Publisher
MEDRESEARCH
www.medresearch.in

Tropical Journal of Pathology and Microbiology

2021 Volume 7 Number 5 September October

E-ISSN:2456-1487
P-ISSN:2456-9887
RNI:MPENG/2017/70771

Research Article
Anemia

Correlation of Automated Cell Counter RBC Histograms and Peripheral Smear in Anemias

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DOI: https://doi.org/10.17511/jopm.2021.i05.08

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Introduction: Automate peripheral blood count for the diagnosis of anemia is a fundamental process, and the instrument can give some of the basic and advanced parameters; however, there is a need to depend on a manual microscopic scan of peripheral smear for the morphological correlation and other clues which the cell analyzers cannot determine. In most laboratory setups, traditional emphasis has been placed on verifying the automated data, an exercise that has outlived its importance. This present study was designed to determine the relationship between Abbott cell dyn ruby- 5 part analyzer automated haematology analyzer histograms and peripheral smear using the blood samples at the department of pathology, HIMS HASSAN. **Objectives:** 1) Interpretation of histograms in normal persons and patients with different types of anaemia 2) Comparison of automated histogram patterns with morphological features noticed on peripheral smear examination. **Materials and methods:** The present study will be conducted in Central Laboratory, Dept Of Pathology, HIMS Hasan. A total of 1000 samples sent for CBC and PS would be used for the present study. Source of data: CBC samples sent for analysis received at Central Laboratory HIMS, Hassan. **Result:** The majority of the age groups were 20-40 yrs, microcytic picture being the most common type. The most common type of histogram was broad base followed by left shift type of histogram. **Conclusion:** When the correct interpretation of the curve is paired with the findings of blood count characteristics such as red cell distribution width and red cell indices, the RBC Histogram becomes a useful diagnostic tool.

Keywords: RBC histogram, Anemia, Automated cell counter

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How to Cite this Article

MR Shashidhar, R Purushotham, KR Nagesha, Correlation of Automated Cell Counter RBC Histograms and Peripheral Smear in Anemias. Trop J Pathol Microbiol. 2021;7(5):261-265.
Available From https://pathology.medresearch.in/index.php/jopm/article/view/533

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Manuscript Received	Review Round 1	Review Round 2	Review Round 3	Accepted
2021-05-15	2021-05-17	2021-05-24	2021-05-31	2021-06-06

Conflict of Interest	Funding	Ethical Approval	Plagiarism X-checker	Note
Nil	Nil	Yes	18	

Introduction

The RBC histogram visualizes particle size distribution that plays a critical role in the initial screening and detection method for haematological disorders in current clinical settings. With the emergence of more powerful haematology analyzers with significant improvement and precision, the manual peripheral smear examination steadily declines. The number of the cells counted by the automated hematology analyzers is much more than the cells measured by manual peripheral smear examination, and computerized analyzers provide far better accuracy and with the usage of histograms. The RBC histogram, along with other CBC parameters like RBC distribution width (RDW) and mean corpuscular volume (MCV), has been discovered to be aberrant in a variety of haematological illnesses and may provide essential clues in the diagnosis and treatment of significant red cell disorders [1,2,3,4].

The automated peripheral blood count for anaemia diagnosis is a fundamental process. The instrument can provide some basic and advanced parameters; however, morphological correlation and other clues that cell analyzers cannot determine must rely on manual microscopic scanning of peripheral smears. The number of cells that can be inspected with a slide is typically significantly lower than the number of cells counted by automated haematology analyzers; additionally, they usually provide superior accuracy. The number of slides to be screened can be considerably reduced with the use of histograms. Traditional emphasis in most laboratory setups has been centred on checking automated data, an exercise that has outlived its usefulness.

This histogram, along with other CBC characteristics like RBC distribution width (RDW) and mean corpuscular volume (MCV), has been aberrant in various haematological illnesses and may provide essential clues in the diagnosis and management of significant red cell illnesses.

A histogram can assist laboratorians in 1) monitoring the accuracy of the results provided by analyzers and 2) examining the possible cause(s) of the erroneous automated outcomes.3) establishing a tentative diagnosis 7 Certain situations, such as the presence of fragmented red cells or red cell agglutination, may now theoretically

Be seen on the red cell histogram, which could not previously be seen without a blood film study. A sequential histogram can also clearly demonstrate the increasing emergence of a new erythrocyte population well ahead of other numerical indicators in patients with iron deficiency anaemia (IDA) or megaloblastic anaemia under treatment [5, 6]. This present study was designed to determine the relationship between Abbott cell dyn ruby- 5 part analyzer automated haematology analyzer histograms and peripheral smear using the blood samples at the department of pathology, HIMS HASSAN.

Objectives:

1) Interpretation of histograms in normal persons and patients with different types of anaemia.

2) Comparison of automated histogram patterns with morphological features noticed on peripheral smear examination.

Materials

Setting: The present study will be conducted in Central Laboratory, Dept Of Pathology, HIMS Hasan. A total of 1000 samples sent for CBC and PS would be used for the present study.

Duration: July 2021 to September 2021

Type: Prospective study

Source of data: CBC samples sent for analysis received at Central Laboratory HIMS, Hassan.

Inclusion criteria: All patients who are diagnosed as anaemic according to WHO definition

Exclusion criteria

1) Patients who are less than five years of age.

2) Inadequate quantity of blood sample for automated analyzer (< 3ml).

3) Pre Analytical errors like clotted sample.

Methodology

This is a prospective cross-sectional study done on all patients diagnosed with anaemia according to WHO definition. The CBC samples received would be analyzed in the ABOTT cell dyn ruby instrument, and a peripheral smear would be made from the same sample, using Leishman stain.

Ethical clearance: Done

Statistical analysis: Analytical method

Results

Out of the 1000 samples, 383 samples were from Males, and 617 samples were from females.

Results are given in tabulated form started from Table no. 1 to Table no. 5

Table 1: Age and gender distribution in the study.

Age group (years)	5-10	11-20	21-30	31-40	41-50	51-60	60-80	Overall
No of cases %	44	56	427	240	128	73	32	1000
Male	26	19	112	75	86	45	20	383
Female	18	37	315	165	42	28	12	617

Table 2: Case Distribution as per types of anemia.

Types of anemia	Number of cases	Percentage (%)
Normocytic	154	15.4
Microcytic	728	72.8
Macrocytic	42	4.2
Dimorphic	76	7.6

Table 3: Distribution of RBC histogram in the present study.

S. No	Type of histogram	Percentage (%)
1	Normal	17
2	Left shift	30
3	Right shift	05
4	Broad base	39
5	Short peak	03
6	Bimodal peak	06

Findings of RBC Histograms: In the present study, we found that the maximum number of cases (72.8%) were of Microcytic hypochromic anemia and showed various histograms. Among all, 17% histograms were normal,30% had a left-shifted curve,39% showed broad-based curve, 03% showed short peak, and Bimodal peaked histogram was demonstrated by 06% of total cases.

Correlation with Peripheral smear findings: In our study, we observe that cases of Microcytic hypochromic anemia with less than normal range of Mean Corpuscular Volume (MCV) &Mean Corpuscular (MCH) Hemoglobinwith normal Mean Corpuscular Hemoglobin Concentration (MCHC) and increased Red cell Distribution Width (RDW) and this finding is correlated with anisopoikilocytosis which was seen on the microscopic examination of peripheral blood smear

The cases of Normocytic Normochromic anemia showed the standard limit of MCV, MCH and MCHC and occasional cases having mildly increased RDW. In the present study, cases of Dimorphic anemia showed a normal range of MCV, MCH and MCHC. At the same time, RDW is increased due to the high degree of anisocytosis and poikilocytosis, which was observed in the PBS. Cases of Macrocytic anemia show an increase in MCV, MCH and RDW with normal MCHC

Table 4: Presentation of RBC histograms in different types of anemia.

Types of anemia	Normal curve	Left shift	Right shift	Broad base	Short peak	Bimodal peak
Normocytic	15	-	-	1	-	-
Microcytic	-	38	-	23	-	-
Macrocytic	-	4	-	-	2	-
Dimorphic	3	1	2	1	-	1

Discussions

A single histogram graph can be equivalent to 1000 numbers. The effect of a vast collection of facts represented as a visual representation is significantly greater than the impact of numbers alone. These data can take numerous forms in haematology, one of which is the RBC histogram. The range, size, shape, and other conspicuous aspects of the red cell morphology may all be seen by scanning the histogram visually [6].

RBC Histogramgives clues in diagnosing the many RBC disorders and also parameters like RDW, MCH & MCV. Usually, the curve is bell-shaped with symmetry & 24fl to 360fl is the range of the RBC histogram. The analyzer can recognize only those Red Blood cells 36fl to 360fl volume sizes as RBCs, and the range 24fl to 36 fl are not considered in the RBC count and not taken into consideration by the counter. The histogram begins above the baseline (36fl) indicates the presence of small particles like microspherocytes, platelet clumps, normoblast, elliptocytes, malaria parasites, bacteria, etc. The RBC count does not affect by WBCs count is increased by beyond 50000 cells / cumm [7-10]. In the present study of 1000 cases, the maximum number of instances are having Microcytic anemia (72.8%) followed by normocytic (15.4%), Dimorphic (7.6%) and Macrocytic (4.2%). Other studies like sandhya al. [11]. BynaSyamSundara Rao et al. [12]. Chavda J et al. [13] were also found similar findings of anemia cases regarding

Distribution. Our survey of RBC histogram showed normal curve (18%), left shift (29%), right shift (2%), Broad base (24%), short peak (1%) and bimodal (2%) and these findings regarding RBC histogram were also correlated with other studies.

When the RBC population is homogeneous, the distribution curve is narrow, and a broad base curve usually indicates the presence of greater anisocytosis, as determined by PBS analysis. The size of the RBC determines the shift of the histogram; if the cell size is more significant than normal (macrocytic RBC), the shift is to the right; if the cell size is smaller than normal (microcytic RBC), the shift is to the left. In this study, microcytic RBCs have a left-sided shifted curve due to their small size, while cases with a broad base curve had a high RDW, indicating anisocytosis.

The most prevalent cause of microcytic RBC is iron deficiency anaemia, which affects mostly women in their reproductive years. Iron deficiency during pregnancy is a significant problem in our nation [13, 14]. In macrocytic anaemia, a right shift with a broad-based curve indicates a low Hb level and a macrocytic blood image. The causes of macrocytosis range from benign to malignant, and determining the aetiology requires a comprehensive approach. Macrocytosis can strike at any age, though it is more common among the elderly [15, 16, and 17].

In our study majority of cases of macrocytic anemia showed a right shift curve. Right shift curve correlated well with increased MCV and MCH. The dimorphic blood picture shows a bimodal curve, along with some cases leading to the left and right shifting of the curve. There are wide reasons for dimorphic blood picture, including nutritional anemia, recent blood transfusion or therapy response to nutritional anemia and sideroblastic anemia. To know the exact cause, a thorough evaluation is necessary [18, 19].

The majority of macrocytic anaemia cases in our study had a right shift curve. Increased MCV and MCH are associated well with the right shift curve. The bimodal curve is visible in the dimorphic blood image, with some cases showing the left and proper shifting of the curve. Nutritional anaemia, recent blood transfusion or therapeutic response to nutritional anaemia, and sideroblastic anaemia are all possible causes of dimorphic blood images. To determine the specific cause, a complete examination is required [20].

Table 5: Histogram curves in various studies.

Histogram	Chavda J et al.[13]	Sandhya et al.[11]	Rao BSS et al.[21]	Present study
Normal curve	19%	15%	17.7%	18%
Left shift	27%	30%	29.0%	43%
Right shift	07%	6%	5.45%	2%
Broad base	38%	40%	37.72%	24%
Bimodal	3%	4%	7.27%	2%
Short peak	6%	5%	2.7%	1%

Conclusion

When the right interpretation of the curve is paired with the findings of blood count characteristics such as red cell distribution width and red cell indices, the RBC Histogram becomes a useful diagnostic tool. We might make a tentative diagnosis of fragments in the blood, microcytic, macrocytic, or dimorphic red cells based on these data. Blood indices and Hb values, as well as histograms, will help us. Histograms are a helpful tool for technologists since they may help them determine which instances require specialist peripheral smear testing. The results of the automated analyzer in our investigation were extremely well associated with the microscopic inspection. The majority of the findings were correlating with the study done by Chavda J et al., Sandhya et al. and Rao BSS et al. When paired with PBS findings, the histogram may be used as a screening tool that can minimize the use of peripheral smear examinations, and by comparing the results of both approaches, we can diagnose the majority of anaemia cases.

Author's contribution:

Dr. Nagesha K R & Dr. Purushotham R being the senior faculty, gave timely input results and discussions. **Shashidhar MR**: concept of study, review of literature, results and discussion.

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