Cytochemical Stains in Haematology

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Cytochemical stains are special stains used for staining peripheral blood and bone marrow smears that help in classifying and differentiating different types of leukaemias. In resource poor countries like ours, role of special stains cannot be ignored as they are cheap, time saving, easy to perform, yet effective tools in differentiating different types of leukaemias.

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Leukocyte Alkaline Phosphatase (LAP)

LAP is present in the cytoplasm of neutrophils, eosinophils, osteoblasts, B lymphocytes and endothelial cells. This activity can be quantified by scoring from 0 to 4+ based on the intensity of the staining. The cell that doesn't stain is given a score of zero and that has strong staining is given a score of 4. The total score is calculated counting the individual score of 100 neutrophils or bands. Normal LAP score ranges from 15 to 130.

Purpose
Helps in differentiating Chronic myeloid Leukemia from leukemoid reaction as both show a left shift with increased precursors. While leukemoid reaction will show increased LAP score, it is very low in CML.

Principle
Alkaline phosphatase activity is present in varying degrees in the neutrophil and band form of the granulocytes.

Interpretation
Count 100 neutrophils and score them (0 to +4), then calculate the final score by adding the total scores.

Grading
Score 0- no stain, 1+means faint staining, 2+ means moderate staining, 3+ means strong staining with cytoplasmic background, 4+ means strong staining without cytoplasmic background.
Normal Range: 15-130

LAP is Elevated in

1. Leukemoid reaction.
2. Pregnancy
3. Other myeloproliferative disorders except CML
4. Aplastic anaemia.
5. Multiple myeloma
6. Obstructive jaundice
7. Hodgkin's disease
8. Oral contraceptive drug intake
9. Growth factor therapy

LAP is Decreased in

1. Chronic myeloid leukemia
2. Paroxysmal nocturnal haemoglobinuria
3. Sickle cell anaemia
4. Hypophosphatasia
5. Myelodysplastic syndromes

Diseases Not Affecting LAP Result

1. Untreated haemolytic anaemia.
2. Viral hepatitis.
3. Secondary polycythaemia.

Myeloperoxidase Stain

Purpose

To differentiate acute myelogenous or monocytic leukemia from acute lymphocytic leukemia.

Principle

Myeloperoxidase is present in the primary azurophilic granules of neutrophils, eosinophils and monocytes & activity is increased with maturation and no activity is found in red cells or lymphocytes.

Interpretation

- Red-brown granules are found in neutrophil and myeloid precursor cells.
- Finely granular staining found in Monocytes.
- Negative stain found in lymphoblast, basophils and plasma cell.

Sudan Black B

Purpose

To distinguish acute myelogenous and monocytic leukemia from acute lymphocytic leukemia.

Principle

Sudan black B dye is fat soluble, then it stains fat particles (Sterols, phospholipids and neutral fats) which is present in the primary and secondary granules of myelocytic and monocytic cells.

Interpretation

- Myelogenous cells show coarse staining granules with faint staining pattern for myeloblast and increase staining with maturation.
- Auer rods are +vely stained.
- Monocytic cells show finely scattered granules.
- Negative staining in lymphocytes except Burkitt`s lymphoma cells, may show +ve staining vacuoles.

Acid Phosphatase (ACP)

ACP is present in all hematopoietic cells

Principle

ACP enzyme is present in myelocytic, lymphocytes, monocytic cells, plasma cell and platelets but in these cells ACP activity will inhibited in the presence of tartrate and give no colour, while in hairy cell leukemia, ACP will not be inhibited and gives positive reaction.

Esterases

There are two types of esterases, specific and non-specific. The specific esterase is naphthol AS-D chloroacetate esterase (CAE). These identify the cells of the granulocytic series. It does not stain lymphocytes and monocytes. It can even be used to identify granulocytes in formalin fixed tissues.

The nonspecific esterase activity is seen in monocytes. Both α-naphthyl butyrate or α-naphthyl acetate can used. The cells of granulocytic series are negative with this stain. The α-naphthyl butyrate stain more specific, while the α-naphthyl acetate stain is more sensitive.

In a suspected acute myeloid leukemia, dual esterase stains can be used as they can simultaneous classify the blasts as either myeloblasts or monoblasts. Monocytic nonspecific esterase is Fluoride sensitive.

Periodic Acid-Schiff

The periodic acid-Schiff (PAS) stain indicates the presence of intracellular glycogen and neutral mucopolysaccharides. Cells of many series are positive for PAS, however the staining pattern differs. Erythroleukemia i.e. AML M6 blasts show an intense diffuse cytoplasmic positivity with PAS, while lymphoblasts show block positivity.

Toluidine Blue

Toluidine blue reacts with the acid mucopolysaccharides in the granules of basophils and mast cells to form metachromatic complexes, however, malignant basophils or mast cells may not show a positive reaction.

Perls Stain (Prussian Blue Reaction)

Siderotic granules are found in the cytoplasm of developing cells in bone marrow in the form of Ferric ions. Ferric iron deposits in tissue (mainly as non heme iron like ferritin and hemosiderin) react with the soluble ferrocyanide present in the stain to form the insoluble Prussian blue pigment (a complex hydrated ferric ferrocyanide substance). These deposits are then visualizable microscopically as blue or purple deposits.

Uses

- To assess iron reserves in bone marrow.
- Hereditary hemochromatosis.