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An Overview Proper Sampling for Peripheral Blood Smear for Accurate Diagnosis

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Abstract

The peripheral blood smear (PBS) is a laboratory procedure that examines the cytology of peripheral blood cells spread on a slide. Despite its simplicity, PBF (Peripheral Blood Film) is crucial in the assessment and identification of several clinical disorders. This essay emphasizes the fundamental principles and creative aspects underlying the PBF. The text discusses the laboratory applications, clinical indications, and interpretations of many clinical disorders. Although there have been advancements in automating haematology and using molecular approaches, the peripheral blood film (PBF) continues to be a crucial diagnostic tool for haematologists. The haemato-pathologist should ensure that a high-quality smear is obtained, followed by a comprehensive examination and accurate interpretation that aligns with the patient's clinical condition. Clinicians should be well-informed about the clinical usefulness and correct implementation of the reports in patient treatment. A manual inspection of the peripheral blood smear (PBS) is now conducted on a subset of samples that are submitted for automated full cell count.

Keywords*:* haematology, peripheral blood smear (PBS), peripheral blood film (PBF).

Introduction

Diagnostic formulations in patient care rely on three essential components: clinical history, physical examination, and laboratory studies. According to the literature, laboratory medicine has a role in supporting up to 70% of clinical choices and diagnoses [1]. The peripheral blood film (PBF), also known as the peripheral blood smear (PBS), is a fundamental and highly informative hematological technique that clinicians use to screen, diagnose, and monitor illness progression and therapy response. A comprehensive comprehension of peripheral blood analysis is crucial for a thriving clinical practice [1].

The initiation of a peripheral blood film (PBF) is typically requested by the attending clinician based on a clinical suspicion, or less commonly, it may be initiated by the laboratory [2]. The laboratory may do a peripheral blood film if abnormal results are detected during an automated count or if a patient's clinical information suggests that a peripheral blood film may help confirm their diagnosis. The latter is determined by specific laboratory protocols or regional regulatory standards [3].

Peripheral blood film analysis is commonly used to investigate various clinical conditions. These include unexplained low levels of blood cells such as anemia, leukopenia, or thrombocytopenia. It is also used to investigate unexplained high levels of blood cells such as leukocytosis, lymphocytosis, or monocytosis. Additionally, it is used to assess conditions like jaundice or hemolysis without a clear cause, and to identify features of congenital hemolytic anemias such as splenomegaly, jaundice, or bone pains. Peripheral blood film analysis is also helpful in diagnosing suspected chronic or acute myeloproliferative diseases like chronic myeloid leukemia, as well as organ failure such as renal or liver disease. It can detect features of hyperviscosity syndrome seen in conditions like paraproteinemias, leukaemic hyperleucocytosis, or polycythemia. Furthermore, it is used in cases of severe bacterial sepsis and parasitic infections, malignancies with potential bone marrow involvement, suspected nutritional anemia, suspected chronic lymphoproliferative diseases like chronic lymphocytic leukemia, and lymphoma with leukaemic spills. Lastly, it is used to evaluate the response to therapy in blood disorders, among other applications [4,5].

Discussion:

The analysis of the peripheral blood smear (PBS) was once an essential component of a comprehensive blood count (CBC). Nevertheless, due to the availability of advanced automated blood cell analysers that can rapidly and precisely detect cellular sizes and differential white blood cell counts, and the continuous rise in workload, the traditional practice of manually examining peripheral blood smears is no longer common in modern laboratories [6]. Alternatively, the initiation of PBS examination might be prompted by either the laboratory responsible for processing the blood sample or the clinician responsible for the patient's care. After meeting a specific criterion, the PBS is initially scrutinized by a laboratory technician. Based on the detected irregularities, the peripheral blood smear (PBS) may or may not be referred to a hematologist for additional analysis at a secondary stage.

Initially, the laboratory-initiated slide review is typically prompted by aberrant blood cell counts or morphologic changes flagged by the analyzer. The International Society for Laboratory Hematology (ISLH) has suggested a set of guidelines indicating when it is necessary to perform a manual inspection of peripheral blood smear (PBS). This is recommended when specific numerical indices deviate significantly from the normal range or when abnormal flags are raised. These rules can be adopted by individual laboratories and adjusted to accommodate the specific traits of local patients [8]. The slide review's quality is contingent upon the expertise of the laboratory staff conducting it, and the potential influence of the microscopic examination on clinical care remains unexplored. Furthermore, it is uncertain if blood smears that do not meet the criteria for manual inspection can nevertheless offer valuable indications for clinical diagnosis.

Clinicians have the option to escalate the case to the next stage by engaging a haematologist for a specialized assessment or by referring the patient to a haematologist for additional evaluation. The peripheral blood smear (PBS) is manually evaluated by the haematologist using both clinical and analytical criteria [6,9].

In order to guarantee precise and dependable outcomes, it is necessary to manage pre-analytical variables that have the potential to impact the quality of the picture. These factors encompass patient readiness and agreement, methodology for collecting blood samples, transportation to the laboratory, and

preservation of the samples. Due to the invasive nature of blood collection, it is essential to provide the patient/client with counseling regarding the procedure. Typically, blood is collected from veins in the outer parts of the body and preserved in a container with anticoagulant. The blood to anticoagulant ratio must be appropriately balanced. Occasionally, it is possible to collect capillary blood through a small puncture in the finger. It is important to use caution in order to minimize tissue harm. The presence of surplus tissue fluid disrupts the dispersion of the cellular components of blood. Ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant. It is imperative to promptly dispatch samples to the laboratory. For optimal analysis, it is recommended to test samples within a time frame of 2 hours from the moment of blood collection. Failure to promptly prepare a blood smear can lead to the deterioration of the cellular components of blood and potentially cause pseudo-thrombocytopenia, which is a falsely low platelet count caused by the creation of platelet aggregates [2,10].

Slide preparation is carried out by skilled individuals, ideally a medical laboratory technologist, who can guarantee the production of high-quality slides suitable for microscopic examination. Additionally, laboratory assistants can receive training in the skill of slide preparation.

To create a peripheral blood film (PBF) smear, one needs slides, a pipette or capillary tube, and a blood spreader. The 'push' or wedge method, often known as the cover-slip method, is employed [5]. The latter is less frequently utilized. Seven The wedge method involves placing a drop of thoroughly mixed blood (after at least 10 gentle inversions) on the base of a slide, near one end (about 1 cm from the edge), using a pipette or capillary tube. A spreader slide, featuring chipped edges, is positioned in front of the blood on the base slide. It is then dragged rearward until it comes into contact with the blood droplet, causing the blood to spread across the entire width of the base slide. The spreader slide should possess a sleek termination to avert any irregularities in the tail end of the smear. Next, a smear is created by positioning the spreader at an angle of approximately 30 to 45 degrees to the blood [11]. It is important to avoid exerting excessive force on the spreader slide during the spreading process. This can result in the occurrence of slide fractures and laboratory mishaps. Smear artifacts can arise due to the presence of unclean slides, fat droplets, or slides of low quality. It is imperative to adhere to laboratory safety measures when handling any clinical specimen. Each blood samples must be regarded as potentially high risk. While intercalating chemicals, which are routinely used stains, are effective in destroying microorganisms, they do not provide any protection against HIV and HBV. The smear should span two-thirds of the length of the base slide and should have a feathered oval end. Typically, a smear that is faster and steeper will be thicker [12]. For example, a more inclined and rapid spreading technique can be employed for weak or pale samples. The smear is adequately desiccated. To accurately measure hypochromia, it is important to avoid high humidity during the process of generating a smear. High humidity can lead to inadequate drying and result in an artificial acute refractile border that demarcates the area of central pallor. Next, proceed to annotate the slide using a pencil or crayon on either the frosted end or the head end. To fix the dried smear, it is treated with either 100% methanol or ethyl alcohol and then stained using a Rowmanosky stain. It is recommended to fix a thoroughly air dried smear within 4 hours of preparation, but it is preferable to do it within one hour[12]. Optimal fixation duration ranges from 10 to 20 minutes. Incorrect fixation leads to the formation of artifactual burr cells, which are crenated red blood cells with refractile edges.

Romanosky stains consist of a combination of acidic dye and basic dyes, which are used to selectively stain certain cellular components [12]. The Leishman stain, commonly employed in our surroundings, consists of polychrome methylene blue as its basic component and eosin as its acidic component. Alternatively, the May-Grunwald Giemsa or Wright-Giemsa stain might be employed [11]. The staining intensity is directly influenced by the duration

of contact time and the concentration of the stain. It is crucial to ascertain the appropriate duration of contact with each new batch of stain produced or obtained.

The smear is immersed in a stain solution for approximately 5-10 minutes, then diluted twice with buffered water and left for an additional 5-10 minutes to allow the cells to absorb the stain. Subsequently, the slide is thoroughly flushed with flowing water. Efforts should be exerted to cleanse the lower surface of the slide using cotton wool in order to eliminate any surplus stain. Ultimately, the slide is positioned on a rack with the end containing feathers inclined upwards in order to facilitate drying. Stain artifacts, such as debris and precipitates, can result from excessive staining (prolonged contact with the stain) and insufficient rinsing with running water. At times, larger cells like monocytes may be displaced towards the outside edge and delicate part of the film, and it is important to take note of this when analyzing the film. Occasionally, smears are created from the buffy layer, which is the white area between the plasma and red cell layer and has a high concentration of white cells and platelets. This is done after a vigorous centrifugation process, particularly in specimens with low levels of neutrophils [12,13].

Preparing slides can be a labor-intensive task, especially when dealing with a high number of specimens. Nevertheless, there are automated slide stainers, such as the dipping-style slide stainer, that are currently accessible [11]. It is recommended to prepare at least two slides for each specimen and evaluate the slide's quality promptly. Slides of substandard quality should be discarded and replaced with new ones. Creating a fresh slide is a more secure option compared to interpreting a slide of low quality. The quality of the film generated is contingent upon the meticulous application of the smearing technique and the high standard of the staining procedure [14]. To obtain high-quality differential staining, it is necessary to ensure that the stain is in contact for an appropriate duration to prevent over or under staining.

Thresholding is a basic method for dividing an image into foreground and background regions based on variations in intensities or colors. Transform methods are employed to discern the characteristics in alternative domains. Prasad et al. [15] created a decision support system that utilizes color image analysis to identify malaria parasites in thin PBS images. Morphological operations were employed for the detection of red blood cells (RBCs), while color image processing techniques were utilized to isolate the region of interest. This technique was able to identify around 96% of the parasites in 200 Giemsa stained photos with a magnification of 100X, using consistent staining and lighting circumstances [16]. Bhavnani et al. [17] introduced a technique for segmenting and quantifying red blood cells (RBCs) and white blood cells (WBCs) with Otsu thresholding and morphological operations. The WBC counting was conducted by enumerating the number of linked components and achieved an average accuracy of 94.25%. The counting of red blood cells (RBCs) was conducted using Watershed segmentation and Circular Hough Transform (CHT), resulting in accuracies of 92.67% and 91.07% respectively. The primary goal of watershed segmentation is to identify the watershed lines that create a continuous course, resulting in uninterrupted boundaries between the regions. It isolates things that are almost identical in shape and size from the background. The Circular Hough Transform (CHT) is a computational technique used to identify and extract circular objects present in an image. By exploring a 3D Hough space, the transform can accurately determine the radius and centroid of each circular object present in an image. In their study, Maji et al. [18] introduced a method for counting red blood cells (RBCs) utilizing Otsu thresholding and mathematical morphology. They also divided the cells into four categories: circular cells, non-circular cells, overlapped cells, and artifacts. The mean accuracy achieved was 96.9% for circular cells and 97.1% for non-circular cells, based on a sample of 146 pictures. Ruberto et al. [19] introduced a technique for detecting malarial parasite-infected blood cells. They utilized the HSV component to measure color similarity and employed the Watershed algorithm. The method was applied to 12 Giemsa stained

images obtained at various magnifications, which exhibited differences in stain and lighting circumstances. Ruberto et al. [20] introduced a technique that utilizes edge boxes for recognizing and measuring red blood cells (RBCs) by region proposal. They achieved an accuracy between 96% and 98% for 180 images from the ALL-IDB dataset. The study groups demonstrated the application of the identical methodology to another malarial parasite, specifically the MP-IDB database, which consisted of 100 photos. The acquired accuracy fell within the range of 89% to 99%. Sharif et al. [21] conducted an initial investigation on a method for segmenting red blood cells (RBCs) using a combination of masking and watershed algorithm. The study involved analyzing 20 images with a magnification of 40X. Nevertheless, this approach need enhancement in segmenting big overlaps. Biswas et al. [22] introduced a technique for segmenting blood cells using the Watershed Transform (WT) and Sobel filter in the spatial frequency domain. They achieved an accuracy of 93% for 30 pictures, as determined by a structural similarity index matrix. Habibzadeh et al. [23] introduced a technique for segmenting white blood cells (WBC) and red blood cells (RBC) by utilizing the YIQ color space and wavelet transform (WT). They reached a 90% accuracy in RBC segmentation by analyzing 10 photos. Nevertheless, they indicated that they would tackle significant fluctuations in blood cells and subpar image quality in their forthcoming research. Cruz et al. [24] introduced a method for counting red blood cells (RBCs) using blob analysis, which relied on the HSV component and WT. They achieved an average accuracy of 95.6% while analyzing 10 blood samples under 40X and 100X magnifications. Several research groups have documented the utilization of Hough Transform (HT) for the segmentation of red blood cells (RBCs) from pictures obtained using PBS. The accuracy was claimed to be between 94% and 96%. Mahamood et al. [25,26] suggested a method for segmenting blood cells based on color in the CIELAB color space and utilized the Circular Hough Transform (CHT) for extracting the cells. The experiment was conducted using the ALL-IDB dataset, which consisted of 108 Wright stain images with magnifications ranging from 300X to 500X. The average accuracy achieved for white blood cells (WBCs) was 81%, while for red blood cells (RBCs) it was 64%. In their study, Sarrafzadeh et al. [27] introduced a technique that utilizes the circlet transform to accurately quantify red blood cells (RBCs). They achieved a minimal error rate when analyzing 100 photos at a magnification of 100X.

Methods for segmentation based on edges

Das et al. [28] introduced a technique that utilizes edge detection algorithms, specifically Canny, Laplacian of Gaussian (LOG), and Sobel, to distinguish red blood cells (RBCs) and other types of white blood cells (WBCs). They achieved an accuracy of 85% for a set of 20 photos. Poomcokrak et al. [29] introduced a method for counting red blood cells (RBCs) using the Canny edge algorithm. The Multilayer Perceptron (MLP) achieved an accuracy of 74% when applied to a dataset consisting of 59 red blood cells (RBCs) and 59 non-RBCs. MLP, short for Multilayer Perceptron, is a straightforward type of neural network that use the backpropagation technique to train its neurons. The system is composed of an input layer, a variable number of hidden layers, and an output layer. The concealed layer processes the input data and delivers it to the output layer. MLPs are commonly utilized for solving supervised learning challenges. Backpropagation is employed to optimize weights and biases by minimizing the error. Hafiz et al. [30] introduced an algorithm for segmenting red blood cells (RBC) by employing boundary-based thresholding and Canny edge detection techniques. They achieved an average accuracy of 87.9% when evaluating the program on five images from the Broad Bioimage Benchmark Collection (BBBC) dataset.

Segmentation methods that rely on clustering Abbas et al. [31] introduced a technique for dividing blood cells into segments by utilizing the YCbCr color space and the K-means clustering algorithm on a dataset of 90 Giemsa stained blood smear images. The method

allowed for the easy identification of blood cells based on the distinct hue of each component. The authors Wei et al. [32] introduced a technique for identifying and quantifying overlapping red blood cells (RBCs) in microscopic pictures of blood smears. The H and S components were utilized to distinguish between white blood cells (WBCs) and segmented red blood cells (RBCs). The H and S components have a strong correlation with human perception of color. Hue is the perceptual quality associated with the wavelength of light. The letter "S" denotes the degree of color purity. The segmentation process involved the application of Watershed and K- means clustering methods. A precision of 92.9% was achieved for a set of 100 photographs stained with Wright-Giemsa. Nevertheless, the authors of this research proposed refining the segmentation algorithm to enhance its resilience. Acharya et al. [10] introduced a technique that utilizes K-medoids to distinguish red blood cells (RBCs) from other constituents of blood. They achieved a remarkable accuracy of 98% when using this method to 1000 Wright stained pictures. In their study, Savkare et al. [33] introduced a technique for blood cell segmentation utilizing the K-means clustering algorithm and wavelet transform (WT). They achieved an impressive accuracy of 95.5% when applied to 78 microscopic pictures stained with Giemsa. Nevertheless, they indicated that the effectiveness of this approach is compromised when cells are inadequately labeled and exhibit poor contrast. Ruberto et al. [20] introduced a method for selecting the best threshold for blood cell segmentation using fuzzy sets. The local threshold was determined by analyzing the histogram, resulting in an average accuracy of 98% and a computation time of less than one second.

Conclusion:

While manual microscopic examination is considered the most reliable method for analyzing peripheral blood smears (PBS), the use of an automated decision system is crucial for achieving rapid and precise diagnosis, as it helps to address the constraints associated with microscopic analysis. An automated system plays a crucial part in the study of PBS. Nevertheless, the research highlights the necessity for a comprehensive PBS database that is accessible to the public, contains accurate annotations, and is supported by a reliable mechanism to aid doctors in disease identification. To make a diagnosis from a peripheral blood smear (PBS), one needs a comprehensive clinical database on the different potential cytological abnormalities, their causes, and extensive laboratory expertise. The findings obtained from a peripheral blood smear (PBS) can provide accurate and definitive information about a disease, such as the diagnosis of sickle cell disease or chronic myeloid leukemia through examination of blood film. In other instances, the evidence is just suggestive and necessitates additional laboratory examinations or more sophisticated investigations, such as cytochemistry, flow cytometry, cytogenetics, or molecular methods, particularly when dealing with malignancies. Although there have been significant advancements in genetic and molecular tools for diagnosing different diseases, the analysis of blood smear morphology continues to be an essential tool in the field of hematology. It continues to be a crucial puzzle in uncovering the underlying causes of puzzling symptoms and indications in primary and secondary blood disorders.

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