ORIGINAL ARTICLE

JIACM 2025; 26 (1-2): 42-46

Artefacts in Haematology: Lessons to be Learnt

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Introduction

Peripheral blood smear (PBS) examination provides invaluable information about morphology of cells, as many diseases manifest with changes in peripheral blood cells. It is an inexpensive method for diagnosis of both haematological and non-haematological disorders. A systematic and thorough examination of PBS is adjuvant to clinical data and, most of the times, sufficient to make a diagnosis like haemoparasite and leukaemia. Recent advancements in the technology of automated cell counters have reduced the work load in laboratories; however, microscopic examination of PBS is still important for the evaluation of cytopaenias or for flagging a report of the automated haematology analyser. The art of PBS preparation and staining is gradually losing its shine. A good PBS needs ideal pre-analytical, analytical and post-analytical variables as important ingredients to work on; if PBS is not made according to standard laboratory practices, it can lead to various artefacts. Artefacts need to be correctly identified as they can be misinterpreted as pathological abnormality.

The present study highlights the importance of various artefacts on PBS to avoid misinterpretation of the smears.

Material and Methods

This study was conducted over a period of one month February to March 2023 at the Department of Pathology, Maulana Azad Medical College, New Delhi, including routine and emergency cases with control slides for assessing the staining characteristics using standard laboratory practices. A total of 500 cases were included in this study out of which 50 cases had artefacts.

Anticoagulants used for blood collection included EDTA, Trisodium Citrate and Heparin. EDTA and sodium citrate remove calcium from blood, hence prevent coagulation. EDTA is the most commonly used anticoagulant.

Excess amounts of EDTA affect both red cells and WBCs, produce shrinkage and degenerative changes. Platelets are also affected. Due to excess EDTA, they swell and then disintegrate leading to pseudo-high platelets count. So, proper blood to anticoagulant ratio is must for PBS. It is used for coagulation studies. This is because anti-EDTA antibodies induce pseudo-thrombocytopaenia. Lithium or sodium salt of heparin at a concentration of 10 - 20 IU/mL is commonly used for chemistry, arterial blood gas analysis and emergency tests. It is not good for blood counts and making PBS because it induces platelets and leucocytes clumping and gives a faint blue colour to the background when films are stained by Romanowsky dyes[1].

Procedure: Staining of slides

The peripheral blood sample is received in a Dipotassium Ethylene Diamine Tetraacetic acid (EDTA) vial. The blood samples are kept at room temperature until the smear making and staining, and for next 24 hours in the refrigerator for delta check. The glass slides are cleaned to ensure they are grease and dirt free. To make a blood film, a small drop of blood is put on the centre line of the slide and about 1 cm from one end. A spreader slide with smooth end is placed at an angle of about 30 degrees to the base of the slide, then moved backwards to touch the drop of blood. The drop should spread quickly along line of contact. Ideally it should cover two-third of slide with oval feathered end. The faster and steeper the smear, the thicker it is. Ideally blood films should be made as soon as blood has been collected. However, in routine practice samples come to laboratories after a variable delay. Smears should be made soon after arrival of samples to prevent changes in morphology of blood films.

Smears should be properly air dried and fixed within 4 hours, ideally within one hour. Smears are fixed with absolute methanol and stained with Romanowsky stain.

Romanowsky stain contains both acidic and basic dyes that gives a differential staining to the different cellular components. Commonly used stains are Leishman stain, MGG (May- Grunwald- Giemsa), and Giemsa stain. We used MGG stain to stain the peripheral blood films.

Fixed films are put into a staining jar containing MGG stain, freshly diluted with equal volume of buffered water. The films are allowed to stain for 15 minutes. Then they are transferred without washing into a jar containing Giemsa stain freshly diluted with 9 volumes of buffered water, pH

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6.8. After 10 - 15 minutes, slides are transferred to a jar containing buffered water, pH 6.8; rapid washing is done 3 - 4 times and then allowed to stand undisturbed for a short time for differentiation to take place. When differentiation is complete, slides are placed upright to become dry[2,3]. The control peripheral smear for comparison and to assess the various artefacts is shown in Fig. 1-A.

Causes of artefacts including *in-vivo* and *ex-vivo* factors with their effects on PBS have been compiled in Table I.

Various artefacts on PBS

Fixation artefact: Fixation artefacts are formed when there is presence of water in methanol used for fixation of the blood film. This is manifested as refractile rings in red cells, interfering in assessment of morphology of red blood cells. To remove this, methanol must be stored in a bottle with tight fitting lid to prevent its exposure to atmosphere. This is very important in humid climates. Even the presence of 1% water in methanol affects the morphology of films. Methylated spirits should not be used for fixation because they contain water (Fig. 1-B)[2,3].

Also, the duration of fixation and dryness of the smear before staining affect the morphology of cells as inadequately fixed and dried smear can lead to poor staining and altered morphology (Fig. 1-C).

Tailing artefacts: Tailing artefacts are produced when excessive pressure is applied while preparing the smear. Due to tailing, there is poor distribution of leucocytes. One should be careful in proper smearing of the smear (Fig. 1-D).

Table I: Causes of artefacts on PBS

***In-vivo* factors**	**Effects on PBS**
Antibodies to blood cells	Agglutination of platelets, erythrocytes and leucocytes
Increased plasma volume	Pseudo-anaemia
Decreased plasma volume	Pseudo-polycythaemia
Treatment-related	Platelets agglutination and Pseudo-Pelger-Huet anomaly
Monoclonal immunoglobulins	Pseudo-thrombocytosis and Pseudo-leucocytosis
***Ex vivo* factors**	**Effects on PBS**
Anticoagulants: EDTACitrate, Oxalate and Heparin	➢ Agglutination of leucocytes; agglutination, satellitism and degranulation of platelets ➢ Agglutination of leucocytes and platelets
Overfilling of tubes	Pseudo-polycythaemia, Pseudo-thrombocytopaenia and Pseudo-leucopaenia
Prolonged storage of specimen	Pseudotoxic changes, pseudo-echinocytosis and platelet degranulation
Temperature of specimen	Agglutination of erythrocytes, leucocytes and platelets

Tailing artefacts: Tailing artefacts are produced when excessive pressure is applied while preparing the smear. Due to tailing, there is poor distribution of leucocytes. One should

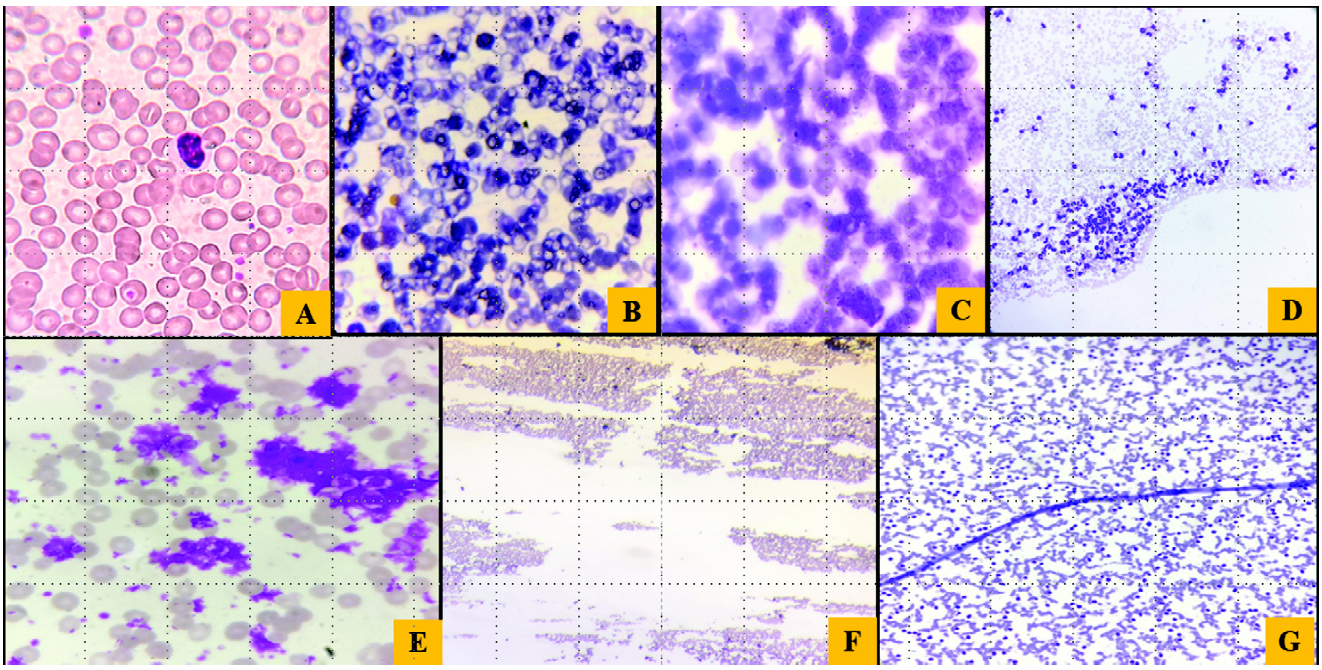

Fig. 1: A) *Control peripheral smear,* ***B)*** *Water artefact,* ***C)*** *Poor Staining,* ***D)*** *Poor spreading leading to Tailing artefact,* ***E)*** *Stain deposits,* ***F)*** *Grease artefact,* ***G)*** *Cotton artefact.*

be careful in proper smearing of the smear (Fig. 1-D).

Stain precipitates: Stain precipitates are formed as a result of evaporation of methanol. They are formed when slides are over-stained and inadequately washed under running water. Washing of slides at proper time, filtration of stain and using fresh working solution are remedies to remove stain deposits (Fig. 1-E).

Grease: Due to presence of grease on slide, there is presence of holes and streaks on smear. Also, RBCs will not take the stain and remain unstained. Properly cleaned slides should be used for making smear (Fig. 1-F).

Cotton: Cleaning of slides with cotton can lead to presence of cotton fibre on the slide. They can mimic microfilariae in stained smears. They can also mimic fungal hyphae. To rectify this, slides should be cleaned with lint cloth (Fig. 1-G)[4].

Storage artefacts: Smears made from stored blood show marked degenerative changes in all cell lines. RBCs show crenation. Cytoplasmic changes observed in WBCs are formation of cytoplasmic vacuoles, blebs and rupture and psuedotoxic changes. Nuclear changes observed are nuclear lobes fusion followed by fragmentation, karyolysis and rupture (Fig. 2). These degenerative changes can be mistaken as toxic changes as a result of infection or inflammatory condition. Absence of dark staining granules help in correct interpretation of these cases.

Platelets can become swollen (Fig. 3-A). If these swollen platelets burst into fragments, it can result in psuedo-thrombocytosis[1].

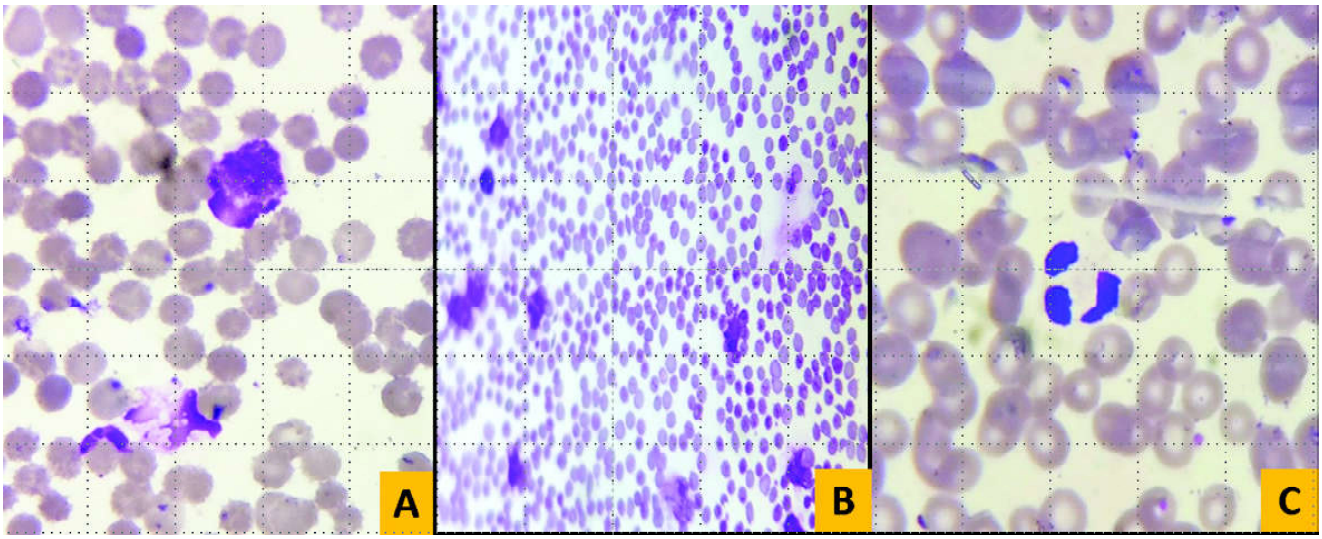

Fig. 2: A) *Cytoplasmic blebs and rupture,* ***B)*** *Cytoplasmic fragments,* ***C)*** *Nuclear lobes fragmentation.*

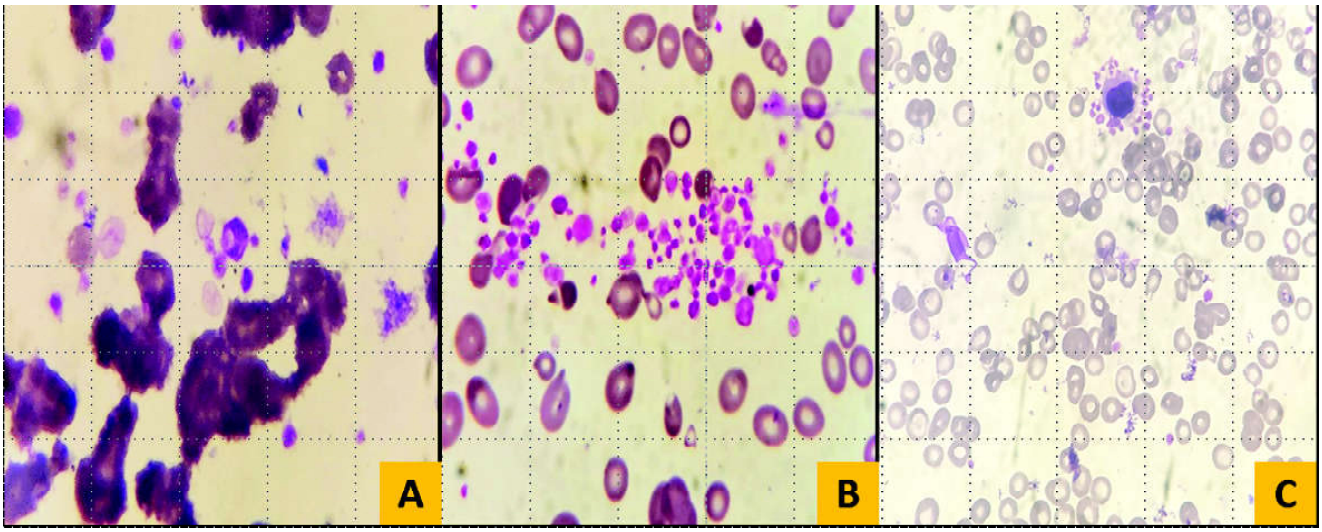

Fig. 3: A) *Platelet swelling,* ***B)*** *Platelet clumps,* ***C)*** *Platelet satellitism.*

Psuedo-thrombocytopaenia and platelets agglutination: It is the commonest quantitative abnormality noted secondary to platelets agglutination. This phenomenon is noted more commonly in severely ill patients, e.g., malignancy, chronic liver disease, autoimmune diseases, infection and cardiovascular diseases. However, it can be seen in patients with no underlying disease. It is caused by an autoantibody against glycoprotein IIb/IIIa present on the cell membrane of platelets. In the presence of EDTA, the epitope of glycoprotein IIb is revealed which normally remains hidden in the glycoprotein IIb/IIIa. Binding of the autoantibody to this epitope, results in aggregation[5] (Fig. 3-B).

Pseudo-thrombocytosis: Falsely elevated platelet count can be seen when the blood contains particulate matter with similar size and scatter properties as platelets; e.g., bacteria, cryoproteins, cytoplasmic fragments. Neutrophilic cytoplasmic debris ranging in size from 2 to 5 µm in diameter is noted in peripheral blood samples from patients with hairy cell leukaemia/ acute myeloid leukaemia or lymphoma or in patients with severe infections. These fragments may also get counted as platelets by automated analysers. As platelet transfusions are frequently required in leukaemic patients with low platelet counts, this masked thrombocytopaenia can delay appropriate transfusion. Therefore, all leukaemic patients should be reviewed with low platelets counts. Cryoglobulins, microcytic red cells, bacteria, red cell inclusions like Pappenheimer bodies, red cell fragments or microspherocytes can also result in falsely elevated platelet count[5].

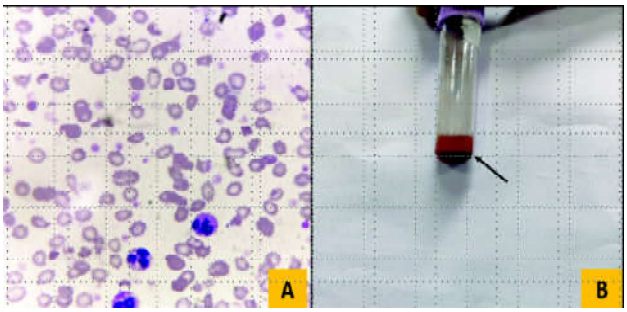

Fig. 4: *Pseudoechinocytes due to low quantity of sample.*

Platelet satellitism

Platelet satellitism is a rare cause of pseudo-thrombocytopaenia due to the adhesion of platelets to the surfaces of circulating leukocytes. It occurs when circulating auto-antibodies bind to cryptic antigens exposed because of the calcium-chelating activity of EDTA. The antibody platelet antigen (antiglycoprotein IIb/IIIa) binds to the Fca receptor (CD16) on the cell surface (Fig. 3-C)[6].

Pseudo-echinocytes

Echinocytes are RBCs with numerous fine, uniform spicules along the periphery. These are formed due to exposure of red cells to high pH while exposure to an acidic pH produce stomatocytes. Exposure of blood to glass (tubes or slides) may cause change in pH because of the release of alkali

Table II: Types of artefacts and their remedial measures.

Types of artefacts	Cases (n = 50) (%)	Causes	Remedial measures
Fixation artefact	6 (12%)	Presence of water in methanol	Usage of tight-fitting lid bottle/ Quality control of Methanol
Tailing	12 (24%)	Excessive pressure during smearing Roughened edges of spreader Dust-ridden slides	Spreader slide with smooth end should be used with appropriate pressure
Stain deposits	10 (20%)	Overstaining and inadequate washing	Usage of fresh working solution, filtration of stain and proper washing
Grease artefact	4 (8%)	Due to grease on slide and improper cleaning	Properly cleaned slides should be used
Cotton	2 (4%)	Cleaning of slides with cotton	Lint cloth should be used for cleaning
Storage artefact: Cytoplasmic blebs and rupture Cytoplasmic fragments Nuclear lobes fragmentation Platelet swelling and fragmentation	12 (24%) 2 (4%) 2 (4%) 5 (10%) 3 (6%)	Prolonged storage	Avoid prolonged storage Smear should be made within 4 hours (ideally 1 hour) of collection of blood
Platelet clumps	5 (10%)	EDTA induced agglutination Certain *in-vivo* factors	Collection in Citrate vial Fresh Finger-prick smears
Platelet satellitism	2 (4%)	Ca-chelating activity of EDTA Infections	Repeat sample in Citrate vial Treat the cause
Pseudo-echinocytes	6 (12%)	Low quantity of sample taken	Optimum blood sample should be taken in EDTA vial as per the insert
Increased hematocrit	3 (6%)	Overfilling of tube	Adequate quantity of blood sample should be taken

from the glass. It is reversible by correction of the pH. Pseudo-echinocytosis can also sometimes be seen on blood films, possibly as a result of flattening of red cells on the glass slide depending on the type of glass used. Other common cause of pseudo-echinocytes is low quantity of blood samples in vials (Fig. 4)[5].

Pseudo-polycythaemia

Pseudo-polycythaemia is due to an increased haematocrit caused by reduction in plasma volume rather than a true increase in red blood cells. Causes are physical stress, severe burns, excess alcohol consumption, diarrhoea or vomiting, insufficient water intake, fluid loss or diuretic therapy. Gaisbock's disease is also a cause of Pseudo-polycythaemia. It is associated with obesity, a history of smoking, hypertension, elevated erythrocyte count, elevated haemoglobin with reduced plasma volume. Overfilling of vacutainers may result in artifactually high hematocrit[5].

In the present study, 50 out of 500 cases showed artefacts. The most commonly encountered artefacts were storage artefacts and tailing artefact, each seen in 24% of cases followed by stain deposits in 20% of cases. Also, in 12 cases (24%), there was overlapping of different artefacts noted. The list of various measures helpful in prevention of these artefacts has been compiled in Table II.

Discussion

The word artefact is derived from a latin word *"arsfactum"* means art (*"ars"*) plus made (*"factum"*). Artefacts are artificially produced feature, introduced in the specimen under study. PBS examination is most useful and crucial for evaluation of anaemia, thrombocytopaenia, morphological assessment of cells such as blasts in leukaemia, and malarial parasites, etc. Poor staining of films hamper the morphology of blood cells. Each laboratory needs to follow standard operating procedure for blood film staining so that artefacts are kept to a minimum. Suboptimal staining leads to loss of RBC morphology and polychromasia.

Artefacts on EDTA are extensively studied as it is the anticoagulant of choice and cellular components and morphology of cells are preserved with EDTA if smears are made within an hour of collection of blood. After 6 hours, marked EDTA changes were noted on PBS, comparable with the study done by Narsimha *et al*[1]. These can range from crenation of RBCs, platelets swelling and nuclear fragmentation, cytoplasmic rupture of WBCs. In the present study as well, the most common artefacts noted were due to prolonged storage of blood due to delay in transport and processing of the blood sample (Table II).

Stain deposits are formed due to inadequate washing and improper staining. Due to stain deposits, it is difficult to diagnose malarial parasite and can even lead to misinterpretation. Cotton fibres can mimic fungal or filarial parasite to an untrained laboratory personnel.

Conclusion

Artefacts can be a serious handicap in day-to-day laboratory practices, and if not identified and rectified, can lead to misinterpretation and wastage of resources.

References

1. Narasimha A, Kumar H, Prasad CS. Anticoagulant induced artefacts in peripheral blood smears. *Ind J Hematol Blood* Transfus 2008; 24: 43-8.
2. Adewoyin AS, Nwogoh B. Peripheral blood film-a review. *Ann Ib Postgrad Med* 2014; 12: 71-9.
3. Sareen R, Kapil M, Gupta GN. Incubation and its effect on Leishman stain. *J Lab Physicians* 2018; 10: 357-61.
4. Dalal BI, Brigden ML. Artifacts that may be present on a blood film. *Clin Lab Med* 2002; 22: 81-100.
5. Bhatia M, Mishra B, Thakur A *et al*. Microscopic artefacts in clinical practice – a diagnostic dilemma. *J Clini Diagnosti Res* 2018; 12: DR01-4.
6. Gioia AL, Fiorini F, Fiorini M. Platelet Satellitism: Just a Laboratory Curiosity? A Case Report of Platelet Satellitism with Multilineage Involvement. *Ann Lab Med* 2022; 42: 606-8.