

**23****LEUKEMIA****23.1 INTRODUCTION**

Leukemia is a disease of unknown etiology and fatal termination characterized by uncontrolled, abnormal and widespread proliferation of leukocytes and their precursors in bone marrow and blood.

**OBJECTIVES**

After reading this lesson, you will be able to:

- classify Acute Lymphoblastic leukemia
- describe FAB classification of AML
- explain Cytochemistry stains in diagnosis of leukemia
- explain Immunophenotyping technique in diagnosis of Leukemia
- describe Chronic myeloid leukemia, Chronic lymphocyte leukemia, Chronic myeloproliferative disorders, Myelodysplastic syndromes

23.2 LEUKEMIA**23.2.1 Definition**

Leukemia is a disease of unknown etiology and fatal termination characterized by uncontrolled, abnormal and widespread proliferation of leukocytes and their precursors in bone marrow and blood.

23.2.2 Classification

This is based on the clinical course of the disease and the type of cell line that is involved.



Notes

In the early 1990s the **French – American – British** group introduced the FAB classification of acute leukemia based mainly on the morphological appearance of blasts in Romanowsky stained smears of peripheral blood and bone marrow.

More recently (2002) the WHO classification of leukemia included in addition to morphology, immunophenotyping of the blasts and cytogenetic studies.

ACUTE LEUKEMIA is of sudden onset and rapid progression unless treated and is characterized by the **presence of blast cells** in peripheral blood and bone marrow.

CHRONIC LEUKEMIA is of gradual onset and slow progression and is characterized by the **presence of more mature cells** in the peripheral blood and bone marrow.

23.2.3 Diagnostic Tools in the Diagnosis of Leukemia

- A. Morphology of the blasts in peripheral blood and bone marrow.
- B. Cytochemistry of blasts in the various leukemias

Leukocyte cytochemistry uses techniques to identify enzymes or other cytoplasmic products in cells. Cytochemistry is useful for

1. Identification of myeloid blasts from lymphoid blasts
2. Differentiation of granulocytic and monocytic components of acute myeloid leukemia
3. Detection of unusual lineages eg basophils.
4. Detection of the absence of certain enzymes in a malignant clone eg Leukocyte alkaline phosphatase in Chronic Myeloid Leukemia.

The common Cytochemical stains used are

- (a) Myeloperoxidase (MPO) This enzyme is present in primary and secondary granules of granulocytes. MPO splits H_2O_2 and in the presence of a chromogenic electron donor forms an insoluble reaction product. Substrates are benzidine substitutes. The reaction product is stable. MPO is not inhibited by heparin, oxalate or EDTA. The substrate is 3,3 diaminobenzidine with a phosphate buffer at pH 7.3. A brown granular deposit is seen in blasts of myeloid origin.
- (b) Sudan Black B (SBB). This lipophilic dye binds with granule components in granulocytes and monocytes irreversibly. Staining reaction is comparable to MPO.
- (c) 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. WBC show diffuse, confluent red colour. ALL blasts show “block” positivity and the method can be used to differentiate ALL from AML.

- (d) Acid phosphatase. This enzyme is present in haemopoietic cells. It is useful for the diagnosis of T cell ALL and Hairy cell leukemia. Air dried smears fixed in methanol, acetone, citric acid buffer are exposed to naphtholAS-BI phosphate substrate at an acid pH of 5.0. The reaction is detected with the use of hexazotised pararosaniline dye. Nuclei are counter stained with haematoxylin. T cells show strong localized polar positivity. Other WBC also show variable positivity. In hairy cell leukemia the cells react equally positive in the presence and absence of tartaric acid.
- (e) Esterase stains. WBC granules contain several types of esterases that hydrolyse acyl or chloroacyl esters of α naphthol or naphthol AS. **Specific esterase** stain with naphthol AD chloroacetate ester (CAE) and is positive in the myeloid series and strong positive in promyelocytes including Auer rods. **Non specific esterase** (NSE) stains with α naphthyl acetate ester (ANAE) or α naphthyl butyrate ester or (ANBE) and is positive in 80% of the monocyte cell line. A stain combining the two substrates sequentially or in combination in one single step may be used to differentiate myeloblasts from monoblasts.
- (f) Neutrophils contain alkaline phosphatase enzyme in their granules. The reaction between leukocyte alkaline phosphatase in cells, naphthol AS phosphate substrate, alkaline buffer at pH 9.0 is detected using a coupling azo dye (Fast Blue BB salt or Fast Garnet GBC) A counter stain is used for nuclei. The azo dye product is blue or brown granules in neutrophil cytoplasm. The neutrophils are scored as 0 = negative, 1+ = occasional granules in cytoplasm, 2+ = moderate granulation, 3+ = heavy granulation and 4+ = heavy granulation overlapping the nucleus.
- A positive control that is used is usually from a pregnant female. The abnormal neutrophils in CML have markedly decreased levels of this enzyme. In CML neutrophils stain negative for the enzyme
- (g) Toluidine Blue stain is used to stain the metachromatic granules in basophils and mast cells.
- C. Immunophenotyping is done to determine whether the cells are of myeloid or lymphoid origin and if lymphoid, whether they are of T or B cell origin. The cells at various stages of development express antigens on the surface.



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Leukemia

Monoclonal antibodies are available commercially to detect these antigens. The antibodies are tagged with a fluorescent label. The test cells are incubated with a series of specific antibodies and by observing the degree of binding and the specificity of the antibody using a flow cytometer it is possible to identify and quantify the cells for diagnosis and for follow up of therapy. Flow cytometry is also used to detect the presence of cytoplasmic or surface immunoglobulins and the presence of certain cytoplasmic enzymes eg myeloperoxidase.

- D. Cytogenetics. Many of the blasts show specific cytogenetic markers which help identify them as well as predict their behavior to chemotherapeutic drugs. Standard cytogenetics, RT-PCT, FISH are some techniques that are used.
- E. Cytospin of Cerebrospinal Fluid is used to detect the presence of small numbers of lymphoblasts in CSF in ALL. The cytospin is a special centrifuge which is gentle on the cells and produces minimum distortion of the blast cells.



INTEXT QUESTIONS 23.1

1. Acute leukemia is characterized by in peripheral blood and bone marrow
2. Chronic leukemia is characterized by in peripheral blood and bone marrow
3. Common cytochemical stains used in diagnosis of leukemia are,,,,
4. reaction method is used to differentiate ALL from AML
5. test is used in diagnosis of T cell & Hairy cell Leukemia
6. is used to differentiate myeloblasts from monoblasts
7. is used for identifying the origin of cells
8. Cytospin of CSF is used to detect in ALL

23.3 ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

23.3.1 Clinical Presentation

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. Childhood ALL is associated with a good prognosis.



Notes

23.3.2 Laboratory Diagnosis

1. Haemoglobin, PCV, RBC count are decreased.
2. MCV, MCH and MCHC are normal RDW is normal. There is normochromic normocytic anaemia.
3. WBC count is elevated ($20.0 - 100.0 \times 10^9/L$). Rarely the count may be low in subleukemic leukemia.
4. Platelet count shows moderate to severe thrombocytopenia
5. The blood film shows normochromic normocytic anaemia, leukocytosis with many blast cells and thrombocytopenia.
6. The differential cells show many blast cells There is neutropenia. The FAB classification describes 3 types of ALL depending on the morphology of the blasts. They are

A. ALL L1

The blast is small, $14 - 16\mu\text{m}$ in diameter, the cytoplasm is barely visible, the nucleus occupies the whole cell, has condensed chromatin and nucleoli are not seen. Figure 23.1.

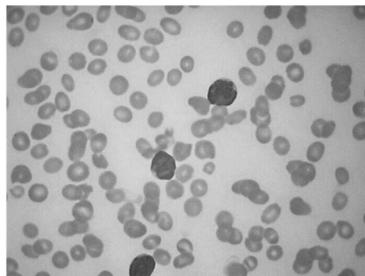


Fig. 23.1: ALL L1

B. ALL L2

The blast is $15 - 18\mu\text{m}$ in diameter, nucleus occupies most of the cell. The nuclear membrane is thick, chromatin is coarse, $1 - 2$ nucleoli are seen. The chromatin around the nucleolus is condensed. The cytoplasm is pale blue, forms a rim around the nucleus and does not contain any inclusions. Figure 23.2.

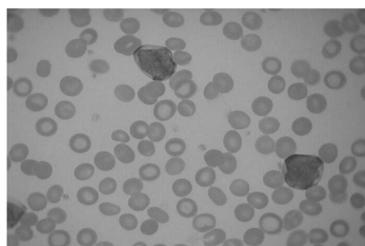


Fig. 23.2: ALL L2

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Leukemia

C. ALL L3

The blast is 18 – 20 μm in diameter, nucleus has reticular chromatin, 1-2 prominent nucleoli, cytoplasm is moderate in amount blue in colour and there are punched out vacuoles in the cytoplasm and overlying the nucleus. Figure 23.3

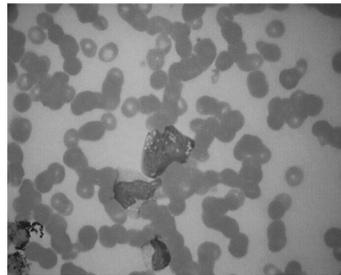


Fig. 23.3: ALL L3

7. Cytochemistry. Special stains are done to differentiate ALL from AML. These are
 - A. Myeloperoxidase stain and Sudan Black B stains are negative in ALL.
 - B. Periodic Acid Schiff stain may show block positive staining in the cytoplasm of ALL blasts with no differentiation between T and B cells.
8. Immunophenotyping differentiates the blasts into T and B cells. B cells are CD19+, CD10+ and may show cytoplasmic CD22. T cells are CD2+, CD5+, CD7+
9. Bone marrow examination shows solidly cellular marrow with suppression of normal erythropoiesis, myelopoiesis and megakaryocytes. The marrow is replaced by proliferating blasts. In the FAB classification there must be >30% blasts in the marrow and in the WHO classification there must be >20% blasts in marrow.
10. Biochemical tests show raised serum uric acid levels, raised serum LDH and serum creatinine may be elevated

23.4 ACUTE MYELOID LEUKEMIA (AML)

23.4.1 Clinical Presentation

AML affects young adults who present with fever, weakness, pallor, infections, bleeding, bone pains and splenomegaly.

23.4.2 Laboratory Diagnosis

1. Haemoglobin, PCV, RBC count are decreased.
2. MCV, MCH and MCHC are normal RDW is normal. There is normochromic normocytic anaemia.

3. WBC count is elevated ($20.0 - 100.0 \times 10^9/L$). Rarely the count may be low in subleukemic leukemia.
4. Platelet count shows moderate to severe thrombocytopenia
5. The blood film shows normochromic normocytic anaemia, leukocytosis with many blast cells and thrombocytopenia.
6. The differential cell count shows many blast cells There is neutropenia. The FAB classification describes 8 types of AML depending on the morphology of the blasts. They are

A. AML M0

The blast is 15 - 18 μ m in diameter, nucleus occupies most of the cell, nuclear membrane is fine, chromatin is fine, 2 - 3 nucleoli are seen. The cytoplasm is pale blue, forms a rim around the nucleus and does not contain any inclusions. These cells are CD 13+, CD 33+ and CD 117+ by flow cytometry <3% of cells show MPO/SBB positivity

B. AML M1

The blast is 15 - 18 μ m in diameter, nucleus occupies most of the cell, nuclear membrane is fine, chromatin is fine, 2 - 3 nucleoli are seen. The cytoplasm is pale blue, forms a rim around the nucleus and may contain thin rod like pink structures called Auer rods. This is formed by the condensation of primary granules and when present are diagnostic of AML. Figure 23.4 Myeloperoxidase and Sudan Black B stains show early positivity in 30 - 100% of cells

C. AML M2

The blast is 15 - 18 μ m in diameter, nucleus occupies most of the cell, nuclear membrane is fine, chromatin is fine, 2 - 3 nucleoli are seen. The cytoplasm is pale blue, forms a rim around the nucleus and contains fine early granulation and thin rod like pink structures called Auer rods. Myeloperoxidase and Sudan Black B stains show positivity in 30 - 100% of cells.

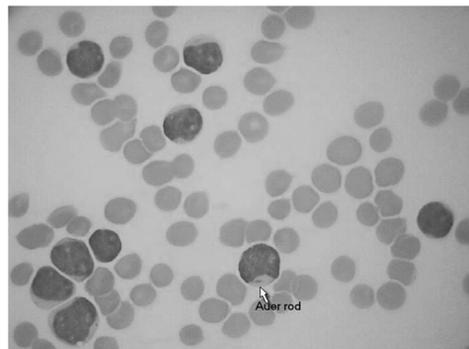


Fig. 23.4: Acute Myeloid Leukemia (AML M2)





Notes

D. AML M3 (Promyelocytic leukemia)

The blast is 18 – 20 µm in diameter, nucleus has reticular chromatin, 1-2 prominent nucleoli. The nucleus appears folded on itself or may be bilobed. The cytoplasm is moderate in amount and filled with fine pink azurophilic granules. Many Auer rods are seen and these cells are called faggot cells. Figure 23.5. Sometimes the granulation is not prominent and the leukemia is called AML M3 Hypogranular variant .The myeloperoxidase stain and SBB stains are strong positive. 90% of the cells show specific esterase positivity. AML M3 is associated with a cytogenetic abnormality namely T(15:17)

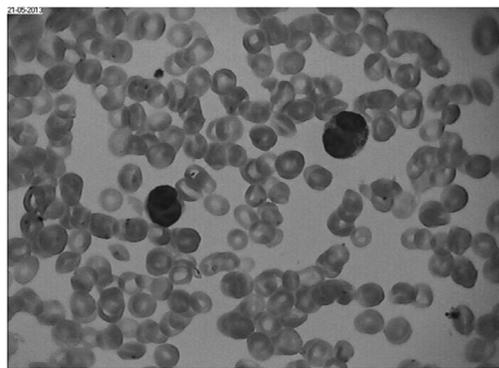


Fig. 23.5: Acute Myeloid Leukemia (AML M3)

E. AML M4 (Myelomonocytic leukemia)

In this variant two types of blasts are present – myeloblasts as described above and up to 20% monocytes and monoblasts. The monoblaste are 20 – 22 µm in diameter, nucleus has reticular chromatin, 2-3 prominent nucleoli and the nucleus may be indented. The cytoplasm is abundant, vacuolated and may contain few azurophilic granules and sometimes Auer rods. MPO, SBB and non specific esterase are positive A variant of this leukemia with prominent eosinophilia and a cytogenetic abnormality inversion16 is known. Figure 23.6.

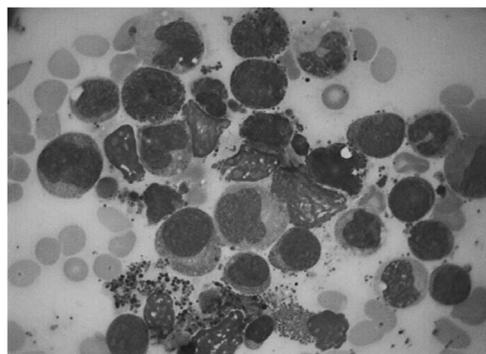


Fig. 23.6: Acute Myelomonocytic Leukemia (AML M4) Eosinophilic



F. AML M5 (Monocytic leukemia)

In this variant the predominant cell line is monocytoid and is a mixture of monocytes and monoblasts which make up >80% of the cells. MPO, SBB and combined esterase are positive. In addition to cytochemistry there is also increase in serum and urinary lysozyme excretion

G. AML M6 (Erythroleukemia)

In this variant there are two cell lines involved erythroid which must be greater than 50% in marrow and myeloblasts which makes up greater than 20% of the non erythroid cells in marrow. Auer rods and dysplasia may be seen. PAS positivity is seen in erythroblasts.

H AML M7 (Megakaryoblastic leukemia)

In this variant the cell line involved is megakaryoblastic. The blasts superficially resemble lymphoblasts. Flow cytometry is needed to identify these blasts which show positivity for CD 41 and CD 61 which are platelet markers.

7. Cytochemistry. Special stains are done to differentiate ALL from AML. These are
 - A. Myeloperoxidase stain and Sudan Black B stains are negative in ALL.
 - B. Periodic Acid Schiff stain may show block positive staining in the cytoplasm of ALL blasts with no differentiation between T and B cells.
 - C. Esterase stains are done to differentiate the monocyte cell line
8. Immunophenotyping is done to identify the myeloid, monocytoid, erythroid and megakaryocyte cell lines.
9. Bone marrow examination shows solidly cellular marrow with suppression of normal erythropoiesis, myelopoiesis and megakaryocytes. The marrow is replaced by proliferating blasts.
10. Biochemical tests show raised serum uric acid levels, raised serum LDH and serum creatinine may be elevated
11. Cytogenetic studies are performed to demonstrate typical abnormalities in certain leukemias and to monitor response to treatment.

23.5 CHRONIC MYELOID LEUKEMIA (CML)

23.5.1 Clinical Presentation

This condition affects all age groups mostly young adults. There is anaemia, fever, weight loss, sweating, bone pain and enlarged spleen. The leukemia runs a chronic course of 2 – 5 years unless treated and transforms into acute leukemia, myeloblastic or lymphoblastic as a terminal event.



Notes

23.5.2 Laboratory Diagnosis

1. Haemoglobin, PCV, RBC count are decreased.
2. MCV, MCH and MCHC are normal RDW is normal. There is normochromic normocytic anaemia.
3. WBC count is elevated ($100.0 - 800.0 \times 10^9/L$).
4. Platelet count is normal or increased.
5. The blood film looks like a bone marrow preparation because of the marked leukocytosis. The red cells show normochromic normocytic anaemia. The entire series of myeloid cells are seen. Myeloblast make up 2 -5% of the cells , myelocytes ~ 20%, neutrophils ~20%. There is an increase in basophils which make up 5 – 10% of the cells. Eosinophils may also be increased. Lymphocytes are normal. Platelets may be normal or increased. Figure 23.7. A falling haemoglobin, increasing blast percentage and increase in basophils indicate blast transformation.

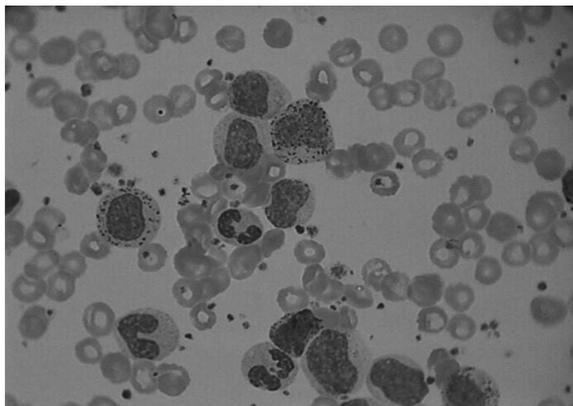


Fig. 23.7: Chronic Myeloid Leukemia

6. The bone marrow is solidly cellular with no fat spaces, There is marked myeloid hyperplasia with all stages of maturation, basophilia and increased megakaryocytes. The erythroid series is normal
7. Cytochemistry Leukocyte Alkaline phosphatase (LAP)

Neutrophils contain alkaline phosphatase enzyme in their granules. The abnormal neutrophils in CML have markedly decreased levels of this enzyme. Blood smears made from finger prick are stained using a phosphate substrate, alkaline buffer at pH 9.0, a coupling azo dye and a counter stain. In the presence of the enzyme the reaction product is seen as blue or brown (depending on azo dye used) granular deposit in neutrophil cytoplasm. In CML neutrophils stain negative for the enzyme.

8. Cytogenetics

A typical translocation between 19 :23 called the Philadelphia chromosome is demonstrated in CML.

9. Biochemistry

Increased serum LDH, serum uric acid and serum vitamin B₁₂ are seen

23.6 CHRONIC LYMPHOCYTIC LEUKEMIA

23.6.1 Clinical Presentation

This condition affects older age groups and is common in Caucasians. There is anaemia, fever, weight loss, sweating, enlarged lymph nodes and spleen. The leukemia runs a chronic course of 2 – 5 years unless treated and may be associated with frequent infections because of depressed immunity.

23.6.2 Laboratory Diagnosis

1. Haemoglobin, PCV, RBC count are mildly decreased or normal
2. MCV, MCH and MCHC are normal RDW is normal.
3. WBC count is elevated ($50.0 - 250.0 \times 10^9/L$).
4. Platelet count is normal. Rarely there may be immune thrombocytopenia.
5. 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. Figure 23.8.

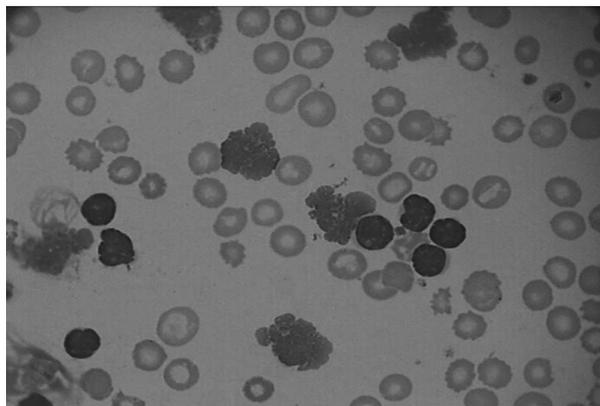


Fig. 23.8: Chronic Lymphocytic Leukemia

6. The bone marrow is solidly cellular with no fat spaces. 90% of the cells in the marrow are mature lymphocytes and a relative decrease in erythroid and myeloid series. Megakaryocytes are normal.



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Leukemia

7. Flow cytometry shows the presence of B cell markers with one aberrant T cell marker, namely CD5
8. Biochemistry
Increased serum LDH, serum uric acid may be seen

23.7 CHRONIC MYELOPROLIFERATIVE DISORDERS

This is a group of disorders where there is proliferation of the various components of the marrow leading to a chronic disease. These include:-

- (a) Chronic myeloid leukemia – as described above
- (b) Polycythemia rubra vera – erythrocytosis, leukocytosis and thrombocytosis
- (c) Myelofibrosis - abnormal megakaryocyte proliferation leads to deposition of fibrous tissue in marrow. The normal haematopoiesis takes place in other organs like liver and spleen (extramedullary haematopoiesis) resulting in a leukoerythroblastic blood picture.
- (d) Essential thrombocythemia – proliferation of abnormal megakaryocytes giving rise to thrombocytosis with abnormal platelets which also have platelet dysfunction
- (e) Chronic myelomonocytic anaemia

23.8 MYELOYDYSPLASTIC SYNDROMES OR PRELEUKEMIA

In this group of disorders the maturation of cells is abnormal and dysplastic and may involve one or more cell line in the marrow. These conditions usually progress to acute leukemia There are several types of MDS which include

- (a) Refractory anaemia – persistent unexplained anaemia in older age group patients. Bone marrow shows evidence of erythroid hyperplasia with dysplastic maturation.
- (b) Sideroblastic anaemia – persistent unexplained hypochromic microcytic anaemia in older patients with the presence of increased iron stores and ringed sideroblasts in marrow.
- (c) Refractory anaemia with excess blasts (RAEB) Marrow shows 5 - 20 % myeloblasts and the cell lines show dysplasia
- (d) RAEB –T refractory anaemia with excess blasts in transformation. This condition is reclassified as AML with dysplasia in the WHO classification



INTEXT QUESTIONS 23.2

1. Basophils are increased in
2. Inclusions diagnostic of Acute Myeloid Leukemia are called
3. A special cytochemical stain done in Chronic Myeloid Leukemia is
4. A Chromosomal abnormality seen in Chronic Myeloid Leukemia is
5. A Special stain used to differentiate myeloblast from lymphoblast is
6. FAB classification stands for
7. Promyelocytes contain granules.
8. The characteristics of Chronic Lymphocytic Leukemia in peripheral blood are and



WHAT HAVE YOU LEARNT

- Leukemia is characterized by uncontrolled, abnormal & widespread proliferation of leukocytes and their precursors in bone marrow and blood
- French – American – British classification is based on morphological appearance of blasts in peripheral blood & bone marrow
- WHO classification of leukemia includes morphology, immunophenotyping of blasts & cytogenetic studies
- Acute leukemia is of sudden onset & slow progression and is characterised by presence of more mature cells in peripheral blood and bone marrow
- Leukocyte cytochemistry uses techniques to identify enzymes or other cytoplasmic products in cells
- Common cytochemical stains used are Myeloperoxidase, Sudan Black B (SBB), Periodic Acid Schiff reaction (PAS), Acid Phosphatase, Esterase stain, Azo dye, Toluidine Blue stain
- Immunophenotyping is done to determine whether the cells are of myeloid or lymphoid origin
- Cytospin of Cerebrospinal fluid is used to detect the presence of small numbers of lymphoblasts in CSF in ALL

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Notes

Leukemia

- Acute Lymphoblastic Leukemia(ALL) affects children but also seen in adults
- In ALL the blood film shows normochromic normocytic anaemia, leukocytosis and megakaryocytes
- In FAB classification there must be >30% blasts and in WHO classification there must be >20% blasts in marrow
- Acute Myeloid Leukemia (AML) affects young adults, blood film shows normochromic, normocytic anaemia, leukocytosis with many blast cells & thrombocytopenia
- Chronic myeloid leukemia affects all groups mostly young adults
- Blood film shows marked leukocytosis, the red cells show normochromic normocytic anaemia
- Cytochemical stain of Leukocyte Alkaline Phosphatase (LAP) is useful in the diagnosis
- Chronic Lymphocytic Leukemia affects older age group
- In CLL, the blood film shows, the red cells are normochromic normocytic and a persistent absolute lymphocytosis



TERMINAL QUESTIONS

1. Classify Acute Lymphoblastic Leukemia
2. Describe FAB classification of Acute Myeloid Leukemia
3. Describe Diagnostic tools in Leukemia
4. Write briefly on Chronic Myeloid Leukemia
5. Write briefly on Chronic lymphoblastic leukemia
6. Write briefly on Myelodysplastic syndromes
7. Write briefly on chronic myeloproliferative disorders.



ANSWERS TO INTEXT QUESTIONS

23.1

1. Presence of blast cells
2. Presence of more mature cells

Leukemia

3. Myeloperoxidase, Sudan Black B, Periodic Acid Schiff reaction, Acid Phosphatase, Esterase, Azo dye, Toluidine Blue stain
4. Periodic Acid Schiff (PAS)
5. Acid phosphatase
6. Esterase stain
7. Immunophenotyping
8. Lymphoblasts

23.2

1. Chronic Myeloid Leukemia
2. Auer rods
3. Leukocyte Alkaline Phosphatase
4. Philadelphia
5. Sudan Black B stain
6. French-American-British
7. Azurophilic
8. Leukocytosis and absolute Lymphocytosis

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