Immature Granulocyte Measurement Using the Sysmex XE-2100

Relationship to Infection and Sepsis

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Abstract

We determined the usefulness of immature granulocyte measurement as a predictor of infection or positive blood culture and compared the results with total WBC count and absolute neutrophil count (ANC). Blood samples from 102 infected and 69 noninfected patients were analyzed using the Sysmex XE-2100 automated blood cell counter (Sysmex, Kobe, Japan). The percentage of immature granulocytes was significantly higher in infected than in noninfected patients and in patients with positive than patients with negative blood cultures. Receiver operating characteristic curves showed that the percentage of immature granulocytes was a better predictor of infection than the WBC count and comparable to the ANC. Automated immature granulocyte measurements reflect a biologically and clinically relevant phenomenon but are not sensitive enough to be used as screening assays for prediction of infection or bacteremia. However, although infrequently encountered, a percentage of immature granulocytes of more than 3 was a very specific predictor of sepsis and might help expedite microbiologic laboratory evaluation of a subset of patients.

Early detection of bacteremia and sepsis facilitates timely initiation of antimicrobial therapy, reduces morbidity and mortality, and decreases health care costs. While there is no true "gold standard" for the detection of sepsis, a positive blood culture in an appropriate clinical context is considered diagnostic.[1] The major disadvantage of blood culture is a relatively long incubation period before establishing the diagnosis. False-negative blood culture results might be caused by inappropriate culturing conditions for fastidious organisms, insufficient quantity of blood, and the presence of antibiotics or other inhibitory factors in the blood sample.[2] Blood cultures are prone to false-positive results arising from errors in collection or processing of the samples.[3] As a result, clinicians have tried to use other laboratory parameters to predict bacteremia and sepsis.

Quantitation of leukocytes, including neutrophils, is important in patients who might have an infection, because of the important role of acute inflammation in combating bacterial infection. Several studies have addressed the usefulness of total WBC count, absolute neutrophil count (ANC), and band count in the prediction of infection.[4-6] The latter value, while a well-known marker of left shift in granulopoiesis, has been notoriously difficult to measure accurately or precisely. A reproducible measurement of immature granulocytes might be a useful parameter to predict the presence of infection or sepsis.

The Sysmex XE-2100 (Sysmex, Kobe, Japan) automated blood counter accurately measures several blood parameters, including 5-part WBC differential count and, unlike earlier counters, also can enumerate, rather than just flag for, immature granulocytes. The goals of the present study were to determine the effectiveness of the immature granulocyte count as a surrogate marker for infection and to determine

whether immature granulocytes could be used as a screening marker to predict positive blood culture results.

Materials and Methods

All blood samples were analyzed within 24 hours of collection. WBC, ANC, percentage of immature granulocytes, and absolute immature granulocyte count were determined from blood samples by the XE-2100 automated blood cell counter.[7,8] The ANC measurement includes total bands and neutrophils. The immature granulocyte measurement, which includes promyelocytes, myelocytes, and metamyelocytes but not bands or blasts, is performed in the differential channel. A lysing reagent causes disruption of mature WBC membranes, leaving bare nuclei, while immature myeloid cells with low cell membrane lipid content remain intact. Subsequently, the cells are analyzed by nucleic acid fluorescence and side scatter; this technology is different from that used in the Sysmex system SE 9500.[9] Data on precision, linearity, carryover, and stability of the XE-2100 have been reported.[7,10,11] Comparison of 465 samples for immature granulocyte count by visual count and by the Sysmex XE-2100 revealed a correlation coefficient of 0.81.[8] Detection of immature granulocytes by the Sysmex XE-2100 showed sensitivity, specificity, and efficiency of 92%, 81%, and 83%, respectively.[12]

The study used samples originally obtained for routine blood count determination. Results of the study were neither documented in patients' charts nor communicated to treating physicians. The study population comprised 142 patients who were admitted to the Johns Hopkins Hospital (JHH) Adult Emergency Department (Baltimore, MD) or already were hospitalized at JHH. For these patients, blood cultures had been ordered by their treating physicians to rule in or rule out sepsis based on various clinical indications. Blood culture bottles were incubated in BacT/Alert 3D (Organon Teknika, Durham, NC), a totally automated system capable of continuous monitoring of aerobic and anaerobic cultures. Blood culture results were finalized after 7 days of incubation. An additional 29 samples were selected randomly from an uninfected outpatient population without available blood cultures. The patients' charts were reviewed retrospectively, and patients were divided into infected (n = 102)

and noninfected (n = 69) groups. Results of blood cultures also were reviewed to determine the presence of sepsis. Not all infected patients were septic. For a selected group of patients with available blood culture results, the WBC, ANC, percentage of immature granulocytes, and absolute immature granulocyte count were measured for 5 consecutive days, and results were correlated with chart review. The study protocol was approved by the JHH Institutional Review Board before initiation.

Results

Usefulness of Immature Granulocyte Measurement in Infection

The WBC count and percentage of immature granulocytes were measured in 102 patients with evidence of infection and 69 noninfected patients ▮**Table 1**▮. WBC counts and the percentage of immature granulocytes were significantly higher in patients with infection. Of the 102 patients with infection, 50 (49.0%) had a percentage of immature granulocytes of 1% or more, whereas the percentage of immature granulocytes was 1% or more in only 14 (20%) of 69 patients without infection (Table 1).

The diagnostic values of the WBC count, ANC, and percentage of immature granulocytes for predicting infection were evaluated by receiver operating characteristic (ROC) curves ▮**Figure 1**▮. These illustrated that the percentage of immature granulocytes had a larger area under the curve than the WBC count and was equivalent to that of the ANC, suggesting that it was a better predictor of infection than the WBC count and comparable to the ANC. However, at 90% specificity, the sensitivity was only about 35% to 40%, and at 90% sensitivity, the specificity was only about 20%.

A subset of 104 patients, including 72 with and 32 without infection, had serial determinations of the percentage of immature granulocytes on each of 5 successive days. Among the noninfected patients, the great majority of those with results less than 1% on day 1 had results that remained low. There was much greater variability among the infected patients, almost all of whom had some therapeutic intervention. However, there was no simple relationship between change in the percentage of immature granulocytes and therapeutic

▮**Table 1**▮
Comparison of WBC Count and Percentage of Immature Granulocytes Measurements as Predictors of Infection

	Infected (n = 102)	Not Infected (n = 69)	*P*
Mean ± SD WBC count, /μL (× 10⁹/L)	10,800 ± 7,000 (10.8 ± 7.0)	8,400 ± 5,000 (8.4 ± 5.0)	<.01
Mean ± SD immature granulocyte percentage	1.6 ± 2.8	0.7 ± 0.8	<.001
Proportion ≥1 %	50 (49.0%)	14 (20%)	<.0001

status; some infected patients who were treated and became afebrile showed a decrease in the percentage of immature granulocytes, but others did not, and still others showed substantial fluctuation during the 5 days without a clear relationship to therapy.

Immature Granulocytes as a Predictor of Positive Blood Culture Result

Blood culture results were available for 142 patients and were positive in 91 patients and negative in 51 patients. ▌Table 2▐ summarizes the WBC count and the percentages of immature granulocytes obtained within 24 hours of the samples submitted for blood culture. On average, there was no significant difference between WBC counts of patients with positive blood culture results and patients with negative results. However, the percentage of immature granulocytes was significantly higher in patients with positive blood cultures. Of the 91 patients with positive blood culture results, 39 (43%) had a percentage of immature granulocytes of 1% or more, whereas the percentage of immature granulocytes was 1% or more in only 12 (24%) of 51 patients with negative blood culture results (Table 2). Although very high percentages of immature granulocytes were uncommon, specificity was high, with 11 (92%) of 12 patients with a percentage of immature granulocytes of more than 3 having proven septicemia. ROC curves also were used to compare the diagnostic value of the percentage of immature granulocytes in predicting infection compared with sepsis; the immature granulocytes test performed better as a predictor of infection ▌Figure 2▐.

Discussion

We evaluated immature granulocytes as measured on the Sysmex XE-2100 automated blood counter as a marker to predict infection and sepsis. The percentage of immature granulocytes was significantly higher ($P < .001$) in infected than noninfected patients, and, among patients clinically suspected of having septicemia, it was significantly higher in patients with positive blood culture results than in patients with negative blood culture results ($P = .005$). These findings indicate
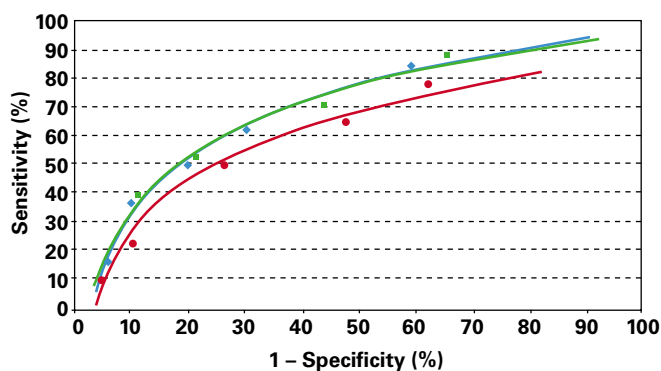

▌Figure 1▐ Receiver operating characteristic curves comparing ability of immature granulocytes (blue), absolute neutrophil count (green), and WBC count (red) to predict infection. The immature granulocyte and absolute neutrophil count curves are superimposable.

that this parameter measures a biologically and clinically relevant phenomenon. However, the percentage of immature granulocytes was neither a sensitive nor a specific assay that could be used to distinguish infected from noninfected patients or to identify patients with positive blood culture results. The use of the absolute immature granulocyte count did not improve the prediction outcome (data not shown).

We compared the performance of the percentage of immature granulocytes with the WBC count and the ANC, 2 parameters that commonly are evaluated in patients suspected of having infection or sepsis. All 3 parameters were significantly higher in patients with infection. However, ROC curves showed that the percentage of immature granulocytes was a better predictor of infection than the WBC count and was comparable to the ANC. ROC curves showed that all 3 parameters have low sensitivity when high specificity is desired and low specificity when high sensitivity is desired. The one possible exception to this general statement would be that the specificity of high percentages of immature granulocytes (>3%) for sepsis was greater than 90%. Although this level was attained in only 12% (11/91) of patients with positive blood cultures, it suggests that such a finding might be used to target careful workup in this subset of patients.

▌Table 2▐
Comparison of WBC Count and Percentage of Immature Granulocytes Measurements as Predictors of Sepsis

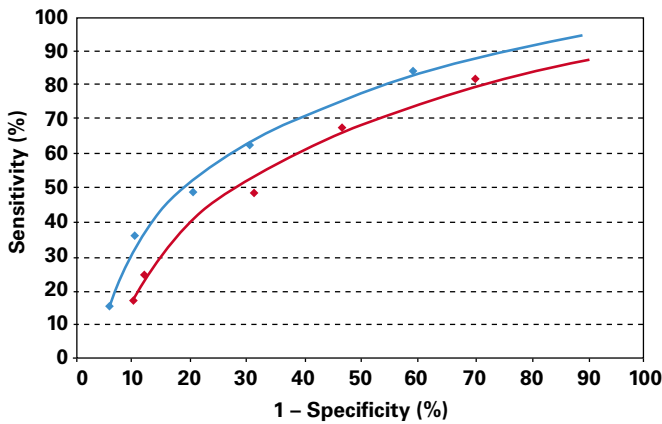|	Culture Positive (n = 91)	Culture Negative (n = 51)	P
Mean ± SD WBC count, /μL (× 10⁹/L)	10,300 ± 7,300 (10.3 ± 7.3)	10,200-5,500 (10.2 ± 5.5)	NS
Mean ± SD immature granulocyte percentage	2.0 ± 3.6	0.7 ± 0.8	.005
Proportion ≥1%	39 (43%)	12 (24%)	.02
Proportion >3%	11 (12%)	1 (2%)	.04

NS, not significant.

Figure 2 Receiver operating characteristic curves comparing ability of the percentage of immature granulocytes to predict patients with infection (blue) as opposed to patients with septicemia (red).

Several studies have assessed the clinical usefulness of quantitative and qualitative changes in leukocytes for predicting infection in pediatric and adult populations.[4-6] Toxic granulation, Döhle bodies, and cytoplasmic vacuoles showed high sensitivity but low specificity for predicting infection.[13] Myeloid progenitor cells were significantly higher in infected than in uninfected patients.[14] Based on ROC curves and multivariant logistic modeling, the combination of automated ANC and band count more accurately predicted bacterial infection than the ANC alone.[15] In 1 report, the manual band count seemed to be superior to the WBC count, the ANC, and the immature to total neutrophil count ratio in detecting infectious disease, although none of the parameters was sensitive enough to be used as a screening tool.[13] However, other reports dispute the clinical usefulness of the band count in the diagnosis of infection.[6] Compared with the band count, the immature granulocyte count and the ANC were better discriminators of infected from noninfected patients with normal total leukocyte counts.[16] Compared with the WBC count and the ANC, the band count was an insensitive indicator of occult bacteremia in infants.[17] In pediatric patients 3 to 36 months of age, the ANC and WBC count were better predictors of bacteremia than the band count.[18]

Overall, the leukocyte-related parameters seem to lack sensitivity or specificity in predicting infection because the elevations of immature granulocyte, neutrophil, and band counts are not specific for infection and may be seen in a variety of other conditions, including chronic inflammatory diseases, neoplasia, acute hemorrhage, hemolysis, tissue damage or necrosis, seizures, metabolic abnormalities, use of certain drugs, and myeloproliferative disorders.[6] In our study, review of medical records showed that 4 of 14 noninfected patients with a percentage of immature granulocytes of 1% or more had received steroids.

Additional markers have been evaluated for the prediction of infectious disease. Acute phase reactants, such as C-reactive protein (CRP),[19-22] and circulating inflammatory mediators, including C3a and interleukin-6, may help predict sepsis.[23] While it is a relatively sensitive marker for infection, CRP has low specificity as a result of elevation in tissue injury and various noninfectious inflammatory conditions.[24] Serum levels of procalcitonin, the prohormone of calcitonin, were shown to have high specificity but low sensitivity for the diagnosis of systemic infection in patients examined in the emergency department.[25] Compared with the leukocyte count and CRP, procalcitonin may be a better diagnostic marker for infection.[25-27] It remains to be seen whether immature granulocyte measurements could be combined with these other markers to create an algorithm with better diagnostic sensitivity or specificity.

Molecular methods are promising and evolving diagnostic tools in microbiology. DNA microarray technology is being studied as a tool for the rapid detection and identification of microorganisms and the prediction of the microbial resistance profile.[28,29] Polymerase chain reaction, which has been used extensively for the diagnosis of infectious agents in various tissue sources, including blood, is more sensitive and a faster test than blood culture for the detection of bacteremia.[30-32] The design of primers to highly conserved DNA regions, such as the bacterial 16S ribosomal RNA gene, permit the detection of gram-positive and gram-negative bacteria in a single test.[30,31] The specificity of the test can be varied based on the design of species-specific primers.[32,33] Because of high sensitivity of the assay, clinically insignificant bacteremia may be detected. However, based on our results, it may be that these expensive and nonroutine tests might be indicated for patients determined to have high percentages of immature granulocytes. Limiting these tests to prescreened patient populations might improve their predictive value, and the rapidity with which they could be performed could result in earlier definitive treatment of patients with clinically significant sepsis.

In summary, the percentage of immature granulocytes correlates better with infection and positive blood culture results than the WBC count and is comparable to the ANC. All of these parameters have low sensitivity and, therefore, are not useful as sole screening tests for infection. Although infrequent, high cutoff levels for the percentage of immature granulocytes may predict positive blood culture results. Finding a high percentage of immature granulocytes might help direct molecular testing and, thus, lead to a more rapid diagnosis of severe infection. Finally, prediction of infection or bacteremia may be improved by incorporating the immature granulocyte assay into an algorithm that includes additional laboratory parameters. Future studies that combine immature granulocyte measurements with those of other

parameters in specific at-risk populations might help in the early detection of infection in patients.

References

1. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med.* 1992;20:864-874.

2. Wilson ML. Blood cultures: introduction. *Clin Lab Med.* 1994;14:1-7.

3. Lorian V, Amaral L. Predictive value of blood cultures [published correction appears in *Infect Control Hosp Epidemiol.* 1992;13:693]. *Infect Control Hosp Epidemiol.* 1992;13:293-294.

4. Wenz B, Gennis P, Canova C, et al. The clinical utility of the leukocyte differential in emergency medicine. *Am J Clin Pathol.* 1986;86:298-303.

5. Shapiro MF, Greenfield S. The complete blood count and leukocyte differential count: an approach to their rational application. *Ann Intern Med.* 1987;106:65-74.

6. Cornbleet PJ. Clinical utility of the band count. *Clin Lab Med.* 2002;22:101-136.

7. Briggs C, Harrison P, Grant D, et al. Performance evaluation of the Sysmex XE-2100^{TM}, automated haematology analyser. *Sysmex J Int.* 1999;9:113-119.

8. Briggs C, Harrison P, Grant D, et al. New quantitative parameters on a recently introduced automated blood cell counter: the XE 2100. *Clin Lab Haematol.* 2000;22:345-350.

9. Pollard Y, Watts MJ, Grant D, et al. Use of the haemopoietic progenitor cell count of the Sysmex SE-9500 to refine apheresis timing of peripheral blood stem cells. *Br J Haematol.* 1999;106:538-544.

10. Gould N, Connell B, Dyer K, et al. Performance evaluation of the Sysmex XE-2100^{TM}, automated hematology analyzer. *Sysmex J Int.* 1999;9:120-128.

11. Tsuruda, K, Tsuji T, Usui T, et al. Evaluation and clinical usefulness of the automated hematology analyzer, Sysmex XE-2100^{TM}. *Sysmex J Int.* 1999;9:129-138.

12. Ruzicka K, Veitl M, Thalhammer-Scherrer R, et al. The new hematology analyzer Sysmex XE-2100: performance evaluation of a novel white blood cell differential technology. *Arch Pathol Lab Med.* 2001;125:391-396.

13. Seebach JD, Morant R, Ruegg R, et al. The diagnostic value of the neutrophil left shift in predicting inflammatory and infectious disease. *Am J Clin Pathol.* 1997;107:582-591.

14. Selig C, Nothdurft W. Cytokines and progenitor cells of granulocytopoiesis in peripheral blood of patients with bacterial infections. *Infect Immun.* 1995;63:104-109.

15. Wile MJ, Homer LD, Gaehler S, et al. Manual differential cell counts help predict bacterial infection: a multivariate analysis. *Am J Clin Pathol.* 2001;115:644-649.

16. Ardron MJ, Westengard JC, Dutcher TF. Band neutrophil counts are unnecessary for the diagnosis of infection in patients with normal total leukocyte counts. *Am J Clin Pathol.* 1994;102:646-649.

17. Gombos MM, Bienkowski RS, Gochman RF, et al. The absolute neutrophil count: is it the best indicator for occult bacteremia in infants? *Am J Clin Pathol.* 1998;109:221-225.

18. Isaacman DJ, Shults J, Gross TK, et al. Predictors of bacteremia in febrile children 3 to 36 months of age. *Pediatrics.* 2000;106:977-982.

19. Krediet T, Gerards L, Fleer A, et al. The predictive value of CRP and I/T-ratio in neonatal infection. *J Perinat Med.* 1992;20:479-485.

20. Ballou SP, Kushner I. C-reactive protein and the acute phase response. *Adv Intern Med.* 1992;37:313-336.

21. Berger C, Uehlinger J, Ghelfi D, et al. Comparison of C-reactive protein and white blood cell count with differential in neonates at risk for septicaemia. *Eur J Pediatr.* 1995;154:138-144.

22. Russell GA, Smyth A, Cooke RW. Receiver operating characteristic curves for comparison of serial neutrophil band forms and C reactive protein in neonates at risk of infection. *Arch Dis Child.* 1992;67:808-812.

23. Groeneveld AB, Bossink AW, van Mierlo GJ, et al. Circulating inflammatory mediators in patients with fever: predicting bloodstream infection. *Clin Diagn Lab Immunol.* 2001;8:1189-1195.

24. Clyne B, Olshaker JS. The C-reactive protein. *J Emerg Med.* 1999;17:1019-1025.

25. Hausfater P, Garric S, Ayed SB, et al. Usefulness of procalcitonin as a marker of systemic infection in emergency department patients: a prospective study. *Clin Infect Dis.* 2002;34:895-901.

26. Bossink AW, Groeneveld AB, Thijs LG. Prediction of microbial infection and mortality in medical patients with fever: plasma procalcitonin, neutrophilic elastase-alpha$_1$-antitrypsin, and lactoferrin compared with clinical variables. *Clin Infect Dis.* 1999;29:398-407.

27. Hatherill M, Tibby SM, Sykes K, et al. Diagnostic markers of infection: comparison of procalcitonin with C reactive protein and leucocyte count. *Arch Dis Child.* 1999;81:417-421.

28. Anthony RM, Brown TJ, French GL. DNA array technology and diagnostic microbiology. *Expert Rev Mol Diagn.* 2001;1:30-38.

29. Heller MJ. DNA microarray technology: devices, systems, and applications. *Annu Rev Biomed Eng.* 2002;4:129-153.

30. Kane TD, Alexander JW, Johannigman JA. The detection of microbial DNA in the blood: a sensitive method for diagnosing bacteremia and/or bacterial translocation in surgical patients. *Ann Surg.* 1998;227:1-9.

31. Cursons RT, Jeyerajah E, Sleigh JW. The use of polymerase chain reaction to detect septicemia in critically ill patients. *Crit Care Med.* 1999;27:937-940.

32. Reno WL III, McDaniel DO, Turner WW Jr, et al. Polymerase chain reaction for the detection of bacteremia. *Am Surg.* 2001;67:508-512.

33. Isaacman DJ, Zhang Y, Rydquist-White J, et al. Identification of a patient with *Streptococcus pneumoniae* bacteremia and meningitis by the polymerase chain reaction (PCR). *Mol Cell Probes.* 1995;9:157-160.

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