Review Article

# ABC of the Peripheral Smear

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**Peripheral blood smear examination is an inexpensive and powerful diagnostic tool to diagnose both hematological and non-hematological disorders. With the advancing newer technology, it is becoming like a "lost art". The peripheral smear often called as a window into the functional status of the bone marrow as it can provides rapid, reliable information about a variety of hematologic disorders. Systematically and thorough review of the smear is an important adjunct to other clinical data and in some cases, often sufficient to establish a diagnosis like hemoparasite infestation. Newly developed automated cell counters are increasingly providing sophisticated data however, only an experienced reviewer can weigh the relative significance of the findings and assess their importance within the context of other clinical data. Even with the availability of cumbersome data by automatic cell counters, there are a number of settings in which interpretation of the peripheral smear is especially important to guide for further management. This concise review provides a systematic approach while evaluating a peripheral blood smear, the findings and its clinical significance in diagnosing variety of the hematological disorders.**

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***Key words*** **: Peripheral blood smear, Preparation, Examination, Interpretation, Blood cells morphology, Reporting.**

A complete blood count (CBC) is the most common and foremost investigation used by physicians in the day to day health care practice for detection of disease and its monitoring. Recent advances in the technology of the automated hematological analyzers had significantly improved the quality by improving accuracy, precision and time per analysis. Although manual intervention has been reduced significantly nowadays, evaluation of the results by qualified clinical laboratory professionals is still essential.[1] The examination of a well prepared and stained peripheral smear is like a "*lost art*" in the modern era. Much valuable information can be gained from examining the blood smear than from any single haematologic procedure.

**Preparation of a peripheral smear[2]:** A well spread peripheral smear can be made using the wedge technique either manually or automated technique. A small drop of non-clotted blood (10µl- capillary or EDTA blood sample) is put on a clean glass slide (dust, dirt, grease, and fingerprint-free) approximately 1.5 cm from one edge. By using another glass slide edge as spreader at an angle of 30-45°, the blood should be dispersed along the length of the slide with the aim to prepare monolayer to disperse cells uniformly. An ideal smear should cover 2/3rd to 3/4th length of slide with free lateral margins, free from holes, streaks, or irregularities and preferably tongue-shaped with feathered edge.

**Staining of a peripheral smear[2]:** A well spread peripheral smearis air-dried and then fixed in methanol to later stained using prototypical stain, Romanowsky stains such as Wright's, Leishman or Giemsa stain as per standard technique. After staining well prepared well-stained slide monolayer is viewed under a microscope at different magnification for a complete

***Editor's Comment :***

- **The peripheral smear is a window into the functional status of the bone marrow**
- **A well prepared and well-stained smear examination under light microscope is particularly important in the evaluation of cytopenia or flagging in abnormalities in automatic hematological analyzer**
- **The peripheral smear should be evaluated in an optimal area to look for number, size, shape, and presence of any inclusions in red cells, white cells, and additional features including the presence of any abnormal cells including blasts or abnormal granulation or hemoparasite**
- **A well-framed format of the peripheral smear report contains the complete patient details, all the relevant information regarding the morphologic characteristic of each series of blood cells and authorized signature.**

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analysis of individual blood cell morphologic characteristics.

**Evaluation of a peripheral smear :** Firstly, an optimal area for the examination of peripheral smear is selected using 4X magnification of the microscope. The optimal area in peripheral smear for evaluation is at the junction of the body and tail of the smear where RBCs just touch each other without significant overlapping. At the next low magnification (10X), RBCs agglutination or rouleaux formation or platelet aggregation and parasites like microfilaria can be detected. At high magnification (40X) total, differential leukocyte count and platelet count can be roughly estimated and also can be correlated with the results of automatic hematological analyzers, if available. A detailed evaluation of blood cell morphology including size, shape, nuclear, cytoplasm or granules characteristics or parasite like malaria parasite detection can be done under oil immersion (100X).

**Role of examination of a peripheral smear:** Peripheral smear evaluation can provide valuable information regarding the diagnosis of various diseases or monitoring of the response of therapy. Special consideration on examination of blood smear by well-trained laboratory professionals should be taken in case of flagging of abnormalities on the automatic hematological analyzer. Systematic evaluation with special attention on the morphology of RBCs can diagnose the cause of anemia while the number and morphology of platelets are essential while evaluating thrombocytopenia. The presence of abnormal cells including nucleated RBCs, blasts or parasites including species identification (in case of malarial parasite) requires evaluation of a peripheral smear. Additional information like variation in shape, size or inclusions in RBCs with special attention to abnormal shaped RBC like sickle cell or spherocytes or schistocytes can guide for the requirement of further procedure/investigation. Abnormal nuclear lobulation or granularity like Auer rods in leucocytes might help in the diagnosis of various syndromes or sepsis or malignancy like Promyelocytic Leukemia or Clonal Hematopoiesis including Myelodysplastic Syndrome. The presence of abnormal cells including myeloblast/ lymphoblast or abnormal lymphoid cells (hairy cell/ Sezary cell) indicates the underlying malignant hematologic disorder (Fig 1).[1-4]

**Interpretation and clinical significance of peripheral smear findings :** Accurate interference of morphologic findings requires the selection of an optimal area by scanning the entire slide. Below mentioned are various morphologic findings of blood cells including red blood cell, white blood cell, and platelets which have clinical significance and need thorough evaluation, even further investigations or procedures like bone marrow evaluation to reach the diagnosis.

**(1) Abnormal cell distribution :**

***(a) Rouleaux Formation :*** The appearance of red cells arranged as a stack of coins in an otherwise optimal area, a phenomenon called rouleaux formation, most commonly seen in conditions with increased total proteins like multiple myeloma.[5]

***(b) Irregular clumps of red cells :*** May signify the presence of certain infections like Hepatitis C or cold agglutinins disease.[5]

***(c) Broken cells or smudge cells :*** Presence of smudge cells signify artifact while preparing the smear or presence of fragile cells like lymphocytes in the case of CLL or even immature lymphoid cells like lymphoblasts.[4]

***(d) Abnormal clumps of platelets :*** Signify normal or increased platelet number. Highly valuable to rule out pseudo-thrombocytopenia.[6]

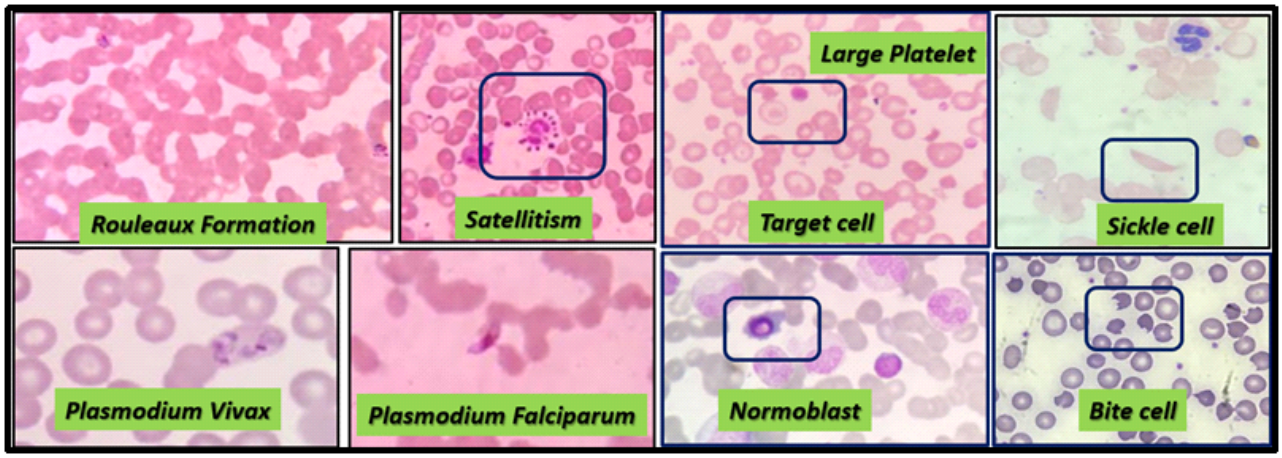


Fig 1 — Morphologic characteristic of RBCs and Platelets

(**2) Red blood cells:** A mature red cell is anucleate with the size of approximate of the nucleus of small mature lymphocyte (7-8µm), biconcave shaped with nearly 1/3rd central pallor and lacks any intracytoplasmic inclusions. Abnormal variations in shape, size or presence of cytoplasmic inclusion bodies signify associated clinical abnormalities.[2] Anisocytosis refers to variation in size and poikilocytosis refers to variation in shape of red cells. Various pathological conditions are associated with the presence of aniso-poikilocytosis of red cells and permutation combinations of other associated morphological findings can be helpful in diagnosing the cause of anemia.[7]

***(a) Anisocytosis :*** Mean corpuscular volume provided by an automatic hematologic analyzer confirms the actual size of red cells (normal MCV- 81-99fl). However, MCV can be misleading in case of variations of size of red cells as in case of dimorphic anemia. A high red cell distribution width (RDW) is suggestive of presence of red cells with different sizes but a definitive appreciation of variation in size requires peripheral smear assessment. Microcytosis (<80fl) and macrocytosis (>100fl) are associated with various hematological and non-hematological conditions as depicted in Table 1.[2]

***(b) Poikilocytosis :*** Various hematological and some non-hematological disorders are associated with the presence of distinctive red cells shape. Table 2 gives a brief review of various shapes and their clinical significance.[2]

The poikilocytes including acanthocytes, codocytes, echinocytes, stomatocytes, drepanocytes, and degmacytes are caused by membrane abnormalities while dacrocytes, spherocytes, schistocytes are caused by trauma to red cells in the vasculature.[6]

***(c) Intracytoplasmic inclusion bodies:*** Inclusion bodies are aggregates of the stainable material. A normal red cell contains no inclusion bodies in the cytoplasm. Table 3 provides various cytoplasmic inclusions found in red cells and their significance.[2,6]

Table1 — *RBCs anisocytosis and associated clinical conditions*

| RBCs Size | Clinical Significance |
|---|---|
| Normocytic | Acute haemorrhage, malignancy associated anemia, Enzyme deficiency |
| Microcytes | Iron deficiency, Thalassemia, Anemia of chronic disease, Lead poisoning, Sideroblastic anemia |
| Macrocytes | Megaloblastic anemia, Liver disease, Drug-induced, Alcohol, Myelodysplastic syndrome, Aplastic anemia |
| Both Microcytes and Macrocytes | Dimorphic anemia |

Table 2 — *RBCs poikilocytosis and associated clinical conditions*

| RBCs Shape | Clinical Significance |
|---|---|
| Schistocytes (Fragmented RBCs) | Microangiopathic hemolytic anemia including TTP, DIC, MAHA |
| Drepanocytes (Sickle cells) | Sickle cell anemia |
| Codocytes (Target cells) | Hemoglobinopathies, Thalassemia, Iron deficiency, Liver disease, Postsplenectomy |
| Spherocytes | Hereditary spherocytosis, Autoimmune hemolytic anemia, ABO incompatibility |
| Acanthocytes (Spur cells) | Neuroacanthocytosis, Abetalipoproteinemia, Liver disease, Post splenectomy |
| Echinocytes (Burr cells) | Artefacts, Uremia, Pyruvate kinase deficiency |
| Stomatocytes (Mouth Cells) | Artifacts, Hereditary Stomatocytosis, Alcoholism, Myelodysplastic syndrome |
| Degmacytes (Bite cells) | G6 PD deficiency |
| Dacrocytes (Teardrop cells) | Myelofibrosis, Marrow infiltration, Osteopetrosis |

Table 3 — *RBCs inclusions and associated clinical conditions*

| RBCs Inclusion | Inclusion | Clinical Significance |
|---|---|---|
| Howell Jolly bodies | DNA | Asplenism, Severe hemolytic anemia |
| Heinz bodies | Denatured hemoglobin | Unstable hemoglobin, G6PD deficiency, Oxidant-Drug injury |
| Pappenheimer bodies | Iron deposits | Thalassemia, Sideroblastic anemia, Post splenectomy |
| Hb H inclusion | Globin chains | Hemoglobin H disease |
| Basophilic stippling | Ribosomes | Lead poisoning, Thalassemia, Megaloblastic anemia, Myelodysplastic syndrome |
| Cabot rings | Microtubules remnants | Megaloblastic anemia, Myelodysplastic syndrome |
| Hemoglobin crystals | Hemoglobin | Hb C disease or Hb SC disease |
| Parasite | Hemoparasite | Malaria and Babesiosis |
| Red cell Ghosts | Devoid of hemoglobin | Intravascular hemolysis like fulminant falciparum or Clostridium perfringens infection |

***(d) Polychromatophils :*** Young red cells released from marrow called reticulocytes contain ribosomes and hence give bluish tin to cytoplasm which when stained with Romanowsky's stain appears as bluish-red cytoplasm and hence referred to as Polychromatophils. An increased polychromatophils in smear signify the response of marrow to hemolysis or hematinics.

***(e) Nucleated red blood cells :*** Normal red cells are anucleated. Normoblasts (nucleated red blood

cells) are normally not found in the peripheral smear. However, severe hemolysis, profound stress erythropoiesis, hypoxemia or infiltration of marrow with granuloma, metastatic deposits, myelophthisic conditions like myelofibrosis are associated with the presence of nucleated red cells in the peripheral blood. The presence of nuclear or cytoplasmic irregularity suggests dyserythropoietic associated with conditions like myelodysplastic syndrome, and the presence of the binucleate normoblasts in the peripheral smear of a child suggests congenital dyserythropoietic anemia.[8]

**(3) White blood cells :** Mature leucocytes including granulocytes, monocytes, and lymphocytes are nucleated cells present in peripheral blood, having characteristic morphology. Granulocytes undergo sequential maturation in the bone marrow and only mature forms are released in the peripheral blood. The presence of immature granulocytes in the peripheral blood or any variation in the morphology is associated with various disorders (Fig 2).[8]

The change in the number of leucocytes (decrease in count- Leucopenia and increase in count- Leucocytosis) or change in lobulation or granularity is associated with various systemic disorders including nutritional disorders to hematological malignancies. Table 4 gives a summarize form of various disorders associated with it.[9]

**(4) Platelets :** The cytoplasmic fragments of megakaryocytes are released directly in the peripheral bloodas platelets (anucleated granular), are best visualized under oil immersion. Both the variations in number and size of platelets are associated with different hematological disorders. The evaluation of peripheral smear is most valuable to rule out pseudothrombocytopenia in case of low platelet count on automatic hematological analyzer.[1] The presence of platelet clumps due to technical error while taking a blood sample or sticking of platelets over neutrophils cell membrane, phenomena called satellitism are the most common causes of pseudothrombocytopenia. Table 5 provides the main variation in size and granularity of platelets and their associated clinical significance.[2]

Table 4 — *WBC characteristics and associated clinical significance*

| Characteristic | Clinical Significance |
|---|---|
| Neutropenia | Viral infection, Drug-induced, Chronic idiopathic neutropenia, Cyclical neutropenia, Nutritional deficiencies including folate or Vit B12, Hematological malignancy |
| Neutrophilia | Bacterial infections, stress, Drugs, Myeloproliferative neoplasm |
| Eosinophilia | Helminth infections, Asthma, allergic reactions, Hypereosinophilic syndrome, Chronic eosinophilic leukemia |
| Monocytosis | Tuberculosis, Malaria, Viral infections, CMML |
| Basophilia | Chronic myeloid leukemia and other myeloproliferative neoplasm |
| Lymphocytosis | Viral infections, Autoimmune disease, Drug hypersensitivity, Lymphoproliferative disorder |
| Lymphopenia | HIV, Chemotherapy, Malnutrition, Alcoholism, Autoimmune disorders, Hematological malignancy |
| Hypolobation | Pelger-Huet anomaly, Pseudo-pelger-Huet anomaly seen in dysgranulopoiesis |
| Hyperlobation | Megaloblastic anemia, Uremia, Myelodysplastic syndrome, Drugs induced |
| Hypogranularity | Myelodysplastic syndrome |
| Hypergranularity | Alder Reilly anomaly, Chediak Higashi syndrome, Toxic granules in sepsis |
| Inclusions (Dohle bodies) | Bacterial infection, Sepsis, G-CSF induced, Pregnancy |

**(5) Other cells :**

***(a) Blasts :*** The presence of blasts (myeloblasts or lymphoblasts) in the peripheral smear signify the requirement of further evaluation of such patients including bone marrow examination to rule out underlying hematological malignancies. Myeloblasts are medium to large size with opened up chromatin and 1-2 prominent nucleoli and moderate basophilic cytoplasm with fine granules and Auer rods while lymphoblasts ae small to medium size immature cells with condensed chromatin, inconspicuous nucleoli, and scanty basophilic agranular cytoplasm; presence of either

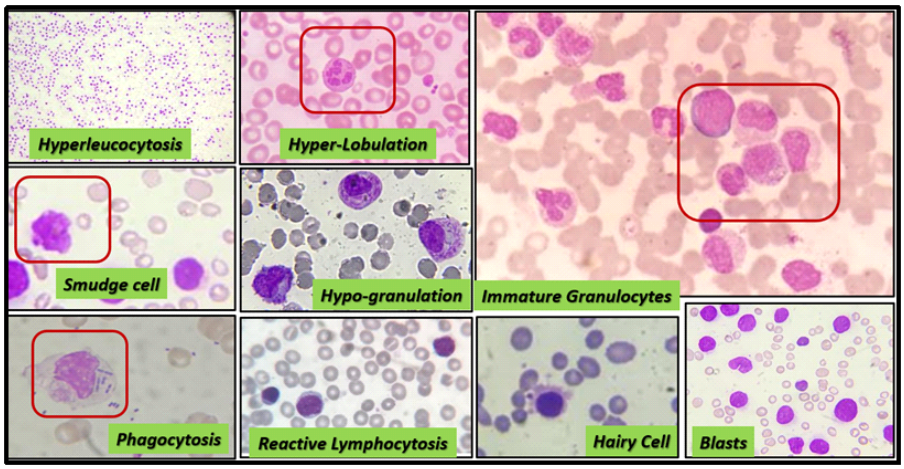


Fig 2 — Morphologic characteristic of WBCs

signifies underlying hematological malignancy including acute leukemia.[8,9]

***(b) Atypical lymphoid cells:*** Apart from the presence of an increased number of small mature lymphocytes (Monoclonal lymphocytosis), presence of other atypical lymphoid cells with characteristic morphology in the peripheral smear warrants the further evaluation of such patient to rule out underlying leukemia or lymphoma. Reactive lymphocytosis is the most common diagnostic challenge in such patients and many times require ancillary techniques to rule out leukemia or lymphoma involvement.Table 6 summarizes the morphology and clinical significance of various atypical lymphoid cells.[1,3]

***(c) Leukoerythroblastic picture :*** The combined presence of early precursors of granulocytes including myelocytes and metamyelocytes, normoblasts (intermediate and late erythroid precursors) and teardrop cells in the peripheral smear suggests marrow infiltration or fibrosis leading to outpouring of immature cells in the peripheral blood.[1,2]

Table 6 — *Atypical lymphoid cell morphologic characteristics and associated clinical significance*

| Lymphoid cell | Morphology | Clinical significance |
|---|---|---|
| Turk's cell | Medium size, round nucleus, prominent nucleoli with abundant basophilic cytoplasm | Infectious mononucleosis, Viral infections |
| Large granular lymphocytes | Large cells with condensed chromatin and abundant fine granular cytoplasm | Viral infections, Large granular lymphocytic leukemia |
| Hairy cell | Medium size cell with oval nuclei, condensed chromatin, abundant cytoplasm, and regular cytoplasmic projections | Hairy cell leukemia |
| Villous lymphocytes | Small mature lymphocytes with bipolar villous projections | Splenic marginal zone lymphoma |
| Buttock lymphocytes | Small lymphoid cells with cleaved nuclei | Follicular lymphoma |
| Cloverleaf cell | Medium size lymphoid cells with hyperlobulated (cloverleaf or flower-shaped) nuclei | Adult T cell leukemia/ Lymphoma |
| Sezary cell | Lymphoid cell with cerebriform nuclei | Cutaneous T cell lymphoma |
| Plasma cells | Mature or immature plasma cell | Plasma cell Leukemia |

**Reporting the peripheral smear :** The typical format of peripheral smear reporting including patient's detail including name, age, gender, registration number with date and time of sample collection and reporting. The main part of the report includes the detailed description of count and morphologic characteristics of each cell line including red cell, white cell, and platelets with special emphasis on the presence of any abnormal cells or hemoparasite. The end part of the report includes diagnosis or differentials in different hematological disorders and further advice regarding recommended laboratory evaluations for definitive diagnosis. The report will be incomplete without authorized signature of concerned hemato-morphologist. The interpretation of blood smear should be reconciled with the clinical features and other hematological or investigation findings.[2,3,9]

Table 5 — *Platelets characteristics and associated clinical significance*

| Platelet Characteristics | Clinical Significance |
|---|---|
| Pseudo Thrombocytopenia | Clumping of platelets, Satellitism, Severe microcytic anemia |
| True Thrombocytopenia | Defect in production (due to marrow failure, infiltration or hematological malignancy) or excessive destruction (Immune-mediated, drug-induced, Heparin-induced, Thrombotic thrombocytopenic states, DIC) or Sequestration (Hypersplenism) |
| Thrombocytosis | Post hemorrhage/surgery, Postsplenectomy, Iron deficiency anemia, Drug-induced, Myeloproliferative neoplasm |
| Small Platelets | Familial thrombocytopenia like Wiskott Aldrich syndrome or X-linked thrombocytopenia |
| Large platelets | Bernard Soulier syndrome, Marrow recovery, ITP, MYH9 related anomalies, Myeloproliferative neoplasm, Alport syndrome |
| Giant platelets | Myelodysplastic syndrome, Myeloprolifeartive neoplasm |

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