

**Association of Automated Cell Counter RBC Histograms and Peripheral Smear in the Diagnosis of Anemia****Jaydeep Gorani<sup>1</sup>, Hitesh Anadkat<sup>2</sup>, Bimal Patel<sup>3</sup>**<sup>1,2</sup>Assistant Professor, Department of Pathology, Gujarat Adani Institute of Medical Sciences, Bhuj, Kutch, Gujarat, India<sup>3</sup>Associate Professor, Department of Pathology, Gujarat Adani Institute of Medical Sciences, Bhuj, Kutch, Gujarat, India

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**Abstract**

**Background and Aim:** 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. The purpose of this study was to examine the association between Abbott cell dyn ruby- 5 part analyzer automated haematology analyzer histograms and peripheral smear utilising blood samples from the pathology department at HIMS HASSAN.

**Material and Methods:** In this investigation, 2000 patients' peripheral blood smears were examined and linked with cell counters produced RBC indices and histograms. Source of data: CBC samples sent for analysis received at Department of Pathology, GAIMS, Bhuj, and Gujarat. RBC indices were observed in conjunction with the histogram pattern. • Microcytic hypochromic anaemia • normocytic normochromic anaemia • macrocytic anaemia • dimorphic anaemia • haemolytic anaemia

**Results:** The majority of those affected by anaemia were between the ages of 31 and 50. That the majority of patients (69.2%) had Microcytic hypochromic anaemia and displayed diverse histograms. In all, 14% of histograms were normal, 27% had a left-shifted curve, 43% had a broad-based curve, 5% had a short peak, and 6% had a bimodal peaked histogram. In our study, we observed cases of Microcytic hypochromic anaemia with less than normal ranges of Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) and increased Red cell Distribution Width (RDW).

**Conclusion:** Technologists can use histograms to help them identify the cases that call for specialised peripheral smear testing. The RBC Histogram becomes a valuable diagnostic tool when the proper interpretation of the curve is combined with the results of blood count features like red cell distribution width and red cell indices.

**Keywords:** Anemia, Automated Cell Counter, Peripheral Smear, RBC Histograms.

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**Introduction**

Anaemia is a serious public health issue across the world, particularly among women of reproductive age in underdeveloped nations. The World Health Organization's global estimates of anaemia prevalence averaged 49% between 1993 and 2003.<sup>1</sup> Anaemias are a significant diagnostic and clinical group of haematological illnesses that affect people all over the world. [1,2]

The RBC histogram depicts particle size distribution, which is important in the early screening and identification of haematological diseases in current clinical settings. The manual peripheral smear test is progressively declining as more sophisticated haematology analyzers with great improvement and precision become available.

The number of cells counted by automated haematology analyzers is far more than the number of cells counted by manual peripheral smear testing, and computerised analyzers enable substantially superior accuracy and use of histograms. [3,4] The RBC histogram, as well as other CBC parameters such as RBC distribution width (RDW) and mean corpuscular volume (MCV), have been found to be abnormal in a range of haematological diseases and may give important insights in the diagnosis and treatment of serious red cell disorders. [5-7]

Be visible on the red cell histogram, which was previously unavailable without a blood film examination. A sequential histogram can also

clearly show the progressive appearance of a new erythrocyte population in patients with iron deficiency anaemia (IDA) or megaloblastic anaemia who are receiving therapy. [8] The purpose of this study was to examine the association between Abbott cell dyn ruby- 5 part analyzer automated haematology analyzer histograms and peripheral smear utilizing blood samples from the pathology department at HIMS HASSAN.

### Materials and Methods

The present research analysis was done in the department of the pathology, medical college and associated hospital. The study period was for one year. The institute ethical committee was informed about the study and the clearance certificate was obtained prior to the start of the study. The data of the included patients were kept confidential. The inclusion and exclusion criteria followed in the study were as follows:

**Inclusion criteria:** As per the WHO references ranges all the cases of anemia were included in the study.

**Exclusion Criteria:** Patients with leucocytosis, leukemoid reaction, leukaemia, parasites and platelet disorders were excluded from the study.

### Method of Data Collection:

Venipuncture was used to obtain the venous sample, which was then placed in EDTA vacutainers. The samples were aspirated into a semiautomatic haematology analyzer while blood smears were made and stained with Leishman stain. Anaemia was classified using data derived from a haematology analyzer (RBC indices with RDW), followed by a review of peripheral blood smear results. The RBC histogram's location and shape were noted. During the reporting of the peripheral smear, however, the haematologists were not given access to the histogram and RBC indices.

RBC histograms were measured for position (normal, left shift, and right shift) and form (normal bell-shaped or Gaussian, broad-based, bimodal peak with skewing to the left and right). RBC indices were observed in conjunction with the histogram pattern. • Microcytic hypochromic anaemia • normocytic normochromic anaemia • macrocytic anaemia • dimorphic anaemia • haemolytic anaemia

### Statistical Analysis

The data was gathered, and the association between the diagnoses made by the peripheral smear versus RBC histogram and indices was statistically evaluated using one-way analysis of variance (ANOVA) to see if the mean difference in various RBC characteristics was significant. A p-value of 0.05 or less was judged statistically significant.

### Results

In this investigation, 2000 patients' peripheral blood smears were examined and linked with cell counters produced RBC indices and histograms. The patients had microcytic hypochromic anaemia, normocytic normochromic anaemia, macrocytic anaemia, dimorphic anaemia, and hemolytic anaemia, as determined by peripheral smear testing. The participants in the research varied in age from one to 95 years.

The majority of those affected by anaemia were between the ages of 31 and 50. The research mostly comprised females, with a F: M ratio of 1.9. Males were impacted more than females beyond the age of 65.

In the current investigation, we discovered that the majority of patients (69.2%) had Microcytic hypochromic anaemia and displayed diverse histograms. In all, 14% of histograms were normal, 27% had a left-shifted curve, 43% had a broad-based curve, 5% had a short peak, and 6% had a bimodal peaked histogram.

In our study, we observed cases of Microcytic hypochromic anaemia with less than normal ranges of Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) and increased Red cell Distribution Width (RDW), and this finding is correlated with anisopoikilocytosis, which was seen on microscopic examination of peripheral blood smear.

MCV, MCH, and MCHC were all within the normal limits in instances of Normocytic Normochromic anaemia, with just a few cases having modestly elevated RDW.

Cases of Dimorphic anaemia in this investigation had normal MCV, MCH, and MCHC levels. Simultaneously, RDW is raised due to the high degree of anisocytosis and poikilocytosis detected in the PBS.

Macrocytic anaemia is characterised by an increase in MCV, MCH, and RDW in the presence of normal MCHC.

**Table 1: Age and gender distribution in the study**

Age groups	Male	Female
5 – 10	52	36
11 – 20	38	74
21 – 30	224	630
31 – 40	150	320

41 – 50	172	84
51 – 60	90	56
61 – 70	40	24

**Table 2: Presentation of the histograms in relation to different types of anemia**

Types of anemia	Normal curve	Left shift	Right shift	Broad base	Short peak	Bimodal peak
Normocytic	45			3		
Microcytic		114		69		
Macrocytic		12			6	
Dimorphic	9	3	6	3		3

## Discussion

A single histogram graph may represent 2000 numbers. The impact of a large collection of data conveyed as a visual representation is far larger than the impact of numbers alone. In haematology, this information can take several forms, one of which is the RBC histogram. By visually scanning the histogram, you can examine the range, size, shape, and other noticeable elements of red cell morphology. [9] RBC parameters such as RDW, MCH, and MCV were acquired, which aided in the diagnosis and classification of anaemia. The normal curve was either symmetrical bell shaped or had a Gaussian distribution. MCV ranges between 80 and 100 fl on the normal curve. The analyzer can only recognise red blood cells with volume sizes ranging from 36fl to 360fl as rBCs, and those with sizes ranging from 24fl to 36 fl are not counted and are not taken into account by the counter. [10] The presence of microscopic particles such as microspherocytes, platelet clumping, normoblast, elliptocytes, malaria parasites, bacteria, and so on is shown by the histogram beginning above the baseline (36fl). The RBC count is unaffected when the WBC count exceeds 50000 cells/cumm. [7,11]

The most common cause of microcytic RBC is iron deficiency anaemia, which mostly affects women throughout their reproductive years. Iron deficiency during pregnancy is a major issue in our country. A right shift with a broad-based curve implies a low Hb level and a macrocytic blood picture in macrocytic anaemia. The causes of macrocytosis range from benign to malignant, and establishing the aetiology necessitates a thorough investigation. Macrocytosis can affect people of any age; however it is more frequent among the elderly. [12,13] On peripheral smear examination, a few instances identified as macrocytic on histogram and RBC indices analysis were found to be dimorphic. The presence of large platelets and platelet clumps, as well as fragmented RBCs in hemolytic disorders, might explain the little variation in the analysis of microcytic anaemias by peripheral smear examination and RBC indices/histogram. As a result, peripheral smear eliminates these inaccuracies. This study agreed with the findings of Poonam and colleagues. [14] When comparing

Histogram and Indices analysis to peripheral smear, only a few instances were identified as hemolytic anaemia because fragmented RBCs were counted as microcytes and polychromatophils found in hemolytic anaemia were counted as macrocytes by cell counters. These data suggest that RBC histograms and RBC indices have limitations in the diagnosis of Hemolytic Anaemia. [15]

## Conclusion

Technologists can use histograms to help them identify the cases that call for specialised peripheral smear testing. The RBC Histogram becomes a valuable diagnostic tool when the proper interpretation of the curve is combined with the results of blood count features like red cell distribution width and red cell indices. We might make a tentative diagnosis of fragments in the blood, microcytic, macrocytic, or dimorphic red cells based on these data. Histograms, blood indices, and Hb values will all be useful.

## References

1. Turawa, E.; Awotiwon, O.; Dhansay, M. A.; Cois, A.; Labadarios, D.; Bradshaw, D.; Pillay-van Wyk, V. J. I. J. o. E. R.; Health, P. Prevalence of anaemia, iron deficiency, and iron deficiency anaemia in women of reproductive age and children under 5 years of age in south africa (1997–2021): A systematic review. 2021, 18, 12799.
2. Sharif, N.; Das, B.; Alam, A. J. P. o. Prevalence of anemia among reproductive women in different social group in India: Cross-sectional study using nationally representative data. 2023, 18, e0281015.
3. Asad, S.; Ahmed, I.; Ali, N. J. J. o. L. P. Utility of peripheral film findings and its correlation with automated analyzer—An audit from tertiary care hospital. 2017, 9, 001-004.
4. Constantino, B. T. J. L. M. Red cell distribution width, revisited. 2013, 44, e2-e9.
5. Wen, Y. J. E.; Cardiology, C. High red blood cell distribution width is closely associated with risk of carotid artery atherosclerosis in patients with hypertension. 2010, 15, 37.
6. Patel, S.; Metgud, R. J. J. o. c. r.; therapeutics. Estimation of salivary lactate dehydrogenase in oral leukoplakia and oral squamous cell

- carcinoma: a biochemical study. 2015, 11, 119-123.
7. Hussain, S.; Frayez, M. J. I. J. o. P. H. R.; Development. Correlation of Automated cell counters RBC Histogram and Peripheral smear in Anemias. 2022, 13, 234-237.
  8. Yčas, J. W. J. A. i. c. c. Toward a blood-borne biomarker of chronic hypoxemia: red cell distribution width and respiratory disease. 2017, 82, 105-197.
  9. Constantino, B. T. J. L. M. The red cell histogram and the dimorphic red cell population. 2011, 42, 300-308.
  10. Garg, M.; Sangwan, K. Comparison of automated analyzer generated red blood cell parameters and histogram with peripheral smear in the diagnosis of anaemia. 2019.
  11. Choudhary, S.; Bordia, S.; Choudhary, K. J. I. J. o. B.; Research, A. M. Sensitivity of Red cell histogram and CBC parameters against peripheral blood smear in various anemias. 2018, 8, 135-141.
  12. Benson, C. S.; Shah, A.; Frise, M. C.; Frise, C. J. J. O. M. Iron deficiency anaemia in pregnancy: a contemporary review. 2021, 14, 67-76.
  13. Abu-Ouf, N. M.; Jan, M. M. J. S. m. j. The impact of maternal iron deficiency and iron deficiency anemia on child's health. 2015, 36, 146.
  14. Sandhya, V.; Rashmi, G. J. I. J. o. P.; Oncology. Correlation of peripheral smear with RBC indices and RBC histograms in the diagnosis of anemia. 2017, 4, 242-246.
  15. Swami, L.; Bhatt, N.; Khumanthem, G.; Bansal, I.; Sale, S.; Mane, V. Correlation of peripheral smear with RBC indices and RBC histogram in the diagnosis of anemia.