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■ Practical bone marrow cytology in the dog and cat

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■ Βασικές αρχές της κυτταρολογικής εξέτασης του μυελού των οστών στο σκύλο και τη γάτα

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ABSTRACT

In companion animal medicine, bone marrow (BM) aspiration cytology is a cost-effective diagnostic tool, which provides excellent morphological detail of cells and infectious agents. The major indications for BM aspiration include unexplained clinical manifestations (e.g., fever, body weight loss), persistent hematological or biochemical abnormalities (e.g., anemia, leukocytosis), diagnosis and/or staging of malignancies (e.g., lymphoma, mast-cell tumor), diagnosis of important infectious diseases such as leishmaniosis (*Leishmania infantum*) and canine monocytic ehrlichiosis (*Ehrlichia canis*) and evaluation of canine iron stores. Complications associated with BM aspiration are rare and the equipment and supplies required minimal, hence accounting for the popularity of this procedure in the clinical setting compared to BM core biopsy. This review focuses on the technical details pertaining to the collection of BM aspirate material, including the usual anatomic sites for sampling, the equipment required, the preparation of the animal, the aspiration technique and the BM material processing for good quality slide preparation. The description of the cellular population normally anticipated in the BM of the dog and cat and the systematic approach in evaluating and interpreting the BM cytological findings are also highlighted. In the last part of this review, the current classification guidelines for the canine and feline BM malignancies are briefly outlined.

Keywords: Bone marrow, cat, cytological evaluation, dog

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ΠΕΡΙΛΗΨΗ

Η κυτταρολογική εξέταση του μυελού των οστών (ΜΟ) αποτελεί ένα πρακτικό διαγνωστικό εργαλείο στην ιατρική του σκύλου και της γάτας, που επιτρέπει τη λεπτομερή μορφολογική εκτίμηση κυττάρων και μικροοργανισμών. Οι κυριότερες ενδείξεις για την κυτταρολογική εξέταση του ΜΟ περιλαμβάνουν τη διερεύνηση ασυνήθιστων κλινικών (π.χ. πυρετός, απώλεια σωματικού βάρους), αιματολογικών (π.χ. αναιμία, λευκοκυττάρωση) και βιοχημικών διαταραχών, τη διάγνωση και την κλινική σταδιοποίηση νεοπλασμάτων (π.χ. λέμφωμα, μαστοκύττωμα), τη διάγνωση σημαντικών λοιμωδών νοσημάτων όπως της λείσμανίωσης (*Leishmania infantum*) και της μονοκυτταρικής ερlichίωσης (*Ehrlichia canis*) του σκύλου, καθώς και την εκτίμηση της παρακαταθήκης σιδήρου στο ΜΟ του σκύλου. Η παρακέντηση του ΜΟ είναι πολύ ασφαλής και ο απαιτούμενος εξοπλισμός ελάχιστα δαπανηρός, γεγονός που εξηγεί τη μεγαλύτερη συχνότητα με την οποία διενεργείται στην κλινική πράξη σε σχέση με την οστεομυελική βιοψία. Στην ανασκόπηση αυτή περιγράφονται οι τεχνικές λεπτομέρειες για την αξιόπιστη συλλογή ΜΟ και κυρίως τα συνήθη ανατομικά σημεία για τη δειγματοληψία, ο απαιτούμενος εξοπλισμός, η προετοιμασία του ζώου, η τεχνική αναρρόφησης του ΜΟ και η διαδικασία παρασκευής κυτταρολογικών επιχρισμάτων. Ιδιαίτερη έμφαση δίνεται στην περιγραφή του φυσιολογικού κυτταρικού πληθυσμού του ΜΟ στο σκύλο και στη γάτα, καθώς και στην οργανωμένη εξέταση και ερμηνεία των κυτταρολογικών ευρημάτων. Τέλος, παρουσιάζεται επιγραμματικά η σύγχρονη κυτταρολογική διάκριση των νεοπλασμάτων του ΜΟ στο σκύλο και στη γάτα.

Λέξεις ευρετηρίασης: γάτα, κυτταρολογική εξέταση, μυελός των οστών, σκύλος

INDICATIONS FOR BONE MARROW (BM) ASPIRATION

After birth and in health, hematopoiesis is normally confined to BM, which occupies the medullary cavities of trabecular bone (Figure 1). Bone marrow comprises of red marrow that contains the abundance of hematopoietic cells (active marrow) and yellow marrow, predominated by adipose tissue. The distribution and relative proportion of red and yellow marrow are age dependent. After birth, the BM cavity is completely occupied by red marrow. With

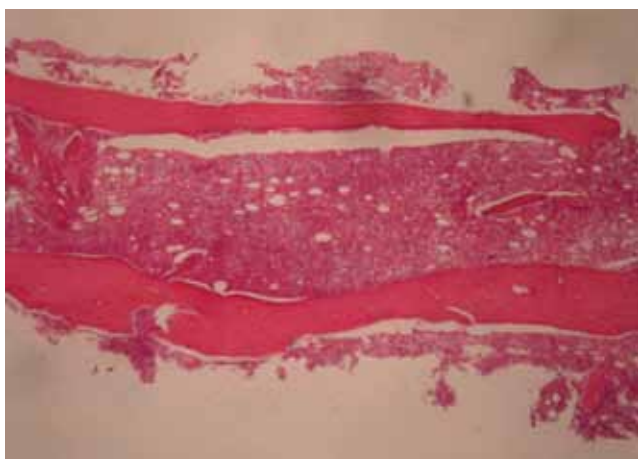


Figure 1: Bone marrow trephine biopsy of normal bone structure showing bony trabeculae and active hematopoietic bone marrow. Paraffin embedded, hematoxylin and eosin x 20.

aging, hematopoietic marrow is gradually replaced by yellow marrow so that during adulthood, red marrow is mainly confined to the vertebrae, skull, ribs, sternum, pelvis and the proximal humeri and femora. This trend, however, can change and with increased demand and appropriate stimuli hematopoietic tissue can expand.

Bone marrow aspiration cytology is a cost-effective diagnostic tool, providing excellent morphological detail of cells and infectious agents (Grindem et al., 2002). Though with BM core biopsy, the architectural pattern of tissue is better reflected, and a range of features including BM cellularity, the presence of myelofibrosis and focal inflammatory or neoplastic lesions are better appreciated (Raskin and Messick, 2012), cellular detail is more difficult to assess; the latter along with a slightly more elaborate procedure for obtaining the sample and longer processing times might contribute to the fact that, in the clinical setting, BM aspirations are more frequently performed compared to core biopsies in the dog and cat (Grindem et al., 2002).

Indications for BM examination include hematological abnormalities that cannot be investigated by other means, investigation, staging and management of hematologic and other neoplasias (e.g., lymphoma,

Table 1: Decision-making variables for bone marrow (BM) examination in dogs and cats with hematologic abnormalities

Variable	Comments
Age	<ul style="list-style-type: none"> BM examination is not necessary in a young animal with neutropenia and anemia and laboratory tests supportive of parvovirus infection.
Single or multi-lineage aberrations	<ul style="list-style-type: none"> Usually single abnormalities do not necessitate BM examination. Multiple abnormalities, especially if morphologic atypia is present, are strong indications for BM examination. BM examination is indicated in the majority of pancytopenic patients.
Anemia	<ul style="list-style-type: none"> BM examination is not indicated if underlying cause is inferred by the history, clinical examination or peripheral blood examination.
Erythrocytosis	<ul style="list-style-type: none"> BM examination not required for secondary or relative erythrocytosis that can be explained by the clinical condition (e.g. hyperadrenocorticism).
Neutropenia	<ul style="list-style-type: none"> Isolated neutropenia, especially with morphological evidence of toxic changes in the blood smear examination, usually does not require BM examination. Marked chronic neutropenia, especially with absence of toxic morphology and no obvious underlying cause probably requires BM examination.
Neutrophilia	<ul style="list-style-type: none"> Neutrophilia with toxic changes and obvious underlying etiology usually does not require BM examination. Marked chronic neutrophilia, with appreciable dysplasia indicates the need for BM examination.
Thrombocytopenia	<ul style="list-style-type: none"> Isolated thrombocytopenia may or (more commonly) may not require BM examination.
Thrombocytosis	<ul style="list-style-type: none"> No BM examination is required when clinical signs suggest reactive thrombocytosis.
Blasts	<ul style="list-style-type: none"> BM examination always indicated.
Other abnormal cells	<ul style="list-style-type: none"> May or may not require BM examination.
Single-multilineage dysplasia	<ul style="list-style-type: none"> Multilineage dysplasia always indicates BM examination for possible myelodysplasia or leukemia (in practice occasionally omitted in FeLV positive cats)

From Foucar 2001 (modified)

mast cells tumors, solid tumors), evaluation of unexplained clinical manifestations (e.g., fever of undetermined origin, body weight loss, lameness), evaluation of a patient that does not respond to treatment after initial diagnosis (e.g., failure of response to therapy in a patient diagnosed with immune mediated thrombocytopenia (ITP)), response to therapy for patients with haemolymphoid neoplasms, diagnosis of diseases such as leishmaniosis (*Leishmania infantum*) and canine monocytic ehrlichiosis (*Ehrlichia canis*) and evaluation of iron stores in the dog (e.g., differentiation of iron deficiency anemia from anemia of inflammation) (Foucar, 2001, Mylonakis et al., 2003, Mylonakis et al., 2004, Saridomichelakis et al., 2005, Raskin and Messick, 2012).

Several variables should be taken into account before making the decision to perform BM examination (Table 1). In conjunction with the clinical history and other laboratory findings, these include the age of the animal, the presence of single or multiple hematological abnormalities, the presence of single or multi-lineage dysplasia or the detection of blast or other abnormal cells in peripheral blood. Marrow aspiration is redundant if the underlying cause of the abnormality is inferred or known from the history (e.g., a chemotherapy session preceding the development of neutropenia), the physical examination (e.g., melena in an anemic animal) or the blood smear evaluation (e.g., regeneration and spherocytosis in an anemic dog, *Babesia* spp. trophozoites in a dog with fever) (Harvey, 2012a). On the contrary, the presence of blast cells or pancytopenia, almost always necessitate BM examination. In any case of uncertainty, the clinician is advised to seek the assistance of a clinical pathologist or a specialized hematopathologist. Bleeding tendency is not a contraindication for BM aspiration if a superficial site is sampled and proper hemostatic measures are applied (Mylonakis et al., 2004).

Overall, BM aspiration is a very safe procedure with rarely appearing complications. Post-sampling tissue injury, hemorrhage, neuropathy or infection may occur, but they are avoided if adherence to technical details is exercised. The collection of

BM from the sternum or the ribs bears the risk of inadvertently penetrating the thoracic cavity and lacerating intra-thoracic organs, especially if material is obtained from small-sized dogs (Harvey, 2012a).

SITES, EQUIPMENT AND TECHNIQUE FOR BM ASPIRATION

The most suitable BM aspiration sites in large-sized dogs include the iliac crest and proximal (greater tubercle) humerus, while in small-sized dogs and cats the humerus and the proximal (greater trochanter) femur are the most accessible (Raskin and Messick, 2012). Alternatively, it was recently shown in healthy anesthetized Beagles that sternal BM aspiration with a hypodermic needle was safe, providing cytological samples of equivalent quality to those from humerus or ilium (Defarges et al., 2013). Historically, aspiration of the proximal rib with special aspiration needles (Silverman-type) has also been found to provide cytological samples of high quality in the dog, although the technique was more risky and labor-intensive compared to the iliac crest and sternum (Penny and Carlisle, 1970). Anecdotally, aspiration of the costochondral junction of the rib with a hypodermic needle in medium-to-large-sized dogs may also be a safe and practical technique for obtaining cytological material. A combined technique has been recently described and evaluated in adult dog cadavers, in which aspiration precedes core biopsy but both procedures are done with the same needle and at the same site (Reeder et al., 2013). Although there was no difference in cellularity, megakaryocyte count, myeloid/erythroid ratio (M/E ratio), iron stores, or the overall diagnostic quality, marrow core length was shorter, hemorrhage artifact was more common and the failure rate in obtaining diagnostic material was higher using the combined technique compared to the standard direct core biopsy method (Reeder et al., 2013).

Postmortem BM aspiration should be performed within 30 minutes from the animal's death, as cellular degeneration (especially of the myeloid



Figure 2: Equipment and supplies for bone marrow (BM) aspiration: Aspiration needle (15-gauge, Illinois-type), 2% lidocaine solution, 10-ml syringe ringed with 0.15 ml of 2-3% EDTA solution, microscope slides, scalpel blade No. 11, EDTA tube for BM storage, sterile gloves, antiseptic soap preparation.

lineage) begins rapidly after death, and the overall quality of the aspirate tends to be poor (Harvey 2012a, Grindem et al., 2014). Ideally, in an animal planned to be euthanized, BM aspiration should be done prior to the administration of the euthanasia solution (Harvey 2012a). If a delay is anticipated until postmortem aspiration, the cadaver is placed under refrigeration but not freezing (Raskin and Messick, 2012).

Equipment and supplies for BM aspiration are minimal (Figure 2). Mild to moderate pain is experienced during the BM aspiration procedure (Guillot et al., 2011); therefore, sedation (e.g., butorphanol [0.2 mg/Kg] and acetylpromazine [40-50 µg/Kg] in one syringe, intramuscularly) may be required in uncooperative animals. In the majority of clinical canine and feline cases, local anesthesia (infiltration of subcutaneous and subperiosteal tissues with lidocaine 2% [1ml/10 Kg]) normally suffices for the procedure. Admixing lidocaine with bicarbonate (9/1 parts, i.e., 45 ml lidocaine with 5 ml bicarbonate), minimizes the pain induced by lidocaine infiltration itself. The authors apply the following aspiration procedure: the patient is placed in a position that facilitates access to the aspiration site (e.g., lateral position for the humerus and proximal femur, and standing or sitting position for the iliac crest). The

area is clipped, surgically scrubbed and infiltrated with lidocaine. Sterile gloves are always used by the operator. A stab incision is made in the skin with a No. 11 scalpel blade. The aspiration needle with stylet in place is passed through the skin incision to the bone. The needle is then rotated and advanced in a clockwise-counterclockwise motion until it is firmly embedded into the bone. The stylet is removed, a syringe ringed with a few drops of ethylenediamine tetra-acetic acid (EDTA) solution (0.1-0.15 ml of 2-5% solution) is attached to the needle, and steady negative pressure is applied to the plunger (5-7ml vacuum pressure) to draw marrow into the syringe. At this stage, vocalization is strong evidence that the marrow cavity has been sampled. As soon as approximately 0.5-1 ml of BM aspirate is collected, the pressure on the plunger is released and the syringe is removed along with the needle. Subsequently, direct pressure is applied at the sampling site to minimize bleeding and the incision is usually not sutured. If BM collection was impossible on the first attempt, the stylet is reinserted and the needle is advanced further in the bone, and negative pressure is reapplied. If sampling is still impossible, the pressure is maintained and the needle is slowly withdrawn, until BM is procured or the needle exits the bone. Repeated failures to obtain BM, indicates the need for aspirating an adjacent site, another bone, or to perform core biopsy.

BM ASPIRATION SAMPLES PROCESSING

BM aspiration samples should be processed as quickly as possible after collection, ideally within 2 hours; for short-term storage (up to 8 hours), they may be kept at room temperature or be refrigerated (Raskin and Messick, 2012). Several smear preparation techniques have been described (Mylonakis et al., 2005, Harvey, 2012a). The most commonly used is the squash technique: one drop of blood is expelled onto a glass slide, the latter is tilted to allow drainage of excessive blood and the slide-attached spicules are squashed by another perpendicularly-placed slide. Alternatively, BM aspirate material is expelled into

a Petri dish and several spicules are picked with a plastic pipette, transferred to glass slides and squashed by a slide or coverslip. The latter technique offers the advantage of allowing the clinician to aspirate a larger volume of sample increasing the likelihood of a successful aspiration procedure, since it helps decrease the effect of blood contamination during the selection and smearing process. Once several smears have been prepared, they are subject to rapid air-drying, and stained with a Romanowsky-type stain such as Giemsa, Wright-Giemsa and Diff-Quik (Mylonakis et al., 2005), usually requiring longer staining times compared to regular blood smears (usually 2 staining cycles for automated stainers). Additional stains, such as Pearls' stain for iron, Periodic acid-Schiff (PAS) for fungi, can be performed on an individual basis. Smears containing adequate BM material will have deep blue-staining material on them. Submission to a diagnostic laboratory should ideally include numerous stained and unstained, unfixed slides.

NORMAL MARROW CELL POPULATION

The recognition of the cells normally appearing in the BM and their expected reference ranges is essential for the proper cytological evaluation of BM in diseased dogs and cats.

Erythrocytic lineage

The consecutive developmental stages of the erythrocytic (erythroid) cells include the rubriblasts, prorubricytes, rubricytes (basophilic and polychromatophilic), metarubricytes, and the polychromatophilic and mature erythrocytes (Figures 3-5) (Harvey, 2012a).

The *rubriblasts* have an almost perfectly round nucleus, with fine chromatin pattern and one or two visible nucleoli. The cytoplasm is intensely basophilic and this stage has the highest nucleus-to-cytoplasm (N:C) ratio of the erythroid precursors. The *prorubricytes* have usually no visible nucleoli, a slightly less basophilic cytoplasm and a lower N:C ratio compared to rubriblasts. The *basophilic rubri-*

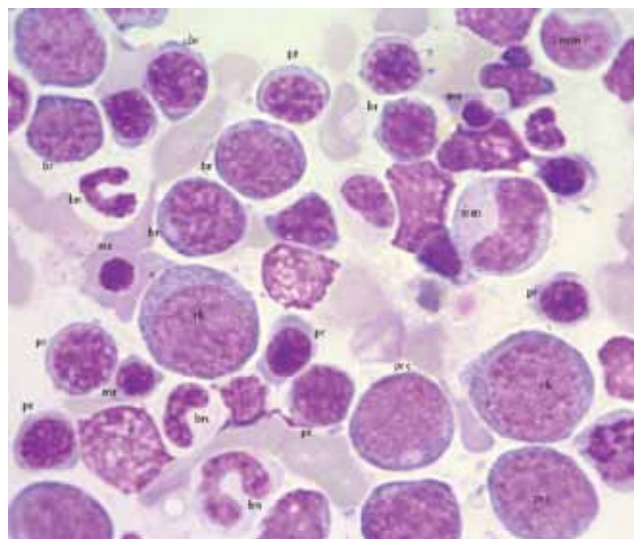


Figure 3: Canine bone marrow aspirate: various developmental stages of the erythroid series are seen, including rubriblasts (rb), prorubricytes (prc), basophilic (br) and polychromatophilic (pr) rubricytes and metarubricytes (mr). A few band neutrophils (bn) and metamyelocytes (mm) are also present (Giemsa, x1000).

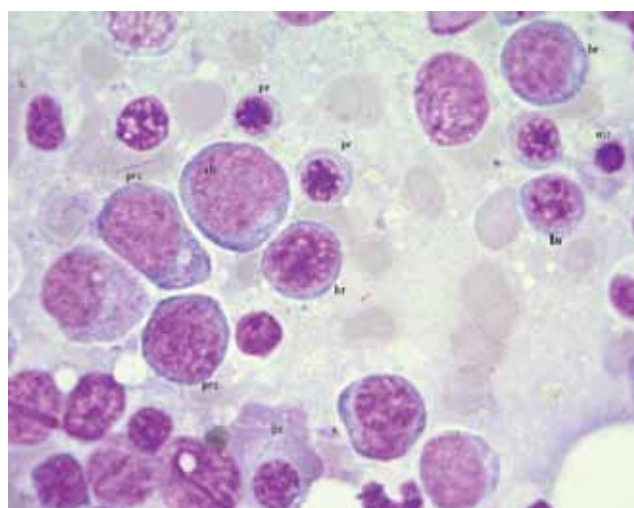


Figure 4: Same dog as in Figure 3. Various developmental stages of the erythroid series, including rubriblasts (rb), prorubricytes (prc), basophilic (br) and polychromatophilic (pr) rubricytes and a metarubricyte (mr). A plasma cell (pc) is also seen at the bottom of the field (Giemsa, x1000).

cytes are smaller than the prorubricytes, have a blue cytoplasm, lower N:C ratio and a very heterogeneous chromatin pattern (dark and light areas). The *polychromatophilic rubricytes* have reddish-blue cytoplasm, are slightly smaller than basophilic rubricytes and have a more condensed nucleus. The *metarubricytes* feature a very pyknotic nucleus and a cytoplasm that is polychromatophilic or orthochromic

(red). The *polychromatophilic erythrocytes* have lost their nucleus and they mostly represent reticulocytes (stained with supravital stains), eventually forming *mature erythrocytes*.

Normally, rubriblasts account for less than 5% of all erythroid cells in the BM (blast pool), the aggregate of prorubricytes and rubricytes for the 65-75% of all erythroid cells (proliferative pool) and metarubricytes for 20-30% of all erythroid cells (maturation-storage pool) (Grindem et al., 2014).

Granulocytic and monocytic lineages

The consecutive developmental stages of the granulocytic cells include the myeloblasts, the progranulocytes, the myelocytes, the metamyelocytes, the bands and the mature neutrophils (segmented neutrophils or segmenters) (Figure 6). The myeloblast, the myelocyte and the metamyelocyte stages of the granulocytic series cannot reliably be differentiated from monocytes (Grindem et al., 2014).

The *myeloblasts* are large round cells (overall, larger than rubriblasts but smaller than megakaryoblasts) with a round to oval nucleus with fine chromatin and one or more visible nucleoli. The cytoplasm is less basophilic than that of rubriblasts and this stage has the highest nucleus-to-cytoplasm (N:C)

ratio of the series. Cytoplasmic granules are not usually observed, but primary (non-specific) granules tend to form in late myeloblasts and therefore a few (<15) magenta-staining granules may be seen in the cytoplasm (Harvey, 2012a). The *progranulocytes* have usually no visible nucleoli and the cytoplasm is typically packed with several primary magenta-staining granules, while the N:C ratio is lower compared to myeloblasts. The *myelocytes* are smaller than progranulocytes and have a round nucleus without visible nucleoli. These cells are deprived of primary granules, but secondary granules appear at this stage. They are not visible in neutrophils due to their neutral staining properties, but are easily seen in eosinophils and basophils. The *metamyelocytes* feature a kidney-shaped nucleus, with indentations extending between 25% and 75% of the nuclear width and cytoplasmic characteristics similar to those of myelocytes. The *band cells* have a curved, rod-shaped nucleus with parallel sides and no area with diameter less than half the diameter of any other nuclear area. The *segmented granulocytes* (mature neutrophils, eosinophils or basophils) demonstrate a nucleus with two or more lobes and areas with intense constrictions.

Normally, myeloblasts account for less than 5% of all myeloid cells (blast pool), the progranulo-

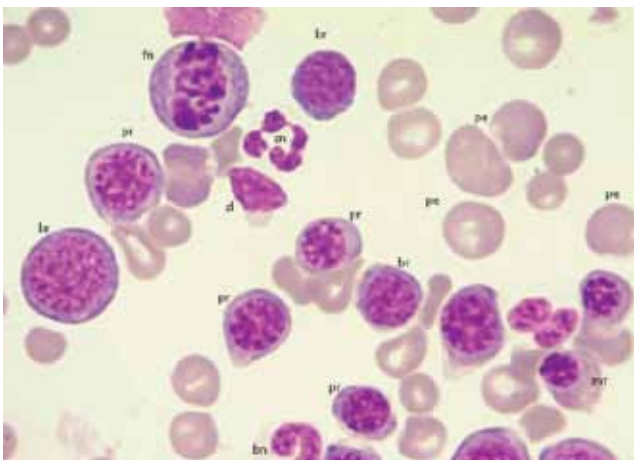


Figure 5: Bone marrow aspirate from a dog with immune-mediated hemolytic anemia. Various late developmental stages of the erythroid series predominate, including basophilic (br) and polychromatophilic (pr) rubricytes and metarubricytes (mr). A small lymphocyte (sl), a few band (bn) and segmented (sn) neutrophils, a presumptive basophilic rubricyte with fragmented nucleus (fn) and several polychromatophilic erythrocytes (pe) are also seen (Giemsa, x1000).

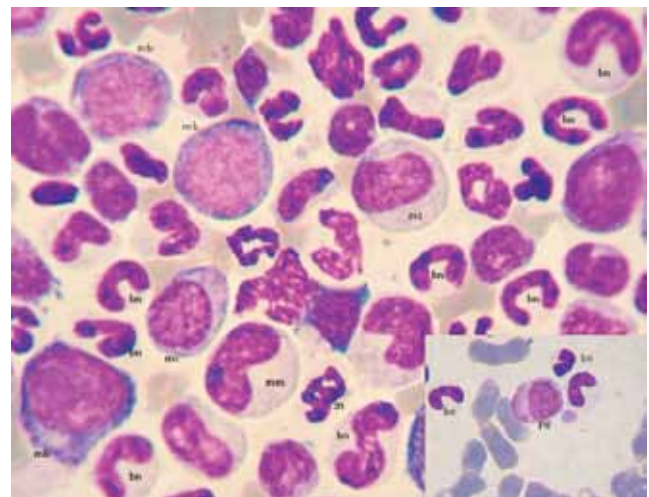


Figure 6: Various developmental stages of the myeloid series, including myeloblasts (mb), myelocytes (mc), metamyelocytes (mm), band neutrophils (bn) and segmented neutrophils (sn). Inset: a progranulocyte is seen amidst band neutrophils (Diff-Quik, x1000).

cytes plus myelocytes for 15% of all myeloid cells (proliferative pool) and the aggregate of metamyelocytes, bands and segmenters for 80-85% of all myeloid cells (maturation-storage pool) (Grindem et al., 2014).

The monocytic series includes the monoblasts, promonocytes and monocytes and account for a small percentage of the BM cells. *Monoblasts* cannot be differentiated from myeloblasts by light microscopy, although they tend to have a more irregular nucleus, while *promonocytes* are similar to neutrophilic myelocytes and metamyelocytes. *Monocytes* are identical to those seen in the blood (Grindem et al., 2014).

Megakaryocytic lineage

The consecutive stages of the megakaryocytic series include the megakaryoblasts, promegakaryocytes, and megakaryocytes (Figure 7). *Megakaryoblasts* are characterized by a single nucleus and deeply basophilic cytoplasm, and they are hardly differentiated from other blast cells. With maturation, megakaryoblasts undergo endoreduplication resulting in the formation of very large multi-

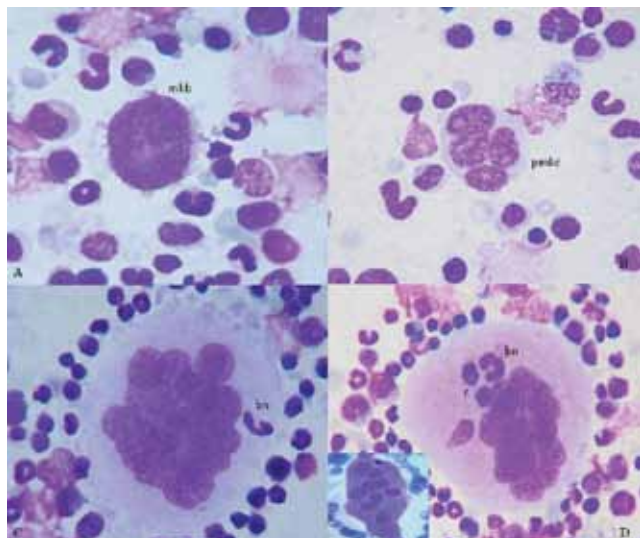


Figure 7: Megakaryocytic developmental stages in bone marrow aspirate smears. A: megakaryoblast (mkb) with single nucleus, B: promegakaryocyte (pmkc) with four nuclei, C: basophilic megakaryocyte with multiple fused nuclei and an intracytoplasmic band neutrophil (bn), D: mature megakaryocyte with a cytoplasmic band neutrophil (bn) and two presumptive rubricytes (r). Inset of figure 7D: a multinucleated osteoclast is visualized (Giemsa, x1000).

nucleated cells. The ploidy level of megakaryocytes ranges from 4N (tetraploidy) to 32 N, with the majority of the cells present as 16N (Bain et al., 2010). *Promegakaryocytes* are defined as clearly larger cells compared to the erythrocytic or myelocytic precursors that display 2-4 distinct nuclei. *Megakaryocytes* are very large cells (50-200 μm in diameter) featuring a multilobulated nucleus. Based on their cytoplasmic characteristics they can be further classified into three distinct stages of maturation. Group 1 cells display strong cytoplasmic basophilia and a very high N:C ratio. Group 2 megakaryocytes display less intense staining basophilic cytoplasm, azurophilic granulation and a lower N:C ratio. Finally, Group 3 megakaryocytes feature abundant pale blue staining cytoplasm and numerous azurophilic granules. Overall, the majority of megakaryocytes (≥ 70 -80%) in BM aspirates are mature (Queisser et al., 1971, Mylonakis et al., 2005).

Lymphocytes and plasma cells

Lymphocytes share a common precursor with myeloid cells. The lymphocytes appearing in the BM are identical to those in the blood. Small lymphocytes predominate, but occasional medium-sized or large-sized lymphocytes are seen. In the majority of dogs and cats, lymphocyte percentages do not exceed 5-10% of nucleated cells; however, as many as 15-20% lymphocytes have been noticed in healthy dogs and especially cats (Grindem et al., 2002).

Plasma cells (Figure 4) are normally larger than small lymphocytes and feature a round eccentric nucleus and densely basophilic cytoplasm. A clear cytoplasmic area adjacent to the nucleus representing the Golgi apparatus, further typifies plasma cells. Plasma cells containing round structures (Russell bodies) are called Mott cells. Both lymphocytes and plasma cells are unevenly distributed in the BM, and their percentages may substantially differ between different areas, but overall, plasma cells do not exceed 2% of BM cells in dogs and cats (Grindem et al., 2014).

Miscellaneous cells

Macrophages do not normally exceed 2% of cells in the BM of dogs and cats. They usually display cytoplasmic vacuolation and may contain phagocytized material such as nuclear debris, hemosiderin and less frequently erythrocytes or leukocytes. Osteoclasts (Figure 7) and osteoblasts are occasionally seen in BM aspiration smears especially in young growing animals. Osteoclasts are giant cells with separate multiple nuclei that may be confused with megakaryocytes; however, the latter have fused nuclei. Osteoblasts are reminiscent of “large plasma cells”, having an eccentric nucleus and foamy basophilic cytoplasm. Mast cells lacking morphological atypia are very rarely seen in the BM of healthy dogs accounting for no more than one per 1,000 nucleated cells (Mylonakis et al., 2006). Vascular and connective tissue cells are uncommonly seen and tend to become more apparent in aplastic BM when the precursors of the other blood cells are depleted. Adipocytes are almost always present in BM aspirates. Mitotic cells, not always identifiable, may account for 2% of nucleated cells in the BM (Harvey, 2012a).

EVALUATION AND INTERPRETATION OF BM CYTOLOGY

The BM cytological examination should always be done in the context of hematological findings from a complete blood cell count and peripheral blood smear morphologic examination performed simultaneously or within hours of BM aspiration. A minimum of 3-4 smears should be examined as the cellularity can differ between the spicules or infiltration by a neoplastic population of cells can be patchy. The outcome of the aspiration procedure itself may offer some clues towards the overall BM quality. As a norm, easily obtained material indicates a normocellular or hypercellular BM, while repeated failure to obtain sufficient material (“dry taps”) may be due to poor technique, hypocellular or fibrotic marrow, or a densely-packed marrow cavity

(Grindem et al., 2014). A step-by-step cytological evaluation of BM is outlined in Table 2.

Low power microscopy

The evaluation starts on the low power (x10 objective) for the assessment of smear quality (including the selection of areas suitable for high power microscopy), BM cellularity, megakaryopoietic activity and the semiquantitation of iron stores. High quality smears have numerous well spread out BM spicules and several monolayers of intact, well-stained cells. The cellularity of BM is evaluated by estimating the proportion of cells to adipose tissue in the flecks (Figures 8 and 9). It is an age-dependent

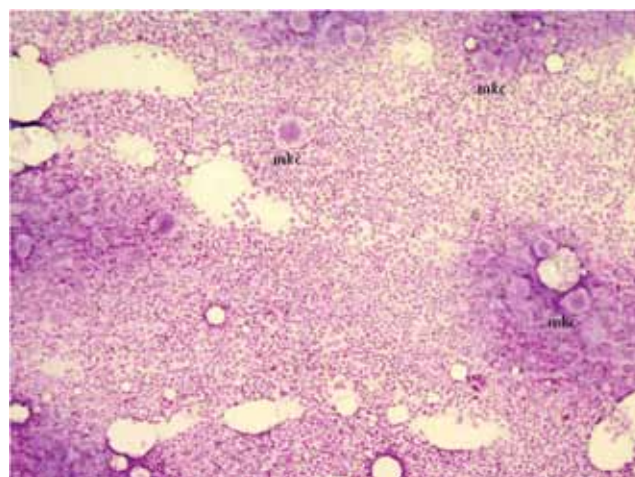


Figure 8: Increased bone marrow cellularity in a dog with acute (non myelosuppressive) monocytic ehrlichiosis (*Ehrlichia canis*). Several megakaryocytes (mkc) are scattered in the field (Giemsa, x100).

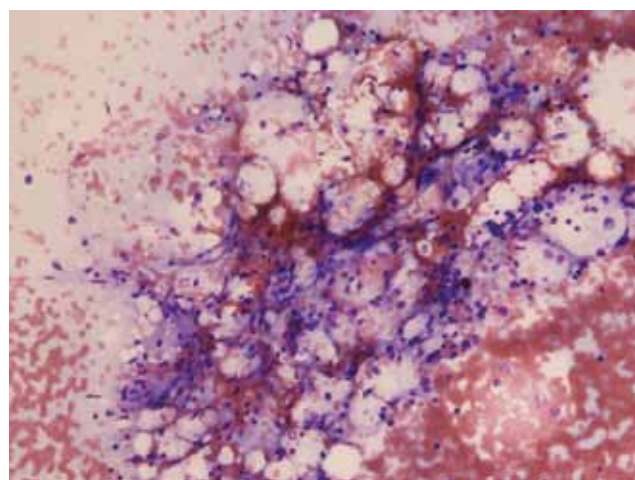


Figure 9: Decreased bone marrow cellularity in a dog with aplastic pancytopenia (modified Wright-Giemsa, x200).

Table 2: Step-by-step evaluation of bone marrow (BM) cytology in the dog and cat

BM FEATURE	SPECIFIC OBSERVATIONS OR COMMENTS
<i>Low power (x10 objective) light microscopy</i>	
Quality of smear	<ul style="list-style-type: none"> • Adequacy of spicules • Monolayers with intact, well-stained cells • Selection of fields for high power microscopy
BM cellularity	<ul style="list-style-type: none"> • Age-dependent variable
Megakaryocytic lineage	<ul style="list-style-type: none"> • Quantification • Sequence and maturation of developmental stages • Dysplasia
Iron stores	<ul style="list-style-type: none"> • Applicable to dogs only
<i>High power (x100 objective) light microscopy</i>	
Myeloid/erythroid (M/E) ratio	<ul style="list-style-type: none"> • 500-cell differential or subjective differential count
Erythrocytic lineage	<ul style="list-style-type: none"> • Sequence and maturation of developmental stages • Dysplasia
Myelocytic lineage	<ul style="list-style-type: none"> • Sequence and maturation of developmental stages • Dysplasia
Percentage of blast cells	<ul style="list-style-type: none"> • Of all nucleated cells (excluding plasma cells, lymphocytes, macrophages and mast cells) • Of non-erythroid cells
Numbers and morphology of various cells	<ul style="list-style-type: none"> • Lymphocytes • Plasma cells • Mast cells • Monocytes and macrophages • Hemophagocytosis
Infectious agents	<ul style="list-style-type: none"> • <i>Leishmania</i> spp., <i>Ehrlichia</i> spp., etc. • Non hematopoietic neoplastic cells

From: Raskin and Messick 2012 (modified)

variable with young animals having cellularity as high as 75% and aged individuals as low as 25% in health. Cellularity varies among smears and between flecks; therefore, several smears and flecks should be examined to reach a reasonably accurate estimation. Hypocellular BM is usually seen in aplastic disorders (e.g., myelosuppressive canine monocytic ehrlichiosis, viral feline leukemia, estrogen toxicity) or in myelofibrosis (Weiss, 2003). A hypercellular

BM indicates either erythroid or myeloid hyperplasia, myeloid, lymphoid or metastatic neoplasms, or myelodysplasia (Grindem et al., 2014).

Quantitative assessment of megakaryopoiesis is largely subjective, due to the relatively low numbers and uneven distribution of platelet precursors in the BM smears, being more abundant within or near the BM particles or the smear edges (Grindem et al., 2014). Published normal canine megakaryocyte

numbers vary considerably, mainly due to differences in smear preparation technique and in the way they are quantified (Mylonakis et al., 2005). Overall, 2–7 cells per spicule, or 2–4 per low-power field in squash preparations, represent a likely normal megakaryocyte count (Mylonakis et al., 2005, Silva et al., 2012, Grindem et al., 2014). More than 10–20 cells per low-power field may suggest megakaryocytic hyperplasia (Grindem et al., 2014), indicating a regenerative thrombocytopenia, usually due to increased destruction (e.g., immune-mediated thrombocytopenia) or utilization (e.g., disseminated intravascular coagulation) of platelets, reactive thrombocytosis (e.g., iron deficiency) or essential thrombocythemia. When the percentage of mature megakaryocytes is less than 50%, a regenerative response or a maturation arrest is inferred (Grindem et al., 2014). Megakaryocytic hypoplasia is usually observed in the context of generalized BM suppression (Mylonakis et al., 2004), but uncommonly, selective aplasia of the megakaryocytic series may be seen, presumably due to immune-mediated mechanisms (Lachowicz et al., 2004). Engulfed neutrophils or other blood cells may temporarily be found superimposed on the cytoplasm of mature megakaryocytes, a phenomenon called emperipoiesis (Figure 7). The significance of this finding in the dog and cat is currently unclear. Dysplastic changes of megakaryocytes (dysmegakaryocytopoiesis) include dwarf megakaryocytes (small megakaryocytes with one or two condensed nuclei and mature staining cytoplasm), large but hypolobulated or hyperlobulated megakaryocytes, and increased numbers of promegakaryocytes. Dysplasia may occur in primary (neoplastic) or secondary (nonneoplastic) myelodysplastic syndromes mainly due to drugs, immune-mediated disease and various hematopoietic neoplasms (Harvey, 2012b).

Stainable iron (hemosiderin) may appear within macrophages or extracellularly as gray to black aggregates in routinely stained cytological smears (Figure 10). Reliable assessment of BM iron stores necessitates the use of Prussian blue stain. Unlike dogs, healthy cats lack stainable iron in the BM. As a rule, diminished iron stores in the dog suggest iron deficiency, while increased stores may be associated

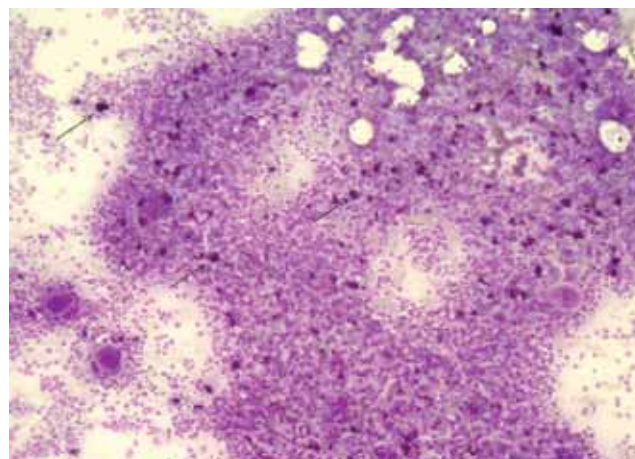


Figure 10: Bone marrow iron stores in a dog with hemolytic anemia. Hemosiderin appears as several black globules (arrows) (Giemsa, x100).

with hemolytic anemia, anemia of chronic disease or increased age (Mylonakis et al., 2010, Harvey, 2012a).

High power microscopy

Unlike megakaryocytic cells, a thorough morphologic examination of the remaining cell lineages, and the estimation of the differential counts and myeloid/erythroid ratio (M/E) require the use of x50 or x100 objectives.

The M/E ratio (also referred to as granulocytic/erythroid ratio) is calculated by identifying 500 cells (originating equally from the marrow flecks and inter-fleck areas) as granulocytes (including segmenters) or nucleated erythrocytes (Moritz et al., 2010). In the clinical setting, subjective estimation of M/E ratio usually suffices. In the majority of healthy animals, the M/E ranges from 0.5 to 3 (Bienzle, 2012, Harvey, 2012a, Raskin and Messick, 2012, Grindem et al., 2014). The interpretation of the M/E ratio should be made in conjunction with marrow cellularity (Grindem et al., 2002).

In healthy dogs and cats, all stages of erythrocytic series are represented in the normal proportions, with the vast majority of cells (>80%) being in the mature stages (rubricytes and metarubricytes). Isolated erythrocytic hyperplasia implies that the M/E ratio is decreased, the BM cellularity is normal or increased and the neutrophil count in the blood is

normal and may be effective (increasing hematocrit) or ineffective (Harvey 2012b). Effective hyperplasia usually occurs in hemorrhagic or hemolytic anemias (Figure 5), while ineffective hyperplasia may commonly appear in chronic iron deficiency anemia and in non-regenerative immune-mediated hemolytic anemia (Stokol et al., 2000). Isolated erythrocytic hypoplasia denotes that the M/E ratio is increased, the BM cellularity is normal or decreased and the neutrophil count in the blood is normal or decreased (Harvey, 2012b). When the M/E ratio exceeds 75, pure red cell aplasia or selective erythrocytic aplasia is defined (Harvey, 2012b). Acquired erythrocytic hypoplasia or aplasia is usually the result of an immune-mediated response that may entirely inhibit the production of erythrocytes or result in a maturation arrest at any stage of the maturation process (Stokol et al., 2000, Harvey, 2012b). It may also be associated with myeloid neoplasms, FeLV-infected cats and in a subset of animals given recombinant human erythropoietin (Mills, 2012). Dysplastic changes of erythrocytic cells (dyserythropoiesis) include increased numbers of rubriblasts, megaloblastic cells, multinucleated cells, abnormal nuclear shapes or fragmentation (Figure 5), nuclear and cytoplasmic asynchrony, maturation arrest and siderotic inclusions (Harvey, 2012b). Dysplasia may occur in primary (neoplastic) or secondary (non-neoplastic) myelodysplastic syndromes due to drugs, various hematopoietic neoplasms, FeLV-infected cats, and in immune-mediated hemolytic anemia (Harvey, 2012b).

In healthy dogs and cats, all stages of myelocytic series are represented in normal proportions, with the vast majority of cells (>80%) being in the mature stages (metamyelocytes, bands and segmenters). Myelocytic (most commonly neutrophilic) hyperplasia implies that the M/E ratio is increased, the BM cellularity is normal or increased and the hematocrit is normal or increased, and may be effective (accompanying blood neutrophilia) or ineffective (persistent blood neutropenia) (Harvey, 2012b). Effective hyperplasia (Figure 6) usually occurs in bacterial infections, immune-mediated diseases, paraneoplastic syndromes,

early estrogen toxicity and chronic myeloid leukemia (Lucroy and Medewell, 1999, Lucroy and Medewell, 2001). The percentage of myeloblasts is not usually increased out of proportion, with the exception of chronic myeloid leukemia, in which myeloblasts may be up to 20% of all nucleated cells (Harvey, 2012b). Ineffective neutrophilic hyperplasia may commonly appear in myelodysplastic syndromes, acute myeloid leukemias and FeLV and FIV infections in cats. Granulocytic (most commonly neutrophilic) hypoplasia denotes that the M/E ratio is decreased, the BM cellularity is normal or decreased and the hematocrit is normal (Harvey, 2012b). Selective neutrophilic hypoplasia or aplasia may result from chemotherapy drugs, griseofulvin in cats, or occur in a subset of

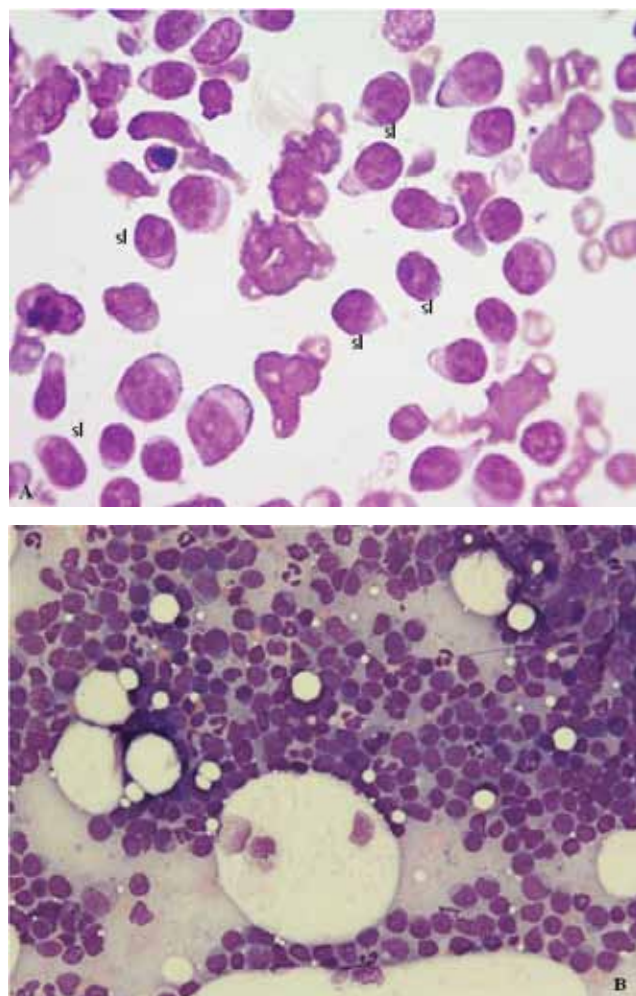


Figure 11: A) Canine chronic lymphocytic leukemia. Small lymphocytes (sl) account for more than 30% of all nucleated cells (Giemsa, x1000). B) Feline small cell lymphoma: small lymphocytes efface the bone marrow (modified Wright-Giemsa, x500).

cases in immune-mediated neutropenia in the dog, and parvoviral infections in dogs and cats (Kalli et al., 2010, Harvey, 2012b). Dysplastic changes of granulocytes (dysgranulopoiesis) include increased numbers of myeloblasts, abnormal nuclear shapes (e.g., donut-shaped nuclei) or multinucleated cells, giant metamyelocytes, bands and segmented neutrophils, maturation arrest, abnormal mitoses, abnormal granulation, hyposegmentation or hypersegmentation (Harvey, 2012b). Most commonly dysgranulopoiesis occurs in primary (neoplastic) myelodysplastic syndromes or in secondary (nonneoplastic) myelodysplasia in acute myeloid neoplasms, in FeLV and/or FIV-infected cats, and in various drugs (Harvey, 2012b).

Increased numbers of small lymphocytes, sometimes combined with plasmacytosis, may occur in immune-mediated hemolytic anemia in the dog and cat and pure red cell aplasia, thymoma, cholangiohepatitis and pyrexia of unknown origin in the cat (Weiss, 2005), in animals with chronic lymphocytic leukemia or small cell lymphoma, in which mature-appearing lymphocytes exceed 30% of nucleated cells (Figures 11, A and B) (Workman and Vernau, 2003). Increased numbers of medium-sized to large lymphocytes imply acute lymphoblastic leukemia or stage V metastatic lymphoma (Figure 12) (Harvey, 2012b).

Benign causes of increased percentages of plasma cells include infectious diseases including canine monocytic ehrlichiosis (particularly in the chronic phase of the disease), leishmaniosis (Figure 13) and feline infectious peritonitis and frequently the immune-mediated hemolytic anemia (Mylonakis et al., 2006, Harvey, 2012a). Neoplastic proliferation of plasma cells, demonstrating or not morphologic atypia, defines multiple myeloma (Figure 14) (Weiss, 2006a).

Abundant mast cells, especially when they present atypia and tend to form aggregates are strongly suggestive of metastatic mast cell tumor especially in the cat (O'Keefe et al., 1987) (Figure 15). Mast cell hyperplasia may occur in BM hypoplasia or aplasia of various etiologies (e.g., myelosuppressive canine monocytic ehrlichiosis) (Mylonakis et al., 2006).

Macrophages may be increased in BM necro-

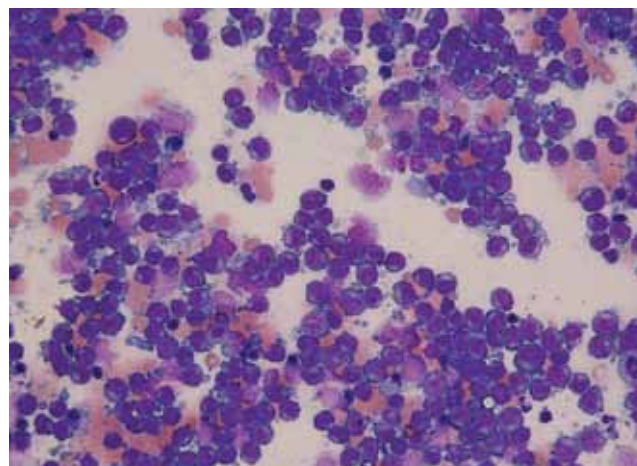


Figure 12: Canine acute lymphocytic leukemia/large cell lymphoma. Large atypical lymphocytes with prominent nucleoli and increased mitotic rate efface the bone marrow (modified Wright-Giemsa, x 200).

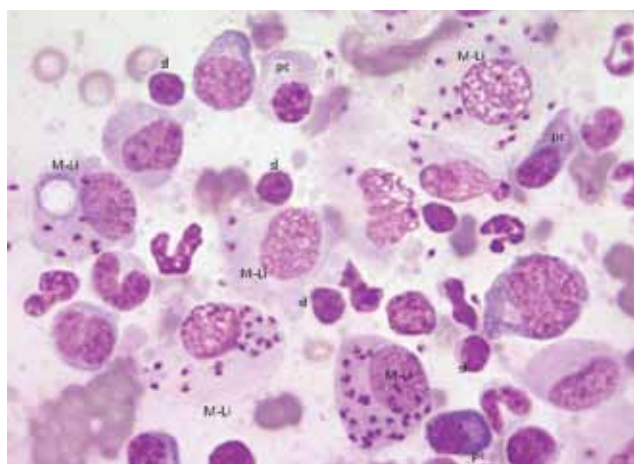


Figure 13: Bone marrow smear from a dog with leishmaniosis (*Leishmania infantum*). Several macrophages with cytoplasmic amastigotes of *L. infantum* (M-Li) are seen. Small lymphocytes (sl) and plasma cells (pc) are also variably present (Giemsa, x1000).

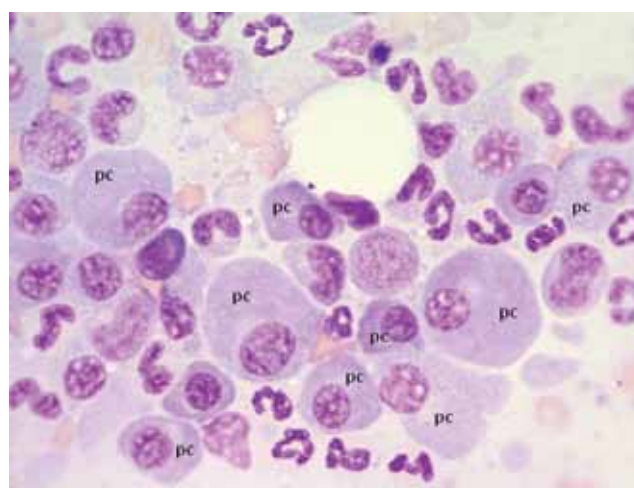


Figure 14: Bone marrow smear from a dog with multiple myeloma: the predominant cell population consists of plasma cells (pc) with eccentrically placed nuclei and basophilic cytoplasm (Giemsa, x1000).

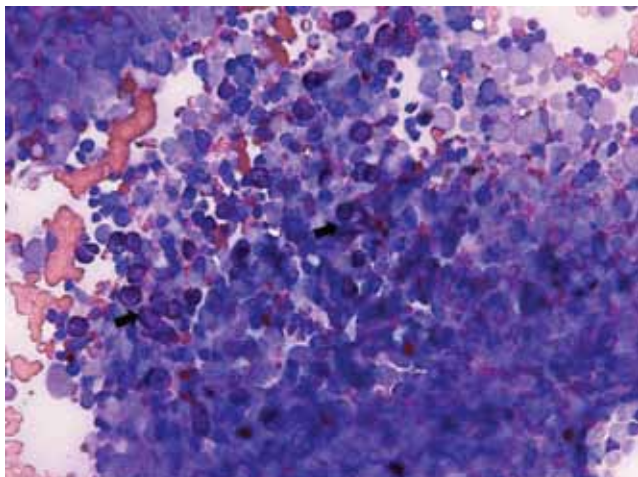


Figure 15: Neoplastic mast cells (arrows) showing no morphologic atypia, infiltrating a canine bone marrow. Note the clustering of the cells. No circulating mast cells were identified in the examination of a peripheral blood film and buffy-coat of this patient (modified Wright-Giemsa, x500).

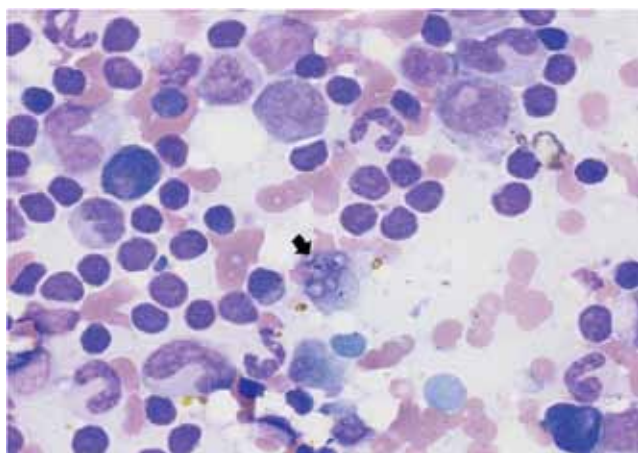


Figure 16: Macrophage containing numerous *Histoplasma capsulatum* organisms (arrow) from an aspirate of canine marrow in a patient with disseminated histoplasmosis (modified Wright-Giemsa, x1000).

sis, granulomatous inflammation or histiocytic sarcoma (Weiss, 2006a, Harvey, 2012a). Infectious agents may be seen in the cytoplasm of macrophages (e.g., *L. infantum*, *E. canis*, *Histoplasma capsulatum* (Figure 16), *Mycobacterium* spp., *Cytauxzoon felis*) (Mylonakis et al., 2003, Saridomichelakis et al., 2005, Harvey, 2012b).

Phagocytosis of blood cells and their precursors is rare in the BM of healthy animals and generally involves only mature erythrocytes (Harvey, 2012b). Frequent BM erythrophagocytoses may occur in immune-mediated hemolytic anemias, in erythrocytic

parasites (e.g., *Babesia* sp., hemoplasmosis), hematopoietic neoplasms, after blood transfusion, and in histiocytic neoplasia, including the hemophagocytic histiocytosis (Mylonakis et al., 2012), while idiopathic cases have also been reported (Weiss, 2007).

BONE MARROW NEOPLASMS

Primary BM malignancies include acute myeloid leukemias (AML) (i.e., neoplasms arising from the erythrocytic, myelocytic and megakaryocytic series), myeloproliferative neoplasms (MPN) (formerly termed chronic myeloid leukemias), primary myelodysplastic syndromes (MDS), acute and chronic lymphoid leukemias and multiple myeloma (McManus, 2005, Juoperi et al., 2011, Harvey, 2012b). Common metastatic marrow neoplasms include lymphoma, mast cell tumor, histiocytic sarcoma and various carcinomas (McManus, 2005, Raskin and Messick, 2012). The presence of blast cells in the BM in excess of 20% of all nucleated cells (ANC) is strongly suggestive of acute myeloid leukemia (Figures 17 and 18), if an exaggerated hyperplastic response has been ruled out (Harvey, 2012b, Raskin and Messick, 2012, Grindem et al., 2014). According to the classification

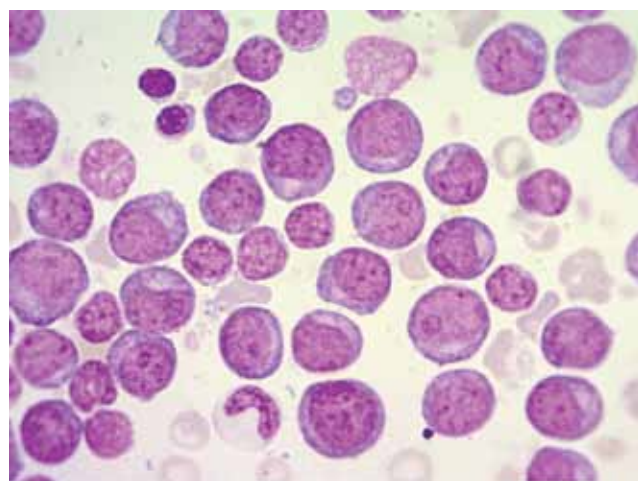


Figure 17: Presumptive erythremic myelosis (AML-M6Er) in a dog. The combination of light microscopic cytomorphology (blast cells with perfectly round nuclei, consistent paranuclear clearing, and deeply basophilic cytoplasm devoid of granules) and the negative immunophenotyping using antibodies to myeloperoxidase, cluster of differentiation (CD) 3, CD79a, CD11b and CD45 were strongly suggestive of AML-M6Er (pure erythroid leukemia) (Giemsa, x1000). [From: Mylonakis ME et al., (2012) Presumptive pure erythroid leukemia in a dog. J Vet Diagn Invest, 24: 1004-1007].

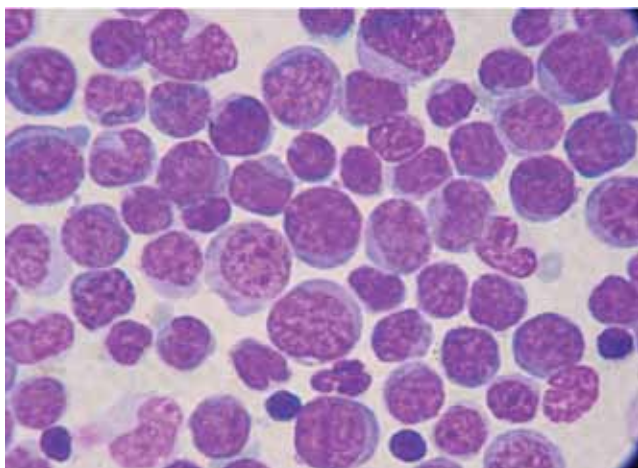


Figure 18: Acute myeloid leukemia in a FeLV-infected cat. A diagnosis of acute myelomonocytic leukemia (AML-M4) was made on the basis of the results of bone marrow cytology (blast cell percentage in excess of 20% of all nucleated cells) and immunochemistry (cluster of differentiation (CD) 3 and CD79a negative, positive for lysozyme, and myeloperoxidase) tests (Giemsa, x1000). [From: Mylonakis ME et al., (2008) Acute myelomonocytic leukaemia with short-term spontaneous remission in a cat. *Aust Vet J*, 86: 224-228].

established by the American Society for Veterinary Clinical Pathology Animal Leukemia study Group, canine and feline acute myeloid leukemias include AML-M1 (myeloblastic leukemia without maturation), AML-M2 (myeloblastic leukemia with maturation), AML-M4 (myelomonocytic leukemia), AML-M5 (monocytic leukemia), AML-M6 (erythroleukemia) or AML-M6Er (erythremic myelosis), AML-M7 (megakaryocytic leukemia) and acute undifferentiated leukemia (Jain et al., 1991, McManus 2005). Blast cells less than 20% of ANC indicate MPN or primary MDS (McManus, 2005). Myeloproliferative neoplasms include chronic myeloid leukemia, eosinophilic leukemia, basophilic leukemia, primary erythrocytosis and essential thrombocythemia (Harvey, 2012b). In MDS more than 10% of the cells in one or more BM cell lineages demonstrate dysplastic changes (Weiss and Aird, 2001, Weiss, 2006b, Harvey, 2012b). In dogs and cats MDS have been classified in three subtypes, namely MDS with erythroid predominance (MDS-Er, M/E<1, blasts<20% ANC), myelodysplastic syndrome refractory cytopenias (MDS-RC, M/E>1, blasts<5% ANC) and myelodysplastic syndrome-excess blasts (MDS-EB, M/E>1, blasts 5-19% ANC) (McManus, 2005, Harvey,

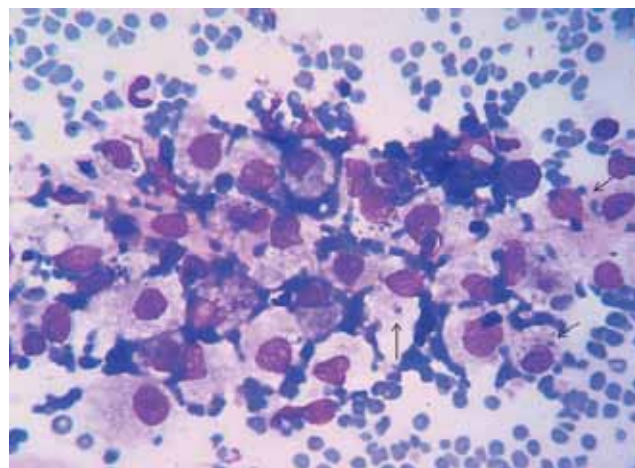


Figure 19: Presumptive histiocytic sarcoma in a persistently anemic cat. Bone marrow aspirate smears were predominated by a monomorphic population of individual or aggregated round-to-oval cells, likely of histiocytic origin that often phagocytosed erythrocytes (arrows) and leukocytes. These features were also demonstrated in spleen aspirates (not shown) (Giemsa, x400). [From: Mylonakis ME et al., (2012) Presumptive histiocytic neoplasm with unusual immunophenotype in a cat. *Comp Clin Pathol*, 21: 231-236].

2012b). Dogs and cats with MDS are prone to develop AML or lymphoid neoplasms (Harvey, 2012b). Mature lymphocytes or lymphoblasts in excess of 30% of ANC support the diagnosis of chronic lymphocytic or acute lymphoblastic leukemia, respectively, provided that a metastatic lymphoma has been ruled out (Workman and Vernau, 2003). The diagnosis of multiple myeloma (Figure 14) is highly suggestive if 15% or more plasma cells occur in the BM (Harvey, 2012b). Large cohesive cell clusters are usually associated with metastatic mast cell tumor, histiocytic sarcoma (Figure 19) or carcinomas (Grindem et al., 2014).

CONCLUDING REMARKS

Bone marrow aspiration cytology is a cost-effective diagnostic tool, which provides excellent morphological detail of cells and infectious agents. Complications associated with BM aspiration are rare and the equipment required minimal, which account for the popularity of this procedure in the clinical setting of companion animal medicine.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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