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Pathology

AN ANALYTICAL STUDY OF HISTOGRAMS AND THEIR CORRELATION WITH PERIPHERAL BLOOD SMEAR FINDINGS

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ABSTRACT **BACKGROUND:** Histograms produced by automated hematology analyzers are the graphical representation of numerical data of different cell populations. The varying shapes and patterns are useful in identifying the underlying pathology.

AIMS: To correlate RBC, WBC and Platelet histogram abnormalities with peripheral blood smear findings.

METHODS: A descriptive cross sectional study was undertaken on 500 patients with abnormal histograms and/or abnormal peripheral smears. Alterations in RBC, WBC and platelet histograms were analyzed with their respective peripheral smear findings. Pearson's Chi square test and Fisher exact test were used for statistical analysis.

RESULTS: Of 500 cases, 85.2% showed RBC histogram defects (n=426), 4.2% had WBC histogram defects (n=21), and 3.6% showed platelet histogram defects (n=18). In RBC histogram defects, majority had a left shift (66.8%) followed by right shift (17.8%) and bimodal peak (0.6%) respectively. WBC defects included WU (n=5), WL (n=1), F1 (n=4), F2 (n=1), F3 (n=3), T1 (n=3) and T2 (n=3). Platelet histograms revealed lower peak, PL (n=4), upper peak, PU (n=3) and multiple peak, MP (n=1). PBS showed RBC defects (n=420), WBC defects (n=171) and platelet defects (n=117). Correlation between RBC, WBC and platelet histogram defects with PBS was statistically significant (P 0.0001). The number of pathological samples picked up by the automated analyzers were significantly less for WBC and platelet abnormalities as compared to RBC.

Conclusion: Histograms should be used as a screening method to pick up pathological samples. These samples should then be followed by a peripheral smear examination for confirmatory diagnosis.

KEYWORDS : Histograms, Rbc, Wbc, Platelet, Peripheral Blood Smear.

INTRODUCTION

Histograms are the graphical representation of numerical data of different cell populations; produced by automated hematology analyzers. In a histogram X axis represents the cell size and Y axis represents the number of cells.[1] Histograms when combined with absolute blood counts provide valuable information regarding various abnormalities in the blood samples. The varying shapes and patterns of histograms are useful in identifying the underlying pathology even before a peripheral blood smear is examined. Identification of shifts of these histogram curves in one direction or the other can be of utmost diagnostic importance.[2] Microscopic examination of a peripheral blood smear also has its advantages.[3] Specific diagnosis of WBC disorders such as Leukemia are possible only with the help of a peripheral smear examination (PBS). Over the past few years it is seen that complete blood count (CBC) performed by the automated hematology analyzers and microscopic examination of their respective peripheral smears have very well complemented each other. This helps in providing a comprehensive report on the patient's blood sample.[4] In this study we have compared the abnormal RBC, WBC and Platelet histograms with their respective peripheral smear findings and analyzed their utility in diagnosing various hematological conditions. There have been various studies to compare RBC histograms with PBS but very few studies are done on the utility of WBC and Platelet histogram comparison with their respective smear findings. This study is unique and one of the first of its kind in English literature to have compared all three histograms with peripheral blood smears.

SUBJECTS AND METHODS

A descriptive cross sectional study was carried out at a tertiary care hospital and research centre in Pune which included 500 inpatients (IPD) of medicine, surgery and OBGY departments. An institutional ethics committee clearance (IECC) was obtained. Data analysis was done based on records of the investigations obtained in Central Clinical Laboratory (CCL) over a period of two years from October 2017 to September 2019. All adult patients with abnormal histograms and/or abnormal peripheral smears were included in the study. All patients with normal histogram and normal PBS along with pediatric patients were excluded from the study. The venous blood samples were obtained in an EDTA tube and run in automated hematology analyzers which included the three part (Sysmex KX-21).

Histograms were studied based on their shape, size, and centre of spread along with the starting and end points of curve. Presence of any histogram defects in RBC (left shift, right shift and bimodal peak), WBC (WU, WL, F1, F2, F3, T1, T2) and platelet (PL, PU and MP) were noted. These were followed with their respective peripheral smear examination prepared according to the standard operating procedures and stained by Leishman stain. The shape, size and chromia of RBCs were studied. The count, morphology and presence of any immature cells were noted for WBCs. Platelets were examined for count, giant platelets and platelet clumps. Study of hemoglobin (Hb) along with indices such as Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RDW), Mean platelet volume (MPV) and Platelet distribution width (PDW) were also included in study. A qualitative analysis of the data was done using Pearson's Chi square test and Fisher exact test wherever appropriate.

RESULTS

The histograms and peripheral smears of 500 patients were analyzed. The age group of patients ranged from 18 to 67 years. Majority of patients were between 31–40 years of age (n=134). 50.4% (n=252) were males. Majority of females were in the reproductive age group. Association of age and sex with histograms and peripheral smears in our study was not found to be statistically significant (P 0.16).

In our study, majority of histograms showed RBC defects (85.2%, n= 426), followed by WBC (4.2%, n=21) and platelet (3.6%, n= 18). Both histogram and peripheral smears revealed combined defects in 24 (4.8%) and 323 (64.6%) cases respectively. Maximum defects were found in RBC and WBC. The detailed distribution of combined defects were as shown in Chart 1. Anemia emerged as the commonest disease entity in our study population and was picked up easily by both peripheral smears and on histograms. The abnormalities of platelets and WBC being less common and more challenging to diagnose, were diagnosed more on peripheral smears than histograms.

The distribution of cases based on the RBC histogram defects, showed that the majority had a left shift (66.8%, n=334) followed by right shift (17.8%, n=89) and bimodal peak (0.6%, n=3) respectively. Based on the peripheral smear examination, microcytic hypochromic anemia was the most common type (62.2%, n=311). The distribution of anemia cases according to PBS features and their respective histograms obtained are as shown in Table 1 (Figure 1). Correlation between the RBC histograms and their peripheral smears was found to be statistically significant (P 0.001). Descriptive statistics of RBC indices in the various types of anemia were as shown in Table 2.

In a normal WBC histogram lymphocytes are distributed between 50-100 fl, mixed cell population (monocytes, basophils and eosinophils) between 100-150 fl, and neutrophils between 150-300 fl.[V] We found 21 WBC histogram defects whereas PBS examination revealed 114 cases with leukocytosis and 57 cases with leukopenia. Correlation between WBC histogram defects and WBC abnormalities on PBS was found to be statistically significant (P 0.001) (Table 3) (Figure 2).

Platelet distribution curves had three discriminators, the lower discriminator (LD) which is set at 2-6 fl, the upper discriminator (UD) at 12-30 fl and a fixed discriminator at 8-12 fl.[5] Other parameters such as MPV, PDW and PCT provide a multitude of information on platelet morphology. Platelet histogram showed three types of flags PL, PU and MP. The mean MPV and PDW in flagged cases were 8.32 fL and 14.05 fl respectively which were within the normal range. The distribution of platelet histogram defects (PL, PU and MP) and their PBS findings were as shown in Table 4 (Figure 3). Correlation between platelet histogram defects and platelet abnormalities on peripheral smears was found to be statistically significant (P 0.001).

DISCUSSION

Our study comprised of adult patients ranging from 18 to 67 years of which 50.4 % (n= 252) were males. Anemia affecting RBC histograms formed the most common histogram defect in our study. Majority of anemic patients were between 21-40 years. Among females, 85.08% (n=211) patients were in the reproductive age group. According to WHO, approximately 1.62 billion people (24.8% of the total population) were affected by anemia worldwide, among which pregnant women are the most affected (41.8%).[6] Nearly 50% of women in the reproductive age group and around 26% of men in the age group of 15-59 years were known to be anemic.[7] Thus anemia being the most prevalent disease entity formed the majority of cases in our study. In a study done by Jain A. et al, the age group of patients ranged from 5 to 70 years. Majority (53.6%) were in the age group of 21-50 years.[8]

PBS revealed microcytic hypochromic anemia in 62.2% (n=311) cases. Of these 94.21% (n= 293) showed a left shifted curve, 4.50% (n=14) showed a normal curve, 0.96% (n=3) showed a right shifted curve and 0.32% (n=1) showed a bimodal peaked histogram respectively. As evident from our results, microcytic anemia was easily picked up the analyzers as left shift of histograms. The mild difference in the analysis of microcytic anemias by PBS and RBC histograms can be explained by the presence of inter observer variations, early microcytic change and patients receiving iron therapy. A thorough peripheral smear examination and clinical correlation will help in arriving at definitive diagnosis. Our study was in concordance with the study done by Poonam Radadiya et al.[9]

Of 18.6% (n=93) cases of macrocytic anemia diagnosed on PBS, 87.09% (n=81) showed a right shift in curve, 9.68% (n=9) showed left shifted curve and 3.22% (n=3) cases showed a normal histogram. Although majority of cases had a right sided shift, the slight variation can be explained by the fact that macrocytic anemia includes megaloblastic anemia and various non megaloblastic causes such as hemolytic anemia, obstructive jaundice, aplastic anemia, alcoholism, hypothyroidism and many others in which the RBC's may be mildly microcytic due to hemolysis or overall normal in size. Patients receiving B12 and folic acid therapy will also show a population of normal sized RBCs. These cases could have been excluded by the coulter machines.[10]

Normocytic normochromic anemia formed 16% (n=80) of total anemia cases on PBS. Histogram analysis displayed a normal curve in 62.5% (n=50) cases, left shift of curve in 31.25% (n=25) cases and right shift of curve in 6.25% (n=5) cases. Our results revealed that the automated analyzers are more sensitive in detecting variations occurring in the size of RBCs. Slight disproportion in the size of RBCs on peripheral smears may be reported as normal by the observer.

Out of 3.2% (n=16) cases of dimorphic anemia diagnosed on PBS, 43.75% (n=7) showed normal curve, 43.75% (n=7) showed left shift in curve and 12.5% (n=2) cases showed bimodal curve. Dimorphic anemia is characterized by two deficiencies, iron deficiency along with nutritional Vitamin B12 and Folic acid deficiency. It is morphologically characterized by two cell population. Dimorphic anemia is purely detectable only on a PBS.[10] The wide variations obtained in the histogram patterns can be due to causes like iron deficiency anemia responding to iron therapy, after transfusion of normal blood to a patient with a hypochromic anemia, sideroblastic anemia and in cases of delayed transfusion reactions.

The WBC histogram defects give a clue to the presence of any imma ture cell or presence of any particular cell population in excess or low amounts. WBC morphology can be studied using a peripheral blood smear. It helps to determine if the WBC count is adequate, increased or decreased. Peripheral blood smear provides information regarding the size, shape and number of neutrophils, lymphocytes, eosinophils, basophils and monocytes along with presence of any immature precursors or blast cells. Thus a combined study of the histogram and peripheral smear will help in the rapid detection of WBC disorders.

We found 21 WBC histogram defects in our study. Of these, five cases were found to have WU defects and four cases had F1. There were three cases each of F3, T1 and T2 defects. two cases showed WL histogram defect and F2 histogram defect was found in only one case.
WU defect occurs when the height of the upper discriminator (UD) is greater than the preset 10% on the Y-axis.[11] The cause for this defect was extreme leukocytosis which was evident on the peripheral smears. All five cases of WU histogram defects were that of leukocytosis between 17,900- 47,600 cells per cubic mm. Three cases had neutrophilia in the range of 82%- 90%. Two cases revealed lymphocytosis of 60% and 62% respectively.

WL defect appears when there is an abnormal curve in front of the lower discriminator. The common causes being unlysed RBC (osmotic resistance), platelet clumps, EDTA-incompatibility, coagulated sample, erythroblasts (NRBC) and cold agglutinins.[12] There were two cases of WL defects in our study. One case had leucopenia with a WBC count of 3,600 cells per cubic mm and the other case had leucocytosis with a WBC count of 34,200 cells per cubic mm with 66% lymphocytosis. The disparity could be because of preanalytic and analytic human errors.

F1 flag appears when the relative height of T1 trough exceeds preset limit of 40%. It indicates that the small cell and middle cell data may be inaccurate.[12] The four cases showing F1 histogram defects had a WBC count ranging from 14,000- 21,500 per cubic mm. Eosinophilia of 12-18% was seen in three cases and the remaining one case showed monocytosis of 15% on PBS.

F2 flag appears when the relative heights exceed the preset of T1 (40%) or T2 (50%). It also means that the middle cell data is inaccurate.[11] This occurs in eosinophilia, acute myeloid leukemia and monocytosis. The case showing F2 defect was a case of 20% eosinophilia.

F3 flag appears when T2 exceeds the preset limit of 50%. It means that the large cell data inaccurate. This occurs in cases of eosinophilia and acute leukaemias.[11] The three cases of F3 were showing a high WBC count between 14,000 to 91,400 cells per cubic mm. One case from them being that of acute leukemia showing 80% blast cells. Of the two remaining cases 1 case had 25% eosinophilia while the other had a differential count within the normal range.

T1 and T2 are valley discriminator defined by the plateau. It separates leukocyte population into three sub populations. The T1 and T2 discriminators are flexible and can be set automatically according to the sample.[11] In extreme pathological condition discrimination between these three populations is not possible. Flag T1 occurs when discrimination between lymphocytes and mid cell population is not done as in abnormal leukocytosis like chronic myeloid leukemia. Flag T2 appears when discrimination between mixed cell and neutrophil could not be done, as in chronic lymphocytic leukemia.

The three cases showing T1 and T2 defects were cases of leukemoid

reactions with a WBC count between 21,700- 33,200 cells per cubic mm and PBS showing shift to left. The peripheral smears of all 500 cases were evaluated for any abnormality in the WBC count or morphology. 114 cases showed an increased count and 57 cases showed a decreased count. Correlation between WBC histogram defects and WBC abnormalities on PBS was found to be statistically significant (P 0.01).

PBS showed abnormality in 171 out of 500 cases whereas their respective histograms were found abnormal in only 21 cases. This can be due to the fact that only extreme cases of leukocytosis and leukopenia are flagged by the coulter machine. We do not deduce the fact that these histograms and suspect flags are efficiently diagnostic by themselves. Morphological defects and other chronic leukemias may be misinterpreted by the analyzers and be considered normal. If any abnormality is found in the WBC histogram, it should ideally be followed by a peripheral smear examination.

Platelet histogram may show three types of flags.[11] PL flag occurs when the lower discriminator exceeds the preset height by 10%. The platelet count, MPV and P-LCR will show the PL flag. It occurs due to noise. PU flag occurs when the upper discriminator exceeds the preset height by more than 40%. It occurs in hemolytic anemia with fragmented red cells and large platelets. MP flag occurs when there are more than one peak in the same curve. The common causes being platelet transfusion, platelet aggregates and recovery from thromboc ytopenia.

Histogram analysis of platelet graph showed a lower peak (PL) in four cases, upper peak (PU) in three cases and multiple peak was found in one case. Three out of four cases showing PL flags were cases of thrombocytopenia and one case had thrombocytosis. Of these four cases with PL flags, two cases had platelet clumps while one case had giant platelets. Of three cases with PU flags, two cases were that of thrombocytopenia and one case was of thrombocytosis. MP flag was found in one case of severe thrombocytopenia on platelet transfusion. Various other histogram defects were found such as graphs having a very small peak, graphs ending abruptly on the axes and in some cases graphs were not formed at all. Such cases had severe thrombo cyt openia with platelet counts below 20,000 per cu mm.

However on PBS examination of 500 cases, 85 cases showed thrombocytopenia. Of these 85 cases histogram defects were found in only 15 cases. Of 32 cases of thrombocytosis in PBS only 3 cases showed histogram defects. Thus platelet histogram defects are very rare and found only in extreme cases of thrombocytopenia and thrombocytosis. Therefore to diagnose diseases of platelets, PBS examination should be considered gold standard. Majority of PBS had normal platelet count, which revealed no histogram defect. Correlation between platelet histogram defects and PBS abnormalities was found to be statistically significant (P value 0.01).

CBC histogram analysis is usually a neglected part of automated haemograms. These if well interpreted, can have a good potential in providing diagnostically relevant information about various disease process. Our study revealed an important correlation between RBC histograms and peripheral smear diagnosis in cases of Microcytic Hypochromic, Normocytic Normochromic and Macrocytic Anemia. The relationship between histogram patterns and peripheral smear diagnosis in dimorphic anemia however posed queries regarding the rationality of these histocytograms. All WBC defects except WL correlated well with the peripheral smear findings. Platelet histograms also proved very useful in easy sampling of pathological cases. Based on our findings we conclude that even in the era of automation and molecular analysis, peripheral smear study alongside clinical history and examination is an important diagnostic tool while managing the various patients of hematological disorders. Their combined interpretation may prove helpful in marking the suspect samples. In the high output laboratories this is therefore a vital contribution by the automated data ensuring that the suspect samples are not uninten tionally missed or delayed in analysis. As a thumb rule we should use histograms as a screening method to pick up pathological samples quickly and follow them up with peripheral blood smear examination for definitive diagnosis.

Chart 1: Distribution of combined defects on histograms and PBS in study group

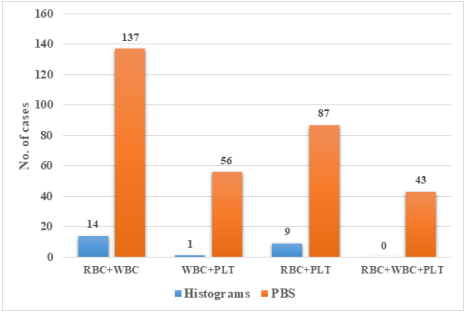

Table 1: Correlation between RBC histogram and type of anemia on PBS

Type of anemia on PBS	RBC histogram changes				Total
	Right shift	Left shift	Bimodal	Normal	
Microcytes	3	293	1	14	311
Macrocytes	81	9	0	3	93
Dimorphic	0	7	2	7	16
Normal	5	25	0	50	80
Total	89	334	3	74	500

Chi-square = 608.03, P<0.0001

Table 2: Descriptive statistics of RBC indices in the various types of anemia

Type of anemia	RBC parameters (Mean ± SD)					
	Hb (gm/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (gm/dl)	RDW (fl)
Normocytic normochromic	10.44 ± 3.02	33.77 ± 9.06	83.41 ± 14.27	25.81 ± 5.14	30.82 ± 3.44	52.28 ± 13.48
Microcytic hypochromic	8.78 ± 2.16	29.23 ± 6.22	67.82 ± 9.99	20.40 ± 3.99	30.06 ± 3.39	46.96 ± 11.35
Macrocytic	10.07 ± 3.43	32.92 ± 11.96	109.20 ± 18.49	32.95 ± 6.55	30.25 ± 3.43	64.92 ± 24.49
Dimorphic	9.69 ± 2.47	31.08 ± 7.84	74.10 ± 11.32	23.34 ± 4.92	31.36 ± 3.46	45.91 ± 7.39

Table 3: Correlation between WBC histogram defects and WBC abnormality on PBS

WBC histogram defects	PBS abnormalities (count)			Total
	Increased	Decreased	Normal	
F1	4	0	0	4
F2	1	0	0	1
F3	3	0	0	3
T1	3	0	0	3
T2	3	0	0	3
WL	1	1	0	2
WU	3	0	2	5
Normal	96	56	327	479
Total	114	57	329	500

Chi-square = 28.29, P<0.0001

Table 4: Correlation between platelet histogram defects and platelet abnormality on PBS in study group

Type of histogram	PBS Abnormalities (count)			Total
	Increased	Decreased	Normal	
Peak Lower	1	3	0	4
Peak Upper	1	2	0	3
Multiple Peak	0	1	0	1
Others	1	9	0	10
Normal	29	70	383	482
Total	32	85	383	500

Fisher exact test: P<0.0001

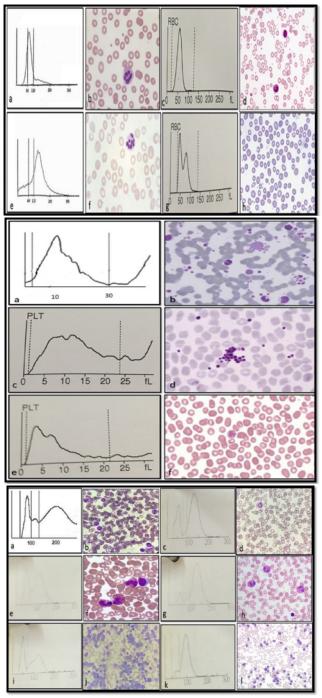

FIGURE LEGENDS

Figure 1. Correlation of RBC histograms with PBS.

a. RBC histogram showing a normal Bell Shaped Curve in normocytic normochromic anemia
b. PBS showing Normocytic normochromic anemia
c. RBC histogram showing Shift to left in microcytic hypochromic anemia
d. PBS showing Microcytic hypochromic anemia
e. RBC histogram showing Shift to right in macrocytic anemia
f. PBS showing Macrocytic anemia
g. RBC histogram showing a bimodal peak in Dimorphic anemia
h. PBS showing Dimorphic anemia

Figure 3. Correlation of WBC histograms with PBS.

a. WBC histogram showing WU defect (Abnormal Curve in Front of the Upper Discriminator)
b. PBS showing Leukocytosis (neutrophilia)
c. WBC histogram showing WL defect (Abnormal Curve in Front of the Lower Discriminator)
d. PBS showing leucopenia
e. WBC histogram showing F1 defect (Flag appearing when the relative height of T1 exceeds preset limit of 40%)
f. PBS showing eosinophilia
g. WBC histogram showing F2 defect (Flag appearing when the relative heights exceed the preset of T1 (40%) or T2 (50%)
h. PBS showing eosinophilia
i. WBC histogram showing F3 defect (Flag appearing when the T2 exceeds the preset limit of 50%)
j. PBS showing acute leukemia
k. WBC histogram showing T1 defect (Abnormal Curve at T1 Level (Trough 1)
l. PBS showing leukemoid reaction

Figure 2. Correlation of platelet histograms with PBS.

a. Platelet histogram showing PL defect (Abnormal height at lower discriminator)
b. PBS showing giant platelets
c. Platelet histogram showing PU defect (Abnormal height at upper discriminator)
d. PBS showing platelet clumps
e. Platelet histogram showing MP defect (Multiple peaks)
f. PBS showing severe thrombocytopenia

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