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Bone Marrow Cytologic and Histologic Biopsies: Indications, Technique, and Evaluation

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KEYWORDS

- Bone marrow • Marrow cytology • Marrow histology
- Biopsy • Indications

Bone marrow evaluation is indicated when the routine examination of a blood smear has failed to provide an answer to the question: What is causing an observed hematologic abnormality? If the blood already clearly indicates an immune mediated hemolytic anemia or regenerative anemia for which an etiology is suspected/known, a bone marrow evaluation is likely superfluous. Similarly, an inflammatory response or leukocytosis for which a cause is suspected or known may not warrant a bone marrow evaluation.

SPECIFIC INDICATIONS FOR BONE MARROW EVALUATION

The most common indication for bone marrow evaluation is cytopenia of one, two, or all three hematopoietic cell lines for which an underlying etiology cannot be found.

- Nonregenerative anemia without evidence of polychromasia (reticulocytes). Persistent, poorly regenerative or nonregenerative anemia requires bone marrow examination particularly via a core biopsy to assess the severity and prognosis of various erythroid hypoplastic or aplastic conditions.
- Persistent neutropenia without a left shift or evidence of regeneration. Regeneration should involve a shift toward immaturity of the granulocytic line (ie, bands, metamyelocytes, and myelocytes) within 3 to 5 days following neutropenia.
- Thrombocytopenia is best evaluated by an assessment of the number and morphology of megakaryocytes via bone marrow core and aspirate biopsy. Levels as low as 10,000/μL often do not present a contraindication for bone marrow evaluation as bleeding is confined within the bone space. It is recommended that a

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coagulation profile be performed first to rule out disseminated intravascular coagulopathy when thrombocytopenia is present.

- The presence of abnormal cell morphology in the blood will often warrant review of the marrow for evidence of leukemia. Dysplastic changes such as megaloblastic rubricytes, neutrophil hypersegmentation, or giant platelets may be subtle indicators of myelodysplastic syndrome or certain myeloid leukemias.
- The suspicion of marrow malignancy warrants bone marrow evaluation. Leukemia may be occult (ie, blast cells may be numerous in the bone marrow, but few or no blast cells are seen in the peripheral blood [subleukemic or aleukemic leukemia]).
- Unexplained elevations in blood cell numbers should suggest bone marrow evaluation. Note that relative erythrocytosis may result from splenic contraction, hypovolemia, physiological induced cardiac disease, and high-altitude hypoxia and in certain dog breeds (eg, greyhounds), all of which do not require bone marrow examination. Certain neoplasms can produce excessive erythropoietin (leading to erythrocytosis) or granulocyte and/or granulocyte-macrophage colony-stimulating factors (leading to leukocytosis) as a result of renal hypoxia or a paraneoplastic secretion. A reactive leukocytosis due to a nidus of inflammation is more common and should be excluded before pursuing the possibility of a paraneoplastic or neoplastic process to explain the elevation. Marked thrombocytosis can result from increased thrombopoietin or thrombopoietic cytokine stimulation, which may occur in chronic iron deficiency anemia and in various inflammatory/infectious conditions.[1] The possibility that persistent thrombocytosis may indicate an underlying myeloproliferative disorder such as a myelodysplastic syndrome or essential thrombocythemia must also be ruled out, particularly if an underlying etiology cannot be found. The latter may be independent of thrombopoietin stimulation; bone marrow examination in these conditions often reveals marked megakaryocytic hyperplasia with morphologic abnormality. Lymphocytosis may indicate antigenic stimulation but marked elevations in lymphoid cells should suggest possible malignancy. Chronic lymphocytic leukemia (CLL) of B-cell immunophenotype in the dog has significant infiltration of the bone marrow in contrast to CLL of T-cell origin, which circulates in peripheral blood but originates from the spleen.[2] T-CLL is more common than B-CLL in the dog compared to the human.
- Hypercalcemia may occur without obvious etiology such related to the presence of an anal mass or hyperparathyroidism. In these situations, occult lymphoid neoplasia with only bone marrow involvement should be considered.
- Focal lymphoma or plasma cell myeloma produces bone lysis and may only be recognized with early disease through core biopsy procedure.
- Hyperproteinemia with either a monoclonal or polyclonal gammopathy supports bone marrow evaluation for neoplasia or an infectious agent. Both B-CLL and plasma cell neoplasia may present with a monoclonal gammopathy. Systemic fungal and protozoal infectious agents such as *Histoplasma* and *Leishmania* may infiltrate the bone marrow and present with hyperglobulinemia. Others may not be visible but still result in gammopathy such as *Ehrlichia canis.*

Fever of unexplained origin often arises from immune-mediated causes but may also reflect a non–immune-mediated etiology where bone marrow evaluation becomes a useful diagnostic tool. Primary bone marrow abnormalities accounted for 22 of 101 canine cases of pyrexia of unknown origin, with myelodysplasia and lymphoid leukemia being most responsible.[3]

- Leukoerythroblastosis identified by concurrent immature granulocytes and nucleated red cells in circulation may indicate bone marrow damage caused by neoplastic infiltration of the bone marrow. While the presence of nucleated red cells in circulation may reflect bone marrow damage, the presence of a concurrent and significant left shift was key in supporting examination of the bone marrow leading to the discovery of a disseminated adenocarcinoma in a dog.[4]
- Therapeutic monitoring of chemotherapy administration and determination of clinical staging for malignancies such as lymphoma or mast cell tumor require bone marrow evaluation. Aspiration and core biopsies are recommended to determine changes in hematopoietic cell prevalence as well as the pattern of neoplastic cell infiltration. A focal type of metastasis is the most difficult to determine by blood smears or marrow aspirates alone. Core biopsy is useful to determine whether mast cells are in their normal perivascular location or abnormally in sheets of cells. Core biopsy examination of the bone marrow in canine lymphoma affords greater sensitivity than aspirate smears due to the manner of metastasis seen histologically.[5]
- Evaluation of iron stores is helpful in determining iron sequestration within macrophages. Marrow evaluation for iron in cats may be unproductive, because their bone marrow normally lacks discernible storage levels. Similarly, neonatal animals often rapidly use iron for erythropoiesis, and storage amounts are low. While both anemia of inflammatory disease and iron deficiency anemia in dogs present with low serum iron, there is a different appearance relative to iron stores within the bone marrow. Iron deficiency anemia has low to absent stores while anemia of inflammatory disease has normal to excessive amounts of hemosiderin in the bone marrow.[6]

CYTOLOGIC BIOPSY VERSUS HISTOLOGIC BIOPSY

Cytologic biopsy provides excellent morphological detail of bone marrow cells. It is relatively cheap and easy to perform and has a rapid turnaround time, providing diagnostic information within minutes of collection. The histologic biopsy provides similar information relative to cell types but more importantly allows architectural evaluation of fat and fibrous connective tissue relative to cellular content. Focal changes of the stroma and determination of overall cellularity are best appreciated by histologic biopsy samples. Such changes, which may be missed by aspiration biopsy alone, include inflammation, neoplasia, marrow necrosis, osteolysis, or myelofibrosis. For maximum information, the two techniques are concurrently performed and interpreted along with the complete blood count (CBC) data obtained within 1 day of the biopsy.

SITES OF BIOPSY

Antemortem

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

Humerus

For obese or very muscular dogs, the craniolateral part of the greater tubercle of the humerus is the site preferred because of lack of muscle, fat, or substantial subcutaneous tissue in this region (**Fig. 1**). For humeral samples, both aspiration and core biopsy needles are directed posteromedial. The pronounced fascial covering of the bone permits the needle to slip easily; therefore patience is advised when inserting the

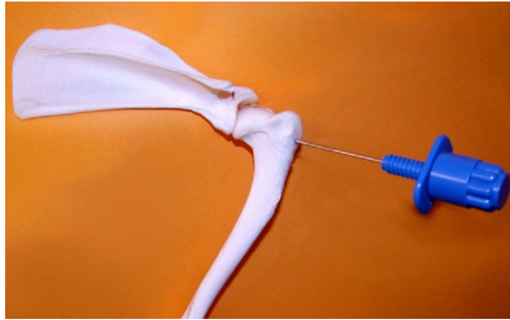

Fig. 1. Placement of the marrow biopsy needle is shown into the craniolateral part of the greater tubercle of the humerus. This site is preferred for obese and muscular adult dogs and cats.

needle into the bone. Lateral recumbency is preferred for collection. Young growing animals, typically less than 6 months of age should **not** be sampled in this location due to the proximity of the epiphyseal growth plate.

Ilium

For thin or nonobese dogs, the dorsal iliac crest is a popular location because it is readily accessible (**Fig. 2**). In small dogs and cats with a thin dorsal ilial crest, transilial samples for core samples are preferred over parallel placement of the needle within the ilium (**Fig. 3**). Patients may be positioned in sternal or lateral recumbency for ilial

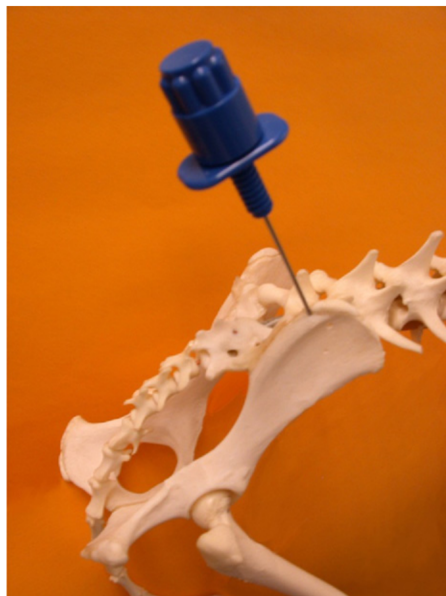

Fig. 2. The dorsal iliac crest is a popular location in thin or nonobese dogs, because it is readily accessible.

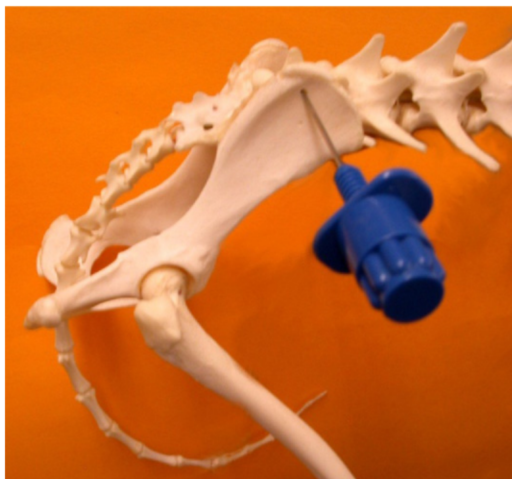

Fig. 3. One or more samples may be taken via a transilial procedure. This site may be helpful in young dogs and cats that have a dorsal ilial crest, too narrow to attempt parallel placement of the biopsy needle.

samples. The needle is introduced at the widest part of the dorsal ilium and directed ventromedial to conform to the concavity of the pelvic bone. If placed too laterally, the biopsy instrument may enter only cortical bone or slip off the sides into surrounding muscle or adipose tissue.

Femur

For small dogs and cats, penetration made just medial to the greater trochanter and parallel to the shaft of the proximal femur may be used to obtain marrow samples, but this area may be less accessible (**Fig. 4**). When obtaining femoral samples, avoid the sciatic nerve located medial and posterior to the greater trochanter. The animal is placed in lateral recumbency during the procedure. Large quantities of bone marrow may be obtained from this site. Bone trabeculae are minimal in this site, providing larger quantities, but the core biopsy may be more easily lost during collection related to the limited dense tissue available to retain the specimen.

Postmortem

Samples should be obtained within 30 minutes after death to ensure intact cell morphology as tissue breakdown is rapid at room or body temperature. Refrigeration, not freezing, is recommended to slow deterioration if a time delay is anticipated. Aspiration material is best obtained within minutes after death. If time delay prohibits aspiration collection, wedge sections from necropsy specimens using a scalpel blade should be taken from the metaphyseal region of long bones (femur or humerus) (**Fig. 5**). Avoid taking diaphyseal or mid-bone samples, especially in older animals, as there is likely to be much fat infiltration and the samples are less likely to be representative of active hematopoiesis within the bone marrow. Alternatively, tissue sections involving the costochondral joint of the ribs or the wing of the ilium may be used, depending on the size of the animal. Cytologic material from cut surfaces may be possible if collection is performed shortly after death but cellular detail is not likely to be optimum.

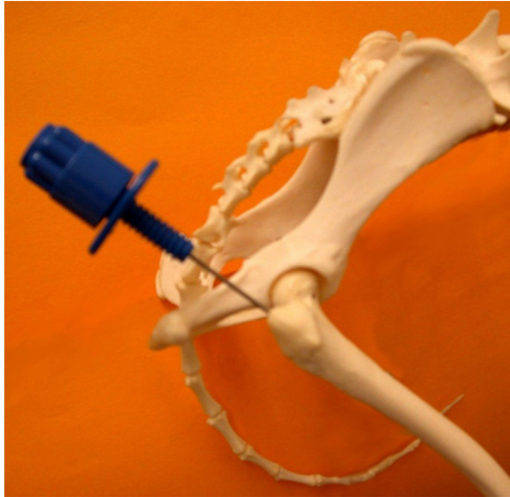

Fig. 4. The proximal femur may be most useful for small animals. Penetration is made just medial to the greater trochanter and parallel to the shaft of the samples, avoiding the sciatic nerve located more lateral and posterior.

PATIENT PREPARATION AND EQUIPMENT

Animals need not be placed under general anesthesia. Sedation with local anesthesia (eg, lidocaine) is often sufficient for aspiration and core biopsies. Pain relief medication may be given when indicated. The area is clipped and surgically scrubbed; then ½ to 2 mL of 2% lidocaine is infiltrated intradermally and deep to the periosteum. The area may be draped and sterile gloves are used. A stab incision is made in the skin with a No. 11 scalpel blade. It is recommended that aspiration biopsy be performed first, followed by core biopsy of the same bone. The core biopsy should be taken a

Fig. 5. Triangular wedge sections may be taken from necropsy specimens using a scalpel blade within the metaphyseal region of long bones (femur or humerus).

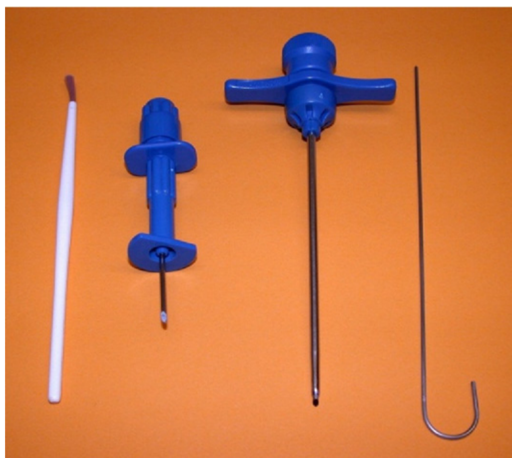

Fig. 6. Bone marrow biopsy equipment is shown. From left to right: paint brush, Illinois sternal aspiration biopsy needle, and a Jamshidi core biopsy needle along with its associated shepherd hook for specimen removal.

short distance away from the aspirate biopsy to avoid collecting the previously damaged tissue.

Bone marrow aspirate biopsy equipment and supplies are minimal (**Fig. 6**) consisting of a special biopsy needle,[a] 12-mL syringe, 5% EDTA solution, scalpel blade, and glass slides. Optionally, Petri dishes with hematocrit capillary tubes or glass pipettes are used to collect unit particles.

Core biopsy materials include a specialized biopsy needle,[b] scalpel blade, glass slides, and a tissue fixative (10% buffered formalin).

Postmortem cytologic and histologic collection materials consist of a fracturing technique such as a bone rongeur or a saw to assist in exposing the metaphyseal region. A cytologic sample can be acquired by gently scraping or brushing the medullary cavity with a hobby paint brush (**Fig. 6**). The acquired specimen can then be transferred to a glass slide by creating 2 or 3 linear streaks. Using the same exposed medullary cavity, a histologic sample is created with a No. 10 scalpel blade by cutting a triangular wedge within the spongy but firm tissue (see **Fig. 5**), which can be then placed into fixative fluid.

BIOPSY PROCEDURES

Aspirate Biopsy

The aspiration needle with stylet in place is passed through the skin incision to the bone. The needle is then rotated to penetrate the cortex several millimeters so that it becomes firmly embedded. Once embedded, the stylet is removed; the syringe containing EDTA is then attached to the needle, and quick, full, successive pulls are applied to the plunger to draw marrow into the syringe. Relax the pressure on the syringe plunger and remove the syringe and needle together. If direct smears are to

[a] Illinois sternal disposable needle, 15 to 18 gauge, 1 to 2 inches long; Cat. Nos. DIN1515X and DIN1518X, Cardinal Health, www.cardinal.com.

[b] Jamshidi biopsy/aspiration disposable needle, 11 to 13 gauge, 2 to 4 inches long; Cat. Nos. DJ2013X, DJ3513X, DJ4011X, Cardinal Health, www.cardinal.com.

be made, avoid excessive blood contamination. Touch a drop of marrow to the slide and quickly make your bone marrow preparations by either a "push" or "squash" technique.

A preferred method involves adding approximately 0.5 to 1 mL of 5% EDTA to the syringe before starting the procedure. Following collection of blood contaminated marrow, squirt it into a Petri dish or onto a slide and allow blood to drain off particles by tipping the dish or slide. Alternatively, marrow can be transferred to a glass slide so particles can adhere. Particles, which appear as tiny glistening flecks or granules, may be picked up with microhematocrit tubes or a glass pipette.

Following collection of a droplet, it is gently expelled onto a glass slide. Smears of the particles are best produced by the squash method. Extra smears should be made for in-house viewing or for special cytochemical or immunocytochemical stains.

Core Biopsy

The technique for acquiring a core biopsy is similar to the aspirate biopsy except that the stylet is removed prior to the instrument becoming embedded in bone. The needle is advanced about 1 cm 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 in 360-degree revolutions in both clockwise and counterclockwise directions. The needle is withdrawn with a twisting motion. The section is removed from the Jamshidi biopsy needle by pushing the sample out the wider end with a probe that enters the narrower cutting end. The core biopsy may be rolled over a glass slide to allow exfoliation of marrow material before it is placed into the tissue fixative. The sample must remain in the fixative for a minimum of 2 to 3 hours. Following mild decalcification for 4 to 8 hours in formic acid/sodium citrate solution, sections of the bone marrow core are prepared and sent to a histopathologist for evaluation. Recommended thickness involves 3-μm hematoxylin and eosin (H&E) sections as well as sections for reticulin and immunohistochemistry stains. Formalin materials should be kept separate (not even close by) from aspirate smears to avoid fixation artifacts to the cytologic preparations. If mailing, send aspirate and core biopsy materials in separate boxes.

Biopsy Complications

Rare complications are encountered during bone marrow aspiration or core biopsy. Tissue injury is minimal unless the sciatic nerve is damaged when obtaining a femoral sample. Excessive bleeding is rarely encountered, even in markedly thrombocytopenic patients. Rare reports have described tumor seeding of hematopoietic neoplasms in humans as a result of bone marrow biopsy attempts. No such cases have been reported in animals.

TECHNICAL ASPECTS OF BONE MARROW PROCESSING

Storage of Bone Marrow Aspiration Samples

Bone marrow samples should be processed as quickly as possible after collection. The best results are obtained when films are prepared within 2 hours of their collection. For short-term storage ($<$8 hours), it is recommended that marrow samples be kept dry and cooled at 4°C; however, storage at room temperature (25°C) is acceptable. Prior to the making of bone marrow films, the sample should be gently and completely mixed by inversion a minimum of 15 to 20 times. Degenerative changes such as cytoplasmic vacuolization, nuclear lobation or fragmentation, and apoptotic changes are often caused by prolong or inappropriate sample storage.

Processing and Staining of Bone Marrow Films

To avoid artifactual changes, bone marrow films also must be rapidly and completely air dried before they are fixed and stained. Slow drying of the marrow preparations causes cell to contract or shrink, whereas excessive water in methanol fixing solution (>3%) can lead to cellular swelling and distorted morphology. In the latter scenario, the cellular features will not be "crisp," nuclear contents may appears to leak into the cytoplasm, a feature in erythroblasts that may be misinterpreted as dyserythropoiesis, and artificial cytoplasmic vacuolation may occur.

Prior to shipping (or delivery) the marrow sample and unstained films to the laboratory, the adequacy of the marrow preparations should be evaluated on a stained preparation. Bone marrow films can be stained with a Romanowsky stain, such as May-Grünwald-Giemsa, Wright-Giemsa, or aqueous-based Wright. Because of their thickness, aspirate smears require a longer staining period with Romanowsky-type stains than blood films. Rapid evaluation of the marrow smear can be made within minutes of collection to ensure adequate material has been obtained. Once stained, a cover slip may be applied. All slides, stained and unstained, should be labeled with date and patient identifying information.

COMPLETE MARROW EVALUATION

For a complete marrow evaluation (CME), testing should include a CBC, bone marrow aspirate, and bone marrow biopsy. A CBC gives excellent quantitative and morphologic information; it should be collected at the same time or within 24 hours of the bone marrow aspirate. Careful review of the blood film by the pathologist reading the aspirate is strongly recommended as it may provide additional information to support marrow findings or help to direct marrow evaluations. Depending on the CBC findings, it may be also useful to obtain a current reticulocyte count, particularly for determining whether erythroid hyperplasia is associated with effective or ineffective erythropoiesis.

The bone marrow aspirate allows excellent morphologic evaluation of cells, differential count and myeloid:erythroid (M:E) ratio. However, if the cellularity of a bone marrow aspirate is low, the finding may be due to "real" pathology or just hemodilution of the sample. A histologic section of a biopsy sample gives the best quantitative information on the cellularity of the marrow as well as revealing myelofibrosis, architectural patterns, and focal lesions. Neglecting one or two parts of the CME often leaves unanswered questions and performing a test several days after the other may also leave some questions. It is recommended that all three tests be done and at the same time.

Assessing Bone Marrow Films

A guideline for the assessment of bone marrow aspirate cytology is shown in **Box 1**. Marrow films should first be examined under low power (×10 objective). The number of particles and overall degree of cellularity, megakaryocyte numbers, as well as presence of low numbers of abnormal cells such as carcinoma cells or focal accumulations of round cells, are assessed at this power. For normocellular marrow particles, the hematopoietic cells should be piled up on the stroma of the spicules, but vacuoles of fat are still present; the overall cellularity should be greater than 25% but less than 75%. Hypocellular marrow has readily visible stromal cells and abundant fat (>75% of the particles area is fat) with few hematopoietic cells. On the other hand, hypercellular marrow contains no or rare fat vacuoles and an abundance of cells (>75% of the particles are composed of cells). An absence of particles in films of the

Box 1
Steps in assessing bone marrow aspiration cytology

1. Particles and/or overall degree of cellularity
2. Content of particles
   a. Iron stores
   b. Megakaryocytes
   c. Stromal cells
   d. Hematopoietic cells
   e. Other cells
3. Myeloid:erythroid ratio (M:E ratio)
4. Megakaryocytic lineage
   a. Numbers
   b. Sequence and completeness of maturation
   c. Dysplasia
5. Erythroid lineage
   a. Sequence and completeness of maturation
   b. Dysplasia
6. Myeloid lineage
   a. Sequence and completeness of maturation
   b. Dysplasia
7. % Blasts
   a. Of all nucleated cells
   b. Of the nonerythroid lineage
8. Other cells
   a. Lymphocytes
   b. Plasma cells
   c. Histiocytic cells
   d. Mast cells
   e. Neoplastic cells (nonhematopoietic)
9. Morphologic interpretation: must have a recent CBC
10. Comments

bone marrow aspirate may preclude valid estimation of cellularity and megakaryocyte number in most instances. Megakaryocytes are often identified within or very near the particles and estimates of their numbers are quite subjective. If more than a few megakaryocytes are found, then the number is designated as "adequate"; if almost none are present, then decreased; if many are present, then increased.

A systematic assessment of the content of particles and a differential count from which the M:E ratio can be calculated are made using the ×40 or ×50 objectives. Since the marrow film is minimally diluted by peripheral blood in the "trails" of cells

Table 1
Differential cell counts from bone marrow for the dog and cat

CELL TYPE	DOG	CAT
Myeloblasts (%)	0.4–1.1	0–0.4
Promyelocytes (%)	1.1–2.3	0–3.0
Myelocyte neutrophils (%)	3.1–6.1	0.6–8.0
Metamyelocyte neutrophils (%)	5.3–8.8	4.4–13.2
Band neutrophils (%)	12.7–17.2	12.8–16.6
Segmented neutrophils (%)	13.8–24.2	6.8–22.0
Eosinophils (%)	1.8–5.6	0.8–3.2
Basophils (%)	0–0.8	0–0.4
Rubriblasts (%)	0.2–1.1	0–0.8
Prorubricytes (%)	0.9–2.2	0–1.6
Basophilic rubricytes (%)	3.7–10.0	1.6–6.2
Polychromatic rubricytes (%)	15.5–25.1	8.6–23.2
Metarubricytes (%)	9.2–16.4	1.0–10.4
Granulocytic to Erythroid Ratio or M:E	0.9–1.8	1.2–2.2
Lymphocytes (%)	1.7–4.9	11.6–21.6
Plasma cells (%)	0.6–2.4	0.2–1.8
Monocytes (%)	0.4–2.0	0.2–1.6
Macrophages (%)	0–0.4	0–0.2
Mast cells	Rare	Rare

Data from Harvey JW. Atlas of veterinary hematology. Philadelphia: WB Saunders; 2001.

adjacent to the particles, the differential counts should be performed in these areas. At the very least, several hundred cells should be counted; granulocytes, nucleated erythroid cells, lymphocytes, plasma cells, and other cells are enumerated. The majority of cells in a marrow smear are differentiating granulocytes, monocytes, and erythroid cells. In normal marrow, neutrophils are the most numerous with monocytes, eosinophils, and basophils collectively accounting for only 5% to 10% of the total myelomonocytic cells. The marrow report should indicate the relative number of granulocytic and erythroid cells in the form of an M:E ratio. In health, the M:E ratio is approximately 1 but can range from 2 to 0.5, depending on the species (**Table 1**). The balance and completeness of maturation in the three hematopoietic cell lines should be assessed. In each series, cells in the late stages of differentiation, the postmitotic pool, should be far more numerous (approximately 85% of the total cells in that series) than the immature stages. A 500-cell differential with all cell types enumerated on several different bone marrow films should be performed if marrow features indicate the possibility of an acute leukemia or myelodysplastic syndrome.

Abnormally high numbers of nonhematopoietic cells, such as plasma cells, lymphocytes, macrophages, and mast cells, in marrow should be noted and reported. It is important to recognize that the accumulation of cells of one particular lineage may be focal; scanning the blood film at low power as well as examining multiple blood films at higher magnifications is necessary to be certain these areas are not missed. Lymphocytes and plasma cells and macrophages are commonly present in low number, scattered throughout the marrow and collectively are fewer than approximately 10% of all the nucleated cells (see **Table 1**). In the cat, small/mature

lymphocytes may represent up to 20% of all the cells. These cells are also slightly more numerous in immature animals than in adult animals.

Fine cytological details of hematopoietic or other cells should be assessed using an oil immersion ×100 objective. Differentiating hematopoietic cells in all series should be evaluated for morphologic evidence of disturbed maturation, such as dysplastic and toxic changes, and left shift. Toxic changes are apparent only in neutrophils and are associated with stimulated granulocytopoiesis, particularly in inflammation. Dysplastic change or (morphologic evidence of abnormal maturation) in erythroid cells and megakaryocytes most commonly are associated with neoplastic disorders of hematopoiesis-myelodysplastic syndromes and acute myeloid leukemias. A pronounced left shift in a series can represent a "maturation block," which can result from destruction of cells in later stages of maturation or from intrinsic defect in regulation of differentiation (hematopoietic neoplasia), or can represent a transient stage in a wave of regeneration after acute injury to marrow, such as myelotoxic drug, acute radiation, or feline panleukopenia infection. Feline leukemia virus (FeLV) infection in cats causes a wide range of dysplastic changes in hematopoietic cells and/or alterations in their numbers—these findings are often disparate, ranging from nonregenerative anemia to erythremic myelosis, neutropenia to leukemia, and thrombocytopenia to megakaryocytic leukemia. Thus, it is strongly recommended that any cat with a hematologic disorder should be tested for FeLV as well as feline immunodeficiency virus (FIV) infection.

Stainable Iron

The quantitative and qualitative assessment of cells in Romanowsky-stained films should be followed by the evaluation of marrow iron stores. While imprecise, the amount of hemosiderin (stainable iron) in marrow helps to support a diagnose iron deficiency anemia (absence) or anemia of inflammation (increased). Adult dogs, horses, and cows should have hemosiderin; however, cats normally have no stainable iron in marrow.

Assessing Bone Marrow Histology

The components involved in bone marrow histology are cortical and trabecular bone and stroma, vasculature, sinus wall layers, nerves, hematopoietic cells, and nonhematopoietic interstitial cells. Refer to **Boxes 2** and **3** for a checklist and list of histochemical stains used to assess bone marrow histologic specimens.

Connective Tissue Elements

Young animals generally present with hypercellular bone marrow, defined as having greater than 75% cellular elements with the remainder of the space occupied by adipose tissue, if present. As the animal ages or when hematopoiesis decreases with disease, fat cells, fibrous tissue, necrosis, or metastatic tumor cells often replace normal cellular elements. The adventitial-reticular (A-R) cell or stromal cell that lines most marrow sinuses influences the change in adipose or fibrous tissue content. The A-R cell will project its cell processes into hematopoietic cords for support and produce reticulin (argentiphilic) fibers during early bone marrow injury. Fibroblasts that produce collagen may also arise from the A-R cells during severe marrow injury or in response to cytokines (platelet-derived growth factor, transforming growth factor-β) released from monocytes and neoplastic megakaryocytes. Reticular cells stain alkaline phosphatase positive and produce interleukin-7. Actin and stem cell factor are expressed by these cells. Ultrastructurally, microtubules, microfilaments,

Box 2
Checklist for bone marrow histologic evaluation

1. Sample integrity; contour/quantity of bone and presence of other elements e.g., muscle.
2. Overall cellularity of hematopoietic elements relative to fat content; estimate proportion.
3. Number of megakaryocytes in high power field (×40 or ×50 oil), their distribution, and general maturity.
4. Cellular patterns in core biopsy (focal or diffuse)
   a. Focal infiltrates with high density areas within adipose
   b. Metastatic populations typically found in paratrabecular location
5. Cell types
   a. Normal heterogeneity of cell types
   b. Monomorphism of a cell type
6. Maturation sequence of granulocytes and erythroid precursors
   a. Majority cells are normally late-stage forms (segmented granulocytes, metarubricytes)
   b. Maturation arrest at any stage? All stages seen?

7 Estimation of myeloid:erythroid ratio comparing granulocytes and erythroid precursors.

8 Iron content may be noted if increased when viewed under H&E staining as coarse orange-brown granules within macrophages; best evaluated with Prussian blue stain. Erythrophagocytosis when noted should be reported.

9 Provide a morphologic interpretation and compare the biopsy findings to the peripheral blood results to see if an appropriate bone marrow response is present. Best if the CBC is performed within 24 hours of bone marrow biopsy.

and intermediate filaments are commonly found to provide structural support. Connected to the cortex is a meshwork of trabecular bone that forms the support for the hematopoietic compartment. It is lined by endosteum along with osteoblasts and osteoclasts that are prominent in young animals or actively remodeled bone. It is thought that the paratrabecular area exerts an inductive effect on granulopoiesis since myeloblasts are most prominent in this area.

Vasculature

The vascular supply to the bone marrow arises from two sources. A minor source occurs from the nutrient artery, which enters the midshaft through the cortex dividing

Box 3
Suggested additional histochemical stains for bone marrow evaluation

Giemsa—eosinophils, mast cells; highlights erythroid precursors especially late stages

Periodic acid–Schiff (PAS)—granulocytes, histiocytic, megakaryocytic, and plasma cells

Prussian blue—hemosiderin deposits

Argentiphilic stain—reticulin fibers

Trichrome—collagen fibers

into ascending and descending medullary arteries from which form the radial arteries. The radial arteries enter through the cortex along the endosteum leading into cortical capillaries, which communicate with capillaries coming from the major source, namely periosteal and muscular arterioles. From these capillaries, blood flows into terminal sinuses within the marrow space. The sinuses are lined by a thin interrupted basement membrane visible with PAS staining. Facing the lumen is a continuous layer of endothelium joined by junctional complexes that reacts with anti-CD34, the stem cell marker. Small apertures in the endothelium allow for cell passage into the lumen from the interstitium. Hypoxia or erythropoietin influences produce wider apertures leading to the early release of precursor erythroid cells. Returning blood drains into a central sinus that exits through the nutrient foramen. Associated with the blood vessels are vasomotor nerves that may react to the discomfort caused by bone marrow aspiration.

Hematopoietic Elements

The major cellular component consists of hematopoietic elements and generally accounts for 25% to 75% of the marrow space. Hematopoiesis occurs in an organized fashion within the marrow.

Granulopoiesis occurs primarily adjacent to trabeculae and is easily visualized with the use of PAS stain. The myeloblasts have large round to oval vesicular nuclei with often a single large nucleolus. As they mature, metamyelocytes and later stages crawl away from this site and may be found anywhere in the interstitium. PAS will stain all stages of granulocytes; however, it is more intense in the myelocyte and later forms. For the monocytic cells, monocytes and macrophages stain more intensely with PAS compared with the promonocyte stage.

Erythropoiesis is evident as erythroblastic islands that contain a central macrophage. These islands are found adjacent to the sinus endothelium. In this location, erythroblasts can mature by pitting the nucleus of the metarubricyte and releasing the remaining polychromatic erythrocyte through openings in the endothelium into the sinus lumen. An exception to this location is found in the avian and reptilian species, whereby erythroblasts line the inside of sinus and all developing stages progress toward the center of the sinus for release into circulation.

Thrombopoiesis, similar to erythropoiesis, occurs adjacent to the sinus so that megakaryocytes can extend their cytoplasmic processes directly into the sinus lumen and fragment into platelets.

Lymphopoiesis is based on their B or T immunophenotype. Small follicles or nodules of B- lymphocytes have been found in people, dogs, and cats. These are small well-differentiated lymphocytes that may respond with formation of a germinal center and demonstration of centrally located immunoblasts. In the cat, these are more prominent in the femur and in conditions such as FIV infection. T-lymphocytes and plasma cells may be concentrated around radial arteries.

Other cells such as mast cells are located perivascular and adjacent to lymphoid nodules. These are in low numbers and scattered individually, not in sheets.

Mononuclear Phagocytes and Iron

Macrophages are present in low numbers often associated with erythroblastic islands as "nurse cells" and may be quite evident with ingested iron or hemosiderin. Additionally, during pathologic conditions such as immune-mediated hemolysis, some infections, histiocytic malignancies, and benign histiocytic proliferations, macrophages may display prominent erythrophagocytosis.

Young animals will have more rapid red cell turnover and therefore less deposits of iron in their marrow compared with adult animals. Species differences also exist, as

for example scattered Prussian blue–positive granules within macrophages are found normally in the dog but are generally absent in the cat, except under pathologic conditions. Ferritin represents a better gauge of body iron content but this minimally stains with Prussian blue unless present in aggregates. Acidic chelating agents, which are present in the decalcifier reduce stainable iron. In these circumstances, review of a cytologic preparation will help determine iron content.

Interpretation of Marrow Findings

The interpretation of bone marrow aspiration and histology should provide quantitative information as well as a morphologic assessment of individual cells and cell lines. Most of the bone marrow abnormalities relate to the overall cellularity and/or presence of cell types. **Table 2** lists disorders to consider based on the cell numbers or morphologic appearance of cells in the blood and cellularity, morphologic abnormalities, or stromal changes in the bone marrow. An explanation of the terms often used when interpreting the aspirate is provided next.

Erythroid, myeloid, and *megakaryocytic hyperplasia* are terms that indicate increased numbers of precursor stages of red cells, granulocytes/monocytes, and platelets, respectively. This is the expected marrow response to loss or destruction of these mature cells from the peripheral circulation. If the response is effective, increased numbers of the end product should be evident in blood (ie, reticulocytosis, rising white blood cells, hematocrit, and/or platelet count).

The bone marrow under conditions of strong regeneration is characteristically hyperplastic such as following recovery from feline panleukopenia. The regeneration may be anticipated from examination of the peripheral blood with the presence of macrocytic polychromatic erythrocytes, left-shifted granulocytes, or megaplatelets. The bone marrow may be hyperplastic relative to megakaryocyte numbers in consumptive or destructive platelet disorders such as subacute to chronic disseminated intravascular coagulopathy or immune-mediated thrombocytopenia. Immune-mediated destruction of platelets has been associated with drug therapy and various neoplasms (mast cell, hemangiosarcoma, mixed mammary tumor, or nasal adenocarcinoma).[7] Therefore, bone marrow hyperplasia may be expected in the presence of these conditions. Granulocytic hyperplasia as a paraneoplastic syndrome has been documented in several tumors.[8]

Neoplasia refers to new or uncontrolled cell growth that within the bone marrow can arise primarily from hemolymphatic tissue or secondarily from metastasis of a nonhematopoietic tumor. Common malignancies that metastasize include lymphoma, mast cell tumor, and various carcinomas.[4] Primary malignancies include acute or chronic lymphoid or myeloid leukemias. Acute myeloid leukemia (AML) is applied to the situation in which the hematopoietic precursors are arrested in an early stage of development. The mechanism of this arrest is believed to involve the activation of abnormal genes through genetic abnormalities. The presence of greater than 20% blast cells in the bone marrow is strongly supportive of an interpretation of AML; however, clinical and laboratory information is essential to distinguish this from an exuberant hyperplastic response.

Dysplasia refers to abnormal growth of cells. Cytologically, these abnormalities include asynchronous maturation of the nucleus and cytoplasm resulting in megaloblastic erythroid precursors, dwarf megakaryocytes, or abnormally segmented neutrophils. Dysplasia may affect one cell line (eg, erythroid, as occurs in lead toxicity or poodle macrocytosis) or two or more cell lines may be affected as may occur with nutritional deficiencies or drug-induced toxicosis.[9] These secondary myelodysplastic cases should be distinguished from the primary myelodysplastic syndrome.[10]

Table 2
Selected causes of bone marrow disorders based on blood and bone marrow examination

Peripheral Blood Examination			
Cytopenia	***Hematocytosis***	***Dysplastic Cells***	***Blast Cells***
Bone Marrow Evaluation			
Aplasia/Hypoplasia	***Hyperplasia***	***Myelodysplasia***	***Neoplasia***
Infections:	Infections:	Myelodysplastic syndrome	Primary lymphoid leukemia
Viral	Bacterial		Primary myeloid leukemia
Rickettsial	Mycoplasmal	Drug-induced dysplasia	Metastatic neoplasia
Protozoal	Rickettsial	Lead toxicosis	
Fungal	Protozoal	Infections:	
Drugs or chemicals	Fungal	FeLV	
Hyperestrogenism	FIV (early)	FIV	
Organ failure	Parvovirus (recovery)	Nutritional deficiencies	
Chronic	Immune-mediated damage	Pelger-Huet anomaly	
disease/inflammation	Iron deficiency	Macrocytosis (poodles)	
Endocrine disorders	Oxidative injury	Myeloid neoplasia	
Irradiation	Zinc toxicosis		
Hereditary cytopenia	Hereditary enzyme deficiency		
Nutritional deficiencies	Parasitic infections		
Myelophthisis	Allergic reactions		
Myelofibrosis	Inflammation/hypersensitivity		
Marrow necrosis	Paraneoplastic syndrome		
Immune-mediated damage	Mature lymphoid leukemia		
	Myeloproliferative neoplasia		
	Plasma cell neoplasia		

Abbreviations: FIV, feline immunodeficiency virus; FeLV, feline leukemia virus.

Myelodysplastic syndrome (MDS) is a term used to designate a group of disorders characterized by peripheral blood cytopenias; however, the marrow is either hypercellular or normocellular for the corresponding cell lineage and there is morphologic evidence of dysplasia in one or more cell lines. Blast cell forms compose less than 20% of nucleated cells.[11] It is believed that normal blood cell maturation, differentiation, function, and survival are impaired, leading to the development of peripheral blood pancytopenia; patients may be at increased risk to transform to AML.[12] Primary MDSs are irreversible acquired developmental disorders of bone marrow stem cells unrelated to concurrent disease, nutritional deficiency, or drug-induced toxicosis.[10] MDSs may be divided into two subtypes (MDS-refractory cytopenia and MDS-excess blasts) depending on the percentage of blast cells in the bone marrow.[13] Cases of MDS-excess blasts, those with 5% or greater percentage of myeloblasts, demonstrate shorter survival and poor response to treatment.[10] MDS is sometimes referred to as *preleukemia* as patients with this syndrome often suffer from chronic debilitation that may continue unchanged or evolve into acute leukemia. The cause is often unknown, but the condition has been associated with FeLV infections.[14]

Ineffective erythropoiesis, granulocytopoiesis, and *megakaryocytopoiesis* are terms applied to the situation in which there is hyperplasia of a cell line in marrow but persistence of the corresponding cytopenia in blood with no evidence of a cause of peripheral loss. Intramedullary death of precursor cells, usually by apoptosis, is inferred from this finding; the most common cause is immune-mediated destruction of precursor cells.[15]

Erythroid, myeloid, and/or *megakaryocytic hypoplasia* are terms applied to the situation in which there are fewer precursor cells than appropriate for the number of mature cells in peripheral blood. For example, the absence of erythroid hyperplasia in an anemic patient would be called erythroid hypoplasia denoting some degree of suppression of erythropoiesis. While the number of erythroid cells in marrow from patients with anemia of inflammatory disease or renal failure may be within normal limits for a nonanemic animal, it is hypoplastic in light of a low hematocrit. Dogs with Sertoli cell tumors may have severe bone marrow hypoplasia due to suppressive effects of estrogen produced by the tumor.[16] Hypoplasia may also result with the effects of infectious agents including viruses (eg, FeLV, FIV, feline coronavirus, feline parvovirus, canine parvovirus, canine distemper virus), rickettsia, protozoa, and fungi. Drugs are often responsible for bone marrow damage and include anticonvulsants, antineoplastic agents, estrogen, and antibiotics such as chloramphenicol and trimethoprim-sulfadiazine.[17] Patients with endocrine disturbances, chronic renal failure, or intestinal malabsorption may have hypoplasia of the erythroid line alone.[18] Hypoplasia of granulocytic lines can result from various infections and toxic insults.[19] Animals admitted for tumor resection and receiving antineoplastic agents should be evaluated for the presence of drug-related myelosuppression. Less common causes involve hereditary disorders, marrow necrosis, immune-mediated disease, myelophthisis, and irradiation. Cytologically, when the hematocrit or CBC is not provided but the marrow particles appear hypocellular with more fat than cells and there is an increased M:E, one is not certain whether erythroid production is less than expected. In this case it can be said that there is a relative erythroid hypoplasia compared to the granulocytic response.

Aplastic anemia is a term applied to the situation in which the marrow is devoid of hematopoietic cells and has been replaced by fat. Destruction or suppression of multipotential hematopoietic stem cells is implicated by this finding. Causative mechanisms may include myelotoxic substances, immune-mediated mechanisms, and some infectious diseases (eg, canine ehrlichiosis).[20,21] Selective absence of one

cell line with normal production of cells in the other two lines is called *pure red cell aplasia, pure white cell aplasia,* or *amegakaryocytosis*.[22] Immune-mediated attack (eg, young cats with pure red cell aplasia) on committed progenitor cells of the affected series is the apparent cause in most cases.[23]

Plasmacytosis, lymphocytosis, histiocytosis, and *mastocytosis* are descriptive terms for increased numbers of plasma cells, lymphocytes, macrophages, and mast cells in marrow samples. Whether the increase is reactive or neoplastic must be determined in each case from other clinical and laboratory findings. When marrow injury is mild, with minimal cell destruction, a reactive response usually occurs. *Reactivity* is a nonspecific immune response involving an increase in such cells as plasma cells, mast cells, macrophages, and eosinophils. B-cell hyperplasia has been associated with FIV infection in cats.[24]

Necrosis and *myelofibrosis* are morphologic changes associated with bone marrow destruction with subsequent healing response. Infection, drugs, chemicals, neoplasia, radiation, or immune destruction may damage the bone marrow. When the insult is severe enough to damage the microcirculation, causing ischemia or directly destroying the hematopoietic cells, permanent and irreversible necrosis may occur.[25] The prognosis is generally poor because long-term supportive care is necessary. If necrosis or marrow injury is moderate, attempts to repair the affected area may result in increased numbers of reticulin and collagen fibers, a condition termed *myelofibrosis*. This is considered a secondary response and may be reversible.[26] In both necrosis and myelofibrosis, bone marrow aspirates often contain particles with low cellularity, much blood contamination, and occasional fibrocytes present. For this reason, a core biopsy sample obtained concurrently with the aspirate biopsy is necessary for confirmation of these conditions. In some cases, the suspicion of connective tissue infiltration may need to be confirmed with special histochemical stains. The peripheral blood may reveal few changes or show severe cytopenias.

Hemosiderosis is considered when iron stores accumulate within macrophages thus appearing as dense aggregates with large coarse granules that react strongly with Prussian blue stain. The accumulation implies increased erythrocyte destruction or ineffective erythropoiesis. This situation is present in cases with anemia of chronic disease related to decreased iron utilization or with pure red cell aplasia related to immune-mediated destruction of erythroid precursors. Increased hemosiderin levels have also been associated with feline myelofibrosis and myelodysplastic conditions.[27]

Reporting Bone Marrow Findings

Key features of the CBC and blood film, as well as bone marrow cytologic and histologic findings should be included in the final report. The white cell count, hemoglobin concentration, and red cell indices (MCV and MCHC), and platelet count should be routinely reported and for some patients, the reticulocyte count or chemistry abnormalities (eg, increased globulins) should be provided. The body of the report should include an assessment of cellularity, a systematic description of each cell lineage and their sequence of maturation, the M:E ratio, and evaluation of marrow iron stores. The presence of abnormal marrow components, including abnormal cells or cell numbers and matrix material, should be also described. The presence of focal lesions, identified only in the histologic sections must also be included in the final bone marrow report. If the patient has a previous bone marrow aspiration, comparison should be provided with previous findings to assess disease progress or response to treatment. While it is possible that a definitive diagnosis can be made

based on the marrow aspirate, if it cannot, a list of additional tests (eg, immunophenotyping, protein electrophoresis, and infectious disease testing) should be provided.

It is also important for the pathologist to relate what his/her level of certainty is in the diagnosis. For example, varying levels of confidence are provided by the following statements: the findings are consistent with an AML; the marrow features are suggestive for, but are not alone conclusive of a diagnosis of AML; the marrow aspiration cytology supports a diagnosis of AML, however it is essential to assess these findings in light of clinical and other laboratory data; or marrow aspiration cytology does not support a diagnosis of AML. Finally, if a bone marrow aspirate yields only peripheral blood or cellularity is too low for an adequate evaluate, this should be reported so that it become part of the patient's record.

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