Manual differential leukocyte count in the presence of large immature cells detected by automated hematology analyzer

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ABSTRACT

Introduction: Large immature cells (LICs) are myeloid or lymphoid cells normally present in bone marrow which may be released in peripheral blood in various conditions. The automated hematology analyzers though have good correlation with microscopy for mature cells, give flag messages when immature cells are present and manual microscopy remains the reference method. The objective of this study was to find types of immature cells comprising LICs and see correlation of automated and manual Differential leukocyte count (DLC) in presence of such cells. **Methods**: This cross-sectional study was conducted in the Central Clinical Laboratory, College of Medical Sciences, after ethical approval. A total of 100 EDTA-anticoagulated blood samples with LIC >3% on automated DLC using the Horiba Yumizen H550 analyzer were included. Repeat CBC samples and leukopenic cases without automated DLC were excluded. CBC and DLC parameters including Hb, TLC, platelet count, and differential counts were recorded. Pearson correlation was used to assess relationship, with $p<0.05$ considered significant. **Results:** Total leukocyte count ranged from 790 to 479190/cumm. Myeloblasts were seen in 6%, promyelocytes in 6%, myelocytes in 41%, metamyelocytes in 73%, bands in 84%, lymphoblasts in 2%, atypical lymphocytes in 30% and atypical monocytes in 5%. There was strong correlation between automated and manual DLC for neutrophils, lymphocytes and eosinophils, fair correlation for monocytes ($p<0.001$) and no correlation for basophils ($p=0.175$). **Conclusions:** Manual microscopy is required to evaluate all the cases in presence of LIC more than reference range.

Key words: Automated hematology analyzer, DLC, LIC, manual microscopy.

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INTRODUCTION

Complete blood count (CBC) which includes total blood cell counts like red blood cell count, total leukocyte count (TLC) and platelets count and also differential leukocyte count (DLC) is the most frequently requested hematologic test and is central to the diagnosis of hematologic diseases.[1, 2] Performing a DLC has two objectives; first is to see the relative distribution of various white blood cells (WBCs) and second is to see their morphologic abnormality.[3]

The only method for performing a DLC was manual microscopic examination of a Romanowsky stained smear for many years. Manual examination is more time consuming, needs more labor, and requires skilled personnel and may have errors due to sampling and performance errors.[4] Multiparametric hematology analyzers have evolved to perform automated DLC over the past decades.[5] Introduction of these automated instruments capable of performing DLC and providing morphologic and quantitative flags have reduced the errors and costs when compared to microscopic evaluation.[6]

Six-part differential (6-Diff) automatic hematology analyzers, in addition to five types of leukocytes, gives absolute count and percentage of large immature cells (LICs). LICs are immature myeloid

and lymphoid cells normally present in bone marrow, but may be released in peripheral blood in various pathologic and physiologic conditions like reaction to stress, infection, bone marrow regeneration or neoplastic disorders like leukemias, myeloproliferative and lymphoproliferative disorders. These cells are larger than normal mature cells and immature in their nuclear complexity and the extent of cytoplasmic granularity. LICs include blasts (myeloblast, lymphoblast, monoblast), promyelocytes, myelocytes, metamyelocytes, prolymphocytes, promonocytes and megakaryocytes.[1]

The automated hematology analyzers have good correlation with manual microscopy for mature cells. However, most of these instruments give flag messages when immature cells are present and manual microscopy remains the reference method when such cells are present.[7,8] Hence, this study aimed to find the different types of immature cells comprising LICs, to see correlation of automated and manual DLC in presence of LICs, and to evaluate the necessity of slide review when such cells are present.

METHODS

This cross-sectional study was conducted in Department of Central Clinical Laboratory in College of Medical Sciences from May 1 to July 31, 2023. Ethical approval was taken from Institutional Review Committee (Ref. No. COMSTH-IRC/2023-10). The study was conducted on EDTA anticoagulated blood samples sent for CBC examination. Automated DLC was performed on Horiba Yumizen H550 6-diff automated hematology analyzer based on the principle of impedance and flow cytometry. 100 CBC cases with LIC > 3% in automated DLC were included. Repeat CBC of same cases and leukopenic cases where automated DLC was not available were excluded. Slides were prepared and stained with Leishman stain.[9] Data was collected in a predesigned proforma and entered into Statistical Package for Social Sciences (SPSS) 20. Age, sex and relevant clinical history were noted. Hemoglobin (Hb), Platelet count, Total leukocyte count (TLC) and DLC with individual neutrophil, lymphocyte, monocyte, eosinophil and basophil percentages and also LIC percentage were noted from automated hematology analyzer print out report. Cases were classified as: anemia, normal Hb and polycythemia for age and gender, leukocytosis, leukopenia and normal TLC for age and thrombocytopenia, thrombocytosis and normal platelet count for age according to standard reference range.[9] Manual DLC was performed by an expert hematology technician/pathologist and DLC findings were noted in separate headings as blasts (myeloblasts, lymphoblasts, monoblasts), promyelocytes, myelocytes, metamyelocytes, band forms, neutrophils, prolymphocytes, lymphocytes, atypical lymphocytes, plasma cells, promonocytes, monocytes, atypical monocytes, eosinophils and basophils. Age, gender, most frequent clinical presentation, Hb, TLC and platelet count were expressed as percentages. Cells other than normal neutrophils, lymphocytes, monocytes, eosinophils and basophils were as expressed as percentages in number of cases seen. For correlation of automated and manual DLC, myeloblasts, promyelocytes, myelocytes, metamyelocytes and band forms were added to neutrophils, prolymphocytes, atypical lymphocytes and plasma cells were added to lymphocytes and promonocytes and atypical monocytes were added to monocytes in manual DLC. Correlation was done between TLC and LIC % and Pearson Correlation Coefficient was calculated and p value less than 0.05 was considered significant at 95% confidence interval with further regression analysis to establish the relationship between them and adjusted R^2 value was calculated. Correlation was done between automated and manual DLC and Pearson Correlation Coefficient was calculated and p-value less than 0.05 was considered significant at 95% confidence interval.

RESULTS

The age of the patients ranged from 1 day to 94 years with mean age of 42.9±28.5 years with maximum 15% cases in 51-60 years age group. There were 61% males and 39% females with male female ratio of 1.6:1. (Figure 1)

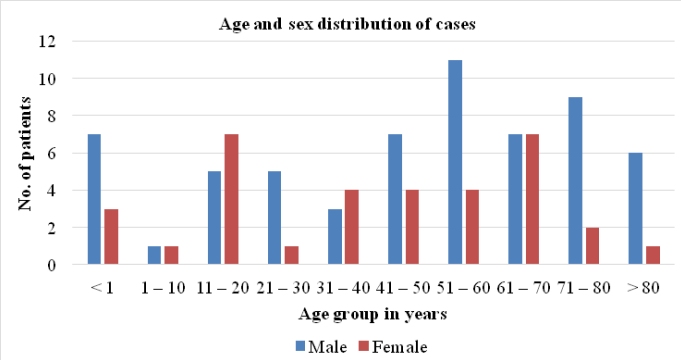

Figure 1: Age and sex distribution of cases

The result showed that 86% cases had presented with fever as most common presenting complaint. Hemoglobin (Hb) ranged from 1.2 to 19.2 g/dl and 67% cases had anemia, 32% cases had normal level and 1% case had polycythemia. Platelet count ranged from 15000 to 660000/cumm with 61% cases having thrombocytopenia, 37% cases had normal platelet count and 2% cases had thrombocytosis.

TLC ranged from 790 to 479190/cumm, in which 39% cases had normal TLC followed by 37% cases with

leukocytosis and 24% cases had leucopenia. LIC percentage ranged from 3.1 to 76.3. There was significant correlation between LIC and TLC as Pearson correlation coefficient was 0.535 and p value was <0.001 at 0.01 level (2 -tailed). On further regression analysis, relation between LIC and TLC was established as LIC = 7.861+0.0123 TLC and adjusted R^2 was 0.279. Further, the correlation of LIC with TLC was statistically more significant for increased TLC. (Table 1, Figure 2)

Table 1: Correlation between LIC % and TLC (n=100)

TLC	LIC % Pearson correlation coefficient	p-value
Decreased	-0.128	0.552
Normal	-0.216	0.186
Increased	0.834	<0.001

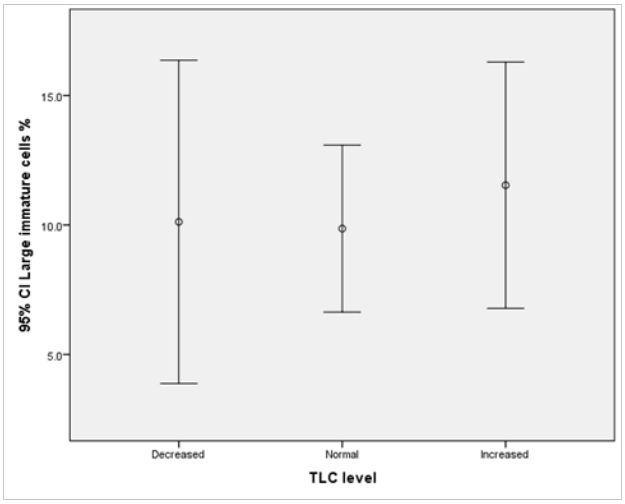

Figure 2: Range of LIC % in normal, increased and decreased TLC

In Manual DLC, myeloblasts were seen in 6% (Figure 3a), promyelocytes in 6%, myelocytes in 41%, metamyelocytes in 73%, band forms in 84%, lymphoblasts in 2% (Figure 3b), atypical lymphocytes in 30% (Figure 3c) and atypical monocytes in 5% cases. 3% cases had plasma cells and 1% case had hypersegmented neutrophils (Figure 3d) without presence of any other immature cells. 7% cases were hematopoietic neoplasms including 4% cases of Chronic myeloid leukemia (Figure 3e), 2% cases of Acute myeloid leukemia and 1% case of Acute lymphoblastic leukemia.

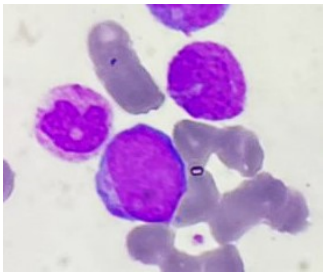

Figure 3a: Myeloblast with Auer rod (Leishman stain x 1000X)

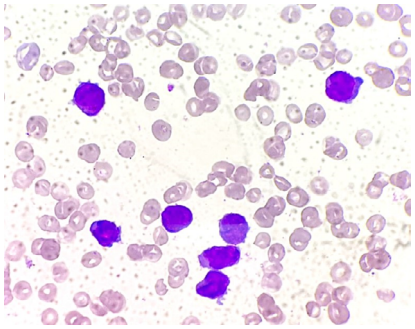

Figure 3b: Lymphoblasts (Leishman stain x 1000X)

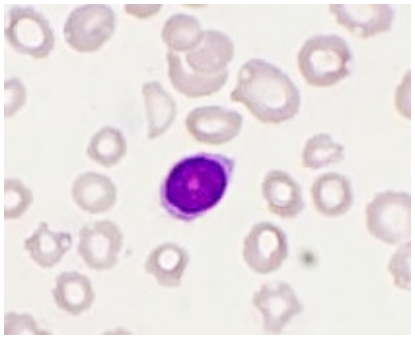

Figure 3c: Reactive lymphocyte (Leishman stain x 1000X)

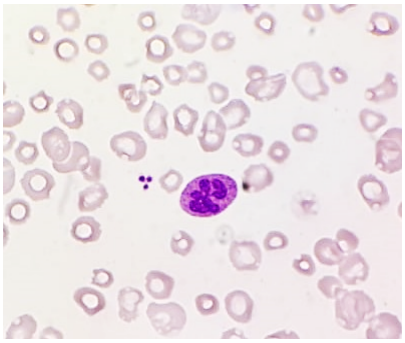

Figure 3d: Hypersegmented neutrophil (Leishman stain x 1000X)

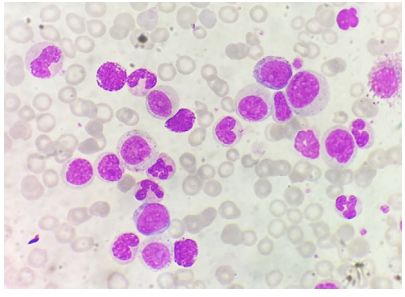

Figure 3e: Chronic Myeloid Leukemia (Leishman stain x 1000X)

There was statistically significant correlation between automated and manual DLC for neutrophils, lymphocytes, monocytes and eosinophils. However, there was no correlation for basophils. (Table 2)

Table 2: Correlation of automated and manual DLC in different types of WBCs (n=100)

Type of WBC	Pearson correlation coefficient	p-value
Neutrophil	0.832	<0.001*
Lymphocyte	0.791	<0.001*
Monocyte	0.504	<0.001*
Eosinophil	0.714	<0.001*
Basophil	-0.137	0.175

*The correlations were significant at 0.01 level (2-tailed)

DISCUSSION

Blood is the easiest and least invasive circulating tissue to sample in human body. A CBC is therefore one of the most common clinical laboratory tests. CBC can be equated to biopsy obtained through venipuncture. First examination of this body fluid was possible after the development of microscope which allowed visual examination as red globules. Later in 1870s, Paul Ehrlich applied dyes to differentiate between types of WBCs. It was known by then that the number of these blood cells vary according to disease, thus making necessary to quantify by blood count. Manual counting of each cell in the counting chamber under microscope was introduced, but chances of error were high as this method was both labor intensive and time consuming. Hence, development of automated blood count became a necessity.[10] Practice of modern laboratory hematology using automated blood cell counting was possible only after invention of Coulter principle based on electronic impedance to count and size blood cells suspended in fluid.[11]

Modern hematology analyzers use multiple techniques like absorption spectrometry, impedance, conductivity measurement and flow cytometry for cell counting and differentiation. With the improved performance by new generation hematology analyzers, automated method is rapid and cost effective for CBC and DLC.[12] However, the automated method has a drawback of inability to accurately classify immature and abnormal cells. In such cases, microscopic examination becomes necessary as triggered by "flags" from these instruments. Microscopic examination being labor intensive and expensive, modern-day laboratory needs to optimize the samples needing such review as well as avoiding false negative results. The microscopic review rate was found out to be 21% in one of the studies.[12,13] Automated analyzers are now widely used to perform reliable DLC, but they need evaluation of their flags to be sure of their efficacy in diagnostic hematology.[14] LIC is one such parameter which gives the flag for immature population of WBCs and is used by Horiba Yumizen analyzers for research purpose only and has to be interpreted carefully.[1]

This study was conducted on EDTA anticoagulated CBC samples run on 6-diff automated Hematology analyzer Horiba Yumizen H550 with LIC >3%. Age ranged from first day neonate to 94 years. 8% cases were seen in neonates. In neonates, interpretation of LIC has to be done carefully because of their immature immune system and greater number of immature cells in circulation.[1] However in a study conducted by Nigro et al., increased immature granulocyte count was significantly associated with positive blood culture rate implying it as a predictor of neonatal sepsis.[15]

In the present study, 78% cases showed immature granulocytes comprising promyelocytes, myelocytes and metamyelocytes and reactive monocytes as an inflammatory response. In 7% cases of hematopoietic neoplasms, LIC% ranged from 9.2 to 50.1. In infection, inflammatory processes, LIC% ranged from 3.1 to 76.3. Promyelocytes, myelocytes, metamyelocytes, band forms, activated monocytes can be released from marrow into circulation as an early response to infection/inflammation which is known as left shift.[1] In a study conducted by Ansari-Lari et al., they found that immature granulocyte percentage more than 3 was a specific predictor of sepsis.[16]

In present study, there was strong correlation between automated and manual DLC for neutrophils, lymphocytes and eosinophils, fair correlation for monocytes and no correlation for basophils. Immature cells were present in all the cases except 1% case where there were hypersegmented neutrophils. In a study conducted by Meintker et al., where they correlated DLC of four different instruments with manual DLC, they found very good correlations for neutrophils and eosinophils, fair correlation for monocytes and lymphocytes, poor reliability for basophil counts and moderate sensitivity of flagging for blasts and immature granulocytes and concluded to take CBC data and clinical information to take into account for detection of leukemic blasts rather than depending on flagging.[12] In a study conducted by Arroyo et al., where they evaluated the efficacy of automated hematology analyzer by comparing their results with manual microscopic counts, another automated analyzer and flow cytometric immunophenotyping, they found good performance especially for the enumeration of neutrophils, lymphocytes, eosinophils and LICs.[17] In a study conducted by Butarello et al., where they compared DLC from four different hematology analyzers with manual microscopy, they found good correlation for neutrophils, lymphocytes and eosinophils, less for monocytes and poor for basophils and concluded that manual microscopy be more directed to flagged samples.[3]

CONCLUSIONS

Automated hematology analyzer can detect immature cells. There is a good correlation between automated and manual DLC for neutrophils, lymphocytes and eosinophils. Manual slide review is necessary in CBC samples showing LIC% more than three.

CONFLICTS OF INTEREST: The abstract of the manuscript was presented as poster in the 62nd IAP-Thailand Annual Meeting 2023 and the abstract was published in official journal of IAP Thailand, Asian Archives of Pathology in Volume 6, Number 1, January – March 2024 (Supplement Issue).

https://www.asianarchpath.com/storage/issue/AAP_Volume_6_Number_1_January-March2024Supplement.pdf

However, the full manuscript with detailed methodology, results and discussion has not been published elsewhere.

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AUTHORS' CONTRIBUTIONS

BG designed the concepts, definition of intellectual content, literature search, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review. AS was involved in data acquisition and manuscript editing.

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