Laboratory Tests in Hematological Malignancies

White blood cells: White blood cells (or leukocytes) are divided into two main groups: granulocytes and agranulocytes. The granulocytes receive their name from the distinctive granules that are present in the cytoplasm of neutrophils, basophils, and eosinophils.

Polymorphonuclear neutrophil: Neutrophil measures 14-15 µm in size. Its cytoplasm is colorless or lightly eosinophilic and contains multiple, small, fine, mauve granules. Nucleus has 2-5 lobes that are connected by fine chromatin strands. Nuclear chromatin is condensed and stains deep purple in color.

Eosinophil: Eosinophils are slightly larger than neutrophils (15-16 µm). The nucleus is often bilobed and the cytoplasm is packed with numerous, large, bright orange-red granules. On blood smears, some of the eosinophils are often ruptured.

Basophils: Basophils are seen rarely on normal smears. They are small (9-12 µm), round to oval cells, which contain very large, coarse, deep purple granules. It is difficult to make out the nucleus since granules cover it.

Monocytes: Monocyte is the largest of the leuko- cytes (15-20 µm). It is irregular in shape, with oval or clefted (kidney-shaped) nucleus and fine, delicate chromatin. Cytoplasm is abundant, blue- gray with ground glass appearance and often contains fine azurophil granules and vacuoles. After migration to the tissues from blood, they are called as macrophages.

Lymphocytes: On peripheral blood smear, two types of lymphocytes are distinguished: small and large. The majority of lymphocytes are small (7-8 µm). These cells have a high nuclear-cytoplasmic ratio with a thin rim of deep blue cytoplasm. The nucleus is round or slightly clefted with coarsely clumped chromatin. Large lymphocytes (10-15µm) have a more abundant, pale blue cytoplasm, which may contain a few azurophil granules. Nucleus is oval or round and often placed on one side of the cell.

Morphology of abnormal leukocytes (Fig. 22.12):

1. *Toxic granules*: These are darkly staining, blue- purple, coarse granules in the cytoplasm of neutrophils. They are commonly seen in severe bacterial infections.
2. *Döhle inclusion bodies*: These are small, oval, pale blue cytoplasmic inclusions in the periphery of neutrophils. They represent remnants of ribosomes and rough endoplasmic reticulum. They are often associated with toxic granules and are seen in bacterial infections.
3. *Cytoplasmic vacuoles*: Vacuoles in neutrophils are indicative of phagocytosis and are seen in bacterial infections.
4. *Shift to left of neutrophils*: This refers to presence of immature cells of neutrophil series (band forms and metamyelocytes) in peripheral blood and occurs in infections and inflammatory disorders.

5. Hypersegmented neutrophils: Hypersegmentation of neutrophils is said to be present when >5% of neutrophils have 5 or more lobes. They are large in size and are also called as macropolycytes. They are seen in folate or vitamin B12 deficiency and represent one of the earliest signs.
6. Pelger-Huet cells: In Pelger-Huet anomaly (a benign autosomal dominant condition), there is failure of nuclear segmentation of granulocytes so that nuclei are rod-like, round, or have two segments. Such granulocytes are also observed in myeloproliferative disorders (pseudo-Pelger-Huet cells).
7. Atypical lymphocytes: These are seen in viral infections, especially infectious mononucleosis. Atypical lymphocytes are large, irregularly shaped lymphocytes with abundant cytoplasm and irregular nuclei. Cytoplasm shows deep basophilia at the edges and scalloping of borders. Nuclear chromatin is less dense and occasional nucleolus may be present.
8. Blast cells: These are most premature of the leukocytes. They are large (15-25 µm), round to oval cells, with high nuclear cytoplasmic ratio. Nucleus shows one or more nucleoli and nuclear chromatin is immature. These cells are seen in severe infections, infiltrative disorders, and leukemia. In leukemia and lymphoma, blood smear suggests the diagnosis or differential diagnosis and helps in ordering further tests (Fig. 22.13 and Box 22.3).

Differential leukocyte count (DLC): DLC refers to relative proportion of different leukocytes expressed as a percentage.

Principle: Leukocytes are counted on a blood smear and percentage of each type of leukocyte is recorded.

Uses of DLC

1. To support the diagnosis of infectious, inflammatory, or allergic disorders.
2. Diagnosis of malignant blood disorders.

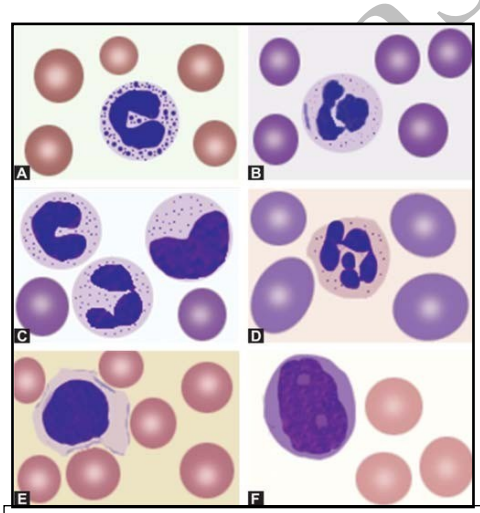
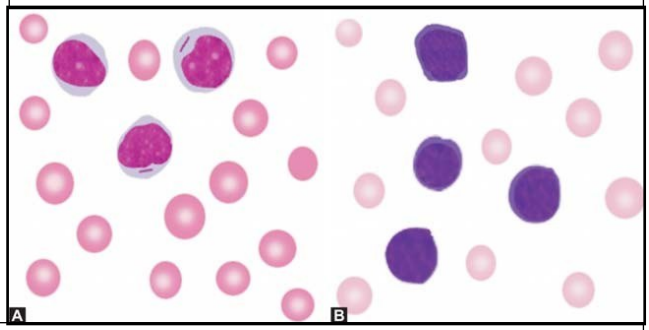


Fig. 22.12: Morphological abnormalities of white blood cells:
(A)Toxic granules; (B) Döhle inclusion body; (C) Shift to left in neutrophil series; (D) Hypersegmented neutrophil in megaloblastic anemia; (E) Atypical lymphocyte in infectious mononucleosis; (F) Blast cell in acute leukemia

Fig. 22.13: Comparison of blood smears in (A) acute myeloid leukemia and (B) acute lymphoblastic leukemia



Neutrophilia

An absolute neutrophil count greater than 7500/µl is termed as neutrophilia or neutrophilic leukocytosis.

Causes

1. Acute bacterial infections: Abscess, pneumonia, meningitis, septicemia, acute rheumatic fever, urinary tract infection.
2. Tissue necrosis: Burns, injury, myocardial infarction.
3. Acute blood loss
4. Acute hemorrhage
5. Myeloproliferative disorders
6. Metabolic disorders: Uremia, acidosis, gout
7. Poisoning
8. Malignant tumors
9. Physiologic causes: Exercise, labor, pregnancy, emotional stress.

Leukemoid reaction: This refers to the presence of markedly increased total leukocyte count (>50,000/cmm) with **immature cells in peripheral blood resembling leukaemia** but occurring in non-leukemic disorders (Fig. 22.15). Its causes are:

- Severe bacterial infections, e.g. septicemia, pneumonia
- Severe hemorrhage
- Severe acute hemolysis
- Poisoning
- Burns
- Carcinoma metastatic to bone marrow

Neutropenia: Absolute neutrophil count less than 2000/µl is neutropenia. It is graded as mild (2000-1000/µl), moderate (1000-500/µl), and severe (< 500/µl).

Causes

1. Decreased or ineffective production in bone marrow:
2. Infections
3. Bacterial: typhoid, paratyphoid, miliary tuberculosis, septicemia
4. Viral: influenza, measles, rubella, infectious mononucleosis, infective hepatitis.
5. Protozoal: malaria
6. Overwhelming infection by any organism
7. Hematologic disorders: megaloblastic anemia, aplastic anemia, aleukemic leukemia, myelophthisis.
8. Drugs: diosyncratic action: Analgesics, antibiotics, sulfonamides, phenothiazines, antithyroid drugs, anticonvulsants.
9. Dose-related: Anticancer drugsIonizing radiation
10. Congenital disorders: Kostman's syndrome, cyclic neutropenia, reticular dysgenesis**.**

Eosinophilia

This refers to absolute eosinophil count greater than 600/ µl.

Causes

1. Allergic diseases: Bronchial asthma, rhinitis, urticaria, drugs.
2. Skin diseases: Eczema, dermatitis herpetiformis.

3. Parasitic infection with tissue invasion: Filariasis, trichinosis, echinococcosis.
4. Hematologic disorders: Chronic myeloproliferative disorders, Hodgkin's disease, peripheral T cell lymphoma.
5. Carcinoma with necrosis.
6. Radiation therapy.
7. Lung diseases: Loeffler's syndrome, tropical eosinophilia
8. Hypereosinophilic syndrome.

Basophilia*:* Increased numbers of basophils in blood (>100/µl) occurs in chronic myeloid leukemia, polycythemia vera, idiopathic myelofibrosis, basophilic leukemia, myxedema, and hypersensitivity to food or drugs.

Monocytosis

This is an increase in the absolute monocyte count above 1000/µl.

Causes

1. Infections: Tuberculosis, subacute bacterial endocarditis, malaria, kala azar.
2. Recovery from neutropenia.
3. Autoimmune disorders.
4. Hematologic diseases: Myeloproliferative disorders, monocytic leukemia, Hodgkin's disease.
5. Others: Chronic ulcerative colitis, Crohn's disease, sarcoidosis.

Lymphocytosis

This is an increase in absolute lymphocyte count above upper limit of normal for age (4000/µl in adults, >7200/µl in adolescents, >9000/µl in children and infants) (Box22.4).

Causes

1. Infections*:*
2. Viral: Acute infectious lymphocytosis, infective hepatitis, cytomegalovirus, mumps, rubella, varicella
- Bacterial: Pertussis, tuberculosis
- Protozoal: Toxoplasmosis

3. Hematological disorders: Acute lymphoblastic leukemia, chronic lymphocytic leukemia, multiple myeloma, lymphoma.
4. Other: Serum sickness, post-vaccination, drug reactions*.*

Leukemia

Leukemia is cancer of the blood cells. It starts in the bone marrow, the soft tissue inside most bones. When you have leukemia, the bone marrow starts to make a lot of abnormal white blood cells, called leukemia cells. They don't do the work of normal white blood cells, they grow faster than normal cells, and they don't stop growing when they should. This can lead to serious problems such as anemia, bleeding, and infections. Leukemia cells can also spread to the lymph nodes or other organs and cause swelling or pain.

Genetic damage is believed to involve several key biochemical steps resulting in (i) an increased rate of proliferation, (ii) reduced apoptosis and (iii) a block in cellular differentiation. Together these events cause accumulation in the bone marrow of early haemopoietic cells known as blast cells.

ACUTE LEUKEMIAS

Acute leukemias are malignant clonal hematopoietic stem cell disorders characterized by proliferation of **blast cells in the bone marrow** and rapidly progressive fatal course if untreated. Malignant cells in acute leukemias are primitive cells with very little differentiation into functioning mature cells and are called as blast cells.

Clinically, patients with acute leukemia present with

- Suppression of normal hematopoiesis like anemia (pallor, weakness, fatigue), neutropenia (infections), and thrombocytopenia (purpura and mucosal bleeding),
- Accumulation of blast cells in marrow (bony pain and tenderness)
- Infiltration of various organs by blast cells (lymphadenopathy, splenomegaly, hepatomegaly, swelling of gums, central nervous system manifestations, and skin infiltrations).

Classification of Acute Leukemias

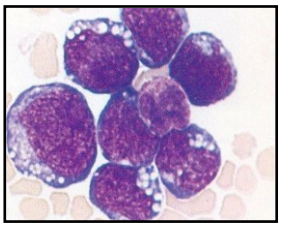
Acute leukemias are divided into two major types: acute myeloid leukemia (AML)and acutelymphoblastic leukemia (ALL).

Table 28.5: Differences between acute lymphoblastic leukemia and acute myeloid leukemia

Parameter	*Acute lymphoblastic leukemia*	*Acute myeloid leukemia*
1. Predominant age	Children (peak 3-4 years)	Adults
2. Morphology of blasts		
• Size of blasts	Small	Large
• Cytoplasm	Scanty	Moderate
• Granules in cytoplasm	Absent	May be present
• Nuclear chromatin	Coarse	Fine
• Nucleoli	0-2	>3
• Auer rods	Absent	Pathognomonic, if present
3. Cytochemistry		
• Myeloperoxidase	Negative	Positive
• Periodic acid Schiff	Block-like	Diffuse
4. Immunophenotyping	B or T lymphoid markers	Myeloid markers
5. Prognosis	Curable in majority of children	Curable in minority of adults

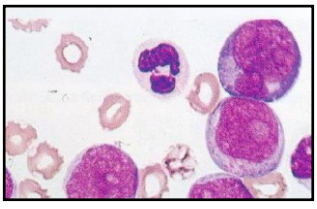
- **Acute lymphoblastic leukemia, or ALL.**

ALL affects children in the 2 – 10 years' age group commonly but is also seen in adults. The patient presents with fever, weakness, pallor, infections bleeding and enlarged lymph nodes and spleen.



The blood film shows normochromic normocytic anaemia, leukocytosis with many blast cells (a primitive, undifferentiated blood cell, often found in the blood of those with acute leukemia) and thrombocytopenia. The differential cells show many blast cells There is neutropenia.

Acute myelogenous (myeloid) leukemia, or AML.



•The blood film shows normochromic normocytic anaemia, leukocytosis with many blast cells and thrombocytopenia.
The differential cell count shows many blast cells
There is neutropenia

CHRONIC LEUKEMIAS

Chronic leukemias are heterogeneous disorders characterized by neoplastic proliferation of maturlooking cells of myeloid or lymphoid lineage. General differences between acute and chronic leukemias are outlined in Table 28.7.
Chronic leukemias are of two main types:

- Chronic myeloid leukemia
- Chronic lymphoid leukemias (Table 28.8).

Table 28.7: General differences between acute and chronic leukemias

Parameter	*Acute leukemias*	*Chronic leukemias*
Clinical presentation	Usually sudden and fulminant	Usually incidental or insidious onset
Hematological features in marrow and blood	Immature blast cells	Mature and differentiated cells
Course	Aggressive	Indolent

Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a chronic myeloproliferative neoplasm originating from a pleuripotent hematopoietic stem cell and characterized by predominant proliferation of granulocytic cells.
There are three phases of CML: chronic phase (3-5 years), accelerated phase (6-12 months), and blast crisis (2-4 months).

Pathogenesis

1. All leukaemia cells in patients with **chronic myeloid leukemia contain** a specific **cytogenetic marker**, described originally in 1960 by workers in **Philadelphia** and now known as the **Philadelphia** or **Ph** chromosome.
2. **The Ph chromosome** is derived from a **normal 22 chromosomes** that has **lost part of its long arm** as a result of a balanced **reciprocal translocation of chromosomal material involving one of the 22 and one of the 9 chromosomes.**
3. **The Ph chromosome** carries a specific fusion gene known as **BCR-ABL**, which results from juxtaposition with part of the **ABL proto-oncogene** (from chromosome 9) with part of the **BCR gene on chromosome 22**.
4. This fusion gene is **expressed as a specific messenger RNA** (mRNA), which in turn generates a protein called **p210BCR-ABL**.
5. **BCR-ABL activates a cascade of proteins** that **control the cell cycle**, speeding up **cell division**. Moreover, **the BCR-ABL protein inhibits DNA repair**, **causing genomic instability and making the cell more susceptible** to developing further genetic abnormalities.

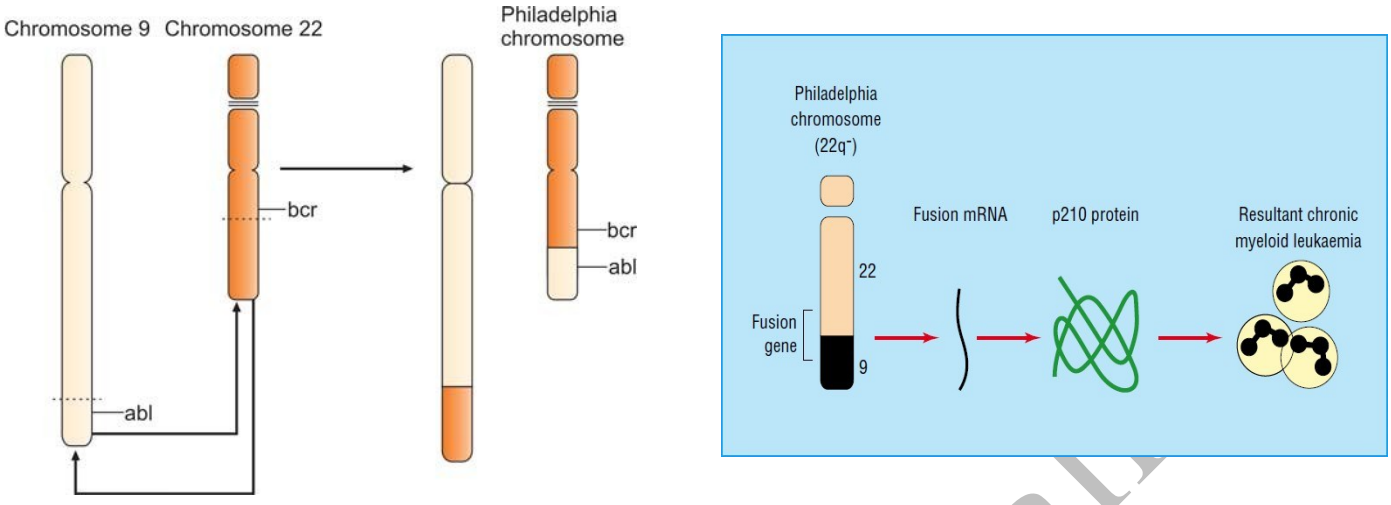


Fig. 28.7: Translocation between chromosomes 9 and 22 causing formation of Philadelphia chromosome

Chronic phase: The average age at diagnosis is 45 years. In chronic phase, the usual presenting features include weakness, weight loss, abdominal fullness, easy bruisability, and splenomegaly. Blood smear in chronic phase of CML shows marked leukocytosis, immature white blood cells, basophilia, eosinophilia, anemia, and thrombocytosis (Fig. 28.8).

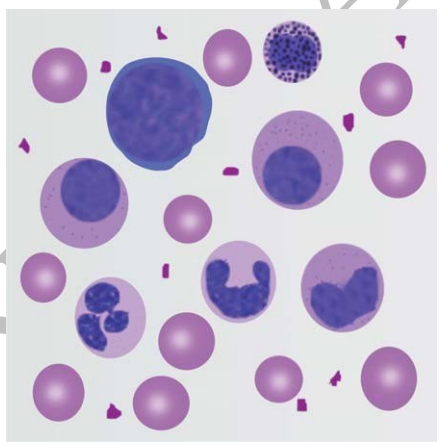
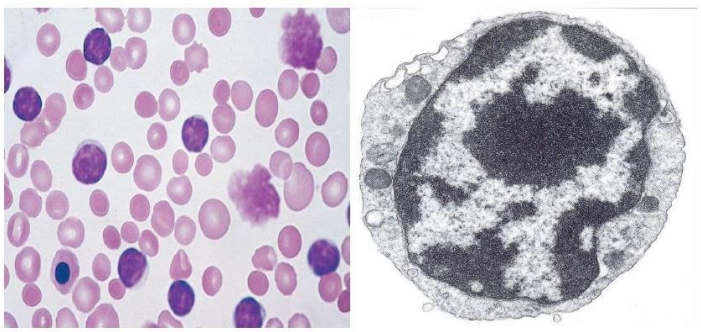


Fig. 28.8: Blood smear in chronic phase of chronic myeloid leukemia showing all forms of immature white cells and a basophil

Chronic lymphocytic leukemia, or CLL.
The blood film shows **leukocytosis**. The red cells are **normochromic normocytic**.
70 – 90 % of the WBC are **mature lymphocytes**. Many cells are smear cells. There is a persistent absolute **lymphocytosis**. **Platelets are normal**



DIAGNOSIS

In most patients the Ph chromosome is seen by **karyotypic** examination of tumour cells. but in a few the Ph abnormality cannot be seen under the microscope but the same molecular rearrangement is detectable by more sensitive techniques: fluorescence *in situ* hybridization (**FISH**) or polymerase chain reaction (**PCR**).

The common Cytochemical stains used are is used to Identification of myeloid blasts from lymphoid blasts

1. Myeloperoxidase (MPO): This enzyme is present in primary and secondary granules of granulocytes.

2. Sudan Black B (SBB): This lipophilic dye binds with granule components in granulocytes and monocytes irreversibly.

3. Periodic Acid Schiff reaction (PAS): Periodic acid oxidizes 1-2 glycol groups to produce dialdehydes. Dialdehydes give a red reaction when exposed to the Schiff reagent which is leucobasic fuchsin. **A positive reaction is seen with carbohydrates especially glycogen, polysaccharides, mucoproteins, glycoproteins etc.** Blood smears are fixed with formalin vapour, exposed to Periodic acid and then Schiff reagent and counter stained with **Haematoxylin**.

4. Acid phosphatase: This enzyme is present in haemopoietic cells. It is useful for the diagnosis of T cell ALL and Hairy cell leukemia