Comparison of Automated Analyzer Generated Red Blood Cell Parameters and Histogram with Peripheral Smear in the Diagnosis of Anaemia

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ABSTRACT

Introduction: Red blood cell (RBC) indices and histogram obtained from automated analyzer give an idea about morphological changes in anaemia. Along with the peripheral smear, they can be used to interpret the cause of anaemia. The aim of the present study was to correlate typing of anaemia based on RBC indices and histograms obtained from automated analyzer with peripheral smear examination and to give a better approach in the diagnosis of anaemia and analyse their limitations.

Material and methods: Blood samples collected from 1575 anaemic patients, over a period of one year, in the Hematology laboratory, were typed for anaemia, first by parameters obtained by haematology analyzer (RBC indices with red cell distribution width) and then by peripheral blood smear findings. The correlation between the diagnosis made by peripheral smear versus RBC indices and histogram were analysed to find out the significance of the mean difference in various parameters.

Results: Microcytic hypochromic anaemia (50.86%) was the most common followed by normocytic normochromic (25.14%), macrocytic (2.29%), dimorphic (15.43%) and haemolytic anaemia (6.29%) as diagnosed on peripheral smear examination. Analysis by RBC indices showed 59.43%, 32%, 3.43%, 2.86% and 2.29% of microcytic hypochromic, normocytic normochromic, macrocytic, dimorphic and haemolytic anaemia respectively. Out of 801 (50.86%) cases of microcytic hypochromic anaemia on smear, 513(32.5%) revealed left shift while 288 (18.3%) showed broad-based curve on the histogram. In normocytic normochromic anaemia, anormal bell-shaped curve was seen in 378 cases (25%) while 18 cases (1.1%) showed the broad curve. Histograms in macrocytic anaemia show a right shift in 27 cases (1.7%) while 9 cases (0.6%) showed left shift. In dimorphic anaemia, the histogram was bimodal only in 2.9% of cases whereas a majority (11.4%) showed broad-base histogram.

Conclusion: Histogram and RBC indices give valuable information, but peripheral smear examination remains the important diagnostic tool in haematological disorders.

Keywords: Anaemia, Automation, Erythrocytes, Hemolytic, Hypochromic, Indices, Macrocytic, Microcytic

INTRODUCTION

Anaemia represents a major public health burden worldwide with high prevalence in developing countries like India. According to the World Health Organization (WHO), almost two billion people have anaemia in the world and half of them is attributable to iron deficiency.The estimated prevalence of anaemia in developing countries is 39% in children <5 years, 48% in children 5–14 years, 42% in women 15–59 years, 30% in men 15–59 years, and 45% in adults >60 years.[1] These staggering figures have important economic and health consequences for low- and middle-income countries. Red blood cell parameters and histogram areindispensable for diagnosis and management of anaemia.The conventional microscopic examination of peripheral blood smears for red blood cell (RBC) morphology provides crucial information about patients' clinical condition.[2] Nowadays sophisticated haematology analysers are being used in almost all large diagnostic and clinical settings but the peripheral smear examination has always been the cornerstone of diagnostic haematology. Analyzing the blood films routinely has facilitated interpretation of various haematological disorders and has been a major diagnostic tool for workup of most commercial laboratories. The advent of automation has reduced subjective errors,improved accuracy, precision and safety in handling of blood samples. Nevertheless, microscopic examination is essential for primary calibration and for presumptive diagnosis of anaemia, leukaemia and other related disorders.[3]

RBC histogram along with other CBC parameters like Red-cell Distribution Width (RDW) andMean Corpuscular Volume (MCV),are crucial for diagnosis and management of various RBC disorders. RBC histogram provides valuable information in addition to the numerical data obtained from CBC as in megaloblastic anaemia with developing iron deficiency.The RBC histogram is often used with peripheral smear examination as an aid in monitoring and interpreting abnormal morphologic changes, particularly dimorphic red cell population.This should be correlated with CBC

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numerical data for better interpretation of results.[4,5] A fair knowledge of histogram pattern can also help to identify and differentiate subclinical anaemia. Only a limited amount of published studies have compared the utility of histogram, CBC parameters and peripheral smear examination in diagnosis of anaemia. The present study was carried out to correlate these parameters in diagnosis of different types of anaemia in a rural health setting.

MATERIAL AND METHODS

The present study was carried out in the Haematology Laboratory of PGIMER Chandigarh Satellite Centre, Sangrur, Punjab over a period of one year.

Inclusion criteria: All cases of anaemia as per WHO reference rangewere included in the study.WHO (2011) recommendation of haemoglobin (Hb) levels to diagnose anaemia at sea level (g/dl)[6,7] is as follows:

- Children ½ – 5 years (Hb< 11)
- Children 5 – 11years (Hb < 11.5)
- Children 11 – 15years (Hb < 12)
- Pregnant females (Hb <11)
- Non-pregnant females (15 years and above) (Hb <12)
- Men (15 years and above) (Hb<13)

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

The identity of patients was not revealed during the course of the study.

Method of data collection: The venous sample was collected by venepuncture in EDTA vacutainers. The samples were aspirated into haematology analyzer SYSMEX XP-100 series and simultaneously blood smears were prepared and stained with Leishman stain.

The present study wasa double-blind study. Anaemia typing was done first by parameters obtained by haematology analyzer (RBC indices with RDW) and then peripheral blood smear findings were noted. The position and the shape of the RBC histogram were recorded. The haematologists, however, were not privy to histogram and RBC indices during the reporting of the peripheral smear.

Categorization of anaemia based on RBC indices by autoanalyzer

- microcytic hypochromic anaemia with increased RDW
- microcytic hypochromic anaemia with normal RDW
- normocytic normochromic anaemia with increased RDW
- normocytic normochromic anaemia with normal RDW
- macrocytic anaemia
- dimorphic anaemia
- haemolytic anaemia

Position (normal, left shift and right shift) and shape (normal bell-shaped or Gaussian, broad-based, bimodal peak with skewing to left and right) of RBC histograms were recorded. RBC indices were seen in association with histogram pattern.

Morphological typing of anaemia based on peripheral smear findings

- microcytic hypochromic anaemia
- normocytic normochromic anaemia
- macrocytic anaemia
- dimorphic anaemia
- haemolytic anaemia

STATISTICAL ANALYSIS

The data was collected and the correlation between the diagnoses made by the peripheral smear vs RBC histogram and indices wereanalysed statistically using one-way analysis of variance (ANOVA) to find out whether the mean difference in various RBC parameters was significant. The p-value of 0.05 or less was considered statistically significant.

RESULTS

In this study, peripheral blood smears in different types of anaemia were evaluated and correlated with cell counter generated RBC indices and histogram of 1575 cases (Table-1). The cases consisted of microcytic hypochromic anaemia, normocytic normochromic anaemia, macrocytic anaemia, dimorphic anaemia and haemolytic anaemia as diagnosed by peripheral smear examination.

The age group of patients included in the study ranged from 1–95years.Out of total anaemia cases reported, most affected people were in the age group of 31-50years.The

Age groups (years)	Male		Female		Total	
	N	%	N	%	N	%
0-10	18	3.1	45	4.5	63	4.0
11-20	27	4.6	90	9.1	117	7.4
21-30	99	16.9	135	13.6	234	14.9
31-40	126	21.5	252	25.5	378	24.0
41-50	72	12.3	252	25.5	324	20.6
51-60	99	16.9	126	12.7	225	14.3
61-70	126	21.5	63	6.4	189	12.0
71-80	9	1.5	18	1.8	27	1.7
81-90	0	0.0	9	0.9	9	0.6
91-100	9	1.5	0	0.0	9	0.6
Total	585	37.1	990	62.9	1575	100.0

Table-1: Age and sex distribution of anaemia cases (n=1575)

Typing of anaemia (PBF)	Males		Females		Total	
	n	%	n	%	n	%
Microcytic hypochromic	225	38.5	576	58.2	801	50.86
Normocytic normochromic	135	23.1	261	26.4	396	25.14
Macrocytic anaemia	27	4.6	9	0.9	36	2.29
Dimorphic anaemia	117	20.0	126	12.7	243	15.43
Hemolytic anaemia	81	13.8	18	1.8	99	6.29
Total	585	37.1	990	62.9	1575	100

Table-2: Typing of anaemia based on peripheral blood smear findings(PBF)

Typing of anaemia	Males		Females		Total	
	n	%	n	%	n	%
Microcytic Hypochromic with raised RDW	216	36.9	441	44.5	657	41.71
Microcytic Hypochromic with normal RDW	45	7.7	234	23.6	279	17.71
Normocytic Normochromic with raised RDW	99	16.9	99	10.0	198	12.57
Normocytic Normochromic with normal RDW	117	20.0	189	19.1	306	19.43
Macrocytic anaemia	36	6.2	18	1.8	54	3.43
Dimorphic anaemia	36	6.2	9	0.9	45	2.86
Hemolytic anaemia	36	6.2	0	0	36	2.29
Total	585	37.1	990	62.9	1575	100

Table-3: Typing of anaemia based on erythrocyte indices by auto analyzer parameters

Type of anaemia	Peripheral smear findings		Erythrocytes indices (analyzer)		p value (ANOVA)
	n	%	n	%	
Microcytic hypochromic	801	50.86	936	59.43	0.107
Normocytic normochromic	396	25.14	504	32.00	0.156
Macrocytic anaemia	36	2.29	54	3.43	0.522
Dimorphic anaemia	243	15.43	45	2.86	0.000
Hemolytic anaemia	99	6.29	36	2.29	0.065
	p = 0.004				

Table-4: Correlation of PBF findings with erythrocyte indices obtained by autoanalyzer

Type of anaemia	Histogram patterns (p = 0.172)										Total (%)
	Normal curve		Left shift		Right shift		Bimodal		Broad		
	M	F	M	F	M	F	M	F	M	F	
Microcytic hypochromic	0	0	108	405	0	0	0	0	117	171	801
Normocytic normochromic	135	243	0	0	0	0	0	0	0	18	396
Macrocytic anaemia	0	0	9	0	18	9	0	0	0	0	36
Dimorphic anaemia	9	0	0	9	0	0	45	0	63	117	243
Hemolytic anaemia	0	0	0	0	0	0	9	0	72	18	99
Total	144	243	117	414	18	9	54	0	252	324	1575

Table-5: Correlation of PBF findings with Histogram patterns obtained by autoanalyzer

study predominantly included females, with F: M ratio of 1.7. After 60 years of age, males were affected more than females. (Table-1)

Correlation of peripheral smear findings with analyser generated RBC indices: In our study based on peripheral blood smear findings, following morphological subtypes of anaemia were reported: were 801 cases (50.86%), 396 cases (25.14%), 36 cases(2.29%), 243 cases(15.43%) and 99 cases(6.29%) of microcytic hypochromic anaemia, normocytic normochromic anaemia, macrocytic anaemia,dimorphic anaemia and haemolytic anaemia respectively. Maximum numbers of cases in both sexes were of microcytic hypochromic anaemia followed by normocytic normochromic anaemia. (Table-2)

Based on the findings of autoanalyzer derived RBC indices, most cases in both sexes were of microcytic hypochromic anaemia with raised RDW (41.71%) followed by normocytic normochromic anaemia with normal RDW (19.43%) and microcytic hypochromic anaemia with normal RDW(17.71%) (Table-3).

When compared the anaemia diagnosis based on RBC parameters and by manual examination of the peripheral smear, there was a statistically significant difference. It was mainly due to dimorphic anaemia. Dimorphic anaemia cases were included in the normal range of MCV i.e. 80–100fl. Dimorphic anaemia showed a significant discrepancy between peripheral smear findings and RBC parameters. (Table-4)

Type of anaemia		Microcytic	Normocytic	Macrocytic	Dimorphic	Hemolytic	Total
n		**936**	**504**	**54**	**45**	**36**	**1575**
RBC	min	2.5	1.7	1.2	1.5	0.8	0.8
	max	6.1	4.8	2.6	3.9	5.5	6.1
	mean	4.6	3.8	1.7	3	2.5	4.1
Hb	min	4.3	5.5	4.7	5.3	3.3	3.3
	max	12.5	11.8	9	9.8	9.8	12.5
	mean	9.5	10.2	6.5	7.5	6.5	9.5
Hct	min	17.1	15.5	13.9	14.2	9.5	9.5
	max	39.3	39	26.8	26.3	34.4	39.3
	mean	31.5	31.1	18.7	21.7	20.2	30.4
MCV	min	51.2	72.6	101.5	52.6	72.9	51.2
	max	87.7	93.9	132	105.9	115.9	132.0
	mean	69.1	82.4	112.5	80.3	100.8	75.9
MCH	min	13.6	22.1	32	18	17.8	13.6
	max	29.1	33.3	40	34.9	40.3	40.3
	mean	20.9	27.4	34.8	27.3	33.4	23.9
MCHC	min	21.1	29.8	33.5	25.5	28.5	21.1
	max	34.4	37.3	36.8	33.8	35.2	37.3
	mean	29.8	33	34.4	30.4	33.4	31.1
RDW	min	11.7	12.3	15	25.3	22.3	11.7
	max	29.9	30.7	27.3	41.7	41.7	41.7
	mean	17.7	15.7	21.4	31.3	34.5	18.0

Table-6: Distribution of RBC parameters in various anaemia cases

Correlation of peripheral smear findings with analyser generated histogram: In our study out of 801 cases of microcytic hypochromic anaemia 513showed left shift curve while 288 showed the broad-based curve. Out of 396 cases of normocytic normochromic anaemia, 378 showed normal bell-shaped curve while 18 showed the broad-based curve. Out of 36 cases of macrocytic anaemia, 9 showed left shift curve while 27 showed a right shift. Out of 243 cases of dimorphic anaemia, 9 showed the normal curve,9 showed left shift curve, 45 showed bimodal curve while180 showed the broad-based curve. Out of 99 cases of haemolytic anaemia, 9 showed bimodal curve while 90 showed the broad-based curve. When we compare anaemia diagnosed by histogram and by manual examination of the peripheral smear. The histogram pattern correlated with most cases of microcytic hypochromic, normocytic normochromic, macrocytic and haemolytic anaemia. However, variations of histogram pattern were seen in dimorphic anaemia, but that was not statistically significant. (Table-5)

DISCUSSION

Anaemia is a global problem affecting the population in both developing as well as developed countries, and there is a debate on which haemoglobin level limits should be used to define anaemia in general population.[8] About one-third of the global population is anaemic.[9]

Since decades,peripheral smears have been used as a major diagnostic tool for workup of anaemia.[1] The advent of automated haematology cell counter has improved accuracy and precision and has reduced subjective errors. Microscopic examination still is required for primary calibration, for the presumptive diagnosis of anaemia, leukaemia and other related disorders.[3] The present study was done to correlate peripheral smear findings with red blood cell histogram patterns and red cell indices obtained from SYSMEX XP-100(3-part differential automated analyzer). There are limited studies conducted on the utility of both red cell indices and histogram in identification and comparison of anaemia.

The RBC histogram is an integral part of automated haematology analysis and is available routinely on all automated cell counters. TheRBC histogram follows well-known coulter principle of counting and sizing red cells providing the basis for generating the histogram. The histogram in association with other CBC parameters such as RDW, MCV has been found abnormal in various haematological conditions.[10]

It was seen that out of 1575 anaemic cases, a majority of cases fall in the adult age group of 21-50 years and among 990 female patients, majority (39.1%) fall in reproductive age group.It has been seen in many studies that anaemia is prevalent in these age group because of the inadequate iron intake, chronic blood loss, mal absorption, infections and other factors like changes in lifestyle, dietary pattern, behavioural and social changes.[3,11] In our study, we observed an increased prevalence of anaemia in females (62.9%). This was in concordance with the study done by Singhal et al (64.9%).[6]After the sixth decade,males were seen to be affected more than females suggesting that other causesmight have contributed to the development of anaemia in this age group.

The most common morphological type was microcytic hypochromic followed by normocytic normochromic anaemia. The most common cause of microcytic hypochromic anaemia is decreased iron reserve of the body, which may be due to multiple reasons. This may be due to decreased iron in the diet, poor absorption of iron from gut,

acute and chronic blood loss, increased demand of iron in certain situations like pregnancy or recovery from major trauma or surgery. Iron deficiency was the most common cause of microcytic hypochromic anaemia. Out of 1575 cases, microcytic hypochromic anaemia showed MCV in the range of 51.2 – 87.7fl with a mean of 69.1 fl. Normocytic normochromic anaemia showed MCV in the range of 72.6 – 93.9 fl with a mean of 82.4 fl. Macrocytic anaemia showed MCV in the range of 101.5 – 132.0fl with a mean of 112.5fl. These findings regarding distribution of anaemia cases are in concordance with other studies.[3,12] (Table-6)

In microcytic hypochromic anemia, MCHC may be normal, but MCV and MCH are decreased. RBC population with low MCV will be shifted towards left. A broad-based curve, because of high RDW, represents anisocytosis. Out of 50.86% cases of microcytic hypochromic anaemia on smear, 32.5% revealed left shift while 18.3% showed broad-based curve on histogram. When we compare all these findings with data derived from analyzer, microcytic hypochromic anaemia was seen in 59.43% cases. This mild difference can be explained by the presence of giant platelets and platelet clumps, fragmented RBCs in haemolytic diseases, when auto analyser considers it as microcytes. So peripheral smears rule out these errors. These findings are in concordance with study done by Singhal et al (49.8%)[5] and Sandhya et al (46%).[12]

Normocytic normochromic anaemia was seen in 25.14% cases on peripheral smear examination while RBC indices showed 32% cases. In normocytic normochromic anaemia, the red cell indices remain within normal range with few cases showing mild increase indices. The population of cells may be variable in size. There may be some microcytic cells with predominance of normal size cells that results in a higher deviation and hence higher RDW. In our study, a normal bell-shaped curvewas seen in 25% while 1.1% showed broad curve. So diagnosis based on RBC indices and histograms were comparable to diagnosis made by peripheral smear examination. It is comparable to study done by Sandhya et al (21.8%)[6] and Rao et al (19.4%).[13]

Macrocytic anaemia was diagnosed in 2.29% cases on peripheral smears examination while RBC indices revealed 3.43% cases. Histogram findings showed right shift in 1.7% while 0.6% showed left shift. There is remarkable difference in findings of the peripheral smear and RBC indices. The reason may be interpretation of haemolytic anaemia as macrocytic anaemia due to presence of polychromatophilic erythrocytes, giving a high value of MCV. However, MCV by automated blood cell counter is rarely inaccurate. Hyperglycaemia, cold agglutinins and leukocytosis may result in false elevation of MCV. Few cases diagnosed as macrocytic by counter report were diagnosed as dimorphic on the peripheral smear. Similar observation was noticed by Chavda et al (3.6%)[4] and Rao et al(2.2%).[13]

Dimorphic anaemia was diagnosed in 15.43% cases on peripheral smear while RBC parameters revealed only in 3.43% cases. Histogram showed bimodal only in 2.9% of cases whereas majority (11.4%) showed broad-based histogram. The broad-based curve can be explained by the presence of multiple populations of cells of varying sizes (i.e. normocytes, microcytes and macrocytes). The bimodal population is usually associated with therapeutic transfusions and/or hematinic agent response to microcytic and macrocytic anaemia, but they may also indicate other haematological disorders such as early iron deficiency developing microcytic population and folate/vitamin b12 deficiency developing macrocytic population, post iron treatment of iron deficiency anaemia and post iron treatment of iron deficiency with megaloblastic anaemia. The additional features along with bimodal curve points to the diagnosis. A right-skewing corresponds to reticulocytosis, and a trail of erythrocytes population on the far right of the histogram correlates to red cell agglutination. Bimodal curve with right shift and skewing towards left indicates combined nutritional deficiency. MCV represents only mean of distribution curve and is insensitive to small number of microcytes and macrocytes. MCH and MCHC give little information independent of MCV. However since dimorphic anaemia is associated with abnormal red cell population, morphological findings should be correlated with the graphical and numerical data for better interpretation of results. Our findings were in concordance with the study done by Chavda et al (14%)[4] and Sandhya et al(17.6%)[12] and other authors.[14,15]

Only few cases were diagnosed as haemolytic anaemia on histogram and indices(2.29%) analysis as compared to the peripheral smear(6.29%) because fragmented RBCs were counted as microcytes and polychromatophils were counted as macrocytes by cell counter. The similar limitation of RBC histogram and RBC indices in diagnosis of anaemia werealso reported by other authors.[10,12] However, the broad-based histogram with right-skewing and elevated RDW with low Hct and relatively high RBC count certainly points to the presence of element of hemolysis in the anaemia as seen in our study.In WBC histogram, WL flagging with deviation of the baseline at lower discriminator is also seen in these cases.

Thus, we can see those histograms area useful diagnostic aid in microcytic hypochromic anaemia and normocytic normochromic anaemia. However, macrocytic, dimorphic and haemolytic anaemia showed different histogram patterns from simple to complex curves. Therefore, in all patients with decreased haemoglobin peripheral smear examination is a must for identifying red cell changes. Bain[16] in her review on the place of peripheral smear examination in the era of automation in 2005 stated that even in the age of molecular analysis, blood smear remains an important diagnostic tool and sophisticated modern investigations of haematological disorders should be interpreted in the light of peripheral blood smear as well as clinical context.

CONCLUSION

Our study revealed an important correlation between RBC parameters, histogram and peripheral smear diagnosis. Diagnosis of anaemia is supplementedby RBC indices and

histogram though peripheral smear remains the definitive diagnostic test for evaluation.

REFERENCES

1. Alvarez -UriaG, Naik PK,MiddeM,Yalla PS, Pakam R. Prevalence and severity of anaemia stratified by age and gender in Rural India. Anaemia. 2014;1-5.
2. Kumar A, Kushwaha R, Gupta C, Singh US. An analytical study on peripheral blood smears in anemia and correlation with cell counter generated red cell parameters. J Appl Hematol. 2013;4:137.
3. Singla S, Bedi S, Joshi K. Comparative study of anemia cases based on peripheral blood smears and cell counter generated red cell indices. MedPulse – Int Med J. 2017;4:44–8.
4. Chavda J, Goswami P, Goswami A. RBC Histogram as diagnostic tool in anemias. IOSR J Dent Med Sci. 2015;14:19–22.
5. Lee H, Kong S-Y, Sohn JY, Shim H, Youn HS, Lee S, et al. Elevated red blood cell distribution width as a simple prognostic factor in patients with symptomatic multiple myeloma. BioMed Res Int. 2014;2014:145619.
6. Singhal S, Verma N, Rathi M, Singh N, Singh P, Sharma SP, et al. Can peripheral blood smear examination be totally replaced by automated hematology analyser - with special reference to anemia? Int J Res Med Sci. 2016;4:4563–6.
7. Means RT, Glader B. Anemia: General Considerations. In: Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, et al., editors. Wintrobe's Clinical Hematology. 13th ed. Philadelphia: Lippincott Williams & Wilkins; 2014. p. 587–616.
8. Mahajani K, Bhatnagar V. Comparative Study of Prevalence of Anaemia in Vegetarian and Non Vegetarian Women of Udaipur City, Rajasthan. J Nutr Food Sci. 2015;S3:1–6.
9. Mishra P, Ahluwalia S k, Garg PK, Kar R, Panda GK. The Prevalence of Anaemia among Reproductive Age Group (15-45 Yrs) Women in A PHC of Rural Field Practice Area of MM Medical College, Ambala, India. J Womens Health Care. 2012;1:113.
10. Radadiya P, Mehta N, Goswami H, Gonsai RN. Automated red blood cell analysis compared with routine red blood cell morphology by smear review. NHL J Med Sci. 2015;4:53–7.
11. De Andrade Cairo RC, Rodrigues Silva L, Carneiro Bustani N, Ferreira Marques CD. Iron deficiency anemia in adolescents; a literature review. Nutr Hosp. 2014;29:1240–9.
12. Sandhya I, Muhasin TP. Study of RBC Histogram in Various Anemias. J Evol Med Dent Sci. 2014;3:15521–34.
13. Rao BSS, Santhi V, Rao NM, Grandhi B, Reddy VLM, Siresala P. RBC Histogram as supplementary diagnostic tool with peripheral smear examination in evaluating anemias. Ann Pathol Lab Med. 2017;4:A668–72.
14. Constantino BT. The Red Cell Histogram and The Dimorphic Red Cell Population. Lab Med. 2011;42:300–8.
15. Park KI, Kim KY. Clinical evaluation of red cell volume distribution width (RDW). Yonsei Med J. 1987;28:282–90.
16. Bain BJ. Diagnosis from the blood smear. N Engl J Med. 2005;353:498–507.

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