Original Article

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Assessment of Peripheral Blood Smear Preparation Technique in Laboratories with High Sample Load

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ABSTRACT

Background: Examination of the blood film is an important part of the hematological evaluation. The reliability of the information obtained depends heavily on well-made and well-stained films that are systematically examined. Commonly used method of smear preparation in hematology the wedge method. In wedge method two slides are used one slide is placed on a flat surface with all necessary conditions maintained and the other slide is used as a spreader slide with is used to spread the blood film producing a good tongue shaped smear.

In laboratory settings with high case load, same spreader is used to produce blood smears of multiple patients.

Methods: The study was designed to know if there is any carry over of cells if the same spreader is used to make blood smears of multiple patients in laboratories having high patient load.

Same spreader was used to make multiple smears from pancytopenia sample after using it initially to make smears from samples of leukemia and neutrophilic leucocytosis.

Result: Slides were examined and results were tabulated, which showed that there was a definite carry over of cells from one smear to another if the same spreader is used consequetively for multiple patients

Conclusion: Caution need to be used in laboratories with high case load where multiple smears need to be prepared and time constraint is a limiting factor. Use of advanced coulters can help in correlation of cells counts and typing instead of only depending on smears for cells counts and reporting. Collecting clinical details of the patient is an important step which is indispensable

Keywords: *Peripheral Smear, High Case Load, Carry Over Of Cells*

Introduction

Examination of the blood film is an important part of the hematological evaluation.[1] Initiation of a PBF is often a clinical request by the attending clinician on account of a clinical suspicion or less frequently initiated by the laboratory [2]. The reliability of the information obtained depends heavily on well-made and well-stained films that are systematically examined.[1]

The method of making the films are : The two-slide or wedge method, the cover glass method, and the spinner method. Commonly used method of smear preparation in hematology the wedge method. In wedge method two slides are used one slide is placed on a flat surface with all necessary conditions maintained and the other slide is used as a spreader slide with is used to spread the blood film producing a good tongue shaped smear.[1]

In laboratory settings with high sample load, same spreader is used to prepare peripheral blood smears of multiple patients. Automation in smear preparation done by few hemoslider also uses the same technique of smearing but the spreader blade is cleaned each time before making the next smear.[3]

The speed and quality of information have become essential items in the release of laboratory reports [4]. Among the numerous advantages of using automated equipment are the reduce time to release results, high sensitivity, greater accuracy with reduced coefficient of variation, better reproducibility and higher productivity in laboratory testing [5,6]. Due to economic constraints faced by developing countries majority of the labs cannot go for automated slide makers and stainers, requiring strict quality controls and rechecks in manual techniques employed in smear preparation and staining.

So, the study was planned to know if there is any carry over of cells if the same spreader is used to make blood smears of multiple patients in laboratories having high patient load.

Materials and Methods:

One sample was taken each of – 1) Pancytopenia, 2) Leukemia and 3) Neutrophilic leucocytosis

Study was done to see if the use of same spreader used initially for leukemia and later for pancytopenia leads to any changes in TLC and PS findings of the pancytopenia case.

Likewise it was also studied whether the use of same spreader initially for leucocytosis and later for pancytopenia led to any changes in TLC and PS findings of the latter case.

Study was Divided into two Parts-

1 A) One smear was prepared with leukemia sample initially and 6 successive smears were prepared with pancytopenia case using the same spreader, and B) One smear was prepared with neutrophilic leucocytosis sample initially and 6 successive smears were prepared with pancytopenia case using the same spreader

2 A) One smear was prepared with leukemia sample initially and one smear from pancytopenia sample using the same spreader and this step was repeated 6 times, and B) One smear was prepared with neutrophilic leucocytosis sample initially and one smear from pancytopenia with the same spreader and this step was repeated 6 times.

Result

One sample was taken each of – 1) Pancytopenia, 2) Leukemia and 3) Neutrophilic leucocytosis, With values as follows – (table1)

After the study results were tabulated and analysed (Table-2,3,4 and 5).

Discussion:

In the first part of study (Table 2 and 3)-

1. A) By the use of same spreader TLC was raised in the smears made from pancytopenia case. TLC was high in smear numbered L1 and L2, L3 smear also showed a raised TLC with respect to the pancytopenia case. And there was not so significant raise of TLC in smears which came later in the order namely L4 and L5.

   There was a definite carry over of blast cells into successive smears in case of L1 and L2 smears and the blast cells were present with high N/C ratio, scant agranular cytoplasm, indented nuclear membrane and inconscpicuous nucleoli.

   In smears numbered L3 and L4 there were cells with poorly maintained features which could not be clearly typed as blasts and smear L5 showed no blasts.

   Irregular distribution of cells were seen predominantly in the head region of the smear (Table2)

   B) TLC was also raised when successive smears were prepared from pancytopenia case using the same spreader used for leucoerythroblastic reaction casev(Table 3).

   TLC was significantly raised in N1 Smear, TLC was also raised in N2 and N3 case with respect to the pancytopenia case and TLC raise was not so significant in N4 and N5 cases.

   Cells were distributed irregularly mainly in tail end and margins of the smear and predominant population of cells constituted of mature neutrophils, band forms and few neutrophilic precursors. Smear N1 and N2 also showed Nucleated RBCs.

2) Second part of the study includes 5 sets of smears (table 4 and 5).

   A) Same spreader used for making a leukemia smear was used to make one pancytopenia smear and the steps were repeated 5 times. There was a definite carry over of cells in almost all pancytopenia smears. TLC was significantly raised in almost all cases. Blast cells were positive in all the smears. There was irregular distribution of cells predominantly in head and in margins in few smears (Table 4)

   B) Same spreader used for making a leucoerythroblastic smear was used to make one pancytopenia smear and the steps were repeated 5 times. There was a definite carry over of cells in almost all pancytopenia smears. TLC was significantly raised in almost all cases. Predominant population of cells constituted of neutrophils, band forms and shift to left cells. There was irregular distribution of cells predominantly in tail end and in margins (Table 5)

   It was seen from the tabulation that there was clear carry over of the cells from the leukemia case and leucoerythroblastic reaction case to the subsequent smears if the same spreader is used.

Table 1:

Case	Pancytopenia	Leukemia	Neutrophilic leucocytosis
RBC	2.01million/mm3	1.99million/mm3	2.66million/mm3
Hb	6.1g/dl	5.3g/dl	8.6g/dl
HCT	18.1%	18.0%	26.6%

Case	Pancytopenia	Leukemia	Neutrophilic leucocytosis
MCV	90fl	91mm3	100/mm3
MCH	30.4pg	26.8pg	32.2pg
MCHC	33.9g/dl	29.6g/dl	32.2g/dl
RDWcv	14.3%	13.8%	13.4%
RDWsd	46fl	45fl	48fl
PCT	11000/mm3	44000/mm3	115000/mm3
TLC	400/mm3	1.5 lakh/mm3	61900/mm3
DLC	P-23.6 L-69.4 M-6.0 E-0.3 B-0.7	95% blasts 5% lymphocytes	P-90% L-08% M-01% E-01%
PS findings	RBC- normocytic normochromic RBC picture.; WBC- leucopenia seen TLC <1000/mm3 PLT-Decreased Impression-Pancytopenia	RBC-normocytic normochromic RBC picture. WBC-Very high leucocytosis. Predominant population of blast cells which show high N/C ratio, scant agranular cytoplasm,indented nuclear membrane and inconscpicuous nucleoli PLT – decreased Impression –acute leukemia	RBC-normocytic normochromic RBCpicture WBC- high leucocytosis Predominant population of neutrophils seen with moderate shift to left. NRBCS + - 10/100wbcs

Table 2:

Case	TLC	DLC	PERIPHERAL SMEAR REPORTING
Leukemia	1.5 lakh/mm3	95% blasts 5% lymphocytes	RBC-normocytic normochromic RBC picture. WBC-Very high leucocytosis. Predominant population of blast cells which show high N/C ratio, scant agranular cytoplasm,indented nuclear membrane and inconscpicuous nucleoli PLT – decreased Impression –acute leukemia
Pancytopenia	400/mm3	Pred lymphocytes	RBC- normocytic normochromic RBC picture. WBC- leucopenia seen PLT-Decreased Impression-Pancytopenia
L1	65000/mm3	Blasts +	RBC- normocytic normochromic RBC picture WBC-Irregular distribution of cells predominently in base
L2	8000/mm3	Blasts +	RBC- normocytic normochromic RBC picture WBC- Irregular distribution of cells predominently in base
L3	3000/mm3	Few suspicious cells +	RBC- normocytic normochromic RBC picture WBC- few cells predominently in base
L4	1500/mm3	Few suspicious cells+	RBC- normocytic normochromic RBC picture WBC- Leucopenia with Very few cells in base
L5	<1000/mm3	Pred lymphocytes	RBC- normocytic normochromic RBC picture WBC- leucopenia

Table 3:

CASE	TLC	DLC	PERIPHERAL SMEAR REPORTING
Neutrophilic leucocytosis	61900/mm3	P-90% L-08% M-01% E-01% Nrbcs-14/100wbcs	RBC-normocytic normochromic RBC picture; WBC- high leucocytosis. Predominant population of neutrophils seen with moderate shift to left. NRBCS + - 10/100wbcs
Pancytopenia	400/mm3	Pred lymphocytes	RBC- normocytic normochromic RBC picture.; WBC-leucopenia seen; PLT-Decreased Impression-Pancytopenia
N1	30000/mm3	Pred neutrophils. Mature neutrophils-70% Band forms and shift to left cells- 25% NRBCs-5/100wbcs	RBC- normocytic normochromic RBC picture.; WBC-Irregular distribution of cells pedominently neutrophils and precursors in margins and tail end
N2	5000/mm3	Pred neutrophils. Mature neutrophils-80% Band forms and shift to left cells- 20% NRBCs-2/100wbcs	RBC- normocytic normochromic RBC picture. WBC-Irregular distribution of cells pedominently neutrophils and precursors in margins and tail end
N3	4000/mm3	Pred neutrophils. Mature neutrophils-90% Band forms and shift to left cells- 10% NRBCs-nil	RBC- normocytic normochromic RBC picture.; WBC-few neutrophils and band forms seen in tail end
N4	<1000/mm3	Few neutrophils + band forms seen	RBC- normocytic normochromic RBC picture. WBC-few neutrophils and band forms seen in margin and tail end
N5	<1000/mm3	Few neutrophils + band forms seen	RBC- normocytic normochromic RBC picture. WBC-leucopenia

Table 4:

CASE	TLC	DLC	PERIPHERAL SMEAR REPORTING
Leukemia	1.5 lakh/mm3	95% BLASTS 5% lymphocytes	RBC-normocytic normochromic RBC picture.; WBC- Very high leucocytosis. Predominant population of blast cells which show high N/C ratio, scant agranular cytoplasm, indented nuclear membrane and inconscpicuous nucleoli; PLT – decreased. Impression – acute leukemia
Pancytopenia	400/mm3	Pred lymphocytes	RBC- normocytic normochromic RBC picture.; WBC- leucopenia seen; PLT-Decreased. Impression-Pancytopenia
LA+	90000/mm3	BLASTS+	RBC-normocytic normochromic RBC picture; WBC-Irregular distribution of cells mainly in base and margins. High leucocytosis predominant population of blasts
LB+	1lakh/mm3	BLASTS+	RBC-normocytic normochromic RBC picture; WBC- Irregular distribution of cells mainly in base. High leucocytosis predominant population of blasts
LC+	60000/mm3	BLASTS+	RBC-normocytic normochromic RBC picture; WBC- Irregular distribution of cells mainly in base. High leucocytosis predominant population of blasts
LD+	80000/mm3	BLASTS+	RBC-normocytic normochromic RBC picture; WBC- Irregular distribution of cells mainly in base and margins. High leucocytosis predominant population of blasts
LE+	55000/mm3	BLASTS+	RBC-normocytic normochromic RBC picture; WBC- Irregular distribution of cells mainly in base. High leucocytosis predominant population of blasts

Table 5:

CASE	TLC	DLC	PERIPHERAL SMEAR REPORTING
Neutrophilic leucocytosis	61900/mm3	P-90%; L-08%; M-01%; E-01%; NRBSs-14/100wbcs	RBC-normocytic normochromic RBC picture; WBC- high leucocytosis. Predominant population of neutrophils seen with moderate shift to left. NRBCS + - 10/100wbcs.
Pancytopenia	400/mm3	Predominantly lymphocytes	RBC- normocytic normochromic RBC picture.; WBC- leucopenia seen. ; PLT-Decreased. Impression- Pancytopenia
NA+	30000/mm3	Pred neutrophils. P-92%; L-08%; NRBCs-6/100wbcs	RBC- normocytic normochromic RBC picture. ; WBC- Leucocytosis seen with predominant population of neutrophils.
NB+	25000/mm3	Pred neutrophils. P-85%; L-14%; E-1%; NRBC-10/100wbcs	RBC- normocytic normochromic RBC picture.; WBC- Leucocytosis seen with predominant population of neutrophils
NC+	20000/mm3	Pred neutrophils. P-95%; L-55%; Nrbcs-2/100wbcs	RBC- normocytic normochromic RBC picture.; WBC- Leucocytosis seen with predominant population of neutrophils
ND+	32000/mm3	Pred neutrophils. P-90%; L-10%; Nrbcs-nil	RBC- normocytic normochromic RBC picture.; WBC- Leucocytosis seen with predominant population of neutrophils
NE+	18000/mm3	Pred neutrophils. P-91%; L-09%; Nrbcs-1/100wbcs	RBC- normocytic normochromic RBC picture. ;WBC- Leucocytosis seen with predominant population of neutrophils

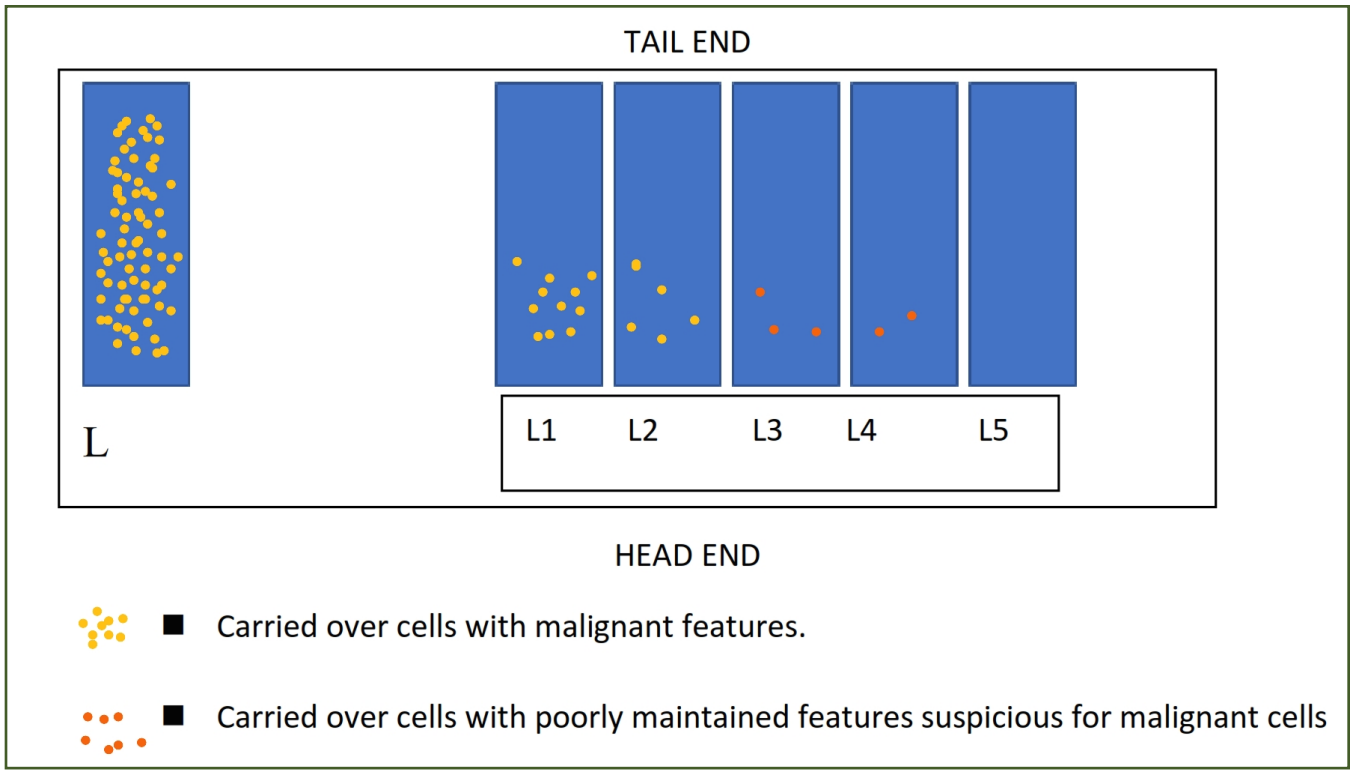

Fig. 1 :

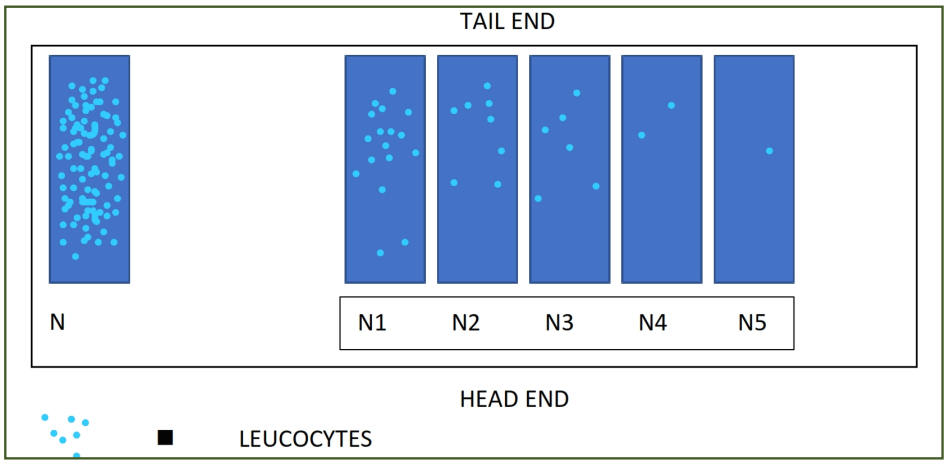

Fig. 2:

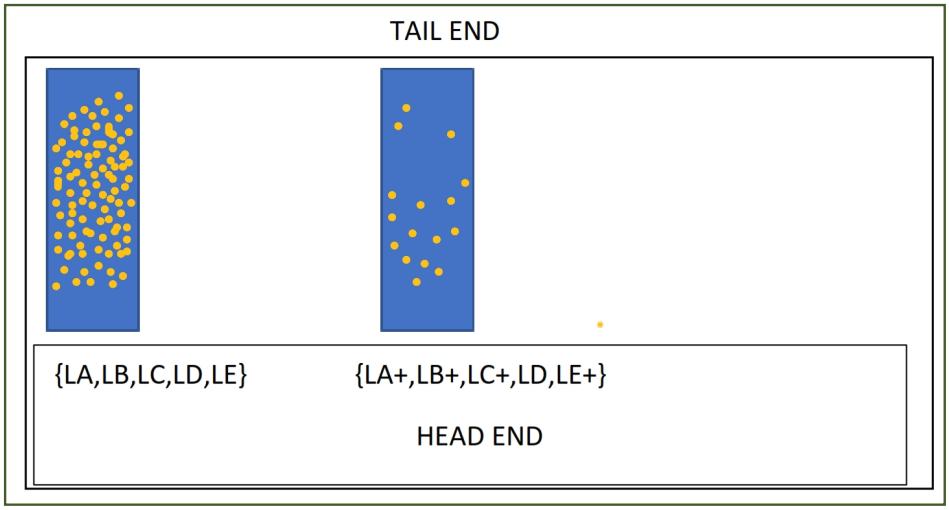

Fig. 3 : Common representational image LA and LA + to LF and LF+

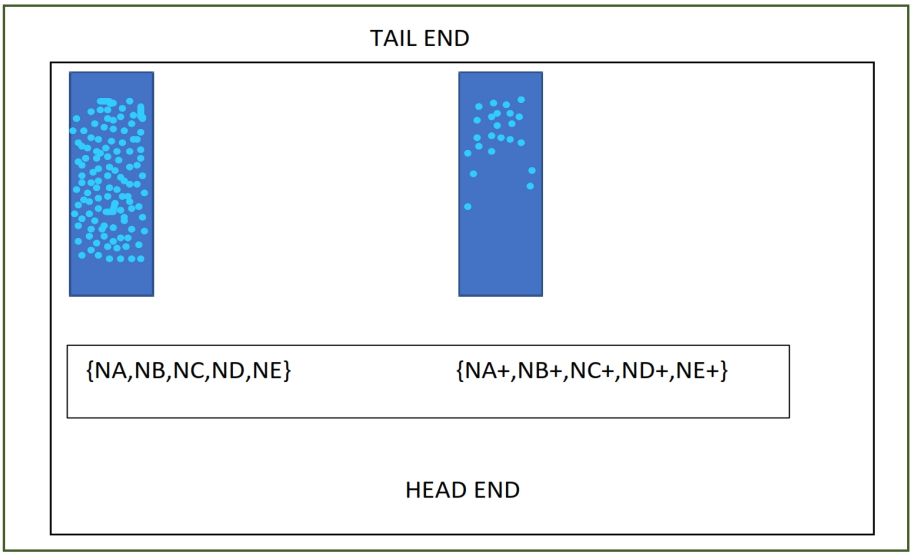

Fig. 4 : Common representational image for NA and NA = to NE and NE+

Conclusion

There was clear carry over of cells to the successive smears by use of same spreader. Carry over was more significant in the successive 3 smears in order. There was irregular distribution of carried cells. In case of malignancy carried over cells were more commonly distributed in head of the smear and in case of neutrophilic leucocytosis carried over cells were more commonly found in tail end and margins of the smear.

Caution need to be used in laboratories with high case load where multiple smears need to be prepared and time constraint is a limiting factor. Use of advanced coulters can help in correlation of cells counts and typing instead of only depending on smears for cells counts and reporting. Collecting clinical details of the patient is an important step which is indispensable.

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