Do the Flags Related to Immature Granulocytes Reported by the Sysmex XE-5000 Warrant a Microscopic Slide Review?

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Key Words: Sysmex XE-5000; IG count; “Imm Gran?” flag; Analytical quality

ABSTRACT

Objectives: The Sysmex XE-5000 instruments (Sysmex, Kobe, Japan) count immature granulocytes (IGs) and use the “Imm Gran?” flag to signal unreliable results. This study investigated the usefulness of the “Imm Gran?” flag and the analytical and diagnostic performance of the IG measurements in a side-by-side evaluation.

Methods: In total, 408 samples were analyzed on three XE-5000 instruments. The IG count and the “Imm Gran?” flag reports from all three instruments were used for reproducibility studies. The diagnostic performance of the automated IGs and the “Imm Gran?” flag were studied by comparing the XE-5000 results with the results of the manual differential.

Results: The reproducibility of the “Imm Gran?” flagging between instruments was poor (κ, 0.75-0.80). The most significant contributor to the report of the “Imm Gran?” flag was bands, and the flag played a minor role in detecting blasts. The interinstrument reproducibility of the IG counts was high (intraclass correlation, 0.99). The IG count reported by XE-5000s was higher than the manual IG count (36%-55%), and the difference and the variability tended to increase with increasing levels of IGs.

Conclusions: The “Imm Gran?” flag has a poor analytical quality and gives no substantial information on the presence of blasts in the sample. We therefore suggest reporting the automated IG count without initial microscopic slide review.

Modern automated hematology instruments, in addition to a fully five-part differential, also provide characterization and counting of abnormal cells in the peripheral blood, such as blasts, atypical lymphocytes, and immature granulocytes (IGs).

The Sysmex XE-5000 (Sysmex, Kobe, Japan) is a fully automated hematology instrument that counts IGs (metamyelocytes, myelocytes, and promyelocytes) in the differential (DIFF) channel based on flow cytometry. In addition, information on immature cells is derived from the immature myeloid information (IMI) channel based on radiofrequency and direct current measuring principles. In the IMI channel, not only IGs but also bands and myeloblasts are detected. If a difference occurs between the numbers of cells detected in the DIFF channel and the IMI channel, a suspect IG flag (“Imm Gran?”) is reported in addition to the immature count derived from the DIFF channel. A flag report indicates the presence of abnormal cells, a finding that, according to the current recommendations, has to be verified by a manual evaluation of cell morphology.

According to our standard procedure, a blood smear shall be examined for (1) all samples with an “Imm Gran?” flag and (2) all samples with a relative IG count of 5% or more (presented as an “IG Present” flag). The purpose of the examination is to verify the presence of IGs and to identify or exclude the presence of other pathologic cells in peripheral blood such as blasts. The microscopic slide review is not supposed to replace the automated IG count with a manual IG count.

To our knowledge, no studies have investigated the quality and the usefulness of the IG-related flags. The aim of this study was thus to evaluate the analytical and diagnostic performance of IG measurements as well as the usefulness of...
the IG flag reports on the Sysmex XE-5000, questioning the need for a manual slide review or a manual count of samples with a flag report. Three XE-5000 instruments were evaluated side-by-side. The interinstrument agreement and the accuracy of the IG counts, as well as the usability of the IG flag reports, were studied by comparing with a manual differential.

**Materials and Methods**

**Sample Selection and Study Design**

At Akershus University Hospital, Oslo, Norway, the routine hematology samples are analyzed randomly on one of three Sysmex XE-5000 instruments (hereafter, XE1, XE2, and XE3) integrated onto a track-based automation system. The instruments were purchased at the same time, calibrated and harmonized by the manufacturer, and used with identical software packages, including firmware upgrades. During a period of 5 months, 408 samples from the daily workload were selected and reanalyzed on all three instruments. For the purpose of this study, all samples were kept anonymous. Only samples reported initially with one or more of the four suspect flags, blasts, IGs, atypical lymphocytes, and abnormal lymphocytes/lymphoblasts were included. The triggering thresholds for the suspect flags were all factory settings. The IG count and the flag report results from all three instruments were used for the study of interinstrument agreement. The diagnostic performance of the IG count and the “Imm Gran?” flag were determined by comparing the results reported by the instruments with the results from a blood smear examination.

**Blood Sampling and Analytical Conditions**

Venous blood samples were collected in Vacuette tubes (Greiner Bio-One, Frickenhausen, Germany) containing dipotassium EDTA. The specimens were transported to the laboratory by a pneumatic tube transporting system and processed on the Sysmex XE-5000 instruments using software version 00-04 in “closed mode” within 4 hours after collection. The instruments were continuously involved in the routine workload during the study period, and their performance was monitored with an internal quality control (QC) system, including Sysmex’s QC material e-Check and an external quality surveillance program. The QC system, based on the e-Check, includes evaluation of the parameter IG number and IG percentage but not the instrument’s flagging performance.

**Sysmex XE-5000**

The Sysmex XE-5000 provides WBC differential counts based on an optical principle, including forward, side scatter, and side fluorescence. The XE-5000 has a DIFF channel, which identifies and counts IG cells in addition to the standard five-part differential populations. The number of cells the instrument routinely counts in each sample to generate a differential cell count depends on the total number of WBCs, the dilution ratio, and the counted volume. At a WBC count value of 5.0 × 10⁹/L, 3,922 cells are counted in the DIFF channel. The IG counts derived from the DIFF channel include metamyelocytes, myelocytes, and promyelocytes. The software reports both the absolute and the relative IG counts.⁴ To alert crucial levels, one can flag the samples with a user-defined flag, termed “IG Present,” which occurs when the absolute or relative IG count exceeds the user-defined threshold. In a separate IMI channel, not only IGs but also bands and myeloblasts are detected. The number of cells the instruments routinely counts using radiofrequency in the IMI channel, at a WBC count value of 5.0 × 10⁹/L, is 5,000 cells. The instrument software examines the scattergrams from both the DIFF and the IMI channels. If there are any disagreements in the information derived from the scattergrams, the IG flag algorithm comes into operation and the “Imm Gran?” flag will be triggered. The IG count derived from the DIFF channel will, however, still be reported. A Q value provides information on the degree of positivity or negativity of the IG flag on a scale of 0 to 300, with increments of 10 arbitrary units. At a given threshold, the instrument will report the “Imm Gran?” flag. The factory setting threshold is preset to an arbitrary value of 100 and is user adjustable. In this study, the factory setting threshold setting was 100 for triggering the “Imm Gran?” flag, and the user-defined IG percentage threshold for triggering the “IG Present” flag was 5%.

**Manual Differential Leukocyte Count**

For each sample, two blood smears were prepared and stained with May-Grünwald-Giemsa using the Sysmex SP-1000. A total of 200 cells were counted by two highly trained technicians, each counting 100 cells. The total count of metamyelocytes, myelocytes, and promyelocytes represented the IG count. The mean IG percentage was calculated for each sample based on the two manual differential counts. The absolute count was computed by the WBC count obtained from the XE-5000. To determine a positive smear finding, we used the criteria listed by the International Consensus Group for Hematology Review.³ The presence of a single myelocyte/promyelocyte (0.5%) and/or two metamyelocytes (1%) qualified for a true-positive smear finding.

**Statistical Analysis**

Statistical analyses were performed using the SPSS software version 16 (SPSS, Chicago, IL) for Windows (Microsoft, Redmond, WA) and Analyse-it version 2.26 (Analyse-it Software, Leeds, England). A two-tailed P value...
of less than .05 was considered statistically significant. To describe the data, the IG counts were summarized as median and range before the analysis of concordance. The intraclass correlation coefficient (ICC [2,1], two-way random, single measures) was calculated to quantify the interinstrument reliability of the IG counts, and κ values were used to determine interinstrument reliability for the “Imm Gran?” flag. The diagnostic performance of the automated IG counts was examined by receiver operating characteristic (ROC) curve analyses and compared for all three instruments with respect to smear findings. Passing-Bablok regression was used for the method comparison between the Sysmex XE-5000s and the manual differential counts. Bland-Altman analysis was used to determine the degree of agreement between the Sysmex XE-5000s and the manual differential IG counts. Pearson correlation coefficient was used to determine the association between the two variables. Logistic regression was used to assess the association between the predictors; band, metamyelocytes, myelocytes, promyelocytes, and blasts analyzed by manual microscopy of the smears; and the IG flag generated by the Sysmex XE-5000 as the dichotomous dependent outcome. All significant variables in the univariate analysis were included in the multivariate analysis to adjust for confounders.

**Results**

The results of the leucocyte counts of the 408 samples performed on the three Sysmex instruments varied from leukopenia to severe leukocytosis (0.5-207 × 10^9/L) with a median of 9.9 × 10^9/L and mean of 20.3 × 10^9/L. A manual differential count of the samples showed metamyelocytes (≥1% cells) in 133 samples, myelocytes (≥0.5% cells) in 280, promyelocytes (≥0.5% cells) in 41, and blasts (≥0.5% cells) in 48. No IGs were found in 31% of the samples, while one, two, or three of the populations of myeloid cells were found in 34%, 38%, and 7% of the samples, respectively. The agreement between the two manual differential IG cell counts in the 408 cases, presented as an ICC, was 0.903 (95% confidence interval [CI], 0.884-0.920).

**Samples With the “Imm Gran?” Flag**

Based on the factory setting thresholds for flagging, the XE1, XE2, and XE3 instruments reported the “Imm Gran?” flag for 81, 80, and 77 samples, respectively. The κ values for agreement between the pair of instruments were 0.79 (XE1/XE2), 0.75 (XE1/XE3), and 0.80 (XE2/XE3) Table 1. One or more instruments flagged for “Imm Gran?” in 102 samples. The three instruments showed full agreement in reporting the flag in 60 (59%) samples. Blast cells were detected by manual counting in 17 (XE1), 17 (XE2), and 18 (XE3) of the samples reported with an “Imm Gran?” flag. For most of these samples (16, 15, and 16, respectively), a “Blast?” flag was also reported. Only one to two of the 48 samples with blasts present in the blood smear were reported with an “Imm Gran?” flag as a lone flag. For these samples, one of the technicians observed one blast by the 100-cell manual differential count, corresponding to the presence of 0.5% circulation blasts in peripheral blood. In addition, in one to two of the 48 samples with blasts present in the blood smear, neither the “Imm Gran?” nor the “Blast?” flag was reported by any of the instruments. For one of these samples, one of the technicians observed one blast by the 100-cell manual differential count, corresponding to the presence of 0.5% circulation blasts in peripheral blood. Case 2 was a leukopenic sample (0.9 × 10^9/L).

The odds ratios (ORs) for association between the myeloid cells observed in the blood film and the “Imm Gran?” flag reported by XE1 are presented in Table 2. The most significant contributor to the report of the “Imm Gran?” flag is bands. The odds for a flag report increase by 8.2% per percentage increase in myeloblasts analyzed by manual microcopy of the smears; and the IG flag generated by the Sysmex XE-5000 as the dichotomous dependent outcome. All significant variables in the univariate analysis were included in the multivariate analysis to adjust for confounders.

### Table 1

<table>
<thead>
<tr>
<th>Pair of Instruments</th>
<th>κ²</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>XE1/XE2</td>
<td>0.79</td>
<td>0.71-0.87</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>XE1/XE3</td>
<td>0.75</td>
<td>0.67-0.83</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>XE2/XE3</td>
<td>0.80</td>
<td>0.73-0.88</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 1: Interinstrument Reliability of the “Imm Gran?” Flag Reports Generated by Three Sysmex XE-5000 Instruments

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bands</td>
<td>1.082</td>
<td>1.046-1.119</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Immature granulocytes</td>
<td>1.103</td>
<td>1.049-1.160</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>1.044</td>
<td>1.019-1.070</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Table 2: Logistic Regression Analyses of the Risk of the “Imm Gran?” Flag Generated by Sysmex XE-5000 (XE1) Based on the Myeloid Cell Proportions (%) in Blood Smears

CI, confidence interval; OR, odds ratio.

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The results from the other two instruments were similar (results not shown).

An IG count was reported for 46% to 52% of the samples with an “Imm Gran?” flag by the XE-5000 instruments. On the XE1, the IG count was 55% higher than the smear-based count \(y = 1.55x \text{ [95% CI, 1.00 to 3.15]} + 0.00 \text{ [95% CI, –0.09 to 0.01]} \). Similar results were found for the XE2 and the XE3 (data not shown). The difference between the two methods as well as the variability tended to increase with the level of the IG counts (data not shown).

Samples With No “Imm Gran?” Flag

For the 306 samples with no “Imm Gran?” flag, an IG count result was reported for 289, 295, and 279 samples analyzed by XE1, XE2, and XE3, respectively. The IG counts ranged from 0 to \(4.7 \times 10^9/L\) with a median of \(0.2 \times 10^9/L\) and a mean of \(0.4 \times 10^9/L\). Table 3 presents the regression analysis of the results of the manual IG count vs the automated count on the three XE-5000 instruments. The coefficient of correlation varied between pairs of instruments indicates poor reproducibility of the “Imm Gran?” flag, supporting the conclusion that the “Imm Gran?” flag is not a sufficient criterion for a microscopic review. The analysis of the same samples on three instruments revealed low concordance in generating the “Imm Gran?” flag. In only 60% of cases, all three instruments reported the IG flag on the same samples. These findings are difficult to explain because all samples were analyzed at the same time and under the same conditions on all three instruments to reduce the factors that usually influence comparative evaluations. In addition, the instruments were under a continuous QC system. It is, however, a problem that the commercial systems’ flagging performance. With a common Q value cutoff at 100, the observed \(k\) value (0.75-0.80) for agreement between pairs of instruments indicates poor reproducibility of the “Imm Gran?” flag, supporting the conclusion that the analytical performance of the flag is low. In general, the usefulness of instrument-generated flags is evaluated by their sensitivity and specificity. However, on the basis of the present findings, we suggest that the interinstrumental reproducibility in reporting flags also has to be taken into account when the usefulness of flag is evaluated.

### Table 3

**Mean, Median, and Range for Immature Granulocyte (IG) Counts Reported by Three Sysmex XE-5000 Instruments**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Mean, (\times 10^9/L)</th>
<th>Median, (\times 10^9/L)</th>
<th>Range, (\times 10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XE1</td>
<td>0.44</td>
<td>0.15</td>
<td>0.4-2.8</td>
</tr>
<tr>
<td>XE2</td>
<td>0.43</td>
<td>0.15</td>
<td>0.4-2.9</td>
</tr>
<tr>
<td>XE3</td>
<td>0.46</td>
<td>0.18</td>
<td>0.4-3.1</td>
</tr>
</tbody>
</table>

*In total, 306 samples were measured on each instrument (XE1, XE2, and XE3).*
An interesting observation in this study is that the most important contributor to the report of the “Imm Gran?” flag is the presence of band cells. It may thus be questioned whether the “Imm Gran?” flag also is a measure of left-shift. Consequently, depending on the actual guidelines, the presence of band cells may result in a significant delay in the report of results.

The present data also show that the “Imm Gran?” flag plays only a minor role in detecting blasts. In samples with an “Imm Gran?” flag, blasts were detected by slide review in approximately 20% of the samples. In 88% to 94% of these, a “Blasts?” flag was reported together with the “Imm Gran?” flag. In accordance with previous results, in approximately 5% of all samples with blasts, no “Blasts?” flag was reported.

The “IG Present” flag is reported when the IG count from the DIFF channel is increased above a given threshold and thus depends only on the analytical quality of the IG count and the level of the threshold. The present data clearly demonstrate that the “IG Present” flag gives almost no information on the presence of blasts in addition to the “Blasts?” flag. In samples with blasts detected by a manual slide review, a “Blasts?” flag in 93% to 100% of the cases accompanied the “IG Present” flag. It is thus questionable whether the presence of the “Imm Gran?” or “IG Present” flag provides sufficient information to warrant a slide review. In this study, up to two of the 48 samples with blasts detected by a manual differential were reported with “Imm Gran?” as the only flag. The consequence of omitting a slide review of samples with “Imm Gran?” is the risk of missing myeloblasts in peripheral blood, which may have an important clinical impact. However, in the two present cases, the smear review revealed only a single blast, observed by one of the two technicians. This discordance could be due to the limitations of the 100-cell manual differential or the fact that one of the

![Figure 1](https://academic.oup.com/ajcp/article-abstract/142/4/553/1767480/DownloadedFromAcademic?view=1)
technicians overlooked the presence of blasts. It is well known that manual cell classification is subjective and has limited application in low-frequency cell populations.\textsuperscript{15,16} Thus, it is difficult to assess the relevance of the single blast detection.

In a previous study, we found that the miss rate of the Sysmex XE-5000 platform in detecting circulation blasts was high, with an observed sensitivity of the “Blasts?” flag from 0.54 to 0.75.\textsuperscript{14} In this context, the loss of clinical value by disregarding the “Imm Gran?” flag seems insignificant. However, the disregard of “Imm Gran?” as the sole reason for manual review will result in a reduced number of smears, simplified routines, reduced use of resources, and a shorter turnaround time. Consequently, there is no obvious rationale for manual examination of blood smears in cases flagged solely for “Imm Gran?”.

The comparison of the Sysmex XE-5000 count with the manual count of IGs (metamyelocytes, myelocytes, and promyelocytes) in samples with no “Imm Gran?” flag revealed a correlation coefficient of 0.82 to 0.89 for the absolute count depending on the three instruments. This is in agreement with previous studies.\textsuperscript{17,18} The comparison between the manual count and the XE-5000 method shows a linear proportional bias, with XE-5000 reporting approximately 35% higher IG counts than the manual method. Similar results were found in samples with an “Imm Gran?” flag. This is in agreement with several previous studies demonstrating that the manual count method underestimates the counting of IGs compared with counting on the Sysmex XE-2100 and by flow cytometry.\textsuperscript{17-19} High imprecision for the manual method at low counts of cells is well known.\textsuperscript{15} However, the present study shows a negative bias as well as a significantly higher variability for the manual method at IG counts of $0.5 \times 10^9/L$ or more. This may be explained by the counting statistics as suggested by Koepke et al,\textsuperscript{16} but it may also be a result of a nonhomogeneous distribution of leukocytes on the blood smears. Wedge techniques for preparing smears may cause nonhomogeneous distribution of leukocytes in the films. Large cells as IGs may be pushed to the edge and to the sides of the blood film and thereby disappear from the counting area.\textsuperscript{20} Another possible reason is that of misclassification. Inadequate classification of metamyelocytes as bands excludes the metamyelocytes from the manual IG count and thereby produces inconsistency between automated and manual IG counting.\textsuperscript{21} It is difficult to assess the significance of the inconsistency since the comparison is halting for many reasons. The instruments have an assumed statistical advantage over the manual count by the larger number of cells counted.\textsuperscript{15} In this study, the agreement observed between the IG counts from the instruments (0.99) was greater than the agreement of IG counts from the two manual counts (0.90). In addition, the XE-5000 measurements had a smaller range of uncertainty. Therefore, although quantitative discordant results are difficult to evaluate, the IG counts from the XE-5000 are supposed to be more accurate than the manual cell counts. Consequently, there is no reason for not using the instrument counts. However, a manual review is needed, which would
yield quantitative results of the separate nonblastic IG types (metamyelocytes, myelocytes, and promyelocytes).

The analytical accuracy of IG counts observed in samples with and without an “Imm Gran?” flag was comparable. These findings indicate that the IG count might be reliable even when accompanied by an “Imm Gran?” flag and can be reported.

The reproducibility of the absolute IG count obtained from the three instruments evaluated by the ICC value was high (0.99). The interinstrument variability between pairs of instruments measured as percent coefficient of variation was also acceptable (14%-19%). These findings are comparable to those of Fernandes and Hamaguchi and Briggs et al for within-run reproducibility of IGs on the Sysmex XE-2100 and indicate that random use of different instruments seems to have a minor influence on the analytical performance with respect to IG counts.

The ability of the instrument count to predict the presence of IGs in smears is very good, with AUCs ranging from 0.918 to 0.931. Manual examination of blood smears does not necessarily count IGs with high accuracy depending on the number of IGs in the sample. In previous studies, difficulties in identifying IG populations by morphology due to a low number of cells have been reported. The manual count of IGs may thus not be the perfect “gold standard” in an ROC curve test. In this study, however, the relatively high numbers of IGs in the samples support the validity of the ROC curve test.

Taken together, the present data confirm a systematic bias in IG counting between the manual slide method and the Sysmex XE-5000 method and that the analytical quality and the diagnostic accuracy of the instrument counting are high. This is an important finding since it has been demonstrated that the IG count may have a central role as an inflammation marker, especially in differentiating between infection and inflammation of other causes.

The agreement between the IG counts from the XE-5000 instruments is good. The IG count from the XE-5000 is higher than that from the manual smear count, and the difference between the two methods increases with an increasing number of IGs. The lack of agreement between the manual IG count and the IG count from the XE-5000 may be linked to errors in the manual counting. The “Imm Gran?” flag has poor analytical quality and gives no substantial information on the presence of blasts in the sample. Therefore, we suggest reporting the IG count from the XE-5000 without smear confirmation.

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