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Hematological investigation

Hematological parameters

- 1. Complete blood count (CBC)
- 2. Blood film (BF)
- 3. Erythrocyte sedimentation rate (ESR):
- 4. Reticulocyte count
- 5. Bone marrow examination

Complete blood count (CBC)

Full blood count including Hb, leucocyte count, Platelets count, red cell indices and leucocyte differential count

ESR (erythrocyte sedimentation rate)

- is the measurement of the sedimentation of red cells in diluted blood after standing for 1 h in an open-ended glass tube of 30 cm length mounted vertically on a stand.
- Rate of sedimentation depended on many factors like difference in specific gravity of RBC and plasma, Rouleaux formation, ratio of RBC and plasma
- > depend mainly on plasma proteins as fibrinogen and other acute phase proteins that increase the red cells Rouleaux and extend of their sinking
- The ESR value is influenced by many factors like age and drugs intake

Clinically useful in dx of many disease

Erythrocyte Sedimentation Rate (ESR)



1hr



The distance, in mm, the RBC fall in 1 hr is the Sed Rate

TABLE 6-1

ERYTHROCYTE SEDIMENTATION RATE RANGES IN HEALTH

Age Range (Years)	ESR Mean (mm in 1 h)
10–19	8
20–29	10.8
30–39	10.4
40–49	13.6
50-59	14.2
60–69	16
70–79	16.5
80–91	15.8
Pregnancy	
Early gestation	48 (62 if anaemic)
Later gestation	70 (95 if anaemic)

Blood film

stained with Leishman stain, is examined for red cells morphology, leucocytes morphology and differential count and for platelets assessment.

Preparing thick and thin films



 Touch one drop of blood to a clean slide.



2. Spread the first drop to make a 1 cm circle.



3. Touch a fresh drop of blood to the edge of another slide.



- 4. Touch the
- drop of blood by spreader slide at 45 degree angle.
- **5**.Pull the drop of blood across the first slide in one motion.
- 6. Wait for both to
- dry before fixing

and staining.

Bloo mear

Definition of Terms in hematological disorders:

- Normocytic
- Normochromic
- Hypochromic
- Anisocytosis
- Poikilocytosis
- Polychromasia
- Leukocytosis
- Leucopenia:
- Thrombocytosis:
- Thrombocytopenia:

Red Cells morphology

	Red cell abnormality	Causes		Red cell abnormality	Causes
\bigcirc	Normal		\bigcirc	Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
\bigcirc	Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia		Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
\bigcirc	Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy	\bigcirc	Elliptocyte	Hereditary elliptocytosis
\bigcirc	Stomatocyte	Liver disease, alcoholism	\bigcirc	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
	Pencil cell	Iron deficiency	\bigcirc	Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
*	Echinocyte	Liver disease, post-splenectomy. storage artefact	L	Sickle cell	Sickle cell anaemia
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Acanthocyte	Liver disease, abetalipo- proteinaemia, renal failure	$\bigcirc$	Microcyte	Iron deficiency, haemoglobinopathy

## Reticulocytes count

- is the percentage of immature circulating RBC as detected by a special stain for them (New Methyelene blue stain),
- normal range 0.2-2.5%.



## **Blood leucocyte morphology**



Monocyte

Plasma cell

Atypical lymphocyte

## Normal blood films



## Hypochromic and microcytic anemia



## peripheral blood in a patient with severe iron deficiency anemia.



## Hereditary elliptocytosis.



## homozygous beta thalassemia

peripheral blood smear in a patient with sickle beta thalassemia.



## Stomatocytes



#### An example of a hypoproliferative anemia is seen in this patient with multiple myeloma.





peripheral blood smear shows sickle cells in a patient with homozygous sickle cell disease



This new methylene blue preparation is from a patient with G-6-PD deficiency



Acquired autoimmune hemolytic anemia

peripheral blood is from a patient with marked acquired autoimmune hemolytic anemia,



#### Striking "burr" and "spur' cell abnormalities



This low oil magnification view of the peripheral blood shows moderate variation in size and shape of the red cells as well as some variation in their degree of hemoglobinization. Several teardrop cells are seen in this field



#### Microangiopathy:MAHA

Thrombotic thrombocytopenic purpura (TTP)



## Bone marrow examination

- There are two types of marrow procedures:
  Bone marrow aspirate:
- Bone marrow sets



#### Common sites for Bone marrow procedures in adults



Manubrium Sternii

**Bone marrow** aspirate smear

11075



## Cells seen in bone marrow





Red Cell precursors (in marrow)

#### Stages of Maturation of the Granulocytic Series

#### In the bone marrow



## Megakaryocyte : the precursor of Platelets in the marrow



#### NORMAL RANGES FOR DIFFERENTIAL COUNTS ON ASPIRATED BONE MARROW

	95% Range	Mean*	Mean [†]
Myeloblasts	0–3	0.4	1.4
Promyelocytes	3-12	13.7*	7.8
Myelocytes (neutrophil)	2-13		7.6
Metamyelocytes	2-6	_	4.1
Neutrophils	22-46	35.5	32.1 ^M ; 37.4 ^F
Myelocytes (eosinophil)	0-3	1.6	1.3
Eosinophils	0.3–4	1.7	2.2
Basophils	0-0.5	0.2	0.1
Lymphocytes	5-20	16.1	13.1
Monocytes	0-3	2.5	1.3
Plasma cells	0-3.5	1.9	0.6
Erythroblasts [§]	5-35	23.5	28.1 ^M ; 22.5 ^F
Megakaryocytes	0-2		0.5
Macrophages	0-2	2.0	0.4

bone marrow aspirate shows relatively normal distribution of both erythroid and myeloid precursors. The former are identified by their somewhat clumped nuclear chromatin in a majority of cases



## Indication of BMA

- in virtually all patients with suspected ALL, AML CML, myelodysplastic syndrome (MDS) or multiple myeloma ,However, a bone marrow aspirate is not necessary for the diagnosis of CLL.
- The diagnosis of promyelocytic leukaemia may be more readily made on an aspirate than on the peripheral blood
- The characteristic cytological features of AML with inv(16) or t(16;16) are apparent in the bone marrow
- In the acute leukaemias and in CML and other myeloproliferative neoplasms, a bone marrow aspirate provides material for cytogenetic analysis as well as for morphological assessment

## Bone marrow biopsy

- here a core of bone marrow tissue is taken, and processed and stained as in histopathological specimens (H&E stain)
- it should be performed only when there is a clear clinical indication.
- only a trephine biopsy shows the architecture of the bone marrow and permits the detection of an abnormal distribution of cells, bone marrow granulomas, and focal lymphoid infiltrates

#### Definite ( indications)

- 1. Investigation of suspected Hodgkin's disease and non-Hodgkin's lymphoma
- 2. Staging of non-Hodgkin's lymphoma
- 3. Diagnosis and follow up of hairy cell leukemia
- 4. Evaluation and follow up of chronic lymphocytic leukemia
- 5. Diagnosis of suspected metastatic carcinoma

#### Possible indications

- 1. Investigation of suspected acute myeloid leukemia
- 2. Investigation of suspected myelodysplastic syndrome
- 3. Staging of Hodgkin's disease
- 4. Evaluation of chronic myeloid (granulocytic) leukemia
- 5. Investigation of suspected primary amyloidosis

Components of the normal bone marrow trephine

- 1. Bone
- 2. Stroma: vessels, reticulin, fibroblasts, fat, iron
- 3. Hematopoietic tissue: granulocytic, erythroid, Megakaryocytic
- 4. Other cells: lymphoid, plasma cells, mast cells

## Bone marrow biopsy slide-core of BM





#### Bone marrow section stained with H&E stain



FIGURE 7-14 Photomicrographs of sections of bone marrow. Iliac crest bone marrow: illustrating the range of cellularity. (A) Hypocellular marrow; (B) normal cellularity; (C) hypercellular marrow.

## Performing the Bone Marrow Aspiration & Biopsy

