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Comparison of basophil count by Beckman Coulter UniCel DxH 800, Sysmex XN-1000, and manual microscopy in cases of suspected chronic myeloid leukemia

Parul Chopra, Sunanda Bhardwaj[1], Anil Arora[1]

Abstract:

INTRODUCTION: Basophilia can help stratify cases of chronic myeloid leukemia (CML) into different phases and monitor response to therapy and has a significant prognostic value. It helps differentiate patients of CML from those with leukemoid reaction. Basophil counts (BCs) given by automated hematology analyzers are often not reliable. Analysis of peripheral blood picture therefore holds its importance in these cases. In this study, we aim to compare the BC in patients with suspected CML using two automated analyzers with manual microscopy.

MATERIALS AND METHODS: Two hundred and sixty-nine ethylenediaminetetraacetic acid samples identified as suspected CML run on Beckman Coulter UniCel DxH 800 and Sysmex XN-1000 were analyzed for BC microscopically on Giemsa-stained peripheral smear slides by two pathologists. The mean of basophil counts obtained microscopically was considered to be standard. They were compared with BC given by automated counters using correlation analysis and Bland Altman plots.

RESULTS: The age of the patients ranged from 4 to 89 years, with a male-to-female ratio of 1.2:1 (148 males; 121 females). BC obtained among both analyzers did not correlate (r^2 = 0.14). Results of microscopically counted basophils correlated well among two pathologists (r^2 = 0.92). Bland–Altman plots showed a mean bias of 2.2% and 2.4% by XN-1000 and DxH 800, respectively, when compared with manual counts. In the frequency distribution analysis, XN-1000 missed all 10 cases with BC >20% whereas DxH 800 missed 3/10 cases with BC >20%. In addition, in the 10%–20% range of BC, XN-1000 identified 6/22 cases whereas DxH 800 identified 12/22 cases. In the 5%–10% range of BC, XN-1000 identified 59/78 cases whereas DxH 800 identified only 43/78 cases.

CONCLUSION: With lower BC, Sysmex XN-1000 and, at higher BC, Beckman Coulter DxH 800 showed better performance. However, BC from none of the analyzers can be used alone without consideration of the microscopic results. All smears should be manually counted for basophils in cases of suspected CML because of its importance in clinical management.

Keywords:

Automation, basophil, chronic myeloid leukemia

Department of Laboratory Medicine, All India Institute of Medical Sciences, [1]Department of Hematology and Immunology, Dr. Lal PathLabs Ltd, National Reference Lab, New Delhi, India

Address for correspondence:
Dr. Parul Chopra, Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India.
E-mail: E mail: parul6588@yahoo.co.in

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Introduction

Basophils are the least common type of granulocyte representing 0.5%–1% of circulating white blood cells (WBCs) playing an important role in immune functions. Basophilia, defined as more than 100 basophils/μL in venous blood, can be seen in various nonneoplastic and neoplastic conditions including chronic

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myeloproliferative neoplasms such as chronic myeloid leukemia (CML), polycythemia vera, or myelofibrosis.

CML is a clonal stem cell disorder characterized by three phases, namely initial indolent chronic phase (CP) followed by an accelerated phase (AP), blast phase, or both. Analysis of the peripheral blood (PB) picture is very important in identifying and classifying the patients into various phases on the basis of which the treatment is given. The other factors indicating progression of CML from CP and transformation to AP or blast phase include clinically declining status of the patient, morphological features such as blast count, increasing basophil count (BC), persistent increase or decrease in platelet and total leukocyte counts (TLCs) along with features such as splenomegaly, clonal cytogenetic evolution, and resistance to tyrosine kinase inhibitor therapy. One of the important laboratory features of patients with advanced CML is marked basophilia. Basophils secrete various mediators contributing to the pathogenesis and evolution of CML. Basophilia can help stratify CML patients into the three phases and monitor patient response to therapy and thus has a significant prognostic value in CML. In addition, it is a very useful parameter to differentiate patients with CML from those with benign leukemoid reaction. Therefore, absolute BC is an important test in clinical decision-making for screening, diagnosis, and monitoring of CML. As the normal circulating levels of basophils are very low, precise counting and determination of reference intervals for circulating basophils can be a challenging task.

BC can be done manually or by automated hematology analyzers. Manual counting on peripheral blood smear (PBS) is considered as the reference method for BC as recommended by the Clinical and Laboratory Standards Institute (CLSI).[1] However, because of the low frequency, manual counting has limitations owing to nonuniform distribution of basophils in the PBS leading to inter-observer differences. For more accurate and precise results, a 200-cell differential count by two observers on two PBS from the same blood sample is recommended.[1] However, since the percentage of basophils is very low in PB, the precision still remains poor. In addition, CLSI does not recommend manual count as a reference method for cells less frequent than 5% of the TLC.

Automated BC performed by different automated hematology analyzers uses different principles such as optical light or fluorescence scattering and electrical impedance-based technology. It is considered to be more reliable due to counting a very large number of cells (8000–10,000 WBC) with higher throughput than counting by manual microscopy.[2] However, some studies have shown a low inter-instrument correlation of BC by automated analyzers and also with the reference manual method.[3-6] In addition, when using differential cell lysis as a basis for basophil detection by automated analyzers, spurious pseudobasophilia can lead to misclassification of abnormal lysis-resistant cells such as plasma cells in multiple myeloma, nucleated red blood cell (RBC), and others as basophils.[7,8] While most studies talk about pseudobasophilia, some report the presence of erratic results or lower BCs given by the automated analyzers than actually present.[4] Not much is known about the falsely decreased BC, especially in cases of CML/suspected CML where the low counts can have implications on diagnosis and prognosis.[9] Hence, the question arises: can we rely on automated BC? Can automated BC substitute the reference manual BC? Are the results similar with different analyzers using different technologies?

To answer these questions, the present study aimed to compare BC in patients of suspected CML using two automated analyzers based on two different principles, i.e. Beckman Coulter UniCel DxH 800 and Sysmex XN-1000, and compare it with reference manual BC on PBS.

Materials and Methods

This is a cross-sectional study carried out at the hematology and immunology department of a national reference laboratory in Delhi, India, between February 2019 and March 2020. All the samples registered for complete blood count were screened to include cases of suspected CML. Two hundred sixty nine patients with TLC >12,000 cells/μL, immature granulocyte flags and basophilia ≥2% on PBS, or absolute BC >500/μL were included in the study.[10,11] Repeat samples from patients already included in the study were excluded.

The 269 samples were run on two automated hematology analyzers Beckman Coulter UniCel DxH 800 (Beckman Coulter, CA) and Sysmex XN-1000 (Sysmex, Kobe, Japan). The time between the analysis of sample on both analyzers was <2 h. The instruments were all in routine use and were regularly used in internal and external quality control programs as per accepted guidelines. Inter-instrument checks were done biannually for our instruments, Coefficient of variation (CV) <5% for all parameters.

Two PBS were also prepared and Giemsa stained within 1 h of running the sample. BC was done manually on these PBS by two hematopathologists (WBC differential counts of 200). The mean of BC obtained by two hematopathologists was considered to be standard and compared with those by automated cell counters.

Statistical analysis was performed using Microsoft Excel and SPSS version 20 IBM SPSS Statistics for Windows (Version 20.0. Armonk, NY: IBM Corp.). Graphs were plotted on Microsoft Excel. Correlation analysis was done, and correlation coefficients were determined using the two-tailed Pearson test. Bland–Altman plots were used to study the agreement between the analyzers and manual BC by microscopy.

Results

Demographic profile

The age of the patients ranged from 4 to 89 years (median: 46 years). There were 148 males and 121 females with a male-to-female ratio of 1.2:1. The TLC of the cases ranged from 12.8 to 648.2 × 10^9/L, hemoglobin ranged from 4.6 to 15 g/dL, and platelet count from 3 to >1000 × 10^9/L. The BCs ranged from 0.4% to 18.6% and 0.0% to 42.8%, by Sysmex XN-1000 and Beckman Coulter DxH 800, respectively. On PBS examination, many cases showed the presence of hypogranular or dysplastic or immature basophils.

Comparison between the two analyzers and with standard manual counts

TLC and hemoglobin correlated well between both the analyzers (R^2 = 0.98 and 0.96, respectively) [Figure 1a and b]. The BC done on PBS by both hematopathologists also showed a good correlation (R^2 = 0.92) [Figure 1d]. The BC by both instruments Sysmex XN-1000 and Beckman Coulter DxH 800 among themselves and on comparing with mean count of both hematopathologists [Figure 1c, e and f] showed a poor correlation (R^2 = 0.14, 0.23, and 0.59, respectively).

When Bland–Altman plots were made, a bias of 2.2% and 2.4%, respectively, was obtained taking the average difference of values obtained by manual BC and XN-1000 [Figure 2a] and difference of values obtained by manual BC and DxH 800 [Figure 2b]. From the plots, it could be made out that at lower percentage of BC, XN-1000 showed better agreement on comparison with manual counts whereas DxH 800 showed less differences and better agreement at higher percentage of basophils. This was also noticed on the frequency distribution analysis [Figure 3] of percentage BC as given by both

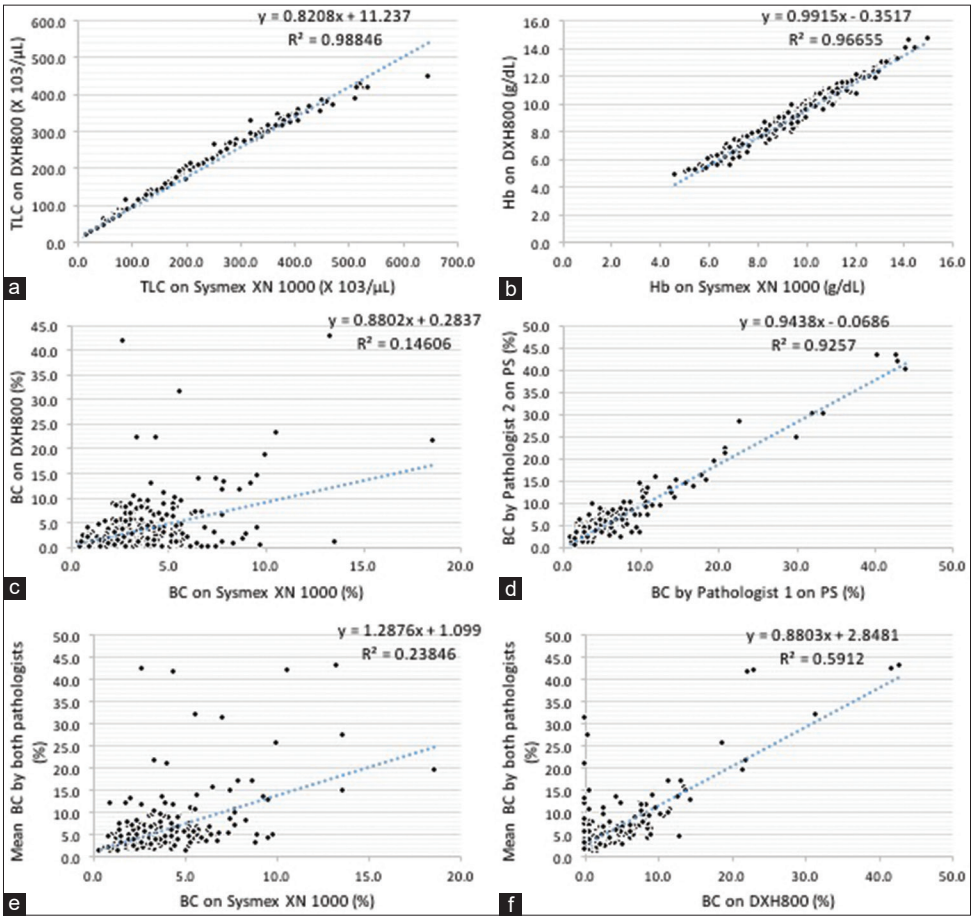

Figure 1: Correlation of total leukocyte count (a), hemoglobin (b), basophil counts (c) between both analyzers Sysmex XN-1000 and Beckman Coulter DxH 800. Correlation of basophil count by both hematopathologists on peripheral blood smear (d). Comparison of mean manual basophil count compared with basophil count by both analyzers, respectively (e and f)

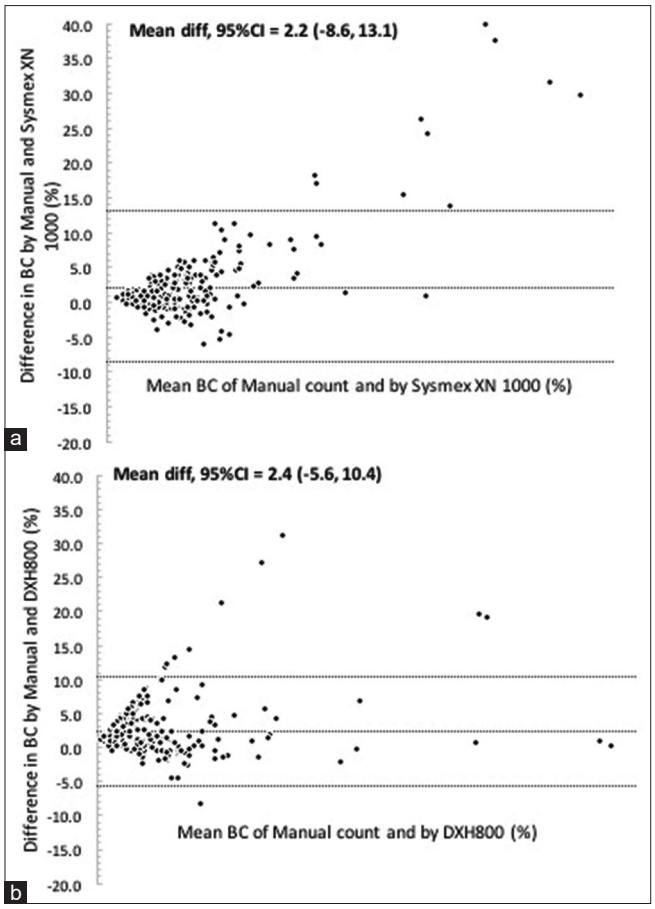

Figure 2: (a and b) Bland–Altman plots obtained by taking the average of values obtained by manual basophil count and XN-1000 (a), manual basophil count and DxH 800 (b) on x-axis and their differences on y-axis showing a bias of 2.2% and 2.4%, respectively

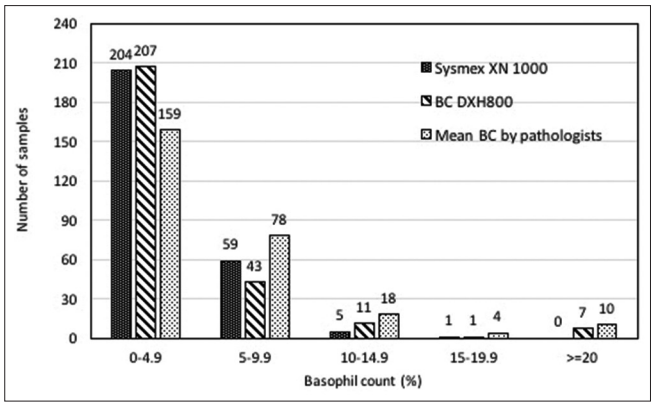

Figure 3: Frequency distribution of percentage basophil count as given by both analyzers and manual count on Peripheral smear (PS)

analyzers and mean count by pathologists; it was seen that XN-1000 missed all ten cases with BC >20% whereas DxH 800 missed three out of ten cases with BC >20%. Furthermore, in the 10%–20% range of BC, Sysmex XN-1000 identified 6/22 cases whereas DxH 800 identified 12/22 cases. In the 5%–10% range of BC, XN-1000 identified 59/78 cases whereas DxH 800 identified only 43/78 cases.

Discussion

The major findings of our study were poor correlation of BCs by both analyzers and manual counts on PS. There was a mean bias in the instruments giving lower counts than those by manual counting. Furthermore, on frequency distribution analysis, it was found that if only the counters were used for BCs, most of the cases of AP would be missed by Sysmex XN-1000 which showed inaccurate counts at higher percentages of basophils. DxH 800, on the other hand, missed three out of ten cases with basophils ≥20%. This may give a clue to the technical personnel to improve the methods of basophil counting in the areas they lack and also the laboratories to define their slide review criteria according to the automated analyzer they use.

Automated analyzers have almost replaced the manual cell counting and differentials due to high throughput, increased precision, and accuracy provided by them. In addition, the graphs and scatterplots provided by them give extra information and help in screening the patients suspected of having hematological malignancies.[12] However, there are certain abnormalities that may not be recognized or if recognized not described/defined clearly by them making it essential for manual smear evaluation. We can set up our limits or use methods to put automated instruments to appropriate use for replacement of manual methods without any compromise of quality.

BC is one parameter which lacks reliability and reproducibility.[4,5] This may be because of the different technologies used by the instrument for differential counts. However, none of the studies available is in cases of CML where actually the BC is of diagnostic and prognostic importance. Due to differences in technologies of measurement of WBC differential count, variations in BC are observed. Flow cytometry-based methods show somewhat better results in terms of giving consistency and correctness.[4,6] However, the imprecision and inaccuracy of automated hematology analyzers may affect the patients in diagnosis and management of patients by putting them in incorrect phase of CML as seen in our study. Most of the other studies available in literature have described pseudobasophilia where there is a false elevation in BCs.[4,8,13,14] Spurious basophilia is seen in samples with degeneration, reactive/atypical lymphocytes, blasts, lyse-resistant cells such as nucleated RBC, platelet clumps, giant platelets, leukocytosis with neutrophilia, and shift to left in myeloid series due to the presence of toxic granules. Not many studies have emphasized the importance of falsely decreased counts where actually

there is basophilia in PB. A study on basophils done by three instruments Sysmex XE-2100 (Sysmex, Kobe, Japan), CELL-DYN Sapphire (Abbott Diagnostics), and ADVIA 120 (Siemens Healthcare Diagnostics) showed that all three of them gave imprecise values of basophils. Sysmex XE-2000 gave pseudobasophilia, and the other two instruments CELL-DYN Sapphire and ADVIA 120 underestimated the BC when compared to flow cytometric detection of basophils.[4] Few other studies on automated cell counters are available in literature. A comparison of the basophil percentages obtained with flow cytometric method and those by ADVIA 120 (Bayer Diagnostics, USA) and the GEN S (Beckman Coulter, USA) in another study demonstrated poor results of BCs by both instruments.[6] Another study comparing automated BCs from four different hematology analyzers CELL-DYN Sapphire (Abbott Diagnostics), Siemens ADVIA 120 (Siemens Healthcare Diagnostics, Germany), Beckman Coulter DxH 800 (Beckman Coulter, CA), and Sysmex XE-2100 (Sysmex, Kobe, Japan) with manual microscopy in normal and abnormal or disease samples including acute and chronic leukemias concluded that basophil numbers from none of the analyzers can be used with confidence without using microscopy.[3] One of the articles suggested that despite the improvement of WBC enumeration and differentials by counting 10,000 cells by automated systems, the screening of PBS by microscopy is required for precise measurement of basophils.[5]

Persistent basophilia is an early sign of myeloproliferative disorder. The presence of basophilia (>250 cells/μL) and eosinophilia (>350 cells/μL) in patients with myelodysplastic syndromes was shown to have poor prognosis and reduced survival.[15] Basophilia is one of the disease progression criteria for AP of CML in the WHO classification. Presence of BCR ABL1 translocation in CML increases the production of histamine by inducing the expression of histidine decarboxylase that in turn leads to increase in basophil production. This is accompanied by the release of fibrogenic and angiogenic cytokines that facilitate the extramedullary spread of myeloid cells along with their precursors.[9]

Keeping in mind the importance of BCs in CML for prognostication and follow-up of cases, ours is the first study to compare the validity of BCs given by automated counters in suspected cases of CML. False-negative/low values given by counters as seen in our study may be due to the presence of dysplastic basophils, hypogranular basophils, and immature basophils in the setting of CML due to rapid production. There are a number of other methods that can be used to count basophils. An old method for staining basophils was with toluidine blue that is not done these days. Flow cytometry can be used as a more sensitive and specific technique to detect basophils by the use of markers such as CD193 (CCR3-eotaxin receptor), CD123 (IL-3R), ectonucleotide pyrophosphatase/phosphodiesterase 3, or CD203c.[4,16] Flow cytometric enumeration of basophils in cases of CML ranging from CP to blast crisis was done in a study that also showed the immunophenotypic aberrations seen in cells of myeloid lineage in these cases.[17] However, this method is expensive and not feasible at all places due to need for increased cost, infrastructure, and trained staff. Biochemical markers, like histamine or tryptase, can also identify the presence of increased basophils but cannot be used for subclassification of CML into various phases due to unavailability of quantitative cutoffs. For identification in paraffin-embedded bone marrow section, immunohistochemical basophil stains like basogranulin (BB1 antigen) can also be used.

BC by automated analyzers can be inaccurate and imprecise. This is why in spite of having important role in diagnosis and prognosis of CML, the BC given by these analyzers cannot be relied upon unless manual counts by microscopy are obtained.

Conclusion

Leukocyte differential counts from different analyzers may be different. With lower BC, Sysmex XN-1000 and, at higher BC, Beckman Coulter DxH 800 showed better performance. However, BC from none of the analyzers can be used alone without consideration of the microscopic results. All smears should be manually counted for basophils in cases of suspected CML because of its importance in relation to clinical management of the patients.

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Conflicts of interest

There are no conflicts of interest.

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