University of Mosul
Lecture No.: 2
College of Veterinary Medicine
Date: November 2024
Unit of Scientific Affairs
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Lecture title: **Bone Marrow Examination**

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Summary:

Bone Marrow Examination: Collection (aspiration) of bone marrow cells for cytological analysis is a valuable consideration when examining cytopenia's (without identifiable cause), identifying blood cell morphologic abnormalities on blood smear, or staging neoplasia, decrease or increase in cellular elements or with appearance of abnormal cellular forms. Most common contraindications may include anesthesia risks and infection of overlying soft tissue. Evaluation of bone marrow cytological samples can provide a more complete overview of the disease process.

Indications for Bone Marrow Evaluation

1. Persistent depression in RBCs, neutrophils, or platelets without evidence of regeneration (i.e., reticulocytosis, bands)

2. Unexplained, persistent elevation in peripheral WBCs.

3. Presence of abnormal cells (e.g., megaloblastic cells, rubricytes, neutrophil hypersegmentation, giant platelets) in peripheral blood.

4. Suspicion of bone marrow dysfunction.

5. Staging for certain hemolymphatic cancers.

6. Unexplained persistent hypercalcemia (dogs).

7. Unexplained monoclonal or polyclonal gammopathy.

8. Fever of unknown origin.

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Bone Marrow Aspiration:

Equipment's needed for sterile aspiration:

- Syringe (20 ml) with metal tip.
- 2 syringe (10 ml) with metal tip.
- 2-3 hemostats.
- Knife handle and blades.
- 2 bone marrow needles, there size and length will depend upon the species of animal
- Numerous clean slides (about 20).
- Different surgical equipment's, and local or general anesthesia.

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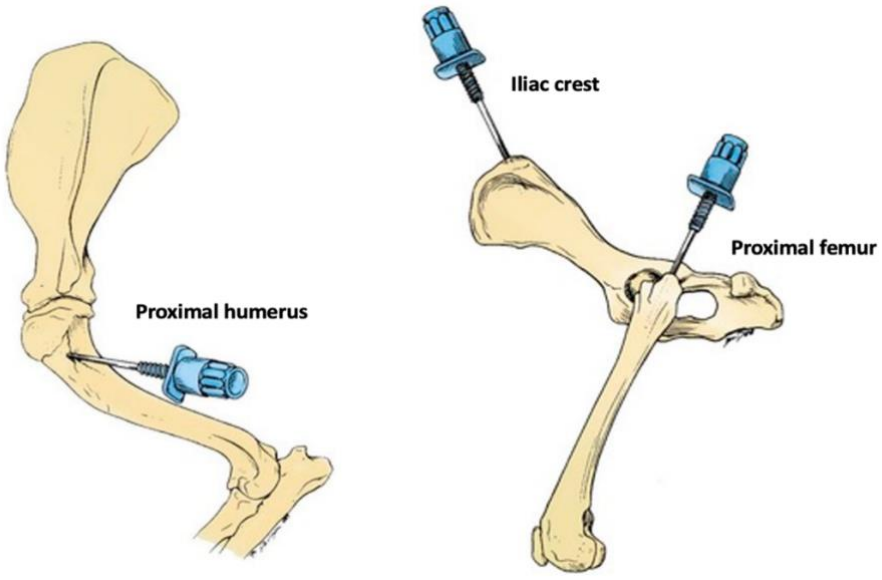
Different sites used for bone marrow aspiration:

Different sites could be used for bone marrow aspirated samples since bone marrow samples is readily obtained either from:

1. Trochanteric fossa
2. Iliac crest
3. Sternum or humerus



How to aspirated bone marrow samples:

1. Under general or local anesthesia and after aseptic surgical preparation of the surface, a short skin incision is made to facilitate penetration.

2. A sterile aspiration needle with a stylet is passed through the skin and muscle. When the needle is forced into the bone by steady pressure accompanied by rotation. When the needle becomes firmly embedded, it has usually penetrated the medullary cavity.

3. A stylet is utilized to free the lumen of the needle of tissue and bone particles, and a dry 20 ml glass syringe is fitted to the needle.

4. The plunger of the syringe should be pulled out a considerable distance to establish a vacuum and withdraw marrow fluid. Only a small amount of fluid (0.5-1.0 ml) should be aspirated.

5. As soon as fluid appears, vacuum should be discontinued, as further negative pressure may result in rupture of a sinusoid and contamination with peripheral blood.

How to Prepare Bone Marrow Smears:

a. After aspiration sonly, prepare a bone marrow smear similar to that used in preparation of blood film.

b. A few drops of marrow are placed on the end of a slide and excess blood is aspirated back into the syringe. If tissue fragments are aspirated, a & quote; squash & quote; preparation is made by placing a second slide firmly down on top of the marrow particles and very carefully drawing the two slides apart. Moreover, slides should be waved in the air for rapid drying.

c. Bone marrow smear stains with any good polychrome stain, such as Wright or Wright-Giemsa, the smear should be exposed to stain for a longer period of time than is necessary for peripheral blood smear.

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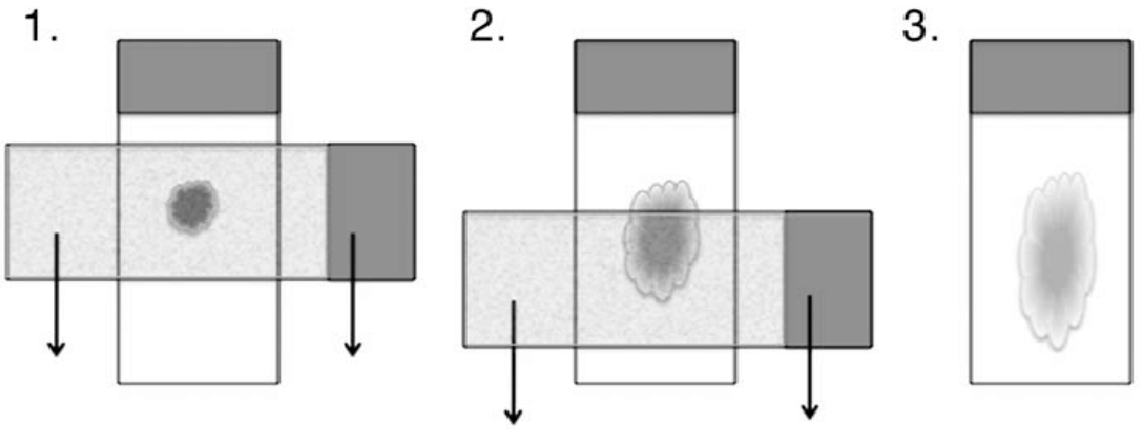


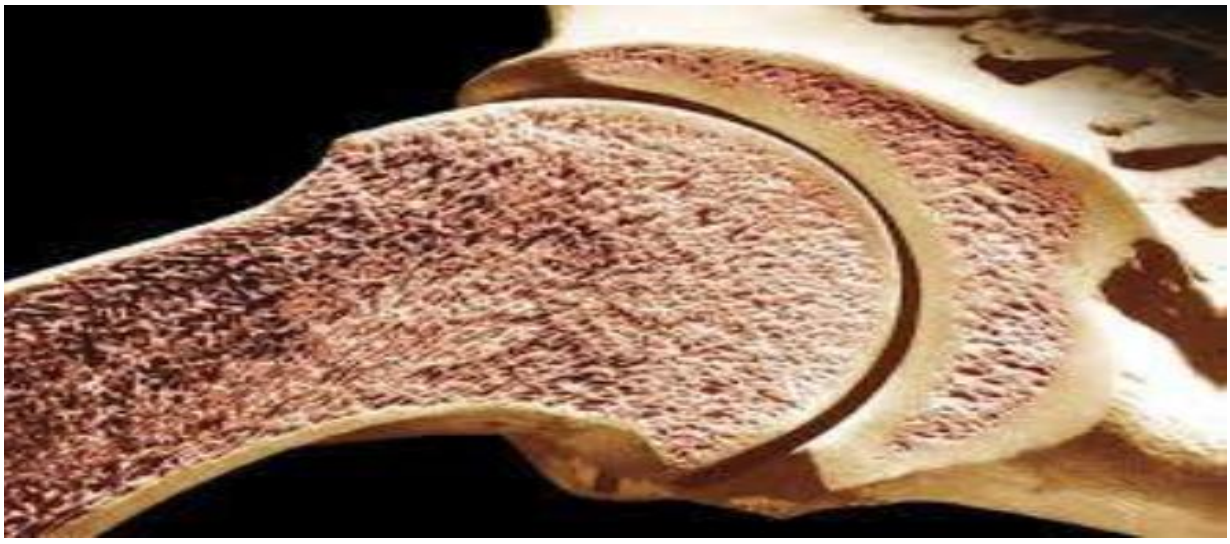
How to Prepare Bone Marrow Section:

Bone marrow sections may be also prepared by one of the following methods:

1. Remove the amount of marrow required for preparation of smears and permit the remainder of the aspirate to clot in the syringe.

2. This clot is placed in a fixative for sectioning.

3. Collect the bone marrow in an anti-coagulant, place it on a slide, and the excess blood is removed from the edge of the slide with gauze. Place the specimen in a fixative for centrifugation and later sectioning.



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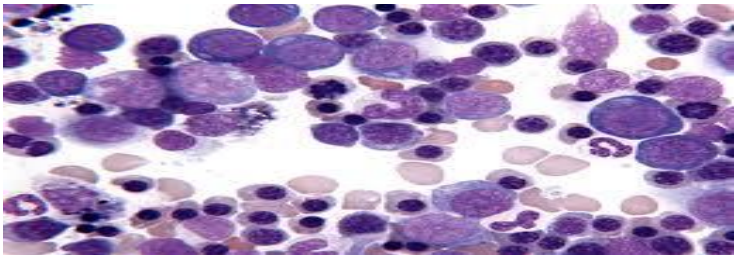
How to examine Bone Marrow Film:

Generally, there are two methods for examination of a marrow film:

1. Scanning the slide under the low power of the microscope, then under the high dry objective, and finally under oil immersion magnification. This method gives the operator possibility to formulate impressions concerning the number and distribution of cells.
2. Making a differential count and calculating the percentage of each cell type. A minimum of 500 cells should be examined, and it is preferable to count 1000 cells.
3. The cellularity of the smear can be evaluated using low power magnification.

Note: Older animals have more fat, while younger's have less fat



Identification of cells :

All cells that develop in bone marrow had been changed morphologically as they progress from primitive to mature types. Primitive cells are usually larger than mature cells, and the nuclei of these young cells are relatively large in relation to the amount of cytoplasm.

Erythrocytes series:

The developmental stages (from immature to mature) of the erythrocytes are:

rubriblast → prorubricyte → rubricyte (from basophilic → polychromatophilic → normochromic) → metarubricyte → reticulocyte →erythrocyte.

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Leukocytes series:

Granulocytic series:

The developmental stages (from immature to mature) of the granulocytes are:

myeloblast → progranulocyte → myelocyte →metamyelocyte → band cell → segmented granulocyte.

Lymphocytic series:

Lymphocytes are formed in lymphoid tissues in many parts of the body, and a few them are formed in the marrow. The developmental stages (from immature to mature) of the lymphocytes are:

lymphoblast → prolymphocyte→ lymphocyte.

Monocytic series:

Young forms of monocytes, particularly mono-blasts, may be difficult to differentiate from other immature cells in marrow.

Myeloid to Erythroid Ratio (M: E):

The most significant information available following bone marrow examination is the ratio of myeloid to erythroid cells (M: E). This ratio is calculated by dividing the number of all granulocytic cells (myeloid) of bone marrow by the total number of nucleated erythroid cells.

Interpretation of the M:E ratio:

It can be made only in relationship to the total leukocyte count of peripheral blood.

The M:E ratio increases when there is:

- Increase in granulocyte production and Erythroid hypoplasia.
  The M:E ratio decreases when there is:
- Decrease in granulocyte production and Erythroid hyperplasia.

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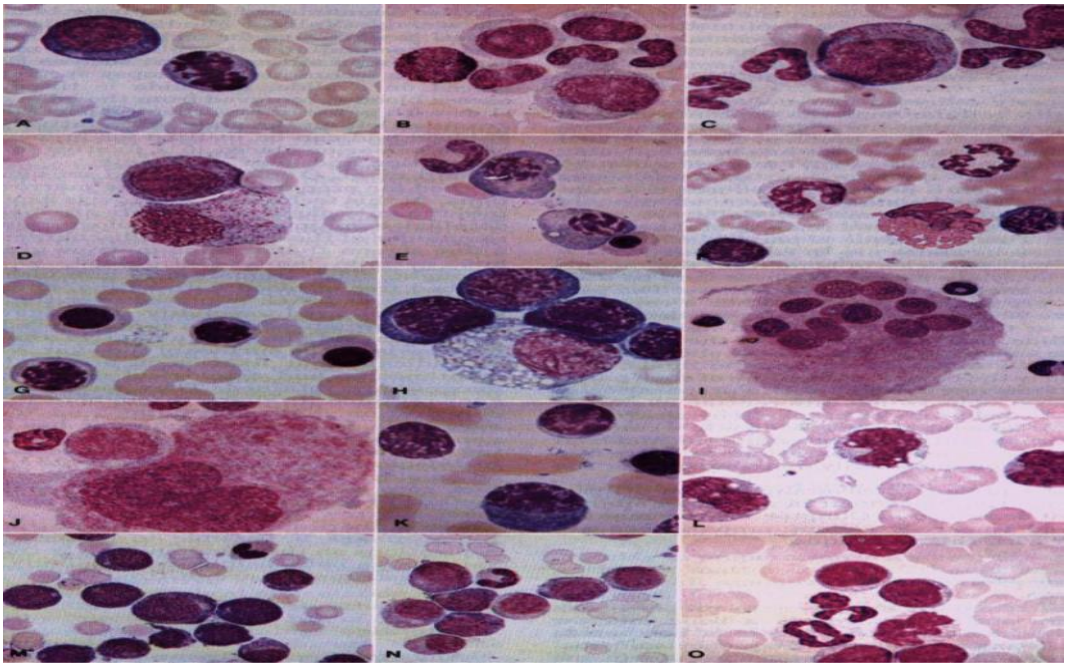

A- Blast cell (500x)
B- Neutrophilic myelocyte (500x)
C- Progranulocytes (500x),
D- 1: early progranulocytes, 2: granular histiocytes (500x),
E- 1: plasma cell, 2: metarubricyte (500x)
F- rubricyte (500x)
G- 1: metarubricyte, 2: polychromic rubricytes (500x)
H- 1: neutrophilic myelocyte, 2: basophilic rubricyte (670x)
I- multinucleated osteoclasts (200x)
J- 1: megakaryocyte, 2: neutrophilic myelocyte (500x)
K- 1: equine lymphocytes, 2: late rubricyte, 3: plasma cell (670x), L- 3 monocytes, note their vacuolated cytoplasm (500x)
M- immature mononuclear cell with a basophilic cytoplasm (peripheral blood film 330x)
N- immature mononuclear cell (330x)
O- peripheral blood film with lyphosarcoma characterized by presence of large lymphocytes.