Getting Beyond the Flags: Quantitative assessment of immature granulocyte (IG) populations may improve the assessment of sepsis and inflammation.

Sysmex America
White Paper
Getting Beyond the Flags: Quantitative assessment of immature granulocyte (IG) populations may improve the assessment of sepsis and inflammation.

Introduction

In reviewing hematological laboratory results, the presence of a left shift has been a diagnostic tool that has been utilized by physicians for decades. Clinically, the presence of a left shift or immature granulocytes in the peripheral circulation can be indicative of a number of disorders (Table 1). The problem is that the term left shift can have a variety of definitions. Does it mean the presence of band neutrophils, or the presence of bands, metamyelocytes, myelocytes and promyelocytes. When the left shift is observed and enumerated using a manual 100-cell differential, the results can be imprecise due to so few cells being counted, particularly in leukopenic samples. Furthermore, results are not reproducible between operators because of differences in morphology interpretation.

In recent years, the ability to count the presence of immature granulocytes (metamyelocytes, myelocytes and promyelocytes) by an automated cell counter has come to fruition. The days when a hematology analyzer would produce a suspect flag indicating the possible presence of immature granulocytes and the need for a manual differential, has come to an end. The Sysmex XE-Series and XT-Series analyzers are equipped with a proprietary software algorithm that counts over 32,000 cells and can generate accurate and reproducible quantitative immature granulocyte counts. The instruments are able to identify and provide an absolute count and percent of the immature granulocyte population comprised of metamyelocytes, myelocytes and promyelocytes. These three cell types are separated from the neutrophil population based on their increased fluorescence emission due to higher levels of DNA and RNA. The measurement is made in less than 25 seconds and is reported automatically with the other CBC results.

Table 1.
Disorders associated with immature granulocytes in the peripheral circulation.

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Infection, esp. bacterial</td>
<td>Myelofibrosis</td>
<td>Metastatic bone marrow malignancy</td>
</tr>
<tr>
<td>Severe inflammatory disease</td>
<td></td>
<td>Trauma</td>
</tr>
<tr>
<td>Myelodysplastic syndromes</td>
<td></td>
<td>Acute organ transplant rejection</td>
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<tr>
<td>Myeloproliferative disease</td>
<td></td>
<td></td>
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<tr>
<td>Chronic Myeloid Leukemia (CML)</td>
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</table>
Correlation studies: gaining agreement

Early studies using flow cytometry (1) showed that it is possible to differentiate immature granulocytes from mature forms and that improved accuracy and reproducibility was possible because 10,000 cells could be interrogated versus 100 manually. These studies used flow cytometry and monoclonal antibody labeling to identify immature granulocytes (IGs). CD45, the leukocyte common antigen, was used to detect cells of hematopoietic origin. Promyelocytes and myelocytes were stained with CD16+/CD11b−; metamyelocytes with CD16−/CD11b+; and mature granulocytes with CD16+/CD11b+. This allowed for the measurement of multiple parameters such as light scatter and fluorescent labels rather than morphological characteristics only. Figure 1 shows the flow cytometry results of a patient with immature granulocytes and a normal, healthy volunteer. The authors suggested that this flow cytometry method might be suitable for following cases of infectious disease.

Figure 1.
Flow cytometric analysis of a patient sample containing IG and a sample from a normal, healthy volunteer after staining with CD45-PerCP, CD16-FITC, and CD11b-PE. In the first step, granulocytes (IG + neutrophils + eosinophils) were identified (A). In the second step, neutrophils and IG were separated from eosinophils (B). In the third step, (C), IG stage 1 (CD16−/CD11b−) and stage 2 (CD16+/CD11b+) were identified as well as mature neutrophils (CD16+/CD11b+). No IG were identified in any samples from normal, healthy adult individuals.
The ability to automate the abnormal white cell differential with quantitative results was reported by Briggs et al (2) in 2003. This team compared manual light microscopy, flow cytometry and an upgraded software for identifying and counting IGs: XE-IG Master (Sysmex America, Inc.). The new software allowed the Sysmex XE and XT-Series hematology analyzers to measure cellular light scatter and fluorescence emission in the leukocyte differential channel. An erythrocyte lysing reagent also acts upon white cells to prepare them for staining. A proprietary nucleic acid dye stains leukocytes, and a laser diode measures side-light scatter and side-fluorescence. IGs are distinguished by their high fluorescence intensity and are reported as a % of the leukocyte population and absolute count.

Figure 2 shows the correlation between Flow Cytometry (IG%) and XE-IG Master (IG%) determinations, and Figure 3 shows the correlation between the manual 400-cell differential and the XE-IG Master (IG%).

Briggs et al also reported on the reproducibility of the XE-2100 instrument using the new Master software using 10 repeat analyses on 8 patient samples. For IG%, the mean CV was 7.02% and for absolute count, the mean CV was 6.93%. Samples were stable for up to 48 hours either at room temperature or refrigerated. Fernandes and Hamaguchi (3) reported excellent correlation between flow cytometric counts [Fujimoto method (1)] and the XE-2100 IG count. Correlation coefficients of 0.93 and 0.95 were determined for absolute count and % respectively. Correlation coefficients between the XE-2100 and manual counts were .82 and .80 for absolute and % determinations. Again, samples were stable at room temperature and refrigerated temperatures.

Figure 2.
The correlation of the percentage of immature granulocytes (IG%) determined by the flow cytometry reference method with the IG% determined by the XE-IG Master Method. Flow cytometric IG counting based on a 3-color method using fluorescein isothiocyanate (FITC)-CD16, FITC-CD11b, and peridinin chlorophyll protein-CD45 was used as the reference method.

Figure 3.
The correlation of the percentage of immature granulocytes (IG%) determined by the manual count reference method with the IG% determined by the XE-IG Master method. The manual IG differential based on a 400-cell leukocyte count was used as the reference method.
Acute Phase Reactants

Table 2 lists positive acute phase reactants (APRs) that may be helpful in the assessment of inflammation and infection. Briggs et al (2) looked at the following APRs in their 2003 study: CRP, ESR, CD64, IL-6 and IG counts as determined on the XE-2100 analyzer. Samples with high IG and normal neutrophil counts were studied (n=43). Of these, 15 patients had an infectious disease and 14 had post-operative bleeding. The mean IG count for these 43 samples was 3.9% (range 2.0% – 11.0%) and the mean neutrophil count was 5.21 x 10⁹/L (range 2.01-7.49 x 10⁹/L). The values for CRP, ESR, PMN cell index and IL-6 were all high, but to varying degrees. Those with a high IG count showed good correlation with CRP and the ESR (84% and 95% respectively), despite neutrophil counts in the normal range. (Figure 4)

Figure 4.
Positive sample rates in samples with high immature granulocyte counts (2.0% or higher) and normal neutrophil levels (2.0-7.5 x 10⁹/L). CPR indicates C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6.

Automated Immature Granulocyte Counts on the Sysmex XE-2100

<table>
<thead>
<tr>
<th>Positive Criteria</th>
<th>Number of Samples</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>CRP ≥ 5.0 mg/dL</td>
<td>42 (84.0%)</td>
</tr>
<tr>
<td>ESR ≥ 20 mm/hr</td>
<td>19 (95.0%)</td>
</tr>
<tr>
<td>CD64 ≥ 3.0</td>
<td>61 (80.3%)</td>
</tr>
<tr>
<td>IL-6 ≥ 5.8 pg/mL</td>
<td>37 (57.8%)</td>
</tr>
</tbody>
</table>

Table 2.
Positive Acute Phase Reactants (3, 7)

- WBC (high, low or normal)
- Immature granulocytes (peripheral blood)
- C-Reactive Protein
- Interleukin I (IL-1)
- Interleukin 6 (IL-6)
- Tumor Necrosis Factor (TNF)
- CD-64
- ESR (“sed rate”)
- Ceruloplasmin
- Components of complement cascade
- Cerum amyloid A
- Fibrinogen
- Alpha-1 antitrypsin
- Haptoglobin
- Ferritin
In another study (4), IG% as a predictor of infection or sepsis was assessed using blood samples from 102 infected individuals and 69 non-infected individuals. Analyzed on the Sysmex XE-2100 instrument, IG% was significantly higher in infected versus non-infected patients (Table 3) as well as patients with positive versus negative blood culture results (Table 4). In 11 of 12 patients with IG% values greater than 3%, the specificity of IG% was greater than 90%.

Similar to Brigg's findings, IG% correlated better than the absolute white cell count and was comparable to the absolute neutrophil count, although neither parameter had the sensitivity to be used as a sole screening tool. Rather, it was suggested that the value of an increased IG% of greater than 3% could be useful as part of an algorithm-driven panel of tests.

Another study, this time in pediatric patients (5), compared CRP, IG ratio (IG%/total white cell count) and IT ratios (IG%/total neutrophil count) to see if they could be used as an early indicator of sepsis. In this study, sepsis [Systemic Inflammatory Response (SIRS)] was diagnosed when a patient presented with two or more of the symptoms shown in Table 5.

The IT ratio is a new way of looking at IG% and the authors suggest that it may be “more specific in diagnosing sepsis since it considers not only the absolute number of immature granulocytes but compares it to the total neutrophil count.” In this way, the parameter may be an indicator of the bone marrow activation seen in sepsis.

A total of 58 patients were evaluated: 31 with positive blood culture results; 7 with negative blood cultures, but clinically-diagnosed sepsis; 20 negative for sepsis. The patients considered “septic” had elevated IG% and IT%, the latter particularly raised and in one case elevated to 37.5%. Negative controls did not have ratios higher than 3%. Another group of patients studied were those with negative blood cultures but clinically diagnosed with sepsis, perhaps as a result of antibiotic treatment. All seven of these patients had elevated IG and IT ratios compared to the negative controls.

To determine a viable cut-off point, the authors decided that eliminating false negatives would be clinically more prudent than eliminating false positives and determined the cut-offs to be 0.35 for IG and 0.65 for IT, which would provide a sensitivity of greater than 70% at the best possible specificity. Using the cut-off values of 0.35 (IG) and 0.65 (IT) values, sensitivities were 74% (Table 5). The IT ratio appeared to be somewhat more sensitive and had better positive and negative predictive values than the IG ratio, perhaps because it is calculated using the neutrophil count rather than the leukocyte count, providing better sensitivity in neutropenic samples. By following patients that had 3-days pre-diagnosis, day of diagnosis and 3 days post-diagnosis data available, the IT ratio showed a rise higher than that of the IG ratio up to 3 days prior to a positive blood culture.

This study of IG and IT ratios also looked at CRP levels in those patients for whom it was available: 21 of 38 septic patients and 17 of 20 in the negative control group. Using a threshold of greater than 11mg/L for CRP, 68% of the septic patients had an increased CRP, although because of the few subjects, this number should be interpreted conservatively. Nonetheless, it was determined that the sensitivity of CRP, in this study at least, was lower than for IT and IG ratios at 71%. The specificity of CRP was also low at 35% compared to the IT ratio of 50% in this pediatric population.
Table 3.
Comparison of WBC Count and Percentage of Immature Granulocytes Measurements as Predictors of Infection.

<table>
<thead>
<tr>
<th></th>
<th>Infected (n = 102)</th>
<th>Not Infected (n = 69)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Mean ± SD WBC count, /µL (x 10³/L)</td>
<td>10,800 ± 7,000 (10.8 ± 7.0)</td>
<td>8,400 ± 5,000 (8.4 ± 5.0)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Mean ± SD immature granulocyte percentage</td>
<td>1.6 ± 2.8</td>
<td>0.7 ± 0.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Proportion ≥ 1%</td>
<td>50 (49.0%)</td>
<td>14 (20%)</td>
<td>&lt;.0001</td>
</tr>
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Table 4.
Comparison of WBC Count and Percentage of Immature Granulocytes Measurements as Predictors of Sepsis.

<table>
<thead>
<tr>
<th></th>
<th>Culture Positive (n = 91)</th>
<th>Culture Negative (n = 51)</th>
<th>P</th>
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<tbody>
<tr>
<td>Mean ± SD WBC count, /µL (x 10³/L)</td>
<td>10,300 ± 7,300 (10.3 ± 7.3)</td>
<td>10,200 ± 5,500 (10.2 ± 5.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ± SD immature granulocyte percentage</td>
<td>2.0 ± 3.6</td>
<td>0.7 ± 0.8</td>
<td>.005</td>
</tr>
<tr>
<td>Proportion ≥ 1%</td>
<td>39 (43%)</td>
<td>12 (24%)</td>
<td>.02</td>
</tr>
<tr>
<td>Proportion &gt; 3%</td>
<td>11 (12%)</td>
<td>1 (2%)</td>
<td>.04</td>
</tr>
</tbody>
</table>

NS, not significant.

Table 5.
Positive predictive values, sensitivities and specifies of both the IG and IT ratios at their established thresholds.

<table>
<thead>
<tr>
<th></th>
<th>sensitivity</th>
<th>specificity</th>
<th>positive predictive value</th>
<th>negative predictive value</th>
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</thead>
<tbody>
<tr>
<td>IT ratio &gt; 0.65</td>
<td>74%</td>
<td>50%</td>
<td>74%</td>
<td>50%</td>
</tr>
<tr>
<td>IG ratio &gt; 0.35</td>
<td>74%</td>
<td>35%</td>
<td>68%</td>
<td>41%</td>
</tr>
</tbody>
</table>

Summary

Diagnosis is complex in sepsis due to a broad range of non-specific symptoms and an equally broad range of laboratory tests that may help the diagnosis. Microbiologic testing in the form of blood cultures may border on being a “gold standard,” but takes up to four days before results are known. The test can also provide false positive and false negative results, frequently due to faulty blood collection techniques. Therefore, the search for quick and economical alternatives to this and the many tests cited here continues.

Now that the laboratory is able to report the immature granulocyte population with a high degree of accuracy and precision, the clinical utility of the WBC count and differential can significantly increase. Physicians will have additional diagnostic parameters to consider when assessing inflammation and acute infection so that timely intervention with appropriate therapeutics can be made.

Hematologic and biochemical tests can contribute to a diagnosis as noted previously in the Briggs et al study, and the fact that the XE-2100 instrument is able to provide highly precise and accurate immature granulocyte information keeps investigators looking at this parameter.

This comprehensive reporting may help improve laboratory productivity by eliminating manual reviews on systems that rely on “flags” to indicate the need for a manual examination for immature granulocytes, some of which will be false positives. As investigators compile information on IG count and IG% and study these parameters along with other acute phase reactants, it may be possible to develop a diagnostic algorithm for laboratory results to aid in the identification of acute inflammation and sepsis.
References


7. http://www.uptodate.com/patients/content/topic.do?topicKey=-22ip2ecYo1zo7Yp

Disclaimer
The uses or clinical applications described in these publications have not been approved or cleared by the FDA. It is the clinician’s responsibility to validate any off-label applications for use in routine clinical practice.

Notice of Intended Use:
“The Immature Granulocyte (IG) parameter on the Sysmex XE-2100® is intended for in vitro diagnostic use to classify and count immature granulocyte cells in EDTA anticoagulated blood.”