (N = 293).

Because the presence of any IGs in a manual differential count is considered abnormal, manual IG counts were dichotomized as 0% and >0% for predictive modeling. For automated IG counts, the distribution of the data suggested the cutoff value of 0.5%. This value was verified by a sensitivity and specificity analysis.

The association of the IG counts with outcome was estimated by using regression techniques for binary outcomes that adjust for multiple observations on the same subject (PROC GENMOD, version 8.1, SAS, Cary, NC). Dichotomous measures for the manual IG counts and dichotomous and log10-transformed continuous measures for automated IG percentage and absolute IG number were used. For these analyses, only the data from nonneutropenic premature infants older than 7 days were used. For all other subsets of infants, the absence or very small numbers of positive blood culture results made it impossible to achieve stable parameter estimates. Day of life greater than 7 was chosen as a clinical variable because many infants receive antibiotics during the first week of life.

Results

A total of 233 blood cultures were obtained from 181 infants. The data for 1 infant with fulminant necrotizing enterocolitis who had persistently positive blood culture results despite appropriate antibiotic therapy were excluded from the
study. There were 121 blood cultures from 110 term infants and 112 blood cultures from 71 preterm infants, ranging in postnatal age from 0 to 253 days, with a median age of 8 days on the day a blood culture sample was obtained.

Only 2 (1.7%) of 121 blood cultures were positive in term infants, and both of these were from the same infant. There were 13 blood cultures from 12 preterm infants who were neutropenic on the day of the blood culture, and the results of all of these blood cultures were negative. There were 99 blood cultures from 62 nonneutropenic preterm infants, all of whom had CBC counts less than 24 hours before the blood culture, except for 1 CBC sample that was obtained 24 to 48 hours before a negative blood culture. Of 44 blood cultures in 41 infants who were 7 days old or younger, none were positive. There were 55 blood cultures from 31 nonneutropenic preterm infants older than 7 days, and 21 (38%) of these were positive. The 21 positive blood cultures were from 16 infants. The bacterial organisms included coagulase-negative Staphylococcus species (n = 14), group B Streptococcus (n = 1), and Enterococcus faecium (n = 1). Fungal organisms were Malassezia furfur (n = 1) and Candida parapsilosis (n = 4). The classifications of infants and blood culture results are shown schematically in Figure 2.

A comparative description of manual and automated IG counts included all CBC counts obtained in association with blood cultures and the CBC counts from infants who had urine or CSF cultures (N = 293). Figure 1 shows the distributions of the manual and automated IG values in our data. The manual IG percentage ranged from 0% to 10.5%, and the manual absolute IG number ranged from 0 to 5.9 × 10⁹/L. More than 70% of the manual IG counts were zeros. The automated IG percentage ranged from 0% to 10.0% (median, 0.3%), and the automated absolute IG number ranged from 0 to 1.64 × 10⁶/L (median, 0.03 × 10⁶/L). A descriptive analysis relating ranges of values for automated IG counts with manual IG counts is shown in Table 1. Although the automated IG ranges and medians rise incrementally with each manual IG percentage category, there is considerable overlap of the ranges, which precludes direct conversion from one measure to the other for an individual result. The percentile ranges suggest that for Sysmex IG values less than 0.15 × 10⁹/L or 0.8%, IGs are unlikely to be observed on manual differential counts for infants.

The estimated performance characteristics of the manual and Sysmex IG counts are shown in Table 2. The calculated sensitivities, specificities, and positive and negative predictive values are the same for both methods, in part because the marginal totals (totals of all positive and negative samples) were the same and also because there were equal numbers of true positive and true negative samples. However, the true positive and true negative samples classified by each method are not identical sets of samples. The relatively low sensitivity and positive predictive value of both IG methods allowed for different combinations of observations to produce the same results by chance. It is important to note that the calculations of sensitivity, specificity, and positive and negative predictive values assume independence of data. Because we have multiple observations from the same subject in many cases, our data do not meet these assumptions. Therefore, these calculations represent an approximation of the true test performance characteristics, unadjusted for any possible intrasubject correlation.

The associations of IG counts with blood culture results are given as odds ratios and 95% confidence intervals in Table 3. These results indicate that values higher than the cut point for the manual IG percentage and the Sysmex IG percentage are associated significantly with positive blood culture results, despite the relatively poor sensitivities of the 2 methods. An infant is about 3.74 times more likely to have a positive blood culture result if any IGs are found in the routine manual differential count than if there are no IGs. Similarly, an infant is 3.63 times more likely to have a positive blood culture result if more than 0.5% IGs are reported by the Sysmex XE-2100. When the Sysmex IG percentage and absolute IG number were analyzed as log₁₀-transformed continuous variables, they also showed significant predictive ability for sepsis, although the confidence intervals were wider. For each increment in the Sysmex IG percentage, the odds of sepsis...
Classification of study population. There is a 1.7% (2/121) positive blood culture rate in term infants, a 0% (0/13) positive blood culture rate in preterm neutropenic infants, a 0% (0/44) positive blood culture rate in preterm nonneutropenic infants 7 days or younger, and a 38% (21/55) positive blood culture rate in preterm nonneutropenic infants older than 7 days.

Table 1
Percentile Ranges of Sysmex Immature Granulocyte Values by Manual Percentage in 293 Neonatal Blood Samples*

<table>
<thead>
<tr>
<th>Manual Immature Granulocytes (%)</th>
<th>No. (%) of Samples</th>
<th>Immature Granulocytes by Sysmex</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>208 (71.0)</td>
<td>Percentage</td>
</tr>
<tr>
<td>0.5-1</td>
<td>50 (17.1)</td>
<td>0.0-0.8 (0.2)</td>
</tr>
<tr>
<td>&gt;1-3</td>
<td>20 (6.8)</td>
<td>0.0-1.3 (0.4)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>15 (5.1)</td>
<td>0.15-5.3 (0.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.60-10.0 (1.2)</td>
</tr>
</tbody>
</table>

* Data are given as range of the 5th to 95th percentile (median) unless otherwise indicated. For proprietary information, see the text.

Table 2
Sensitivities, Specificities, and Predictive Values of Immature Granulocyte Percentages by Manual and Sysmex Methods for Positive Blood Cultures*

<table>
<thead>
<tr>
<th>Immature Granulocyte Value</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual, &gt;0% or automated, &gt;0.5%</td>
<td>7 (TP)</td>
<td>4 (FP)</td>
<td>11</td>
</tr>
<tr>
<td>Manual, 0% or automated, ≤0.5%</td>
<td>14 (FN)</td>
<td>30 (TN)</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>34</td>
<td>55</td>
</tr>
</tbody>
</table>

FN, false-negative; FP, false-positive; TN, true-negative; TP, true-positive.

* Sensitivity, 33%; specificity, 88%; positive predictive value, 64%; negative predictive value, 68%. The 21 positive blood culture results were from 16 infants and the 34 negative blood culture results from 23 infants. The calculated performance characteristics were the same for both methods because there were equal numbers of TP, TN, FP, and FN samples by both manual and automated methods. However, these were not the identical sets of samples. Note: The calculations are not adjusted for multiple observations on the same infant and, therefore, represent estimates of the true values. For proprietary information, see the text.
increased about 6-fold. Neither the Sysmex ANC nor the Sysmex-derived IG/ANC were significant predictors of a positive blood culture result because the confidence intervals around the odds ratios for both of these variables included the null value of 1.

Discussion

Our data posed several challenges for statistical analysis. The first was related to the distribution of the data. The individual values that are recorded from traditional 100-cell manual differential counts are whole numbers and represent true "count" data. The distribution of the IG values obtained by this method can be viewed conveniently as frequencies, as shown in Figure 1. Automated methods, on the other hand, count thousands of cells, and the resulting distribution can be considered continuous data. Both distributions are highly skewed, with the majority of values being zero for the manual method and somewhat greater than zero for the automated method. For these reasons, linear regression, regardless of which particular regression method is used, is not appropriate to compare these data because the prerequisite assumptions are not met.

We chose to compare the 2 IG methods by describing the range of values for automated IG counts that corresponded to manual IG counts. The stepwise progression of the median Sysmex IG count for each increment of the manual IG percentage indicates that there is a relationship between these measures. However, the considerable overlap of the ranges limits direct conversion from one measure to the other. A possible explanation for this phenomenon is that the 2 methods might not be reliably counting the same cells in all cases. The manual method defines IGs by morphologic criteria using light microscopy and is an inherently imprecise method owing to the low number of cells counted. The Sysmex method uses differential nuclear fluorescence patterns to define mature neutrophil and IG clusters and potentially is a more precise method owing to the large numbers of cells evaluated.6-8 However, it is possible that the IG gating strategy used by Sysmex in this version might not be able to adjust for subtle sample-to-sample variation in cell fluorescence and scatter characteristics.

Recently, Sysmex introduced modified IG software, called IG Master, which has been validated against a flow cytometric “gold” standard and is approved for clinical use.8-10 This new software uses a flexible gating strategy, which might produce more accurate IG counts. Weiland et al9 demonstrated a good correlation between 400-cell manual IG counts and the Sysmex IG counts using the IG Master method. The IG Master was not commercially available in the United States at the time that our study began.

Sensitivity, specificity, and positive and negative predictive values traditionally are used in laboratory medicine to characterize the usefulness of a diagnostic test. However, these measures assume independent observations. In our study, we frequently had multiple observations from the same subject, and, therefore, our data did not meet the assumption of independence. Nevertheless, the estimated sensitivity seems to be low, about 33%. Therefore, an elevated IG count seems to be a poor independent predictor of sepsis. The estimated specificity of 88% makes the absence of IGs on manual differential counts or automated IG counts less than 0.5% predictors of negative blood culture results.

Despite the poor sensitivities of both IG methods, we found a significant association between elevated IG counts and positive blood culture results in hospitalized premature infants who are older than 7 days. These infants are more than 3 times as likely to have a positive blood culture result if any IGs are present in the manual differential count or if the Sysmex IG count exceeds 0.5%.

To date, there are few published clinical studies using the updated Sysmex IG Master method. In one study, Briggs et al10 selected CBC samples with normal ANC and elevated IG counts (defined as >2% IGs) for correlation with other markers of inflammation. They evaluated 84 samples from 43 patients, and 28.6% of these samples were from patients with documented infection. The patients with normal ANC and more than 2% IGs had higher mean C-reactive protein values

**Table 3**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual immature granulocyte values (dichotomized as 0% and &gt;0%)</td>
<td>3.74 (1.3-10.84)</td>
</tr>
<tr>
<td>Sysmex immature granulocyte values</td>
<td></td>
</tr>
<tr>
<td>Percentage (dichotomized as ≤0.5% and &gt;0.5%)</td>
<td>3.63 (1.3-10.0)</td>
</tr>
<tr>
<td>Percentage (continuous log10 scale)</td>
<td>6.25 (1.24-31.48)</td>
</tr>
<tr>
<td>Absolute No. (continuous log10 scale)</td>
<td>4.73 (1.17-19.26)</td>
</tr>
<tr>
<td>Absolute neutrophil count (ANC)</td>
<td>2.13 (0.30-14.84)</td>
</tr>
<tr>
<td>Immature granulocyte/absolute neutrophil count (IG/ANC)</td>
<td>2.83 (0.81-9.88)</td>
</tr>
</tbody>
</table>

* For proprietary information, see the text.
† Not significant.
and erythrocyte sedimentation rates than a control group of samples that were selected for normal ANCs and less than 2% IGs. The method of sample selection used in this study⁴⁰ precludes calculation of sensitivity, specificity, or predictive values for inflammation, because only samples with elevated IG counts were evaluated. Their analysis did not adjust for multiple observations on the same subject, which might have biased any apparent relationship between the IG counts and the other markers of inflammation.

Ansari-Lari et al⁹ selected 142 adult inpatients and emergency department patients who underwent sepsis evaluations and 29 randomly selected, presumably uninfected outpatients as control subjects. In their study, the automated IG count performed no better than the ANC as a predictor of infection. Receiver operator characteristic curve analysis indicated a sensitivity of about 35% to 40% with a specificity of 90%, values remarkably similar to those obtained in our study.

There are many reasons why the IG count by the manual or automated method might not perform well as a predictor of infection or sepsis in hospitalized infants. Most premature infants in the NICU have received antenatal steroids, which will increase the ANC and IG count.¹¹-¹³ Elevated IG counts might be a nonspecific response to physiologic stresses associated with prematurity.

A very significant finding in the present study is that not 1 of the premature infants who were 7 days old or younger had a positive blood culture result. The most likely explanation for this striking observation is that the majority of preterm infants are exposed to antenatal and postnatal antibiotics. Preterm infants with neutropenia were excluded from the analysis because none had positive blood culture results. Neutropenia is associated with neonatal sepsis.²,¹⁴-¹⁶ However, none of the neutropenic infants in this study had a positive blood culture result. The use of a positive blood culture result as the criterion for sepsis might have misclassified infants who actually had sepsis but had negative blood culture results owing to insufficient sampling or concurrent antibiotic therapy. If such misclassifications occurred, this might have biased the results, and the direction of the bias cannot be determined.

There are some important ways in which our study differs from previously published studies relating granulocyte counts to neonatal sepsis. First, our descriptive analysis of the patient population allowed us to define a subset of patients for whom the predictive model was likely to be useful. Second, many earlier investigators neglected to adjust for multiple observations on individual subjects in their statistical analyses.¹⁴,¹⁰ The false presumption of independent data can bias the statistical measure of association. A dramatic example of this effect was demonstrated in our own data. We also analyzed the automated nucleated RBC (NRBC) count produced by the Sysmex XE-2100 as a potential predictor of sepsis. When all observations were treated as independent data, the NRBC count seemed to be a highly significant predictor of sepsis, which was an unexpected result. This apparent association disappeared entirely after adjusting for multiple observations on the same subjects. The explanation for this effect is that data for a few infants with positive blood culture results and high NRBC counts skewed the data.

A third difference is in the way in which the manual differential counts were performed. In the large study by Manroe et al² that established the reference ranges for infants in the I/T ratio and that is considered a classic reference in the field, the investigators relied on a single experienced technologist to perform more than 90% of the manual differential counts. However, this approach to achieving consistency does not reflect actual practice patterns in which manual differential counts are more likely to be performed by multiple individuals in any one laboratory. Previous studies using manual differential counts also included band counts, which since have been shown to be unreliable and were not included in manual IG counts in this study.

Our results demonstrate equivalence of the predictive abilities of IG counts produced by the traditional manual differential count and by the automated Sysmex method for neonatal sepsis. Elevated IG counts by either method seem to be rather poor predictors of sepsis. Perhaps their greatest use in this clinical context is the negative predictive value associated with the absence of IGs in manual differential counts or automated values less than 0.5%. Although their predictive abilities are similar, there might not be a one-to-one correspondence of positive and negative results for individual patients by the 2 methods. Additional studies of the Sysmex and other automated IG methods using larger sample sizes of high-risk patient populations are needed to more fully evaluate the clinical usefulness of automated IG counts.

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