Abstract

Bone marrow aspiration is a safe and beneficial procedure performed to help with the evaluation and diagnosis of various conditions affecting the bone marrow in dogs. The procedure is most often indicated when abnormalities are identified in peripheral blood. General anesthesia is recommended for those patients that can tolerate it. The most commonly sampled sites are the proximal humerus and the ilium. Samples to submit to a clinical pathologist include a stained slide, unstained slides, and a peripheral blood smear. If the aspirate is nondiagnostic, a bone marrow core biopsy may be needed.

CLINICAL PATHOLOGY

Bone Marrow Aspiration in Dogs: Indications and a Step-by-Step Tutorial

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Bone marrow is located within the center of bones. It is the main hematopoietic organ at birth that produces blood cells (erythrocytes, leukocytes) and platelets. Active marrow is located throughout most flat and long bones of the body in young animals and will recede from long bones with maturity.[1,2] In adults, active marrow persists in the flat bones of the vertebrae, sternum, ribs, and pelvis, along with the proximal ends of the humerus and femur.[1,2]

Bone marrow aspiration and evaluation are performed in conjunction with clinical findings and diagnostics, including a complete blood count, morphologic changes on peripheral blood smears, other laboratory data, and imaging.[3] Bone marrow cytopathology and histopathology are vital tools for understanding diseases and conditions that affect or arise from the bone marrow. Evaluating aspirate and core bone marrow samples provides information about the status of the bone marrow and its ability to correct abnormalities in the peripheral blood and can indicate infection, myelofibrosis, necrosis, or neoplasia.[4,5]

INDICATIONS

Bone marrow aspiration is safe and beneficial. It is most commonly performed when

Take-Home Points

- Bone marrow aspiration is a safe procedure, most commonly indicated for diagnosing peripheral blood abnormalities.
- Bone marrow aspiration is contraindicated for patients that have metabolic bone disease or are not stable enough to undergo sedation or general anesthesia.
- The most commonly used sites for collection are the proximal humerus or ilium.
- Stain 1 slide in-house to look for diagnostic features (e.g., multinucleated megakaryocytes).
- Send the stained slide, the rest of the unstained slides, and a peripheral blood smear or ethylenediaminetetraacetic acid blood sample to a clinical pathologist.
- A bone marrow core biopsy may be needed if the bone marrow aspirate is nondiagnostic and is recommended in cases of bicytopenia or pancytopenia.

abnormalities are detected in peripheral blood, including persistent neutropenia, unexplained thrombocytopenia, nonregenerative or poorly regenerative anemia, or a combination thereof.[5] Any patient with bicytopenia or pancytopenia without adequate regeneration or with blood cells showing abnormal morphology should undergo bone marrow evaluation to determine the source of the disease.[6] Bone marrow can also be examined to stage neoplastic diseases (e.g., lymphoma, mast cell tumors), estimate levels of body iron stores, evaluate lytic bone lesions, and search for suspected occult neoplasia or infection.[5,6] It can also indicate an underlying cause for hyperproteinemia secondary to multiple myeloma, leishmaniasis, and systemic fungal diseases.[5] Bone marrow evaluation is less helpful if the anemia is regenerative or if the underlying cause of the neutropenia is sepsis.[1]

CONTRAINDICATIONS

Bone marrow aspiration is contraindicated for patients who have metabolic bone disease or are not stable enough to undergo sedation or general anesthesia.[7] Sedation combined with local or systemic analgesia or general anesthesia is required because the procedure is associated with moderate pain, the most commonly reported complication.[6,8,9] General anesthesia is recommended for smaller dogs and cats. Complications are rare but can include pain, bleeding, bruising, and fracture if the bone is weak or compromised.[7] Patients with coagulopathies and severe thrombocytopenia can experience prolonged hemorrhage at the biopsy site, which can be controlled by applying direct pressure to the sampling site for several minutes.[3] A study of 160 bone marrow procedures performed in dogs and cats reported adverse events in 14% (22/160) and an overall complication rate of <1%, excluding pain.[8]

TABLE 1 Bone Marrow Collection Sites[1,5]

ASPIRATION LOCATION	IDEAL PATIENT SIZE	ASPIRATION OR CORE BIOPSY	SITE OF NEEDLE INTRODUCTION	PATIENT POSITION	CONSIDERATIONS
Proximal humerus	Large and small dogs, cats	Both	Greater tubercle	Lateral recumbency	▪ Avoid the articular cartilage ▪ Avoid sampling in young dogs due to the proximity of the growth plate
Proximal femur	Small dogs and cats	Both	Intertrochanteric fossa	Lateral recumbency	▪ Can be difficult in larger, well-muscled, or obese patients ▪ Cortical bone can be too dense in older patients ▪ Avoid the sciatic nerve (located medial and caudal to the greater trochanter) ▪ Use discretion in young animals due to proximity to growth plate
Ilium	Large and small dogs	Both	Dorsal iliac crest	Sternal recumbency preferred; lateral can be considered	▪ Can be difficult in obese patients ▪ Transilial approach useful in cats and small dogs with narrow dorsal iliac crests or in dogs that are obese
Sternum	Large dogs	Aspiration only	Manubrium or sternebrae (typically 2nd through 4th)	Dorsal recumbency preferred; lateral can be considered for 2nd through 4th sternebrae	▪ Carries risk of penetrating the thoracic cavity ▪ Can be performed with light sedation only
Rib	Large dogs	Aspiration only	10th rib above costochondral junction	Lateral recumbency	▪ Carries risk of penetrating the thoracic cavity ▪ Can be performed with light sedation ▪ Samples from older patients with less active hematopoiesis are frequently not representative

BOX 1 Materials and Equipment for Bone Marrow Aspiration (FIGURE A)

- Sterile gloves
- Clippers and scrub solution
- Local anesthetic (e.g., lidocaine, bupivacaine [optional])
- Petri dish with 2.5% to 3% ethylenediaminetetraacetic acid solution (approximately 2 mL) (BOX 2)
- Glass microscope slides
- Microhematocrit capillary tubes (plain) with capillary tube bulbs
- No. 11 surgical blade
- Sterile gauze
- 10- or 12-mL syringe
- 14- to 18-gauge, 1- to 2-inch bone marrow needles with a stylet (e.g., Rosenthal [Jorvet, jorvet.com], Illinois sternal [BD, bd.com], Jamshidi [BD]) (FIGURES B AND C)

(A) Materials and equipment for bone marrow aspiration. **(B)** Aspiration needle with cap and stylet in place. **(C)** Aspiration needle with cap and stylet removed.

COLLECTION SITES

In dogs, bone marrow aspirates are often collected from the proximal humerus or ilium. A special 14- to 18-gauge needle with stylet is placed into the bone marrow cavity, and cells are subsequently aspirated.[6] These needles are designed to penetrate cortical bone without becoming obstructed.[6] Other sites in dogs include the sternum, ribs, and proximal femur. Aspiration from the pelvis and femur is challenging if the region contains abundant adipose tissue.[6]

Historically, the sternum has not been aspirated in animals due to concerns for penetration into the thoracic cavity, especially in smaller dogs.[4,6] However, a study involving 26 systemically healthy beagles showed that collecting sternal bone marrow aspirates by using 20- or 22-gauge hypodermic needles was safe and equivalent in quality to samples from the humerus and ilium.[6] The sternum is a viable site for aspiration due to its softer bone and minimal soft tissue coverage compared with the ilium, femur, or humeral head, and hematopoiesis remains active through adulthood.[4,6,9] TABLE 1 summarizes the common bone marrow collection sites and any special considerations pertinent to each site.

PROXIMAL HUMERUS PROCEDURE

After the needed materials and equipment have been gathered (BOX 1) and prepared (BOX 2), the procedure can proceed according to the following steps:

Step 1 (FIGURE 1A): After administering heavy sedation or general anesthesia, place the patient in lateral recumbency and clip the fur at the site. A local anesthetic (e.g., lidocaine, bupivacaine) can be injected subcutaneously over the collection site. Aseptically scrub the site with an antiseptic soap (e.g., chlorhexidine, iodine). Draping is optional.

BOX 2 Preparing 3% EDTA Solution

Method 1*

Materials

- Dipotassium EDTA 13.8% (Sequester-Sol; Cambridge Diagnostic Products, ecamco.com)
- Sodium chloride (NaCl) 0.9% diluent (sterile)
- 0.2-µm filter
- 1-mL and 3-mL syringes

Directions

1. Swab the top of the Sequester-Sol bottle with isopropyl alcohol. Using a 1-mL syringe, withdraw 60 mg (0.44 mL) of EDTA by inserting a needle into the orifice of the Sequester-Sol vial and tipping the bottle slightly without inverting.
2. Inject the 0.44 mL of EDTA into a 3-mL syringe that has the plunger pulled back to the 2-mL mark with air.
3. After the EDTA has been transferred to the 3-mL syringe, draw sterile water into the syringe to reach a total volume of 2 mL.

**Used at the University of Wisconsin-Madison School of Pharmacy*
EDTA = ethylenediaminetetraacetic acid

Method 2[10]

Materials

- 10-mL EDTA tube
- NaCl 0.9% diluent (sterile)
- 1-mL syringe

Directions

1. Swab the top of the EDTA tube with isopropyl alcohol. Withdraw 0.35 mL of NaCl 0.9% using a 1-mL syringe and add directly into a 10-mL EDTA tube. Gently swirl until contents are thoroughly mixed.
2. Add EDTA solution into a clean Petri dish, which yields a 3% EDTA solution.

Note: If anticoagulant is not used, bone marrow smears must be prepared immediately (within seconds) of collection due to rapid clotting. Smear preparation after the sample has started to clot will not be diagnostic as most nucleated cells will be lysed.[1] Use of an anticoagulant allows sufficient time to make multiple smears of adequate diagnostic quality.

Step 2 (FIGURE 1B): Open and don sterile gloves. A nonsterile assistant should help open and place the aspiration needle, syringe, and surgical blade into the sterile field or present the items sterilely to the clinician performing the procedure. Using the 10- or 12-mL syringe, draw up approximately 1 mL of the ethylenediaminetetraacetic acid (EDTA) solution while maintaining the sterility of the syringe.

Step 3 (FIGURE 1C): Place the syringe within the sterile field. Take the aspiration needle and remove the cap and stylet. Ensure that the needle guard is securely attached to the base of the needle by rotating it clockwise until secure. Attach the syringe containing EDTA and twist it onto the top of the needle. Prime the needle by squirting the EDTA back into the Petri dish, leaving approximately 0.5 mL of EDTA in the syringe. Perform the priming over the Petri dish, not letting the tip of the sterile needle contact the Petri dish. Remove the syringe from the needle and set it aside within the sterile field. Replace the stylet and cap on the needle. Note: The needle guard can be removed in large dogs to facilitate penetration into the medullary cavity.

Step 4 (FIGURE 1D): Palpate and identify the flat area of the proximal lateral humerus, just distal to the greater tubercle. Identify the glenohumeral joint between the scapula and humerus to ensure this space is not inadvertently sampled. Make a stab incision through the skin directly over the flat area.

Step 5 (FIGURE 1E): Tent the skin and "pop" the needle through the stab incision, ensuring that the needle has penetrated the deeper subcutaneous tissue. Reidentify the flat area of the proximal lateral humerus, just distal to the greater tubercle. Place the tip of the needle directly on the bone, perpendicular to the flat surface. Apply moderate pressure in a twisting clockwise and counterclockwise motion until the needle penetrates the proximal bone cortex. After the cortex has been penetrated, reduce the angle so that the needle is aimed toward the elbow, parallel to the medullary cavity. Continue twisting until the needle is entirely embedded within the bone.

Step 6 (FIGURE 1F): Remove the cap and stylet of the needle and quickly attach the syringe containing EDTA. Aspirate until red marrow is seen (2 to 5 mL is adequate) and expel the contents into the Petri dish.

Step 7 (FIGURE 1G): Bone marrow is contained within white spicules and their presence indicates successful aspiration. Use a capillary tube or a Pasteur pipette

with an attached capillary tube bulb or pipette bulb to retrieve and expel these particles for blood smear preparation. A minimum of 1 spicule per slide is required; however, at least 3 to 5 larger spicules per slide are recommended for adequate evaluation.[3] Any remaining bone marrow content can be collected into an EDTA tube and sent with the slides to the lab.

CYTOLOGIC EXAMINATION

After the slides have dried but before sending the samples to the laboratory, a single slide should be stained with a methanolic (e.g., Wright-Giemsa) or aqueous Romanowsky stain to look for diagnostic bone marrow features (**FIGURE 2**). The most notable feature is usually large multinucleated megakaryocytes.[7] These

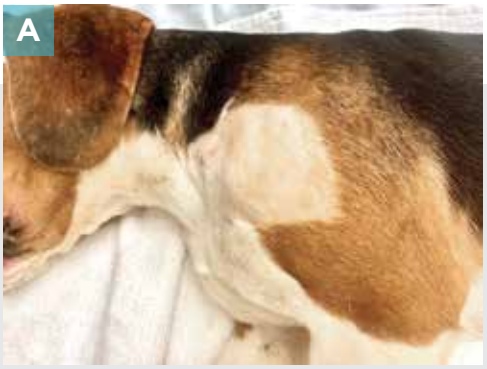
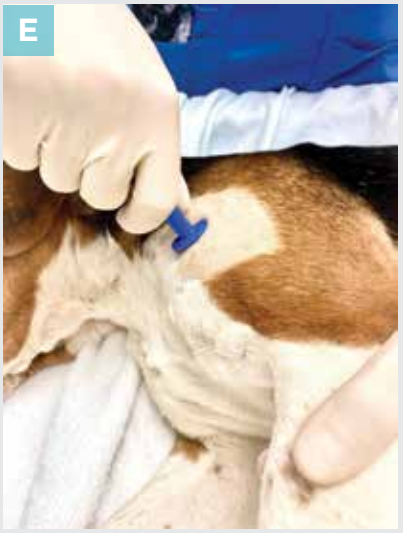
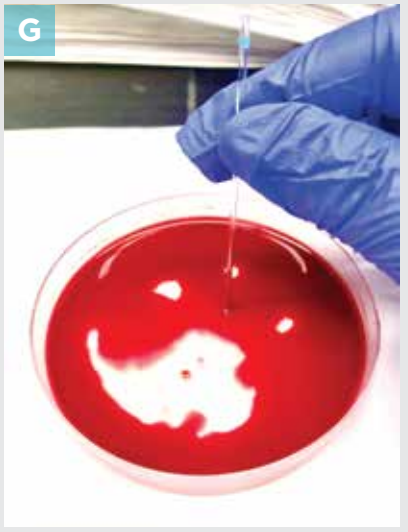


FIGURE 1. Procedure for bone marrow aspiration from proximal humerus. **(A)** Patient in lateral recumbency with fur at the site clipped. **(B)** Drawing up ethylenediaminetetraacetic acid (EDTA) solution from Petri dish. **(C)** Priming the aspiration needle with EDTA solution. **(D)** Making a stab incision over the flat area of the proximal lateral humerus. **(E)** Popping the aspiration needle through the stab incision. **(F)** Aspirating the marrow into the EDTA syringe. **(G)** Using a Pasteur pipette to retrieve particles for blood smear.





are the largest cells in the bone marrow but account for <1% of all nucleated hematopoietic cells.[3] Common hematopoietic cells are those of the granulocytic lineage (e.g., metamyelocytes, bands, segmented neutrophils) and erythroid lineage (e.g., rubriblasts, rubricytes, metarubricytes, mature erythrocytes).[3] Other cells in the bone marrow are monocytes, histiocytes (i.e., dendritic cells, macrophages), fibroblasts, adipocytes, osteoblasts, and osteoclasts.[3] If bone marrow features are not seen, a second aspiration can be attempted at the same site or in the contralateral limb.[7]

SAMPLE SUBMISSION

If the bone marrow sample obtained was adequate, the stained slide, the remainder of the unstained slides, and a peripheral blood smear should be submitted to a clinical pathologist.[7] Ideally, results from a complete blood count performed within 24 hours of collection of the bone marrow aspirate are also submitted to the laboratory, which enables direct comparison of the bone marrow cells to those in the peripheral circulation.

To ensure an accurate diagnosis, provide the clinical pathologist with signalment (patient's age, species, breed, sex, neuter status) and a thorough history.[10] The history should indicate the presence of hypercalcemia or other biochemical changes, current medications, whether the patient receives growth factors or hormones that can alter hematopoiesis, toxin exposure, radiation therapy, and travel to areas where certain infectious diseases (e.g., histoplasmosis, leishmaniasis, coccidioidomycosis) are endemic.[11]

BONE MARROW CORE BIOPSY

A bone marrow core biopsy is performed to obtain a histopathologic sample of the marrow. A bone marrow core biopsy can be performed concurrently with bone marrow aspiration, either a few millimeters away from the original aspiration site or at a separate anatomic

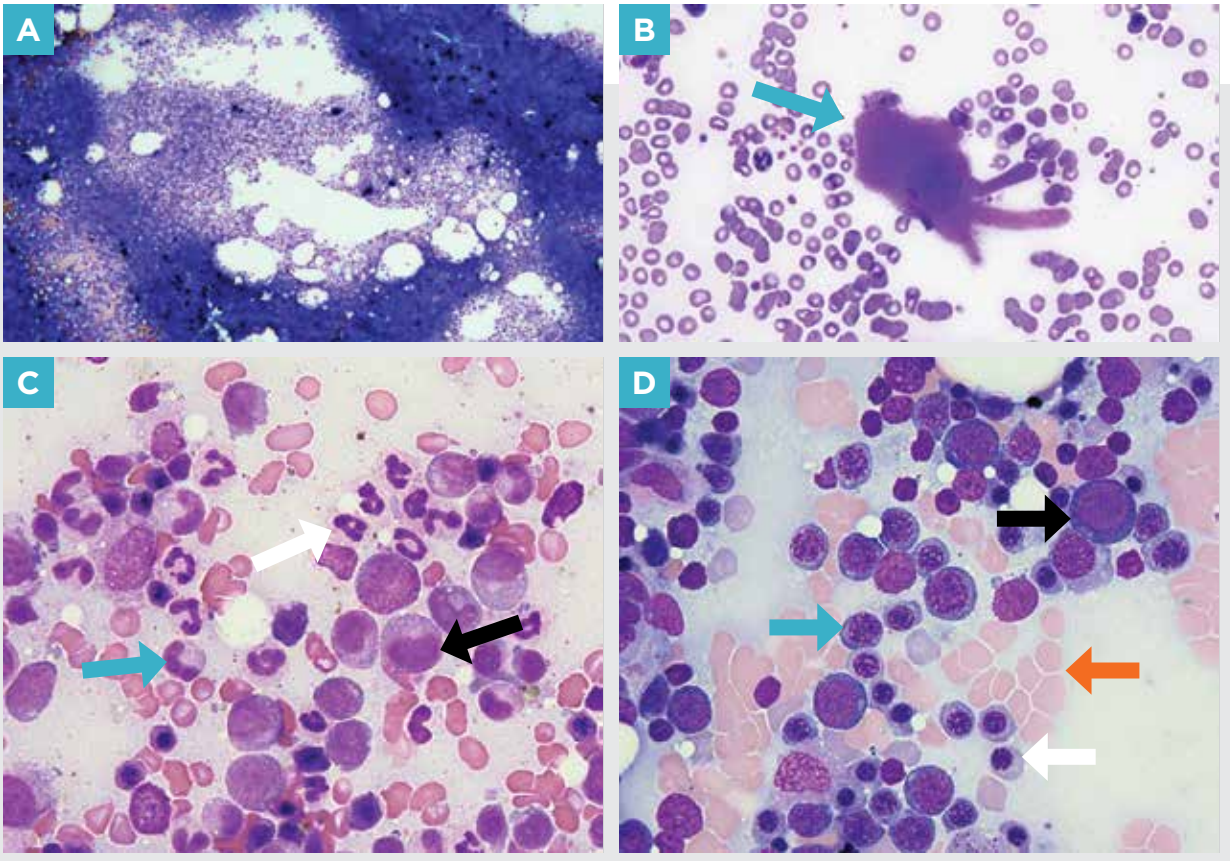


FIGURE 2. Cytology of bone marrow aspirates. **(A)** Normocellular bone marrow; 100× magnification. **(B)** Megakaryocyte **(arrow)**; 600× magnification. **(C)** Myeloid lineage including metamyelocytes **(black arrow)**, bands **(blue arrow)**, and segmented neutrophils **(white arrow)**; 600× magnification. **(D)** Erythroid lineage including rubriblasts **(black arrow)**, rubricytes **(blue arrows)**, metarubricytes **(white arrow)**, and mature erythrocytes **(orange arrow)**; 600× magnification.

Figure 2 (A through D): courtesy Nina Zitzer, DVM, PhD, DACVP

location. Bone marrow aspiration is performed more frequently than core biopsies because it is considered easier, faster, and less expensive.[5]

Cytopathology and histopathology findings complement each other and provide the most accurate assessment of bone marrow when performed concurrently. Cytopathology is preferred for looking at individual cell morphology, assessing maturation and dysplasia, and evaluating for hemoparasites.[3,5] The authors most frequently perform bone marrow cytology and biopsy concurrently in patients with unexplained bicytopenia or pancytopenia. Major disadvantages of aspiration biopsy include the risk of inaccurate representation of cellularity or stromal changes within the bone marrow and missing focal lesions such as foci of metastatic neoplasia.[5]

A bone marrow core biopsy can be used to more accurately evaluate overall cellularity and morphology, especially if aspiration results were unrewarding or inconclusive. A bone marrow core biopsy is preferred for evaluating suspected hypocellularity, megakaryocyte density, myelofibrosis, occult neoplasia, focal lesions (including metastatic neoplasia or granulomatous inflammation), and bony or stromal changes.[3,5]

Bone marrow core biopsy samples are obtained with larger needles (e.g., a Jamshidi needle).[7] The needle is driven into the bone without the stylet. After it is seated in the medullary cavity, it is twisted clockwise and counterclockwise until a sample can be extracted.[7] Bone marrow core samples at least 1.5 cm long are recommended.[3,12,13] Core samples are first rolled over a slide (imprint biopsy), placed in 10% buffered formalin, and submitted along with a bone marrow aspirate and a peripheral blood smear.[7] Aspiration and core samples should be transported in separate boxes as formalin vapors can affect the staining reaction of unstained cells and impede an accurate cytopathologic evaluation when shipped together in the same package.[11] TVP

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