Original Article

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Tweaking the ISLH Slide Making Criteria- Is it worth?

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ABSTRACT

Background: Every laboratory is trying its best to release the report in shortest possible time without compromising its quality. In this era, automated analyzer plays a very important role but manual microscopic review of stained blood film is mandatory to identify morphological abnormalities and to authenticate the results of the analyzer.The aim is to evaluate efficacy of our laboratory criteria using automated analyzer for manual peripheral smear review and to reduce the number of samples requiring microscopic blood film review

Methods: Retrospective study was done on total 526 patients whose samples were collected over a period of 36 months for complete blood count randomly from both inpatient and outpatient population. Based on our experience we have set our slide making criteria which are more stringent than ISLH. We have taken the study population which falls in the range between ISLH criteria and our lab criteria for study. We made comparisons of adapted ISLH criteria with study population laboratory criteria.

Results: Thus, after employing strict screening criteria the yield of true positives was significantly lower than internationally accepted ISLH criteria (Two sample test of proportion, p value < 0.001).

Conclusion: The 41 rules of ISLH for slide review of automated CBC and WBC differential were compared with our study population lab criteria. There was significant reduction in microscopic smear review rate using ISLH criteria. False positive rates are high if cut offs are very narrow. Thus, it was found that tweaking ISLH criteria was an unnecessary exercise.

Keywords: *Manual Blood Smear Review; Screening Criteria; Automated Hematology Analyzers; International Consensus Group of Hematology.*

Introduction

Every laboratory is trying its best to release the report in shortest possible time without compromising its quality. In this new era day by day the different companies are launching their automated hematology analyzers with new features. Automated hematology analyzers have accelerated the speed of reporting with great accuracy and precision, thereby reducing manual hematology procedures without compromising the quality of reports of patients. It is therefore wastage of time and resources to perform manual peripheral smear review for each and every hematology sample provided controls are within acceptable range.

Microscopic examination of stained blood film has complemented the automated analyzer results to provide comprehensive hematology reports but on the other hand it is time consuming, labor intensive and expensive. Microscopic review of the slide is done to provide information in addition to or missing from the analyzer and to provide morphological details and also to verify the results of automated analyzers. To reduce the rate of MSR, the International Society for Laboratory Hematology (ISLH) through the International Consensus Group for Hematology Review (ICGHR) published a set of rules for peripheral smear review following analysis of samples on AHAs. [1] These rules are the manual smear review criteria for automated blood count analysis and considered as international standard. Our objective is to reduce the number of manual smear review as much as possible without compromising the patient's results. Application of the ISLHS criteria reduce the laboratory cost, turnaround time and improving the productivity.

The aim of study is to evaluate efficacy of our laboratory criteria using automated analyzer for manual peripheral smear review and to reduce the number of samples requiring microscopic blood film review

Material and Methods

The study was conducted in the hematology laboratory of Sahyadri specialty Laboratory Pune. The study included a total of 526 blood samples randomly collected from both inpatient and outpatient from all the departments of hospitals during the period of 36 months. First the quality controls were run on the DXH analyzer according to our laboratory standard protocol. If the controls were within range then the sample were run on the hematology analyzer DXH according to lab standard operating procedure.

The blood films were stained with Leishman stain .Microscopic examination of blood films were performed to identify morphologic abnormalities and to authenticate the

results of analyzer. Each sample was reviewed according to our study population lab criteria. (Table 1)

Samples showing errors like hemolytic sample, tiny clots, insufficient blood sample, and wrong container were excluded from the study. Pediatric samples were also excluded from the study as ISLH criteria in the study was with respect to adults. Delta check was also excluded from the study.

A sample which was positive for screening criteria with abnormal findings on peripheral smear was classified as True Positive (TP).A sample which was positive for screening criteria with no abnormal findings on peripheral smear was classified as False Positive (FP). Criteria for positive smear finding: (Table 2)

Total number of true positive, False positive were determined for our study population lab criteria and their percentages were calculated.

Results

In our study population laboratory criteria we get 526 cases which fall outside our Lab criteria but within ISLH criteria. Of all the 526 samples, 35 showed positive smear findings (6.65 %) (True Positive), 491 showed negative smear findings (False positive) (93.35 %).

Of these 526 samples, 166 (31.55%) were having RBC abnormalities, 171 (32.50 %) were having WBC abnormalities, 189 (35.93%) were having platelet abnormalities.

Out of 166 cases of RBC abnormalities,9 cases (7.14 %) were having significant additional findings on slide review.

Out of 171 cases WBC abnormalities 20 cases (11.6 %) were having significant additional findings and out of 189 cases PLATELET abnormalities, 6 cases (3.17 %) were having significant additional findings on slide review.

The most common findings of abnormal RBCs morphology were: Poly-chromatic RBCS (4.2%), Macrocytic RBCs with occasional hyper-segmented neutrophils (1.2 %).

The most common findings of abnormal WBC morphology were: Toxic granules (11.6 %), Shift to left up-to myelocyte (1.16 %).

The most common abnormality in Platelets is Giant platelets (2.6 %), Platelet clumps (0.52 %).

The "Analysis Table" comparing the performance of the adapted ISLH criteria with our study population lab criteria is as follows (Table 3).

Statistical method used is -Two sample test of proportion to test the proportion of true positive by ISLH Criteria and the proportion of true positive by SSL criteria. P-values were two-sided with statistical significance evaluated at the 0.05 alpha level.

In our study population laboratory criteria, we got 526 cases. Of all the 526 positive samples, 35 (6.65%) showed positive smear finding (True positive) and compered this with ISLH True positive 1483 (11.2%), we found significant difference in these two proportions (p-value = 0.001). Thus, after employing strict screening criteria the yield of true positives was significantly lower than internationally accepted ISLH criteria. Hence, we could have saved lot of resources had we adopted ISLH criteria as they are.

Table 1: Adapted international consensus group for hematology review criteria and our study population lab criteria for automated complete blood count

Test Parameter	ISLH criteria	SSL Criteria	Study Population Criteria
WBC Count	<4000 and >30,000	<4000 and >15,000	15,000-30,000
ANC	<1000 and >20,000	<1000 and >11,000	11000-20000
ALC	>5000	>5000	-
AMC	>1500	>1000	-
AEC	>2000	>1500	-
ABC	>500	>500	-
Hemoglobin	<7.0 and >18.5	<10.0 and >18.5	7 to 10
MCV	<75 and >105	<75 and >100	100-105
MCH	<30	<27 and >40	27-30
MCHC	>36.5	>36.5	-

Test Parameter	ISLH criteria	SSL Criteria	Study Population Criteria
RDW-CV %	>22	>17	17-22
Platelet Count	<100,000 and >1000,000	<150000 and >500,000	1,00000-1,50,000 and 500,000-1000,000
MPV	<5 and >12.5	<5 and >10.0	-
FLAGS	All Flags	All flags except NE1	
		Test parameters with	
		and …… (indicates counts are not reliable, ……indicates counts are not available for the parameter)	
		Interpretation of scatterplot	
		Indistinct zones for DLC in scatterplot	

Table 2: Criteria for positive smear finding.

PARAMETER	CELL MORPHOLOGY
RBC MORPHOLOGY	Anisocytosis ≥2 +, Macrocytes ≥2 +, eliptocytes ≥2 +, Stomatocytes ≥3 +, Schistocytes≥2 +, Dacrocytes≥ 2 +, Drepanocytes present, acanthocytes ≥2 +, spherocytes ≥2 +, Howell jolly body present, Cabot ring present, Basophilic stippling ≥1 +, Rouleaux formation present, polychromatophilia≥2+, RBC agglutination present
WBC MORPHOLOGY	Toxic granulation ≥1 +, Cytoplasmic Vaculoation ≥ 1 +, Dohle bodies ≥ 1 +, Hypersegmented neutrophils, Dohle bodies ≥ 1 +, Hyposegmented neutrophils, Hypogranulation present, Pseudo-pelger-huet present, Dusplastic cells
PLATELET MORPHOLOGY	Giant platelets ≥ 1 +, platelet clumps present, Hypogrnaular platelets, Megakaryocyte fragments
Abnormal Cell Types-	
Blast	≥1
Metamyelocyte	≥2
Myelocyte/Promye locyte	≥1
Atypical lymphocytes	≥5
nRBCs	≥1/100 wbcs
Plasma Cells	≥1

Table 3: Comparison of ISLH criteria and study population lab criteria.

Table 3: To test the equality proportions			
	ISLH Criteria N (%)	SSL Criteria N (%)	P-value
True Positive	1483 (11.2%)	35 (6.65%)	0.001
Remains	11815 (88.8%)	491 (93.35%)	
Total	**13298**	**526**	

Discussion

In this new era, various companies are launching their automated cell counters (AHAs) having new features. Automated cell counter accelerate the speed of reporting with great accuracy and precision .They are cost effective and decrease the turnaround time.

However, the results of automated cell counter needs to be confirmed by manual smear review. Also the morphological abnormalities and abnormal cells need to be confirmed by manual smear review.

Manual Smear review is still the gold standard rule to confirm the morphological abnormalities in the cell and to confirm the findings of the autoanalyzer.

Blood smear review allows appropriate interpretation of CBC and manual differential data with other laboratory findings and clinical information. [2] By properly interpreting the CBC and giving the proper diagnosis we can help the clinicians and can suggest the further work up if needed. It also serves as an excellent hematology teaching resource for training students, staff and continuing education of technical staff.

On the other hand, examination of manual smear review is tedious, time consuming, requiring more man power, thereby increasing the turn-around time.

A standard set of criteria are developed by College of American Pathologists (CAP). Although possible, such a set of criteria may not work entirely for all the laboratories. Internationally there were no uniform guidelines applied to automated analyzer for manual smear review. So, Dr.Berend Houwen along with 20 experts defined internationally accepted guidelines or rules. [1] Dr.Berend Houwen published the set of 41 rules for manual smear review. CAP and ISLH recommended that these rules should be validated before executing them on patient's sample. [1]

Till these rules become validated, laboratory professionals should use their own knowledge and experience. They should also take into consideration the need of population in that area, age, gender of patient, automated analyzer used, presence or absence of suspected flags, clinician's requirements and the level of expertise of the technical staff.

In our study we have analyzed only the screened patients which falls in the group between ISLH criteria and our own SSL (Sahyadri Specialty Labs) criteria.

We got only 35 patients out of 526 patients which have additional peripheral smear findings on slide examination and which falls in our screening criteria. i.e. True positive 6.65 %. Thus, even after employing strict screening criteria the yield of true positives was significantly lower than internationally accepted ISLH criteria (Two sample test of proportion, p value < 0.001). Our series also shows that 491 patients out of 526 patients do not have additional peripheral smear findings though they fall in our screening criteria i.e. False Positive 93.35 %.

As stated by Gulati et al. [2,3] review criteria may vary among the institutions but often includes,

1) The patient population in that area
2) Clinicians concerns in specific area of interest of patient's population (e.g. Hematology and oncology patients)
3) Training and experience of laboratory physicians.
4) Training and experience of technical staff performing CBC s and manual diffs.
5) Workload in hematology laboratory
6) There may be minute changes in blood smear which can be missed even by skilled laboratory personal.

The main reasons for adaptation of stringent criteria by our lab as compared to ISLH, was concerns of physicians and hematologists not to miss a single case with abnormal hematological findings. Our lab has one of the most robust training programs for technicians and all staff are well experienced.

Our study population is the population which falls in between ISLH criteria and SSL criteria, hence possibly, we got comparatively less additional findings as compared to Comar et al. [4] (29.49% out of 1977 samples) who reviewed entire ISLH criteria. He concluded that review criteria adapted from ISLH were neither suitable nor safe for use in hematology laboratory. He suggests that local peculiarities should be taken into account during the analysis of samples with positive smear findings so as not to overlook them.

Katyayani Palur et al. [5] reported 61.46% abnormal findings for a range stricter than ISLH range but relaxed than our criteria. The figure of 61.46% appears to be an exception.

After optimizing the smear review criteria, Busadee et al. [6] also finds 17.40 % positive smear results which are comparable but marginally high than our results. This is again because of study group we have selected and their peripheral smear review criteria which includes microcytic RBCs, Hypochromic RBCs, shift to left up-to band cell which we have excluded considering the local requirements.

Among the additional findings, which we have got, polychromatic RBCs is the most common finding. Out of 9, 7 patients have polychromatic RBCs, who were getting treatment for nutritional deficiency.

2 patients of macrocytic RBCs with hyper segmented neutrophils have Vit B12 deficiency, who turned out to be Megaloblastic Anemia.

In WBCs, toxic granules were the most common finding. Out of 20 cases we got toxic granules in all the cases. 2 cases are showing shift to left up to myelocyte with toxic granules. These are clinically significant findings in case of infection, septicemia.

In platelets, out of 6, 5 were showing giant platelets with high MPV, suggesting that the platelet count was within normal range. Thus, we cannot rely on automated cell count as far as platelets are concerned. ISLH has very wide criteria as far as platelets are concerned, viz 1,00,000 to 10,00,000. Our criteria of 1,00,000- 1,50,000 and 5,00,000-10,00,000 appear to be appropriate for our population.

We are having high False positive rate, 93.35 % as compared to ISLH consensus guidelines.

The false positive rate is largely due to instrument generated suspect flagging. The analyzers are intended to be used as screening devices and to flag suspect abnormal samples for further review.

Overcautiously, the auto analyzers are triggered to generate suspect flags so as not to miss potentially important abnormalities and thus minimize the number of false negatives. [7]

El Danasoury et al. [8] depicted that 60.2 % of false positive results using the ISLH criteria were due to the suspected flags.

In case of our laboratory, suspect flags do contribute to increase in false positive rate but the main factor which contribute to the high false positive rate is the narrow cut off of our screening criteria.

Thus, additional abnormal findings, in cases falling in our screening criteria, are comparatively less.

There are few limitations to our study, we have not included pediatric population in our study.

Delta check is also excluded from the study.

Conclusion

In the present competitive era of health care services, and with launching of new analyzers with great accuracy and precision day by day, every lab should take efforts to minimize manual slide review and to improve the productivity and efficiency.

The ISLH criteria are ideal to decrease the number of MSR in the clinical laboratory. In our laboratory, manual smear review rate has increased and there is increase in false positive rates because the cut off of screening criteria are very narrow.

Hence, it is ideal to go with ISLH criteria with appropriate cut offs and with appropriate review criteria for manual smear review after we validate it in our laboratory.

Automated analyzer values and flags complement the manual microscopic examination of peripheral blood smear if the criteria for manual smear review are developed judiciously and validated before use, thereby improving the efficiency.

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Competing Interests

None declared

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