

An Analytical Cross Sectional Study of Comparison between Cell Counter Generated Red Cell Indices with Peripheral Blood Smear Examination in Diagnosing Morphological Type of Anemia

Malar Kodi R¹, J. Nilavu², Rengaraj T³, Aadithya K M⁴

^{1,4}Assistant Professor, Department of Pathology, Government Medical College, Nagapattinam

²Associate Professor, Department of Pathology, Thanjavur Medical College

³Assistant Professor, Department of General Medicine, Government Medical College, Nagapattinam

Received: 25-02-2024 / Revised: 23-03-2024 / Accepted: 26-04-2024

Corresponding Author: Dr. Aadithya K M

Conflict of interest: Nil

Abstract:

Introduction: Anemia affects 24.8% of the global population, and automated hematology analyzers provide accurate RBC indices. However, expertise is required for peripheral blood smear examination, and hematologists only provide incremental helpful information in 4% of cases.

Aim of the Study: To correlate morphological type of anemia based on RBC indices (MCV, MCH and MCHC) and RDW obtained from Mindray BC 6200, a 5 part automated analyzer with morphological type of anemia based on peripheral blood smear examination and to assess the concordance of both investigations in diagnosis of anemia with severity and morphological typing of anemia.

Material and Methods: A total of 200 cases of anemia were studied over a period of two years between June 2019 to June 2021. Anemia typing was done by two methods –Firstly using RBC indices with RDW only. Secondly on peripheral blood smear examination. The results were then correlated.

Results: Morphological typing of anemia in cases of Microcytic hypochromic anemia with normal and raised RDW shows maximum specificity and macrocytic anemia using RBC indices and RDW shows maximum sensitivity. In cases of Normocytic normochromic anemia, the cases with normal RDW showed high specificity and sensitivity but the cases with raised RDW were wrongly typed on indices had a low sensitivity and specificity indicating additional peripheral blood smear examination as an absolutely necessary tool in morphological typing of anemia.

Conclusion: Automated hematology analyzers provide valuable readings for morphological typing of anemia, but peripheral blood smear examination remains the gold standard method. It helps identify the cause of anemia and requires multiple observers for accurate typing. Even today, peripheral blood smear examinations cannot be completely replaced by automated hematology analyzers, as they provide additional information like dimorphic anemia and abnormal cells.

Keywords: Morphological Typing of anemia; Red cell Distribution Width; Mindray BC 6200, automated hematology analyzer; Peripheral blood smear examination.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

Anemia is a global health problem affecting both developed and developing countries and has an important impact on human health and social and economic development. Worldwide, anemia affects 1.62 billion people, equivalent to 24.8% of the total population.

The prevalence of preschool children is the highest [47.4%], and the prevalence of men is the lowest [12.7%], but the most affected population is adolescent girls. One third of the world population suffers from anemia. India is one of the countries with a very high prevalence rate. Anemia is functionally defined as an insufficient RBC mass to

deliver optimal oxygen to peripheral tissues [1]. The World Health Organization (WHO) defines the normal limit of sea level Hb concentration as 12.0 g/dl for women and 13.0 g/dl for men.

Cell count is an important parameter for evaluating anemia. The Cell count can be determined either manually or by automated hematology analyzers. Whether using manual or automated methodologies, the accuracy and precision of counting depend on the proper dilution of the blood sample, uniform distribution of cells, and accurate sample measurement. Manual counting done by using microscope after proper smearing and

staining of peripheral blood. It can count red cells, WBCs, and platelets. Due to the inherent imprecision of manual counting and the amount of technical time required, most cell counts are now performed using automated instruments that improve the accuracy and speed of clinical laboratory analysis, thereby minimizing the level of manual operation, dilution, and analysis. [2] With the increase in automation, some hematology analyzers can be used in combination with instruments that use the same tube of blood for other laboratory tests. [3]

The ability of this Mindray BC 6200 hematology analyzer to perform all RBCs indices, platelet indices, white blood differential counts, particularly those that perform five-part differential (enumerating neutrophils, lymphocytes, eosinophils, monocytes, and basophils), count of nucleated RBCs, reticulocyte count been a remarkable technologic advance [4].

When anemia is established, the morphological type of anemia helps the clinician to approach the cause for anemia. Morphological type of anemia is based on RBC indices – MCV, MCH and MCHC and RDW index. Since years, peripheral blood smear examination was used as window to observe disorders of hematology. Analysis of peripheral blood smear promotes the interpretation of various hematological disorders and it is an important diagnostic tool.

The arrival of automated cell counter improved the precision and accuracy, reduced subjective errors and safety while handling specimens. This study aims to assess possible causes of common discrepancies in establishing diagnosis of anemia and to assess the concordance of both investigations in diagnosis of anemia with severity of anemia in terms of mild, moderate and severe anemia.

Aims and Objectives

1. To do complete blood count and evaluate red cell indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width) with fully automated 5 part CBC counter for the patients with anemia.
2. To do peripheral blood smear for the patients with anemia.
3. To compare the output of both investigations in diagnosing anemia.
4. To correlate morphological type of anemia based on RBC indices (MCV, MCH and MCHC) and RDW with morphological type of anemia based on peripheral blood smear examination.
5. To assess the concordance of both investigation in diagnosis of anemia with severity and morphological typing of anemia

Material and Methods

This cross sectional study was undertaken at the Department of Pathology, Thanjavur medical college. A total of 200 cases of anemia were studied over a period of two years (June 2019 to June 2021). Anemia typing was done by two methods – Firstly using RBC indices with RDW only and secondly on peripheral blood smear examination. The patients were referred from in-patient and out-patient departments in Thanjavur medical college and Hospital. The study participant was selected based on the following inclusion and exclusion criteria

Inclusion Criteria: Both male and female patients more than 18 years of age with hemoglobin below the WHO reference values i.e patients with anemia.

Exclusion Criteria: Patients with hematological malignancies, parasitic infection, pregnant women, patient with history of massive splenomegaly, burn patients, patients with acute blood loss.

Methodology

Blood sample received in central hematology laboratory, pathology department for complete blood count and peripheral blood smear examination. The sample was venous blood which was collected by venipuncture in vacutainer that contains EDTA anticoagulant. The sample is aspirated into the MINDRAY BC-6200, 5PART hematology analyser which analyses the sample and provides with complete blood count, MCV, MCH, MCHC and RDW. With hemoglobin values anemia is graded into mild, moderate and severe anemia. Morphological typing of anemia was done using RBC indices and the above data as

- Microcytic Hypochromic Anemia with normal RDW
- Microcytic Hypochromic Anemia with raised RDW
- Normocytic Normochromic Anemia with normal RDW.
- Normocytic Normochromic Anemia with raised RDW
- Macrocytic anemia

Peripheral blood smear prepared, stained and then examined. PBS morphological type of anemia is done as

- Microcytic hypochromic anemia
- Normocytic normochromic anemia
- Macrocytic anemia
- Dimorphic anemia

The data obtained with hematology analyser was correlated with morphological type of anemia using peripheral blood smear examination. The results were considered as concordant if typing done by both methods indicated the same morphological type of anemia, if the results were different it was

considered discordant. The reference values are taken from Wintrobe's Clinical Hematology – 13th Edition. [10]

Morphological typing of anemia is done using RBC indices with RDW from MINDRAY BC-6200 an automated hematology analyzer and verified then with the typing done by peripheral blood smear examination. The results were considered as concordant if typing done by both methods shows same morphological type of anemia, otherwise results were considered as discordant.

Statistical Analysis

The data were entered using Microsoft Office Excel 2013 and analyzed using SPSS software version 16. Description of categorical variables like Age Category, Sex Distribution, different morphological types of anemia and type of agreement between two methods of typing was expressed as frequency and proportion. Chi square

test and Fisher's exact test was used to compare the frequency between the groups.

Sensitivity, Specificity, Positive Likelihood ratio, Negative Likelihood ratio, Positive predictive value, Negative Predictive value, and diagnostic Accuracy was seen with morphological typing of Microcytic hypochromic anemia and Macrocytic anemia with RBC indices alone. All tests were two tailed and results were considered statistically significant if the p-value is <0.05 at 95% confidence interval.

Ethical Consideration: Ethical principles such as respect to the patient, beneficence and justice were strictly adhered. Ethical committee approval was obtained before starting the study. The approval to conduct the present study was obtained from the Institutional Ethical Committee no. 691, Dated: 09:01:2020. Confidentiality of the study participants was maintained throughout the study.

Results

Table 1: Morphological type of anemia based on RBC indices and peripheral blood smear compared with gender

| Morphological type of anemia | Female (N=106) | | Male (N=94) | | Total (N=200) | | Chi square value, df, p value |
|--|----------------|-------|-------------|-------|---------------|-------|-------------------------------|
| | N | % | N | % | N | % | |
| Morphological type of anemia based on RBC indices | | | | | | | |
| Macrocytic anemia with normal RDW | 0 | 0% | 4 | 4.3% | 4 | 2% | 0.002 |
| Macrocytic anemia with raised RDW | 5 | 4.7% | 15 | 16% | 20 | 10% | |
| Microcytic hypochromic anemia with normal RDW | 9 | 8.4% | 2 | 2.1% | 11 | 5.5% | |
| Microcytic hypochromic anemia with raised RDW | 43 | 40.6% | 24 | 25.6% | 67 | 33.5% | |
| Normocytic normochromic anemia with normal RDW | 26 | 24.5% | 23 | 24.1% | 49 | 24.5% | |
| Normocytic normochromic anemia with raised RDW | 23 | 21.7% | 26 | 27.7% | 49 | 24.5% | |
| Morphological type of anemia based on on peripheral blood smear examination | | | | | | | |
| Dimorphic anemia | 20 | 18.9% | 18 | 19.1% | 38 | 19% | 0.040 |
| Macrocytic anemia | 5 | 4.7% | 14 | 14.9% | 19 | 9.5% | |
| Microcytic hypochromic anemia | 46 | 43.4% | 28 | 29.8% | 74 | 37% | |
| Normocytic Normochromic anemia | 35 | 33% | 34 | 36.2% | 69 | 34.5% | |

Out of 200 cases on which morphological typing of anemia was done with RBC indices and RDW, majority of the case belonged to Microcytic Hypochromic Anemia of raised RDW (33.5%) followed by Normocytic Normochromic Anemia with normal RDW (24.5%) and raised RDW

(24.5%). Among 200 cases in which morphological typing of anemia was done using peripheral blood smear examination, maximum number of the case belonged to Microcytic Hypochromic Anemia (37%) followed by Normocytic Normochromic Anemia (34%).

Table 2: Comparison between types of agreement (Mindray typing vs PBS) with respect to age category and gender

| | Concordant (N=144) | | Discordant (N=56) | | Total (N=200) | | P value |
|---------------------|--------------------|-------|-------------------|-------|---------------|-------|---------|
| | n | % | n | % | N | % | |
| Age category | | | | | | | |
| 18 to 20 years | 19 | 17.9% | 7 | 7.4% | 26 | 13% | 0.007 |
| 21 – 30 years | 18 | 17% | 12 | 12.8% | 30 | 15% | |
| 31 – 40 years | 25 | 23.6% | 24 | 25.5% | 49 | 24.5% | |

| | | | | | | | |
|---------------|----|-------|----|-------|-----|-------|-------|
| 41 – 50 years | 24 | 22.6% | 14 | 14.9% | 38 | 19% | |
| 51 – 60 years | 11 | 10.4% | 22 | 23.4% | 33 | 16.5% | |
| 61 – 70 years | 2 | 1.9% | 11 | 11.7% | 13 | 6.5% | |
| 71 – 80 years | 5 | 4.7% | 3 | 3.2% | 8 | 4% | |
| >80 years | 2 | 1.9% | 1 | 1.1% | 3 | 1.5% | |
| Gender | | | | | | | |
| Female | 76 | 52.1% | 30 | 54.3% | 106 | 53% | 0.919 |
| Male | 68 | 47.9% | 26 | 45.7% | 94 | 47% | |

There is significant association observed between age categories with different types of agreement with the p value shows less than 0.05. Out of 144 concordant cases, 52.1% were females and 47.9% were males. In 56 discordant cases, 54.3% were females and 45.7% were males. The percentage of

female cases was higher in concordant cases when compared to discordant type of agreement and this difference is not significant with the p value shows more than 0.05.

Sensitivity and Specificity

Table 3a: Sensitivity and specificity of morphological typing of Microcytichypochromic anemia with RBC indices alone

| S.No | 2 X 2 diagnostic table | | PBS method - Microcytic Hypochromic anemia | |
|--------------------------|--|-----|--|-----|
| | | | yes | No |
| 1 | RBC indices method - Microcytic Hypochromic anemia | Yes | 56 | 16 |
| | | No | 15 | 113 |
| Diagnostic values | | | | |
| 1 | Sensitivity | | 78.8% (67.5 to 87.7%) | |
| 2 | Specificity | | 87.6% (80.6 to 92.7%) | |
| 3 | Positive Likelihood ratio | | 6.36 (3.9 to 10.2) | |
| 4 | Negative Likelihood ratio | | 0.24 (0.15 to 0.38) | |
| 5 | Positive predictive value | | 77.8% (68.5 to 84.9%) | |
| 6 | Negative Predictive value | | 88.3% (82.7 to 92.2%) | |
| 7 | Accuracy | | 84.5% (78.7 to 89.2%) | |

Total N = 200. Data are expressed as % (95% confidence interval)

Table 3b: Sensitivity and specificity of morphological typing of Macrocyticanemia with RBC indices alone

| S.No | 2 X 2 diagnostic table | | PBS method – Macrocytic anemia | |
|--------------------------|--|-----|--------------------------------|-----|
| | | | yes | No |
| 1 | RBC indices method – Macrocytic anemia | Yes | 19 | 5 |
| | | No | 0 | 176 |
| Diagnostic values | | | | |
| 1 | Sensitivity | | 100% (82.3 to 100%) | |
| 2 | Specificity | | 97.2% (93.6 to 99.1%) | |
| 3 | Positive Likelihood ratio | | 36.2 (15.2 to 85.9) | |
| 4 | Negative Likelihood ratio | | 0 | |
| 5 | Positive predictive value | | 79.1% (61.5 to 90.2%) | |
| 6 | Negative Predictive value | | 100% | |
| 7 | Accuracy | | 97.5% (94.2 to 99.2%) | |

Total N = 200. Data are expressed as % (95% confidence interval).

Table 3c: Sensitivity and specificity of morphological typing of Normocyticnormochromic anemia with RBC indices

| S.No | 2 X 2 diagnostic table | | PBS method - Normocytic normochromic anemia | |
|--------------------------|---|-----|---|-----|
| | | | yes | No |
| 1 | RBC indices method – Normocytic normochromic anemia | Yes | 59 | 26 |
| | | No | 3 | 112 |
| Diagnostic values | | | | |
| 1 | Sensitivity | | 95.2% (86.5 to 98.9%) | |
| 2 | Specificity | | 81.2% (73.6 to 87.3%) | |

| | | |
|---|---------------------------|-----------------------|
| 3 | Positive Likelihood ratio | 5.1 (3.5 to 7.2) |
| 4 | Negative Likelihood ratio | 0.06 (0.02 to 0.18) |
| 5 | Positive predictive value | 69.4% (61.5 to 76.3%) |
| 6 | Negative Predictive value | 97.4% (92.5 to 99.1%) |
| 7 | Accuracy | 85.5% (79.8 to 90.1%) |

Total N = 200. Data are expressed as % (95% confidence interval)

Table 4: Comparison of age category, Types of agreement, gender, Morphological type of anemia by PBS, Morphological type of anemia by RBC indices with RDW with respect to severity of anemia

| | Mild anemia(N=66) | | Moderate anemia (n=58) | | Severe anemia (n=76) | | p value |
|---|-------------------|-------|------------------------|-------|----------------------|-------|---------|
| | n | % | n | % | n | % | |
| Age category | | | | | | | |
| 18 – 20 years | 5 | 7.6% | 1 | 18.9% | 10 | 13.2% | 0.890 |
| 21 – 30 years | 10 | 15.2% | 8 | 13.8% | 12 | 15.8% | |
| 31 – 40 years | 17 | 25.8% | 15 | 25.9% | 17 | 22.4% | |
| 41 – 50 years | 14 | 21.2% | 10 | 17.2% | 14 | 18.4% | |
| 51 – 60 years | 12 | 18.2% | 8 | 13.8% | 13 | 17.1% | |
| 61 – 70 years | 4 | 6.1% | 3 | 5.2% | 6 | 7.9% | |
| 71 – 80 years | 3 | 4.5% | 3 | 5.2% | 2 | 2.6% | |
| > 80 years | 1 | 1.5% | 0 | 0% | 2 | 2.6% | |
| Types of agreement | | | | | | | |
| Concordant | 44 | 66.6% | 41 | 70.7% | 59 | 77.6% | 0.330 |
| Discordant | 22 | 33.4% | 17 | 29.3% | 17 | 22.4% | |
| Gender | | | | | | | |
| Female | 28 | 42.4% | 35 | 60.3% | 43 | 56.6% | 0.110 |
| Male | 38 | 57.6% | 23 | 39.7% | 33 | 43.4% | |
| Morphological type of anemia by PBS | | | | | | | |
| Dimorphic anemia | 12 | 18.2% | 12 | 20.7% | 14 | 18.4% | 0.020 |
| Macrocytic anemia | 4 | 6.1% | 3 | 5.2% | 12 | 15.8% | |
| Microcytic hypochromic anemia | 19 | 28.8% | 21 | 36.2% | 34 | 44.7% | |
| Normocytic Normochromic anemia | 31 | 46.9% | 22 | 37.9% | 16 | 21% | |
| Morphological type of anemia by RBC indices with RDW | | | | | | | |
| Macrocytic normochromic anemia with normal RDW | 1 | 1.5% | 2 | 3.4% | 1 | 1.3% | 0.005 |
| Macrocytic normochromic anemia with raised RDW | 6 | 9.1% | 2 | 3.4% | 12 | 15.8% | |
| Microcytic hypochromic anemia with normal RDW | 5 | 7.6% | 5 | 8.6% | 1 | 1.3% | |
| Microcytic Hypochromic anemia with raised RDW | 13 | 19.7% | 15 | 25.8% | 39 | 51.3% | |
| Normocytic normochromic anemia with normal RDW | 22 | 33.3% | 17 | 29.3% | 10 | 13.1% | |
| Normocytic normochromic anemia with raised RDW | 19 | 28.8% | 17 | 29.3% | 13 | 17.1% | |

Data are expressed as n with %. Fisher's exact test was used to compare the frequency between the groups. In this 200 participants most common age group for mild anemia is 31-40, 25.8% of mild anemia persons in this age group, followed by 41-50 yrs. Common age group for moderate anemia is 31-40 years followed by 18-20 years. Common age group for severe anemia is 31-40 years followed by 41-50 years. In mild anemia 42.4% are female and 57.6% are male, in moderate anemia 60.3% are female and 39.7% are male, in severe 56.6% female, 43.4% male. Moderate and severe anemia more common in female, mild anemia more

common in male. In PBS examination mild and moderate anemia have maximum of normocytic normochromic feature followed by microcytic hypochromic anemia. In severe anemia maximum number of cases belongs to microcytic hypochromic anemia followed by normocytic normochromic anemia. In CBC examination mild anemia have maximum of normocytic normochromic anemia with normal RDW followed by normocytic normochromic anemia raised RDW. In moderate anemia typed as maximum of normocytic normochromic anemia followed by microcytic hypochromic anemia with raised RDW.

In severe anemia typed as maximum of microcytic hypochromic anemia with raised RDW followed by normocytic normochromic anemia raised RDW.

Discussion

It is important for typing of anemia morphologically which provides useful information that aids the clinician/hematologist to further investigate the patient in identifying the cause of anemia so that it can be appropriately treated. In this present study maximum number of patients belongs to microcytic hypochromic anemia followed by normocytic normochromic anemia which in concordance with Swaroop raj study [11] and monika garg et al study [12]. This statistics show the prevalence of iron deficiency anemia because the most common cause for microcytic hypochromic anemia is iron deficiency anemia. Kim et al [13] studied 1500 cases and found that peripheral blood smear examination provided additional information in 28.6% of the cases. The present study showed similar results with 28% of cases having additional information provided by peripheral blood smear examination.

In a study by Japheth E Mukaya et al [14], 60% were female and a study by Swaroop raj shows 63.5% cases are female which was similar to the findings in our study where 53% of the cases were females. This indicates that anemia is still more prevalent in women in India and other developing countries. In the present study anemia is common among 31-40 years (24.5%) which is similar to monika garg et al [12] study. Compared with Arvind et al [15] study our study has more number of severe anemia patients. In view of concordance maximum number of severe anemia cases (41%) was correctly typed in both analyser and PBS examination, whereas maximum review needed for mild anemia cases which are having maximum discordant percentage (39.4%).

Microcytic hypochromic anemia with normal RDW group shows discordance of 27.3% in our study. Out of 3 discordant cases 2 cases were reported in PBS examination as normocytic normochromic anemia both cases having mcv values between 79 to 80 and mch values between 25 to 26 which are borderline. 1 case was reported in PBS as dimorphic anemia may be due to treatment effect. Microcytic hypochromic anemia with raised RDW group shows discordance of 20.3% in our study. Out of 15 discordant cases 2 cases were reported in PBS examination as normocytic normochromic anemia both cases having mcv values between 79 to 80 and mch values between 25 to 26 which are borderline. 13 cases were reported in PBS as dimorphic anemia may be due to treatment effect. Macrocytic anemia with normal RDW group shows discordance of 25% in our study. Only one case was reported in PBS as dimorphic anemia may be due to treatment effect. Macrocytic anemia with

raised RDW group shows discordance of 20% in our study.

Four cases were reported in PBS as dimorphic anemia may be due to treatment effect. Normocytic normochromic anemia with normal RDW group shows discordance of 6.2% in our study. Out of 3 discordant cases 2 cases were reported in PBS examination as microcytic hypochromic anemia both cases having mcv values between 80 to 81 and mch values between 26 to 27 which are borderline. 1 case was reported in PBS as dimorphic anemia. Normocytic normochromic anemia with raised RDW group shows discordance of 61.2% in our study. Out of 30 discordant cases 13 cases were reported in PBS examination as microcytic hypochromic anemia due to polychromatophils analyser may show increased MCV. 17 cases were reported in PBS as dimorphic anemia. Normocytic normochromic anemia with raised RDW group shows maximum number of cases with discordance compared to other group. This is similar with Swaroop raj study and study by Singhal S et al [16].

Macrocytic anemia is having high concordance when compared to other cases. Morphological typing of Microcytic hypochromic anemia with RBC indices alone showed a high specificity (87.6%) and slightly lower sensitivity of 78.8%. Using peripheral blood smear examination will increase the sensitivity in typing Microcytic hypochromic anemia by 22.2% in identifying cases with polychromasia having a normal MCV, which can be mistyped as Normocytic normochromic anemia if typed using RBC indices alone. Decreased specificity may be due to borderline values of MCV cut off. Morphological typing of Macrocytic anemia with RBC indices showed a high sensitivity 100% and specificity of 97.2%. Morphological typing of Normocytic normochromic anemia with RBC indices showed a high sensitivity (95.2%) but slightly lower specificity of 81.2%. Using peripheral blood smear examination will increase the specificity in reducing typing of other morphological types of anemia as Normocytic normochromic anemia by 18.8 by identifying cases with population of RBCs having high degree of anisocytosis but a normal MCV

Conclusion

Most common age group affected by anemia is 31-40 years with female preponderance. Severe anemia is the most common anemia in this study. Morphological typing of anemia in cases of Microcytic hypochromic anemia with normal and raised RDW shows maximum specificity and Macrocytic anemia using RBC indices and RDW shows maximum sensitivity. In cases of Normocytic normochromic anemia, the cases with normal RDW showed high specificity and sensitivity but the cases with raised RDW were

wrongly typed on indices had a low sensitivity and specificity indicating additional peripheral blood smear examination as an absolutely necessary tool in morphological typing of anemia. Cases within the range belongs to <1 or >1 to the cut off point for indices (borderline values) need to be reviewed by 2 to 3 observers for accurate typing.

Cases with mild anemia need peripheral smear review as they have high discordance rate. RBC indices with RDW obtained from automated hematology analyzer gives valuable readings that aid in morphological typing of anemia in most cases of anemia especially present generation of automated hematology analyzers are well on par. However, peripheral blood smear examination, one of the oldest methods used to morphologically type anemia but still remains a gold standard method and cannot be totally replaced by readings from an automated hematology analyzer as the cases with normocytic normochromic anemia with raised RDW show the advantage of being typed using peripheral blood smear which aids in identifying the cause of anemia.

Peripheral blood smear examination even today cannot be totally replaced by automated hematology analyzers as they provide so much additional information such as dimorphic anemia, abnormal cells such as sickle cells, target cells, polychromatophils etc which cannot be summarized completely by the mere numerical calculations of an automated analyzer.

Bibliography

1. E Medicine – Anemia, Chronic: Article by Fredrick M Abrahamian, DO, FACEP. Emedicine.com. 2009-12-07.
2. Bourner G, Dhaliwal J, Sumner J. Performance evaluation of the latest fully automated hematology analyzers in a large, commercial laboratory setting: a 4- way, side-by-side study. *Lab Hematol.* 2005; 11(4):285-297.
3. Chapman M. Hematology analyzers offer new technology and user-friendliness *Lab Med.* 2000; 31:146-150.
4. Davis BH, Bigelow NC. Automated reticulocyte analysis. *Clinical practice and associated new parameters.* *Hematol Oncol Clin North Am.* 1994; 8(4):617-630.
5. John H. Farrant, Grose, Francis (bap. 1731, d. 1791), *Oxford Dictionary of National Biography*, Oxford University Press, Sept 2004; online edn, Oct. 2009.
6. Louis E. Grivetti, "From Aphrodisiac to Health Food: A Cultural History of Chocolate" *Karger Gazette* 6 no. 68
7. Guggenheim, KY. "Chlorosis: the rise and disappearance of a nutritional disease". *The Journal of Nutrition.* 1995; 125 (7): 1822–5
8. Patek, Arthur J.; Heath, Clark W. "Chlorosis". *Journal of the American Medical Association.* 106 (17): 1463–1466.
9. Andral, G. Essaid 'Hematologie Pathologique. Fortin, Masson and Cie, Paris, 184.
10. Wintrobe. Classification and treatment on the basis of differences in average volume and hemoglobin content of red corpuscles. *Arch Intern Med.* 1934; 54: 256- 79.
11. Swaroop Raj "Correlation between morphological typing of anemia based on RBC indices and RDW obtained from XT-2000i, an automated hematology analyzer with peripheral blood smear examination" 2013.
12. Garg, Monika, and Karuna Sangwan. "Comparison of Automated Analyzer Generated Red Blood Cell Parameters and Histogram with Peripheral Smear in the Diagnosis of Anaemia." 2019.
13. Kim SJ, Kim Y, Shin S, Song J, Choi JR. Comparison study of the rates of manual peripheral blood smear review from 3 automated hematology analyzers, Unicel DxH 800, ADVIA 2120i, and XE 2100, using international consensus group guidelines. *Arch Pathol Lab Med.* 2012 Nov;136(11):1408-1
15. Japheth E Mukaya, Henry Ddungu, Francis Ssali, Tim O'Shea, Mark A Crowther.
16. Prevalence and morphological types of anaemia and hookworm infestation in the medical Emergency ward, Mulago Hospital, Uganda *S Afr Med J.* 2009; 99: 881-86.
17. Aravind P, Meghashree V. RBC histograms and peripheral smear study: A comparative analysis. *MedPulse International Journal of Pathology.* March. 2021; 17(3): 49-54.
18. Singhal, Shivangi, 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(10): 4563-6.