

Study of Automated Red Blood Cells Parameters in Correlation with Routine RBCs Morphology by Smear Review

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ABSTRACT

Background: sysmex XT 1800i coulter is a hematology analyzer incorporating new electronic and mechanical design with advanced algorithm technology to perform CBC. The principles of sysmex XT1800 are electrical impedance technology, optical scatter technology, flow cytometry. The sysmex XT-1800i haematology analyser is used in unique fluorescence flow cytometry (FFC) technology. FFC looks at deoxyribonucleic acid (DNA) content, cell size and inner cell complexity rather than cell size alone. This generates remarkably accurate results. Review rates and turnaround time are reduced due to specific fluorochrome labelling. The XT-1800i offers true quantitative immunoglobulin (IG) counting instead of the flagging delivered by other technologies.

Patient and Method: Evaluation of this instrument was performed on 200 samples of inpatient and outpatient people in Ain Shams University Hospitals. We collected samples from all departments of the hospital randomly of any age and sex except those of age less than 18 years old.

Results : this study was centered upon RBCs indices which were mean corpuscular volume (MCV),mean corpuscular hemoglobin(MCH),red cell distribution width (RDW) after comparing sysmex XT 1800 coulter results for RBCs parameters with morphology by smear review under the microscope. We found that the device sensitivity was 74.7% , 75 % ,83 % and specificity was 74.3% , 79.5 % ,78.7% according to RBCs size ,chromasia and anaesocytosis respectively.

Conclusion: at the end of our study we reached to specific criteria at which we must do a blood film smear review to evaluate RBCs abnormalities which cannot be evaluated by the device.

Keywords: hematology analyzer, abnormal RBCs morphology.

INTRODUCTION

Peripheral blood smear (PBS) examination is a very important tool which is used in diagnosis and follows up of abnormal results on a complete blood count (CBC) to evaluate different types of blood cells. It helps to diagnose and monitor numerous conditions that affect blood cell populations [1]. The routine microscopic examination of a well spread, Leishman-stained PBS for RBCs morphology presents a wealth of information regarding red cell size, shape, inclusions and hemoglobin content that are basic and fundamental in hematology testing [2]. During the past three decades, a number of automated hematology analyzers have been developed; most of which directly measure two RBCs parameters, RBCs count and mean corpuscular volume (MCV). The rest of the RBCs indices are calculated from RBC, MCV and hemoglobin (Hb) [3]. Nowadays, hematology automation offers more than CBC testing. There are four immature RBC parameters that can be automatically reported with every CBC to provide the information needed for the physician to assess the state of erythropoiesis. These parameters are number of circulating reticulocytes (RET), immature reticulocyte fraction (IRF), reticulocytes Hb content and nucleated RBCs (nRBC). All these

four parameters are widely available now and can be useful to physicians in diagnosis of iron deficiency anemia because the parameters are direct measures that reflect the iron available for hemoglobin (Hb) synthesis [4]. Owing to the fact that manual microscopic examination of the blood smear is subjective, time consuming and quite misleading impressions can be drawn from inadequately prepared smears, a number of automated hematology analyzers have been developed to manage heavy workload. But, due to the incompleteness of accurate morphological information on individual RBCs from these instruments , 5-10 % of samples in hematology laboratories undergo smear review for abnormal RBCs morphology, from where comes the importance of studying the relationship between RBCs morphology reporting and the results of automated RBCs parameters from hematology analyzers [5]. With the help of different statistical tests and algorithms, the relationship between the complex data obtained from hematology analyzers regarding RBCs parameters and blood smear examination results can be analyzed in order to create a practical guideline that may help predicting RBCs morphology from hematological

parameters of 4 data sets (MCV, MCH, HCT and RBCs)^[6].

PATIENTS AND METHOD

This study was performed in the main hematology laboratory of the Clinical Pathology Department, Ain Shams University Hospitals, a tertiary care university hospital serving a large inpatient and outpatient population. The study samples were routine inpatient and outpatient CBC samples from all departments of Internal Medicine Hospital. The study samples were fulfilled the following inclusion and exclusion criteria:

Inclusion criteria

- 1- Peripheral blood specimens from adults aged over 18 years old.
- 2-Peripheral blood specimens collected from Internal Medicine, Oncology and Surgery Departments as well as Outpatient Clinics.

Exclusion criteria

Pediatric specimens from patients less than 18 years old.

Specimen collection from patients

Two hundred samples of complete blood count (CBC) samples were selected over a 4 months period. These samples were randomly selected from the daily routine workload. According to the laboratory policy the following was done: two mL peripheral venous blood was drawn into Di potassium ethylene di amine tetra acetatic acid (K2 EDTA) vacutainers under complete aseptic conditions and they were delivered to the lab; demographic data including patients' sex, age and clinical condition were collected from the lab request.

A-Automated complete blood count (CBC) and differential counts:

Automated complete blood count (CBC) and differential counts were performed on each sample within 4 hours of collection in open mode using Sysmex XT-1800i system (name of company and country). Samples remained capped at an ambient temperature throughout the study. Sysmex XT-1800i uses the electric resistance detecting method. Red cell distribution width was reported on the Sysmex XT as both standard deviation from the mean red cell distribution width (RDW-SD) and as coefficient of variation from the mean red cell distribution width corpuscular volume (RDW-CV). Procedures for quality assurance and quality control were followed to ensure good performance. The hematology analyzer was regularly calibrated by standardized calibrators Sysmex XT1800 . Daily whole blood controls were used monitor the analyzer's performance (CBC-5D Hematology

Controls). During the course of the study there were no repair in the equipment, change in reagents, calibration standards or controls. Patient samples were never run unless a successful quality control was obtained applying Westgard rules.

B-Manual differential and blood smear review

Blood films were smeared manually from all the samples and stained with Leishman stain, regardless of whether these blood smears had been required or not according to our standard operating procedures. Leishman stain was prepared by adding 0.2 g of the powdered dye to 100 mL of methanol in a conical flask of 200–250 mL capacity. The mixture was kept to warm to 50 C for 15 min with occasional shaking. The flask was allowed to cool and then the solution was filtered to be ready for use^[7]. After the blood film became dry, the slide was flooded with the stain. After 2 minutes, double the volume of water was added to the film for 5–7 minutes, and then it was washed in a stream of buffered water until it has acquired a pinkish tinge (up to 2min). After the back of the slide has been wiped and cleaned, the slide was kept upright to dry^[7]. The 200 blood smear slides were examined under the microscope. Smear review focused on the morphology of red blood cells. The abnormal smear results were reviewed by senior staff.

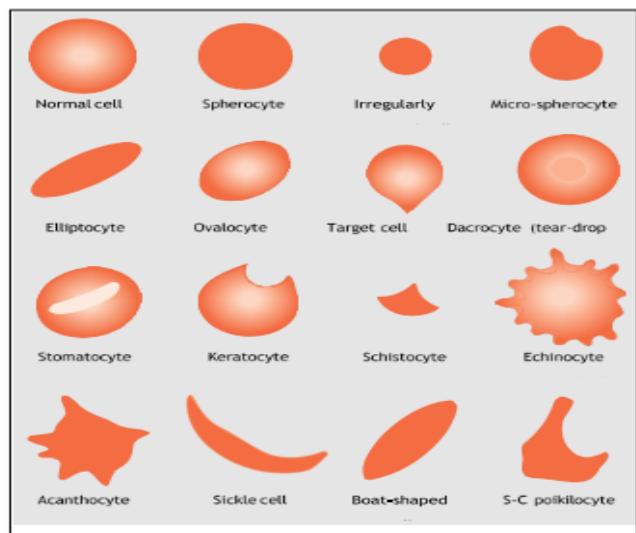


Figure 1- RBCs different morphology

C-Sample classification:

After reviewing smears, the results were compared to the results of red blood cells (RBCs) parameters of Sysmex XT-1800i to classify samples into true positives, false positives, true negatives and false positives. If the smear contained a positive finding correlated with the automated parameters the sample was graded as a true positive. But, if the

smear contained positive findings not correlated to the parameters it was graded as false negative. If the smear result was negative but not correlated with the parameters it was graded as false positive and a negative smear findings correlated with the parameters it was graded as true negative result.

The study was done after approval of ethical board of Ain Shams university and an informed written consent was taken from each participant in the study.

Statistical methods applied were

-**Descriptive statistics:**

1. Mean, standard deviation (\pm SD) and range for parametric numerical data.

2. Frequency and percentage of non-numerical data.

-**Analytical statistics: Chi-Square test:** was used to examine the relationship between two qualitative variables ; **Kappa statistics:** was used to compute the measure of agreement between two investigational methods Kappa's values < 0 as indicating no agreement and 0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial(large), and 0.81–1 as almost perfect agreement.

Recall (sens): is the number of true positives divided by the number of true positives and the number of false negatives. It is also called sensitivity or the true positive rate. Precision is (ppv) is the number of true positives divided by the number of true positives and false positives, but another way, is the number of positive predictions divided by the total number of positive class values predicted. It is also called the Positive Predictive Value (PPV).F measure = $2((\text{precision} \times \text{recall}) / (\text{precision} + \text{recall}))$

P- value: level of significance-P>0.05: non significant (NS).

-P< 0.05: significant (S).

-P<0.01: highly significant (HS).

RESULTS

Red blood cells (RBCs) parameters according to coulter in comparison with blood smear review were:

B- RBCs size, chromasia and degree of anisocytosis as measured by device:

As for RBCs size, normocytic, microcytic and macrocytic cases represented 50%, 47% and 3% of samples respectively. Normochromic and hypochromic cases represented 61% and 39% of samples respectively. Anisocytosis was detected in 68.5% of cases (Figure 2). Anisocytosis grade +1, +2 and +3 was detected among 33%, 16 % and 19.5% of samples respectively (Table 1).

Table 1: description of RBCs size, chromasia and anisocytosis as measured by the device

Parameters	Normal (n %)	Abnormal (n %)
RBCs size	100 (50%)	100 (50%)
	Normocytic (100) (50%)	Microcytic (94) (47%)
		Macrocytic (6) (3%)
RBCs chromasia	Normochromic (122) (61%)	Hypochromic (78) (39%)
	No anaesocytosis (32% of cases)	Anaesocytosis(68%) (33%)1+
	mean = 15.9 ± 3.3	(16%)2+ (19.5%)3+

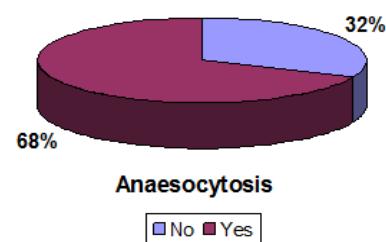


Figure 2

II- Analysis of smear review findings:

A-Description of RBCs size, chromasia and degree of anisocytosis by smear review:

As for RBCs size, normocytic, microcytic and macrocytic cases represented 50.5%, 42% and 7.5% respectively. Normochromic and hypochromic cases represented 66% and 34% of samples respectively. Anisocytosis was detected in 76.5% of cases. Anisocytosis grade +1, +2 and +3 was detected among 54%, 20.5% and 2% of cases respectively (Table 2).

Table 2: description of RBCs size, chromasia and anisocytosis

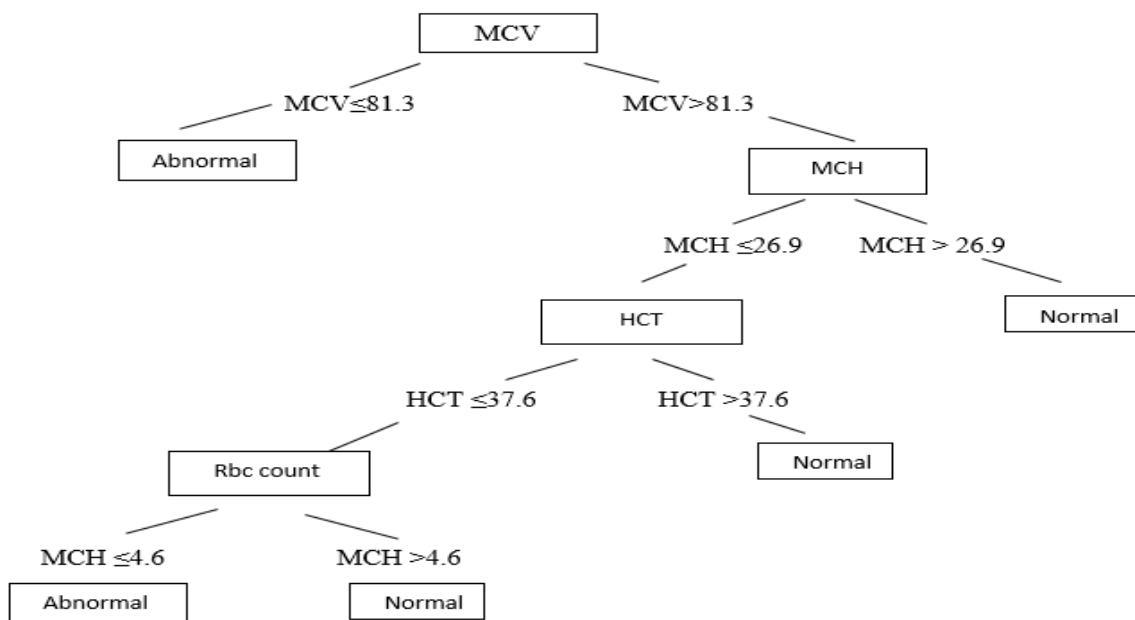
Parameters	Normal (n %)	Abnormal (n %)
RBCs size	101 (50.5%)	99 (49.5%)
	Normocytic (101) (50.5%)	Microcytic (84) (42%)
		Macrocytic (15) (7.5%)
RBCs chromasia	Normochromic (132) (66%)	Hypochromic (68) (34%)
		(108) (54%)1+
		(41) (20.5%)2+
Anisocytosis	47 (23.5%)	(4) (2%)3+

As shown in table 3 and figure 3 the three most common findings of abnormal RBC morphology were acanthocytes (38 occurrences), ovalocytes (21 occurrences) and schistocytes (10 occurrences).

Table 3: agreement between smear review findings and hematology analyzer results

Hematology analyzer results		smear review findings			Kappa	P(sig)	Sensitivity	Specificity	Precision	Accuracy					
		RBCs size					74.70%	74.30%	0.73	0.74					
		Normocytic	Microcytic	Macrocytic											
		N (%)	N (%)	N (%)											
RBCs size	Normocytic	75 (74.3%)		17	8	0.525	0.001 (HS)								
				-20.20%	-53.30%										
size	Microcytic	26 (25.7%)		67	1										
				-79.80%	-6.70%										
	Macrocytic	0 (.0%)		0	6										
				0.00%	-40.00%										
RBCs chromasia		RBCs chromasia				0.529	0.001 (HS)	75%	79.50%	0.71	0.78				
		Normochromic		Hypochromic											
		N (%)		N (%)											
		Normochromic 105(79.5)		17(25%)											
Aniso-cytosis		Anisocytosis				0.552	0.001 (HS)	83	78.7	0.73	0.82				
		0	1	2	3										
		N (%)	N (%)	N (%)	N (%)										
		0 (78.7%)	24 (22.2%)	2 (4.9%)	0(0%)										
		1	10 (21.3%)	52 (48.1%)	4 (9.8%)										
		2	0 (0%)	26 (24.1%)	5 (12.2%)										
		3	0 (0%)	6 (5.6%)	30 (73.2%)										

At the end of the study we concluded that if $\text{MCV} < 81.3$, RBCs it was considered as abnormal RBCs and needed to be followed by a blood smear review, but if $\text{MCV} > 81.3$, we must observe MCH first ; if $\text{MCH} > 26.9$ then it was normal RBCs with no need for smear review, but if $\text{MCH} < 26.9$ we judge by HCT if it was > 37.6 it is normal RBCs, but if $\text{HCT} < 37.6$ then it is needed to be reviewed by a blood smear review (**Figure 3**).

**Figure 3 : the decision tree in predicting RBC morphology**

DISCUSSION

Since the first use of automated analyzers, microscopic examination of a stained blood film has complemented analyzer results to provide a comprehensive hematology report on a patient blood [8]. Despite improving capabilities of automated hematology analyzers, manual slide review is still necessary to identify some morphologic abnormalities that may be relatively unremarkable in automated methods [9]. It is recognized that the automated systems are superior for counting of red blood cells, HB, MCV, MCH and RDW. Whereas, visual microscopy is superior for differentiating cells based on nuances of cytological features, especially for immature cells [8].

These guidelines were formulated with the aims of reducing costs and the turnaround time of the results without sacrificing their quality and justifying the performance and skills of the multiparametric hematology analyzers. So, this study aimed to compare red blood cells morphology by blood smear review with that of the automated device [10]. They are based on the set of screening thresholds for the results given by the analyzers and on the presence or absence of suspect flags. Also, this study aimed to distinguish samples with a high probability of containing relevant morphological alterations for the diagnosis and treatment of patients. When the CBC results do not meet the screening criteria, there were recommended procedures to follow, specifically to prepare an adequate peripheral blood smear for microscopic analysis [8].

Unfortunately, peripheral smear review is very time consuming and requires skill of an experienced morphologist. The ever increasing workload puts the laboratory staff under constant pressure to deliver accurate results to clinicians. So, it was realized that when a patient's automated full blood count is normal, a microscopic evaluation of the blood specimen may not add any value to the information provided by the blood cell analyzer. We found that RBCs morphology is normal in 60 % of samples, where 40 % of them represents abnormal morphology like Acanthocytes which was the most common abnormal shape among the studied samples (19%), followed by ovalocysts(10.5%). This abnormal morphology can be explained by the type of patients involved in the study. There were lots of anemia patients in the Department of Rheumatism,

Radiotherapy, Chemotherapy, Hepatic Disease and Nephropathy and probably in the population in general. These findings are not in agreement with those of Pratumvinit *et al.* [9] and Wei *et al.* [11] who stated that RBCs abnormalities represent the commonest abnormalities in their studies (62.5% and 44%, respectively) and this may be due to large number of samples that they were used. Among the morphological abnormalities of RBCs, anaesocytosis was the most common abnormality in our study while this is consistent with those of Pratumvinit *et al.* [9] who reported that the three most common findings of abnormal RBC morphology were microcytosis, anisocytosis and hypochromia. In the present study, use of the Consensus Group criteria generated a quite high review criteria (74.7%) applying sysmex XT 1800. This review rate is, however, comparable to 46.06% overall review rate reported by Comar *et al.* [10] which applying Consensus Group criteria on their results obtained by 2 analyzers. Actually, it is even closer to the 54.45% microscopic review rate which they reported on using the XT-2000i than to the 43.86% of the XE-2100D. The present study was conducted on inpatients and again this was contributed to the high review rate. Patient composition (males versus females and inpatients versus outpatients) has been recognized as a factor affecting review rate [2]. This probably explains the significantly lower review rate (13.9%) reported by Froom *et al.* [12] in analyzing outpatient sample. We include in our study hemoglobin values and we used more sensitive criteria leading to more peripheral blood smear reviews for RDW, low hemoglobin value. However, our criteria were stricter for MCV, MCH, RDW .Actually, with our laboratory criteria MCV has to be <81.3 fL will trigger the rule of smear review . Theoretically, the use of our laboratory stricter criteria has the potential to avoid some of false positives encountered with the Consensus Group criteria, while it carries the risk of missing some true positives. In our study, MCV as measured by the device was 83.1 ± 8.8 . We found that 84 cases that fell between the two cutoffs for low MCV, 15 cases were of high MCV , 73 were true positives with less than 81 fL criterion, while 26 were false positives by more than 81 fL criterion.

This was the case in Pratumvinit *et al.* [9] study where the false negatives increased after they adjusted the threshold to the stricter <70fL.

However, they did not worry about this increase as they believe that in patients with microcytosis, the detailed blood smear review may not be useful to discriminate between iron deficiency anemia, thalassemia minor and anemia of chronic disease [12]. They rather perform reflex testing using hemoglobin electrophoresis if not previously done in samples with MCV <75 fL and RBCs $\geq 4.5 \times 10^12/L$. This led to a new diagnosis of β-thalassemia minor of 67.8% the patients they tested.

There were a number of limitations in the current study. The study did not include persons less than 18 years old. Moreover, it was conducted on samples from the Department of Internal Medicine that are particularly prone to have more abnormal CBCs and consequently a higher review rate also it included outpatient clinic who prone to have normal CBC.

Samples were collected from routine workload over a period of 4 months, so some uncommon positive findings were not adequately represented in the study such as malaria, RBC auto agglutination and rouleaux formation. The study did not either include cases where diagnosis relies heavily on morphology such as autoimmune hemolytic anemia or microangiopathic hemolytic anemia. So this study required more sophisticated statistical analysis. In addition, optimization of the criteria was beyond the scope of the study.

CONCLUSION

At the end of our study we do a decision tree to create a set of criteria that we can follow to evaluate RBCs morphology by blood smear review. This tree states that if MCV less than 81.3, the RBCs are then abnormal and should be reviewed by a blood smear under the microscope, if MCV is more than 81.3 and MCH more than 26.9 so the RBCs are normal and there is no need for blood smear review, but if MCV is more than 81.3 and MCH is less than 26.9 then this RBCs also should be reviewed by blood smear under the microscope to be more accurate and specific.

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