

Bone Marrow Biopsy Technique and Evaluation

Bone marrow is indicated when the routine examination of a blood smear fails to provide an answer to the question: What is causing an observed hematologic abnormality?

Abnormalities may involve persistent unexplained cytopenias as well as proliferative abnormalities. In general practice, these conditions would be the most common reasons for the submission of a bone marrow aspirate for cytologic examination. It is important to know however that examination of bone marrow may also be used for other conditions such as staging neoplastic conditions (such as lymphomas), the estimation of the adequacy of body iron stores, and the investigation of occult disease in animals with fever of unknown origin, unexplained weight loss and unexplained malaise.

In veterinary medicine, bone marrow aspirates are done more frequently than core biopsies however ideally both samples should be taken and reviewed along with a current CBC (at least within the 24h period of the biopsy). If all three samples cannot be obtained, the best sample combination would include a bone marrow aspirate and a CBC. It is important to note that interpretation of a bone marrow aspirate is severely limited without the CBC, and as such a diagnosis may not be able to be made.

Advantages of taking an aspirate: they are easier, faster and less expensive to perform than a core biopsy and cell morphology can be reviewed (important for example when looking for evidence of dysplasia in any of the hematopoietic cells).

Advantages of taking a core biopsy: these are an accurate way of assessing marrow cellularity and metastatic neoplasia, and allows for assessment of tissue architectural changes (e.g. stromal, such as myelofibrosis and osteolysis).

Whichever sample is taken, cytologic and/ or histopathologic review by a specialist pathologist is/ are advised. With that said, the general practitioner however should also endeavor to develop the ability for assessing adequacy of the sample prior to submission and in, some cases, be able to make preliminary or presumptive diagnoses based on their diagnostic question.

Specific Indications for Bone Marrow Evaluation

- Non-regenerative anemia without evidence of reticulocytosis/ polychromasia. With persistent poorly regenerative or non-regenerative anemia, a core biopsy is very useful, particularly with hypoplastic or aplastic conditions.
- Persistent neutropenia without a left shift (or evidence of regeneration). Note that it usually takes 3-5 days for left shifting of the granulocytic line to be observed in the peripheral blood.
- Persistent thrombocytopenia. Key note that needs to be made here is that severe thrombocytopenia is NOT a contraindication for bone marrow biopsy as bleeding is

confined within the bone space. Platelet levels as low as 10 - 15 X10⁹/L are considered OK. Also, it is best to check coagulation times to rule out DIC as a cause for thrombocytopenia.

- The presence of abnormal cell morphology in the blood smear, which could indicate a myelodysplastic syndrome or leukemia.
- If leukemia is suspected but no evidence is observed within the peripheral blood (i.e. subleukemic or aleukemic leukemia).
- Unexplained increases in blood cell numbers, including normal and abnormal cells. Increases in the number of normal blood cells can be difficult (and costly) as there are many physiologic/ pathologic conditions which need to be investigated and ruled out prior to examination of the bone marrow. Some examples include:
 - Reactive lymphocytosis due to antigenic stimulation. On cats, lymphocyte counts can reach as high as 20 x 10⁹/l!
 - Reactive leukocytosis due to inflammation (common).
 - Erythrocytosis due to dehydration, splenic contraction and cardiac disease.
 - Reactive thrombocytosis due to inflammation or chronic iron deficiency anemia.
- Unexplained hypercalcemia.
- Persistent polyclonal or a monoclonal gammopathy.
- Focal lymphoma or plasma cell myeloma producing bone lysis. With these cases, particularly with early-stage disease, a core biopsy is best.
- Clinical staging for malignancies and chemotherapeutic drug administration monitoring.
- Evaluation of iron stores for determining iron sequestration within macrophages. Low serum iron can be due to inflammatory disease and iron deficiency anemia however in the bone marrow iron stores are low or absent with iron deficiency anemia and normal to excessive with inflammatory disease. Note that marrow evaluation of iron in cats is not possible because their marrow lacks discernable iron. The presence of iron is an abnormal finding in this species (e.g. post blood transfusion, myeloproliferative disorder, hemolytic anemia).

Sites of Biopsy

Antemortem

For small animals, site selection will be determined by the clinician's preference, age, body size and body condition of the animal.

Humerus – Useful site for obese or very muscular dogs. The craniolateral aspect of the greater tubercle is the preferred site due to the lack of muscle, fat and subcutaneous tissue in this region. For both aspirate and core biopsies, biopsy needles are directed posteromedial. The patient is in lateral recumbency for the biopsy procedure.

Ilium – Useful site for thin or nonobese dogs. The dorsal iliac crest is readily accessible with the patient in lateral or sternal recumbency. Biopsy needles are inserted into the widest part of the iliac crest and directed ventromedial (i.e. to conform to the concavity of the pelvic bone).

Femur – Useful site for small dogs and cats. The sample site is just medial to the greater trochanter and biopsy needles are directed parallel to the shaft of the femur. Be careful to avoid the sciatic nerve which is medial and posterior to the greater trochanter! The patient is in lateral recumbency for this site. Because bone trabeculae are minimal at this site, large quantities of bone marrow can be aspirated however core biopsies can easily be lost.

Postmortem

Bone marrow aspiration samples should be taken within 30 minutes (preferably within minutes) of death because cells degenerate quickly. If longer time is needed, refrigeration (not freezing) is recommended. Avoid taking diaphyseal or mid bone samples as there is less active hematopoietic tissue here and in older samples, are likely to mostly contain fat. The metaphyseal region of the long bones is preferable although cut sections of tissue of the costochondral joint of the ribs or the wing of the ilium may also be used. Cytologic material from cut tissue should be performed shortly after death but even then, cellular detail is not optimum.

Patient Preparation and Equipment

Sedation with local anesthesia is often adequate for aspiration and core biopsy procedures. The biopsy site needs to be clipped and surgically scrubbed and 0.5 to 2ml of 2% lignocaine is then instilled intradermally and deep to the periosteum. General anesthesia can also be used. Drape the area and use a scalpel blade to make a stab incision into the skin. An aspiration biopsy is performed first, followed by the core biopsy of the same bone, with the core biopsy being taken a short distance away from the aspiration biopsy site.

Bone marrow aspirate equipment:

- Aspiration biopsy needle (e.g. Rosenthal needle)
- 12ml syringe
- 3-5% EDTA solution
- Scalpel blade
- Glass slides
- Watch glass/ Petri dish
- Hematocrit capillary tubes or glass pipettes

Core bone marrow biopsy equipment:

- Biopsy needle (e.g. Jamshidi)
- Scalpel blade
- Glass slides
- 10% buffered formalin

Procedure for Bone Marrow Aspiration

- Prepare a syringe by aspirating 0.5ml anticoagulant solution into a 12ml syringe and keep ready. ^[1]_[5P]
- A small stab incision is made over the site to allow smooth access for the needle.

- The bone marrow needle with stylet in place should be inserted through the stab incision, the sub-cutis and seated into the periosteum at the desired site. The needle should be advanced by rotating it to and fro under a firm pressure in one plane, angled towards the medullary cavity. This is a procedure that is best not rushed; take a few moments to seat the needle well into the periosteum and outer cortex. Unless this is done it is easy for the needle to slip off the surface of the bone when pressure is applied to it. ^[1]_[SEP]
- Once the bone marrow needle is properly seated in the medullary cavity of the humerus, movement of the limb will result in the same movement of the needle. This is a good method of checking the correct position. ^[1]_[SEP]
- Remove the stylet, attach the syringe containing anticoagulant solution, and apply firm suction. Release the negative pressure as soon as blood starts to fill the syringe. (It is important not to dilute the marrow tissue with too much blood, so aim to collect 1-1.5ml marrow blood into the 0.5ml pre-loaded anticoagulant solution). ^[1]_[SEP]
- Remove the syringe and squirt the contents into a Petri dish or watch glass and tip the dish to allow the blood to drain off. Alternatively, the contents can be squirted onto glass slides and the slides tipped to allow blood to drain and marrow particles to adhere. Marrow particles appear as tiny glistening flecks or granules and these can be picked up with a glass pipette or hematocrit tubes.
- Stain and review one of your smears in-house to check for sample adequacy.
- Once marrow particles have been placed on the glass slide, squash preparations are then made.
- If no bone marrow blood is aspirated it might be because the needle is not seated into the medullary cavity properly or be due to marrow fibrosis and hypoplasia. Try re-positioning the needle but if no sample is aspirated then another site should be used. ^[1]_[SEP]

Procedure for Bone Marrow Core Biopsy

The approach described above is also used to obtain a limited bone marrow biopsy except that the stylet is removed prior to the instrument becoming embedded in bone. This is a core of bone marrow tissue which is preserved in formalin and sent to a laboratory for histopathological interpretation of bone marrow disease. Evaluation of a bone marrow biopsy may provide valuable information about the nature of the hemopoietic disease, especially when the bone marrow aspirate cytology is not very cellular.

To collect a bone marrow biopsy after first completing a bone marrow aspirate, the stylet should be removed from the needle is advanced by about 1cm by smooth twisting motion of the wrist until it is solidly embedded into the bone. To cut and retain the bone sample the needle is sharply rotated 360 degrees in both clockwise and counter-clockwise directions. Finally withdraw the needle (without replacing the stylet) in a twisting motion and once it is out, then gently push the biopsy tissue out of the end of the needle into a glass slide. The core biopsy can be rolled over the glass slide or alternatively it can be tapped multiple times onto another glass slide. The core sample can then be placed into the tissue fixative.

Biopsy Complications

Complications are rare but may include:

- Tissue injury (e.g. sciatic nerve damage).
- Excessive bleeding.
- Possibly tumor seeding of hematopoietic neoplasms (has occurred in humans, no reported cases in animals).