

Bone marrow examination

It is a valuable diagnostic tool it's referring to the pathological analysis of samples of bone marrow obtains by bone marrow biopsy and bone marrow aspiration.

Indication

1. Whenever anemia, neutropenia, or thrombocytopenia is identified and no cause is apparent.
2. Whenever excessive production of neutrophils or lymphocytes is present and no cause is apparent.
3. The examination of the marrow can lend added support to the suspicion that hypoplasia, hyperplasia, or neoplasia of the marrow is associated with the disorder of the peripheral blood.
4. In diseases characterized by abnormalities of the blood when it is impossible to confirm a diagnosis by examination of peripheral blood.

Limitations of bone marrow examination

1. Difficult interpretation of result of bone marrow examination.
2. Bone marrow biopsy it is difficult to obtain a sample that is not contaminated with peripheral blood.
3. The concentration of cellular elements in the marrow may differ in bone from different parts of the skeleton.
4. Impossible to make a quantitative evaluation of cellular elements in bone marrow as no reproducible technique for total count of the nucleated cells in bone marrow.
5. Accurate interpretation of bone marrow examination is dependent up on the experience and ability of individual examining the smear to differentiate various cell types.

Sites used for bone marrow aspiration:

1. Small Animals - Iliac crest.
2. Large Animals - Rib or sternum



Equipment Needed

1. The aspiration of marrow can be accomplished without special equipment by using a Steinman pin to make a hole in the bone cortex and an ordinary syringe and needle to aspirate the marrow.
2. The procedure is greatly simplified by the use of a special bone marrow needle. If one is using the iliac crest, a needle approximately 5/8" long, 18 G is desirable. For the femoral site, a longer needle 1 1/2", 16 G is more useful.

Aspiration Technique

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. The stylet is removed a 10 to 20 ml syringe is attached and with a quick sudden movement the plunger is withdrawn.
4. If the marrow cavity has been entered and the lumen of the needle is free of bone a small amount of marrow will appear in the syringe.
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.
6. Subsequently the biopsy is performed if indicated a different larger trephine needle is inserted in the bony cortex. The needle is there advanced with a twisting motion and rotated to obtain a solid piece of bone marrow.

Preparation of Bone Marrow Smears

As soon as possible after aspiration, prepare a bone marrow smear similar to that used in preparation of blood film.

1. 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 "squash" preparation is made by placing a second slide firmly down on top of the marrow particles and very carefully drawing the two slides apart.

2. Slides should be waved in the air for rapid drying.
3. Bone marrow smear stains with any good polychrome stain, such as Wright's, Wright-Giemsa . The smear should be exposed to stain for a longer period of time than is necessary for peripheral blood smear.

Preparation of Bone Marrow Sections

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. This clot is placed in a fixative for sectioning.
2. 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. Marrow granules left on the slide are relatively free of blood and can be utilized for preparation of the smear or placed in a fixative for centrifugation and later sectioning.

Examination of Bone Marrow Film

In general, there are two ways in which a marrow film is examined:

1. Put 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. Make 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.
4. Older animals have more fat, while younger animals have less fat.

Cell Identification:

All cells that develop in bone marrow alter 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 → metarubricyte → reticulocyte → erythrocyte.

Leukocytes series:

(1) Granulocytic series:

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

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

(2) Lymphocytic series:

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

lymphoblast → prolymphocyte → lymphocyte.

Interpretation of Bone Marrow Examination

Accurate classification of the immature cells of the granulocytic and erythrocytic series requires much study and practice. It is helpful to study color plates of cells of bone marrow and blood films.

1. A differential count is made of 500 nucleated cells. A bone marrow evaluation form should be used to record the names of all cell types.
2. The G:E ratio (**also called the M:E ratio**) is determined by dividing the number of nucleated red cells into the sum of all cells belonging to the granulocyte series, (**proportion of granulocytes to nucleated red cells**).
 - a. An increased G:E ratio indicates that the granulocytes are relatively more numerous than usual. This indicates either granulocytic hyperplasia or erythrocytic hypoplasia. Correlation of the bone marrow findings with the peripheral blood findings usually indicates which of these problems is present.
 - b. A decreased G:E ratio has the opposite significance from an increased ratio, i.e., increased erythropoiesis or decreased granulopoiesis.
 - c. A G:E ratio of 1:1 would mean that nucleated cells of both series are present in equal numbers.