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Validity of Selected WBC Differentiation Flags in Sysmex XT-1800i

Parya Bameni Moghaddam[1], Fatemeh Mahjoub[1], Amirhossein Emami[2], Alireza Abdollahi[3, 4]

1. Dept. of Pathology, Tehran University of Medical Sciences, Tehran, Iran

2. Dept. of Internal Medicine, Tehran University of Medical Sciences, Tehran, Iran

3. Dept. of Pathology ,Imam Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran

4. Thrombosis Hemostasis Research Center, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

***Background*:** Automatic Cell Counter devises make the CBC differential very easy and delivering the results in few second. However, the problem with this device is facing a flag requires a time-consuming microscopic review of the specimen which causes unacceptable wait times for patient as well as costs for laboratories. In this study, we calculated the validity of WBC diff flags in Sysmex XT-1800i. In addition, we verified the correlation between manual and automated samples.

***Methods*:** Overall, 1095 flagged samples were selected in the period of 6 weeks (Imam Hospital complex, Tehran Iran, 2014). The results of both automated and manual counting of the samples were carefully studied and compared. Totally, 624 NRBC flags, 450 Blast flags, 155 abnormal WBC Scatter gram flags, 140 Eosinophilia flags and 468 Monocytosis flags were identified.

***Results* :**Considering NRBC and blast flags there was a significant difference between our manual counted and automated counted NRBCs and blasts (P<0.05). There was no significant difference between automated and manual counting of flags for WBC Scatter gram. A significant difference between automated and manual counting data in flags, eosinophilia and monocytosis was foun (P<0.05).

***Conclusion*:** Maspin expression was reduced in samples with grade II& III of invasive ductal carcinoma. Based on expression of Maspin Inc-erb-B2, it seems that more expression happened in normal group comparing with different scores of it. We could suggest that there was a reverse relationship between tumor formation and Maspin gene expression. These results showed possible role of Maspin as prognostic factor

***Corresponding Information*:** Dr. Alireza Abdollahi M.D.: Keshavarz Blvd, Imam Hospitals Complex, Tehran, Iran. Email: abdollahi_a@tums.ac.ir Tel: +98-9121220588

Introduction

Regarding limited sources (raw materials, manpower, etc.), getting the job done with the least cost must always be considered. Therefore, researchers have always been in searching of finding new ways and inventing new methods to accomplish this goal.

Automatic Cell Counter (ACC) (like Sysmex) devises make the CBC differential very easy and delivering the results in few second (1-4). However, the problem with this device in facing

a flag requires a time-consuming microscopic review of the specimen, which causes unacceptable wait times for patient as well as costs for laboratories (1, 2-5). The advantage of this ACC is that we can save time by not preparing slides and reading them manually and therefore reduce cost. Disadvantage comes when the device shows a flagged specimen, which requires lab personnel perform a microscopic review of samples in a traditional way. Therefore, as the number of the flags increases, cost of the manual process and time will increase, tremendously (2, 5-9).

As it is obvious, a time consuming and slow process facing flagged specimen, which often happens inside the labs, are troublesome, because the manual counting of slides needs specialty and experience. Naturally lab needs more experienced personnel who get paid more than in experience dons. On the other hand, in most cases, after manual counting of flagged specimen, results are not the same as those of ACCs. Therefore, we are able to give suggestions so that the time and cost of the processes be minimized. In this way, we increase the efficiency of our laboratories, use the experienced personnel in rightful pleases and reduce the cost (10-12).

Flagged ACC reports can replace manual ones. For instance, Parham et al. had done conclusive studies with Cell-Dyn 3500 Hematology analyzer device (1). They focused on Absolute Neutrophil Count (ANC), which itself is the index for counting for chemotherapy in oncology clinics. In this study after eliminating invalid dated flags with the Turnaround Time (TAT) had reduced from 45 min to 7 min, suggesting the result must be reached through manual review of slides. Hijiya et al. considered the flags of blasts, immature myeloid cells, monocytosis, NRBCS and platelet clumps, finding very good correlation between manual counting and automated counting of ANC in Coulter Gens and /or HmX devices with (2). Friis-Hansen et al .using ACC and Sysmex XE2100 devices showed that the flag of abnormal WBC Scatter gram with in checking ANC can be disregarded (3). They introduced an algorithm for disregarding the flag. However, they did not check the effectiveness of his algorithm on turnaround time. Antony et al. did extensive study on 296 collected samples in 3 weeks (4).

They studied Friis-Hansen et al. (3) flags in addition to four other flags, NRBC, monocytosis, eosinophilia and blasts, which had the least effect on ANC on basis of lab employee's experiences. Accordingly, correlation between manual and ACC results of these five flags with existed. After determination of these flags, Antony et al. set an algorithm to improve the use of Sysmex (4). They implemented their suggestions clinically and after comparing, they reported the following results:

1. By withdrawing the flags, sample checking was reduced by 57%.
2. 60% of all flagged cases were reported without manual counting.
3. The TAT was reduced to 17.2%.

Before using the suggestions, the time used for differentiation was nearly 115 min but after exercising algorithm, the time reduced to nearly 15 min. In this study, we tried to calculate the validity of WBC diff flags in Sysmex XT-1800i. In addition, we checked the correlation between manual and automated samples and the calculation of the saving cost of ignoring invalid flags.

Material and Methods

Study site

This cross-sectional descriptive study was done in Valiasr Hospital Central Laboratory in Imam Hospital complex in Tehran Iran in 2014. The hospital is a tertiary care academic medical center. It is one of the Tehran University of Medical Science educational hospitals. The lab receives more than 1000 samples daily; more

than 600 WBC diffs are being examined every day. Almost 400 of them are flagged at least with one of our selected flags.The Medical Ethics Committee of Tehran University of Medical Sciences confirmed the study.

Sysmex XT-1800i

The SysmexXT-1800i which serves as automated hematology analyzer for diagnostic use in laboratories, can determine the results of twenty-one parameters of blood samples. The XT-1800i performs analysis of WBCs with an optical detector based on the flowcytometry method. RBCs and platelet count analysis is done by the RBC detector using the Hydro Dynamic Focusing method. Hemoglobin (HGB) is analyzed by the HGB detector by using the SLS hemoglobin detection method. By individual settings the user can adapt the instrument to his/her needs or existing laboratory conditions (4). The ACC devise uses "flags" to indicate whether a sample contains qualitative or quantitative abnormalities.

Manual differential

Undoubtedly, CBC with differentiating is one of the basic and important biochemical laboratory tests in medicine and clinical review of patients. Traditionally, examination of specimens and preparing the slides are done manually; and counting blood cells is done by microscope. For manual differentiation, 4 slides for each sample is taken and stained by Wright-Giemsa stain. Using standard microscopic technique the samples are differentiated by one of the experienced lab personnel.

Auxiliary cycle

Auxiliary cycle is the process that lab personnel take for each flagged sample to be ready and differentiate manually.

Sample size

Our samples were Sysmex XT 1800 flagged peripheral blood sample reports of Vali-Asr Hospital Central Laboratory in Imam Khomeini Complex. We used Cochran formula for number of samples. In this formula, we considered marginal error (e) 0.05, confidence coefficient 95%, z=1.69 and both P and q were considered equal to 0.5. Using the formula with above parameters, sample size was calculated as at least 385 cases.Overall, 1095 flagged samples (flagged with our addressed flags) were selected in the period of 6 weeks. The results of both automated and manual counting of the samples were carefully studied and compared. Out of 1095 samples, 624 NRBC flags, 450 blast flags, 155 abnormal WBC Scatter gram flags, 140 eosinophilia flags and 468 monocytosis flags were identified. Manual counting is considered the valid method of counting performed in optimal condition. We considered lab personnel whom prepare the slides and differentiate them are certified and experienced, the coloring method is done with absolute precision, high quality and within the laboratory standard protocols, working environment and automated cell counter condition (material used for ACC, temperature, humidity, maintenance, power stability and…) are in acceptable margined.

Statistical analysis

We used descriptive (for charts, frequency) and analytic (for hypothesis tests, regression, and correlation) methods, SPSS (Chicago, IL, USA) and Microsoft office excel 2007 for describing our findings.

Results

Identification of flags

Flags were chosen by literature review (2,

3, 6) and our experienced lab personnel. They believed these flags (NRBC, blasts, monocytosis, eosinophilia and abnormal WBC Scatter gram) are the most common flags in our lab associated with WBC diff.

Correlation between data

Overall, 1097 flagged samples (flagged with our addressed flags) were selected in the period of 6 weeks. The results of both automated and manual counting of the samples were carefully studied and compared. Out of 1097 samples, 624 NRBC flags, 450 blast flags, 155 abnormal WBC Scatter gram flags, 140 eosinophilia flags and 468 monocytosis flags were identified. After study of NRBC flags, out of 624 samples, 133 (21.3%) were valid and 491(78.7%) were invalid flags (36% were male and 64% were female). In blast flags, out of 450 flags 113 (25.1%) were valid and 503 (74.9%) were invalid (40.7% male and 59.3% female). In abnormal WBC Scattergrams out of 155 flags 152 (98%) were valid and 3 (2%) were invalid (51.6% male and 48.4% female).

For analyzing NRBC flags data, we used MacNemar test (because both tests were qualitative and they both group of data are depended). Since *P*-value of the analysis was 0.0001, there was a statistically significant difference between our manual counted and automated counted NRBCs.

Out of 624 flagged samples, only 133 (21.31%) of them were valid flags and 491 (78.7%) samples were flagged inappropriately. If we look at the amount of NRBC flags in different wards, we understand the most valid flags belonged to the Neonatal Ward. Over 36% of the samples were rightfully flagged by Sysmex XTi800 (Table 1).

In the blast flags, looking that our data were also qualitative and dependent, we used Mac Nemar analysis again. If *P*-value of analysis is less than significance level, we conclude that our H0 hypothesis is not acceptable and by definition H1 hypothesis is right, and our two set of data do differ statistically. Only 113 out of 450 samples were flagged rightfully (Table 2).

Little over than 25.1%, and over 74.9% of the flags were invalid. In the abnormal WBC Scatter

Table 1
Valid NRBC flags in different wards

		Counted NRBCs Manual		Valid Flags
		Positive for NRBCs	Negative for NRBCs	Positive for NRBC by Manual counting / Positive and Negative for NRBC by Manual counting
Wards	Surgery	3	9	0.25
	ICU	4	51	0.072
	Neonatal	101	177	0.36
	Gynecology	0	8	0
	General and Internal Medicine	18	137	0.116
	Children	1	11	0.083
	Out Patients	0	19	0
	ER	6	78	0.071
	Thoracic Surgery	0	1	0

Table 2
Comparison between manual and automated counting in blast flag

		Automated Counting Method	
		Positive for Blasts	Negative for Blasts
Manual Counting Method	Positive for Blasts	113	0
	Negative for Blasts	450	0

Table 3
Comparison between correlation and SD in automated and manual counting of eosinophilia flag

Methods	Means	Standard Deviation	Correlation	R^2
Manual Counting	11.26	7.13	0.715	0.511
Automated Counting	12.46	7.19	0.715	0.511

gram flags, using Man Nemar analysis the results were as follows: The *P*-value was calculated 0.250. Therefore, our H0 hypothesis is not ruled out. Out of 155 samples that was gathered, only 3 (2%) were invalid and over 98% of the data were flagged accurately.

For eosinophilia flag, using SPSS Paired t-test, we calculated correlation, and compare mean difference between the two sets of data (Table 3). *P*-value was measured 0.000 so our H0 was rejected and null hypothesis was realized and there was a statistically significant mean difference between the two sets of data. The correlation between the two methods is shown in Fig. 1. The correlation between the two data was 0.715 and $R^2 = 0.511$.

Using SPSS paired sample t test, difference between the means and the correlation between the two sets of data was measured in monocytosis flag. The *P*-value is less than our significant level (0.05); therefore, there was a statistically significant difference between the means of the two sets of data which means that our H0 hypothesis was ruled out and H1 hypothesis was realized.

The correlation between the two methods is shown in Fig. 2. The correlation between the two sets of data was 0.719 and $R^2 = 0.517$. The correlation between the two sets of data was 0.719 and $R^2 = 0.517$.

Discussion

There are few studies considering the validity of Sysmex cell counters and even fewer concerning utilization. Most laboratories prefer to have an accurate result even if it means spending more money and time. Whereas this money and time spending on repetitive and less useful tasks can be used for acquiring more accurate cell counters. Interesting point is that we spend a lot of money and time for an auxiliary path that gives us a predictable result.

If we look at the amount of NRBC flags indifferent wards, we understand the most valid flags belonged to the neonatal ward. Over 36% of the samples were rightfully flagged by Sysmex XTi800. In neonates NRBC is used in regards to ichter and anemia. Considering that the NICU ward has the most vali NRBC flags it is a wise

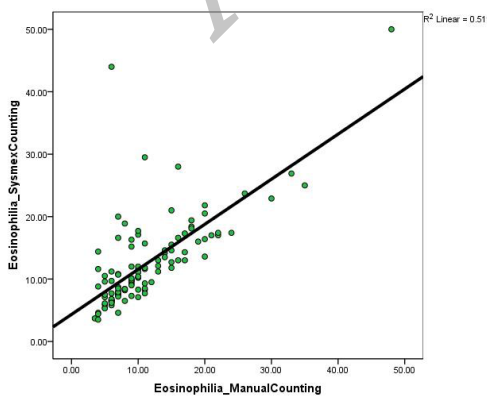

Fig. 1
Comparison between automated and manual counting of eosinophils in Eosinophilia flag

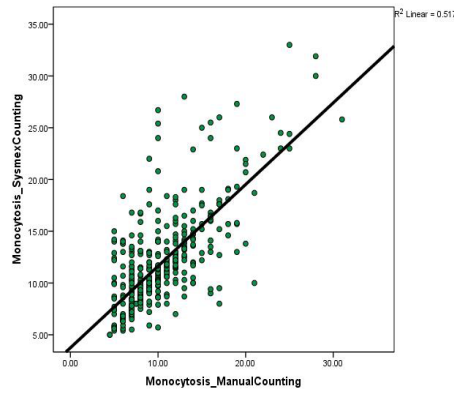

Fig. 2
comparison between automated and manual counting of monocytes in Monocytosis flag

decision to disregard the NRBC flag except in NICU ward.

In previous studies no evaluation was done in validity of the flags themselves (13-18). Furthermore, there was no study on the cost of auxiliary cycle in invalid flags. Indeed correlation between our sets of data in our lab is not the same as other centers. The best correlation in our study was 0.749 with the $R^2 = 0.561$. This result could have different reasons like not meeting the optimal conditions in regards to cell counter (temperature, humidity, power stability, maintenance, standard material and….), lab personnel (accuracy, experience and…) and staining (19-21).

In this study, for the first time (up to our knowledge), we evaluated the validity of the flags themselves individually without the effect of other external elements; and we calculated the cost of invalid flags for lab. In this study we focused mostly on the auxiliary cycle cost rather than TAT (turnaround time) because in Vali-asr Central Laboratory all the samples are reported at the end of the day and the delay in reporting does not affect the curse of treatment in patients and in our country compared to others per capita health budget is considerably low.

Therefore, finding ways to use this limited budget for more efficient use is crucial. Of course in our evaluation we only calculated the tangible costs, not considering the historical cost (the location, maintenance, power, depreciation expense, stockholding cost, set-up cost, cost of capital, indirect costs, personnel disability and…). By estimating all of these costs we can truly discover the depth of flags affect. The cost of 2657644000 Rials (US$ 106305.76) each year like the tip of an iceberg in water is only form 5 flags from the list of WBC flags in one cell counter in one section of our laboratory. If we can save this much money in one of our sections in one cell counter in one flag category, how efficient can we use the asset in our labs if we could find other sources of costs in laboratory. If we can design an algorithm in facing flags (valid or invalid) we can use the extra money for more efficient purposes. Considering our results, we propose the NRBC flags to be ignored and report negative except for the neonatal ward, and the blasts flags to be ignored and report negative in all the cases. The WBC Scatter gram should be considered valid and should be report positive in the patients.

Conclusion

We propose the NRBC flags to be ignored and report negative except for the neonatal ward, and the blasts flags to be ignored and report negative in all the cases. The WBC Scatter gram should be considered valid and should be report positive in the patients.

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Conflict of interest

The authors declare that there is no conflict of interests.

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