European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 09, Issue 02, 2022

Comparison of anemia characterization between automated CBC analysis and conventional peripheral blood smear assessment

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Abstract

Introduction: Complete blood count (CBC) by the automated hematology analyzers and microscopic examination of peripheral smears have traditionally been used in the diagnosis of anemias. The advent of automated hematology analyzers has improved accuracy and precision of test results and has reduced subjective errors. This study aimed at

i) Comparing anemia characterization between automated CBC analysis and conventional peripheral blood smear assessment.
ii) Studying RBC histogram patterns in various categories of anemia.

Materials and methods: Blood samples from 500 adult anemic patients were run in Sysmex XP-100 fully automated, 3-part differential hematology analyzer. In all patients, blinded peripheral blood smear examination by two observers was done. Anemia categorization by peripheral blood smears and automated red cell data was compared.

Results: The number of patients with normocytic normochromic anemia on automated CBC was 280 and on peripheral smear examination was 269. On automated CBC, 137 patients had microcytic hypochromic anemia whereas 107 patients had microcytic hypochromic picture on microscopy. Significantly higher number ($p<.05$) of patients (76) with microcytic normochromic morphology was diagnosed on blood smear compared to automated counts (17). When RBC volume and hemoglobin content were considered together, a Kappa value of. 447 was obtained indicating moderate agreement between the automated and manual (peripheral blood smear) assessment of anemia.

Conclusion: Patients with most anemia types can be accurately diagnosed by automated CBC analysis. The peripheral blood smear has limitations in cases with borderline MCVs and mild hypochromia which may be missed. It, however, still remains the cornerstone in the identification of abnormal RBC morphology seen in hemolytic anemias.

Keywords: Anemia, CBC, peripheral blood smear, automated analyzers

Introduction

Anemia is a very common condition and affects up to one-third population of the world. It is mild and asymptomatic in many cases. It is more common in pregnant women, women of reproductive age and the elderly with a rising prevalence with age [1]. According to the National Family Health Survey 2015-

16, 53% of all women in India are anemic. In India, 53% of women and 23% of males between the ages of 15 and 49 are anemic [2].
Anemia can be easily classified by the use of automated red cell indices generated in a CBC. The study of the peripheral blood smear for the same is cumbersome and is limited by inter-observer variability [3]. Along with automated numerical data, study of red cells histograms is a useful tool in the categorization of anemia [4]. Various shapes of histogram give an indication about the red cell population, sometimes even before the changes are apparent on a blood smear [5, 6]. With the emergence of automated hematology analyzers and accurate red cell indices, microscopic examination of blood smear for the diagnosis of anemia has been challenged especially in high throughput laboratories [7-10].
We carried out this study to compare anemia characterization between automated CBC analysis and conventional peripheral blood smear assessment.

Materials and methods

This cross-sectional study was carried out over a period of one and half years from 1st December, 2019 to 31st May, 2021 in the Department of Pathology, Maharishi Markandeshwar Medical College and Hospital, Kumarhatti, Solan.
Cases for the study were selected by simple random sampling of all patients whose blood samples were sent to the hematology laboratory for CBC analysis. Of these, 500 adult patients (>18 years of age), both in patients and out patients with anemia as per WHO reference range [Hb <13 gm/dl (men), Hb<12gm/dl (women), Hb <11gm/dl (pregnant women)] were included in the study.
The exclusion criteria included: Clotted blood sample, finger prick sample, cases in which no consensus was reached between the observers on microscopy or arte factual changes in red cells obscuring morphological features.
Blood samples collected in evacuated containers containing dipotassium EDTA were run in Sysmex XP-100 fully automatic hematology analyzer or in PCi 20 three part differential hematology analyser within 1 hour of collection. Anemia was categorized as normocytic, microcytic and macrocytic based on MCV. (Microcytic: MCV< 80 f; Normocytic: MCV 80-100 fL; Macrocytic: MCV > 100 fL. Hemoglobin content was categorized as normochromic or hypochromic based on MCH (Normochromic: MCH 27-32 pg, Hypochromic: MCH< 25 pg). RBC histograms in all patients were also studied for left or right shift, widening, double peaks, extended trails or irregular pattern.
To maintain objectivity, recording of red cell morphological features on Leishman-stained slides was done as per standardized criteria [11]. Anemia was assessed by two observers who were blinded to the results of automated CBC data. In case of discrepancy between the observations of both observers, the slides were reviewed jointly and a consensus was reached. Trilevel quality control samples were run daily to ensure instrument precision and accuracy.
In all patients, categorization of anemia by conventional peripheral smear and automated red cell parameters were compared using Chi square and Kappa statistics.

Ethical consideration: The study was undertaken after approval by Institutional Ethical Committee.

Results

The age of the patients in the study ranged from 18 years to 92 years. More than half (57.4%) of the patients were in age group between 18 to 50 years. The mean age was 47.8 ± 18.7 years. Of the 500 patients, 334 were females and 166 males, (M:F=1:2).
Of the 500 patients, 278 (55.6%) had mild anemia (Hb-9.1-12.7 gm/dL; mean Hb: 10.5 ± 0.9 gm/dL), 202 (40.4%) had moderate anemia (Hb-6.1-9.0 gm/dL; mean Hb: 7.9 ± 0.8 gm/dL) while 20 (4%) patients had severe anemia (Hb-3.0-5.9 gm/dL; mean Hb: 4.6 ± 0.9 gm/dL). Nearly 2/3rds of the patients had normocytic normocytic anemia followed by microcytic anemia and macrocytic anemia (Fig 1).

ISSN 2515-8260 Volume 09, Issue 02, 2022

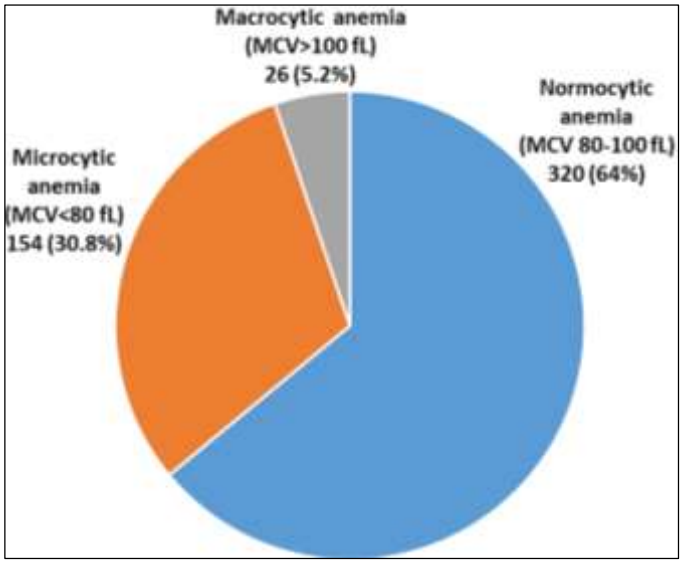

Table 1: Shows comparison of various hematological parameters in normocytic, microcytic and macrocytic anemias

	Normocytic anemia		Microcytic anemia		Macrocytic anemia		p
	Range	Mean ± S.D	Range	Mean ± S.D	Range	Mean ± S.D	
Hemoglobin (gm/dl)	3.9-12.9	9.4 ± 1.7	3.2 - 12.7	9.1 = 1.6	3.0-12.4	7.9 ± 2.4	<.0001
RBC	1.2-5.1	3.4 ± 0.7	2.0-5.9	4.2 ± 0.7	0.8 - 3.9	2.4 ± 0.8	<.0001
Hct	10.4-41.9	30.2 ± 5.6	13.2 -39.9	30.5 ± 5	10.4 - 41.9	25.7 ± 8.3	.0002
MCV (fl)	80-100	88.4 ± 4.9	53.1-79.9	72.9 ± 5.4	100.4 -126.8	108.7 ± 7.1	<.0001
MCH (pg)	20.4-38.8	27.7 ± 2.4	12.4-32.0	21.8 ± 3.3	26.6-44	33.9 ± 3.7	<.0001
MCHC (gm/dl)	25.3-45.2	31.4 ± 2.3	23.2-40.4	29.8 ± 3.2	25.3-35.6	31.2 ± 2.5	<.0001
RDW (%CV)	11.0-28.6	15.1 ± 2.8	10.8-34.8	17.2 ± 3.7	12.3-42.2	21.2 ± 6.5	<.0001
TLC ((/cu.mm)	1300-36400	9100 ± 4700	700-36000	9.2 ± 4.9	2100-6000	5800 ± 3400	.002
Platelets (/cu.mm)	7000-887000	221000 ± 129000	38000-95R000	303000 ± 149000	6000-472000	24000 ± 104000	<.0001

The mean hemoglobin was comparable between patients with normocytic normochromic and microcytic hypochromic anemia while the mean hemoglobin in patients with megaloblastic anemia was significantly lower. RBC count, hematocrit, MCV, MCH, MCHC, TLC and platelet count showed significant intragroup differences ($p<.05$). Mean RDW was lowest in patients with normocytic normochromic anemia and highest in patients with macrocytic anemia. (Table 1)

Table 2: Comparison of automated CBC values and peripheral blood smear findings

Category of anemia	Automated CBC Number (percentage)	Peripheral blood smear Number (percentage)
Normocytic normochromic	280 (56%)	269 (53.8%)
Normocytic hypochromic	40 (8%)	0
Microcytic Normochromic	17 (3.4%)	76 (15.2%)
Microcytic Hypochromic	137 (27.4%)	107 (21.4%)
Macrocytic Normochromic	26 (5.2%)	19 (3.8%)
Dimorphic	0	17 (3.4%)
Megaloblastic	0	10 (2.0%)
Spherocytosis	0	02 (0.4%)

The number of patients with normocytic normochromic anemia, microcytic hypochromic anemia and macrocytic normochromic anemia was comparable between automated CBC and peripheral blood smear findings. Significantly higher number of patients with microcytic normochromic picture were diagnosed

on blood smear compared to automated counts. (Table 2)

Discrepancies between automated CBC analysis and peripheral smear examination

Discrepancies were seen in some cases between the diagnosis made by two methods. There were 8 categories of discrepancies:

i) Normocytic normochromic RBCs on peripheral blood smear and microcytic normochromic on automated CBC.
ii) Normocytic normochromic RBCs by smear examination and microcytic hypochromic on automated CBC.
iii) Microcytic hypochromic anemia by smear examination and microcytic normochromic anemia on automated CBC.
iv) Microcytic hypochromic anemia by smear and normocytic normochromic anemia by automated CBC.
v) Microcytic normochromic anemia by smear and normocytic normochromic anemia by automated CBC.
vi) Microcytic normochromic anemia by smear and microcytic hypochromic anemia by automated CBC.
vii) Microcytic normochromic anemia by smear and macrocytic normochromic anemia by automated CBC.
viii) Macrocytic normochromic anemia by smear and normocytic anemia by automated CBC.

Chi square test showed significant difference ($p<.0001$) between the automated and peripheral blood smear categorization of anemia. Chi square statistic was 81.635. Comparable diagnosis was seen for normocytic normochromic anemia, microcytic hypochromic anemia and macrocytic normochromia. Significant difference was seen for microcytic normochromic anemia and normocytic hypochromic anemia. Megaloblastic anemia, dimorphic anemia and two patients with spherocytosis were diagnosed only on peripheral blood smear.

Table 3: RBC histogram patterns in patients with anemia included in the study (n=500)

RBC Histogram pattern	Number	Percentage
Normal	358	71.6%
Left shift	83	16.6%
Right shift	35	7.0%
Broad	21	4.2%
Dual population	03	0.6%

The most common RBC histogram pattern seen in the study was normal curve (71.6%) followed by left shift and right shift in 16.6% and 7.0% patients respectively. (Table 3, Fig 2)

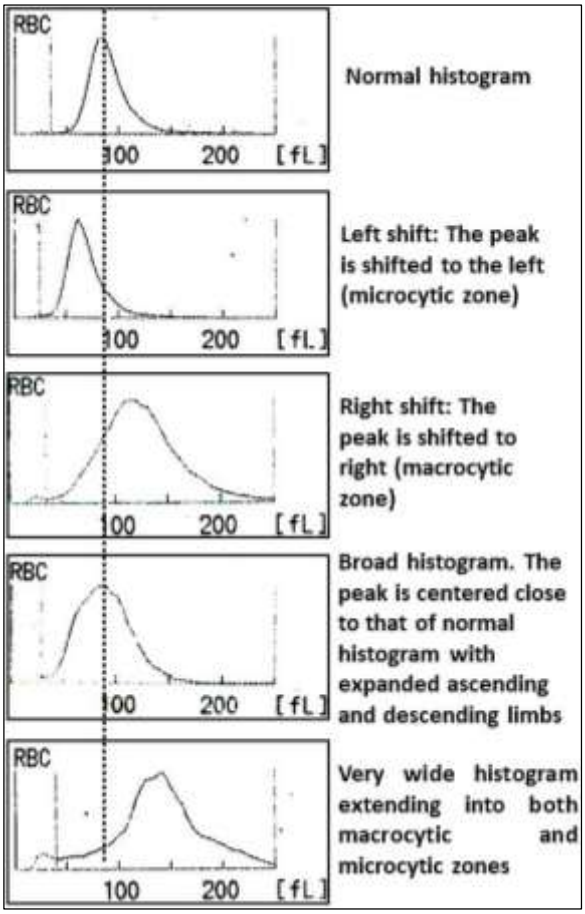

Fig 2: Various histograms in patients with anemia. The dotted line represents the peak position of a normal RBC histogram

Table 4: RBC histogram patterns in different anemias diagnosed on automated CBC (n=500)

RBC Histogram Pattern	Normocytic anemia (n=320) Number (%)	Microcytic anemia (n=154) Number (%)	Macrocytic anemia (n=26) Number (%)
Normal	285 (89.1%)	73 (47.4%)	0
Left shift	05 (1.5%)	78 (50.7%)	0
Right shift	15 (4.7%)	0	20 (76.9%)
Broad	15 (4.7%)	0	06 (23.1%)
Dual population	0	03 (1 9%)	0

Normal RBC histogram was seen in majority (89.1%) of patients of normocytic anemia. Left shift was seen in 50.7% patients with microcytic anemia. Seventy-three (47.4%) patients with microcytic anemia, however showed normal histogram. The details are shown in Table 4.

Table 5: Various histogram patterns along with their mean MCV and RDW in patients with normocytic, microcytic and macrocytic anemia diagnosed on automated CBC (n=500)

	Normocytic anemia (n=320)			Microcytic anemia (n=154)			Nlacrocytic anemia (n=26)		
Histogram pattern	Number	MCV	RDW	Number	MCV	RDW	Number	NICV	RDW
Normal	285	88.1±4.7	14.6±2.2	73	762+3.1	161+-23	-	-	-
Left shift	05	84.8+-2.9	24 2+-3 1	78	693±53	17.9±3.5	-	-	-
Right shift	15	92.6±4.6	17 3±3 0	-	-	-	20	1083-±7.8	20.5±53
Broad	15	913±5.2	19.7±-3.4	-	-	-	06	107.8+4.6	23 5±9 8
Dual population	-	-	-	03	743-±4.9	273±11.4	-	-	-

Out of 320 cases of normocytic anemia diagnosed on automated CBC, 285 (89.1%) showed normal RBC histogram with normal mean MCV and RDW. However patients with normocytic anemia also showed right shift and broad curve in 4.7% cases each. These patients had a normal MCV but with raised RDW and a broad histogram. Cases of normocytic anemia which showed right shifted and broad histograms had higher MCV as compared to those with normal histograms (Table 5). RBC histograms showed a left shift pattern in most patients with microcytic anemia (Fig 3B and 3C) and right shift in patients with macrocytic anemia (Fig 3 F). Overt dimorphic anemia can be suspected from automated RBC data by a wide RBC histogram pattern and very high RDW reflecting the dual RBC population. (Fig 3 G,H,I)

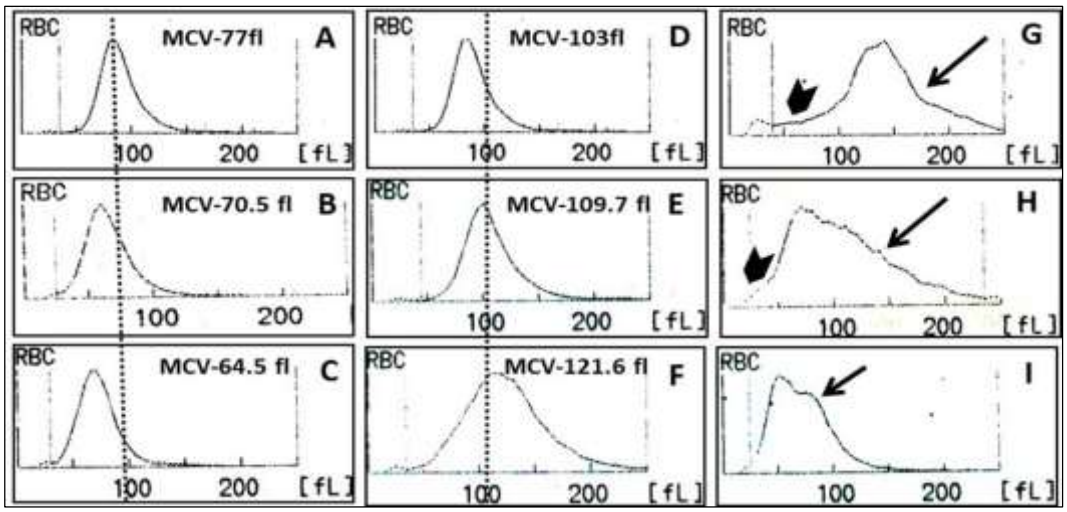

Fig 3: RBC histograms in patients with iron deficiency anemia (A, B, C) show increasing left shift with lower MCVs. Figures D, E and F show right shift corresponding to rising MCVs in patients with macrocytic anemia. Figures G, H and I show histograms in patients with dimorphic anemia with dual RBC population-predominantly macrocytic (arrows) and a minor microcytic population (arrow head). The histogram in Figure I is from a patient with iron deficiency on treatment. The iron replete normocytic normochromic RBCs appear as a minor 2nd population (arrow)

Table 6: Shows comparison of RBC parameters in all anemias except normocytic normochromic anemia

	Microcytic normochromic anemia		**Microcytic hypochromic anemia**		**Macrocytic normochromic anemia**	
	Automated (n=17)	**Manual (n=78)**	**Automated (n=137)**	**Manual (n=110)**	**Automated (n=26)**	**Manual (n=33)**
Hb (gm/dL)	9.6±1.8	9.2±1.6	9.0±1.6	8.7±1.8	7.9±2.4	8.0±2.3
MCV (fl)	77.5±2.3	83.7±9.7	72.3±5.4	75.3±8.5	108.6±7.1	101.0±11.9
MCH (pg)	27.8±2.2	25.8±3.7	21.0±2.5	22±3.5	33.9±3.7	32.4±4.2
RDW (%CV)	14±2.2	15.5±3.0	17.6±3.7	17.7±4.1	21.1±6.5	32.4±4.2

Seventy eight patients were identified to have microcytic normochromic anemia on automated CBC. However, only 17 cases were diagnosed as microcytic normochromic on peripheral smear examination. More patients were diagnosed to have microcytic hypochromic anemia by automated compared to those diagnosed on peripheral smear examination. The latter had slightly higher MCV values. The peripheral blood smear examination overestimated macrocytosis compared to automated counts. (Table 6)

Comparison of agreement (Kappa statistic) between red blood cell automated data and peripheral blood smear findings

When MCV based categorization of anemia was compared with peripheral blood smear findings, moderate agreement was seen. Kappa value of .430 was obtained.

When MCH based categorization of anemia was compared with peripheral blood smear red cell

hemoglobin content (normochromic or hypochromic RBCs), moderate agreement was seen. Kappa value of .555 was obtained.
When RBC volume and hemoglobin content were considered together, a Kappa value of .447 was obtained indicating moderate agreement between the automated and manual (peripheral blood smear) assessment of anemia.

Discussion

Our study has shown significant differences in anemia characterization by automated CBC analysis and peripheral blood smear interpretation.
Nearly two thirds (64%) of the 500 patients in the present study had normocytic anemia followed by microcytic anemia in 30.8% patients and macrocytic anemia in 5.2% patients. Similar to the present study, in a study by Samly *et al*., 61 (55.4%) out of 110 patients had normocytic anemia [12]. In a study by Choudhary *et al*. [8], Ashok *et al*. [13], Sandhya *et al*. [10] and Singla *et al*. [14] majority of the cases were found to be microcytic (70.6%, 53%, 46.8% and 49.2% respectively) by automated indices.
In the present study, red cell indices in patients with normocytic normochromic anemia were comparable to those on peripheral smear. Similar findings were reported by Sandhya *et al*. [10], Nanwani *et al*. [6], Samly *et al*. [12] and Jain *et al*. [15].
In the present study, 107 cases were diagnosed with microcytic hypochromic anemia on peripheral smear examination while automated CBC showed microcytic hypochromic anemia in 137 cases. Another study by Choudhary *et al*. diagnosed microcytic anemia in 70.6% cases on automated CBC and in 52.6% cases on smear [8]. In another study by Jain *et al*., microcytic hypochromic anemia was diagnosed in 40% of cases on smear and in 44.4% cases on cell counter [15]. In the present study, macrocytic anemia was observed in 19 cases on smear examination and in 26 cases by automated red cell indices. Significant difference in the diagnosis of this category were seen in studies by Sandhya *et al*. [10], Samly *et al*. [12] whereas studies by Choudhary *et al*. [8], Nanwani *et al*. [6] and Jain *et al*. [15] showed comparable results.
In the present study, patients with overt megaloblastic anemia, dimorphic anemia and spherocytosis were identified only on peripheral blood smear.
Sandhya *et al*. diagnosed 88 cases (17.6%) of dimorphic anemia by smear examination while 15.8% were diagnosed using an automated counter [10]. Samly *et al*. diagnosed 6 cases of dimorphic anemia in their study [12]. Nanwani *et al*. [6] and Choudhary *et al*. [8] found dimorphic anemia in 14.4% and 8.3% cases respectively on automated and in 6% and 8.1% patients on peripheral smear examination.
In the present study, there was significant difference in the number of patients in normocytic hypochromic and microcytic normochromic groups when anemia categorization by automated method and peripheral blood smear analysis was compared. This is explained by the fact that the RBCs are likely to be either normochromic or mildly hypochromic in early stages of anemia. The mild hypochromia detected by the automated indices is difficult to view with the naked eye. The extension of the one third normal central pallor of the RBC population may not be appreciable till the cellular hemoglobin drops further and hypochromia become visible microscopically. Also, early microcytosis, with borderline MCV values is not appreciable on microscopic examination. In such cases, however, RDW rise may be the first indicator of a significant emerging microcytic RBC population.
Our study showed significant difference ($p<.0001$) between the automated and peripheral blood smear for overall categorization of anemia. However, comparable diagnosis between the two methods was seen for normocytic normochromic anemia, microcytic hypochromic anemia and macrocytic normochromic anemia. There was moderate agreement (Kappa .447) between the automated RBC parameters and peripheral blood smear findings for the categorization of anemia. Similar to our study, study by Sandhya *et al*. found the correlation between the diagnosis made by the two methods to be statistically significant, $p<0.0001$ and the agreement between the two methods was moderate (0.518) [10].
In the present study, 10 (2%) patients were diagnosed to have megaloblastic anemia. Patients with megaloblastic anemia, although suspected on automated RBC data with raised MCV and RDW, still need

peripheral blood smear examination for confirmation of the classical findings of macrovalocytes and hypersegmented neutrophils. RBC inclusions too can be identified only on blood smear examination [16].
In the present study, two patients suspected to have hereditary spherocytosis on blood smear had normocytic anemia based on RBC indices. Unless the number of spherocytes is significantly increased on the blood smear, the MCHC may remain normal thus evading suspicion of this condition on automated analysis [17].
Overt dimorphic anemia can be frequently suspected from automated RBC data by a wide RBC histogram pattern and very high RDW reflecting the dual RBC population.
In the present study, normal RBC histogram was seen in 85.9% of normocytic normochromic anemia cases, 61.7% of microcytic anemia cases, 27.6% of macrocytic anemia and 23.5% of dimorphic anemia. Similar to the findings of the present study, Shrivastava *et al*. [7], Chavda *et al*. [5], Rao *et al*. [18] and Sandhya *et al*. [19] reported normal RBC histogram in normocytic normochromic anemia in 72.2%, 63.2%, 60.5% and 47.1% respectively.
In the present study, the RBC histograms showed a left shift pattern in 54.4% patients with microcytic hypochromic anemia. In the 45.6% patients with microcytic hypochromic anemia in which the left shift was not prominent, 44.1% had normal histogram and 1.5% (two patients) showed dual population. The patients showing normal histogram had borderline MCV (75-80 fl) which did not result in an appreciable shift of the histogram. In the two patients showing dual population in histograms, microcytes and a small population of macrocytes was seen.
In patients with dual deficiency in our study, RBC histograms clearly showed the two populations separately with very high RDW in tune with the varied cell volumes.
In the present study, automated red cell data was found to be useful for categorization of most anemias. The peripheral blood smear was found to have limitations in cases with borderline MCVs and mild hypochromia. In such cases, mis-classification of anemia is possible due to subjectivity in morphological assessment in these patients.
Since the automated CBCs are now the norm in most laboratory settings, time consuming and labor-intensive blood smear analysis can be avoided in cases where the RBC parameters point to a definitive diagnostic category of anemia. In cases where the clinical profile is indicative of specific categories (hemolytic anemia), peripheral blood smear examination is mandatory as the role of automated red cell indices in such conditions is limited.
When the RBC indices in different anemias are significantly deranged, RBC histograms are also indicative of specific diagnosis. Borderline or slightly abnormal values do not cause a visible shift of the RBC histogram.

Conclusion

CBC data including RBC histograms from automated hematology analyzers are as reliable as the standard manual method for the diagnosis of anemia. Automated method scores over manual smear review in patients with borderline or mildly reduced RBC indices. The manual scan of peripheral smear can be diagnostic in select anemias in which abnormal RBC shapes are present which are not easily detected by instruments.

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