

BONE MARROW SAMPLING IN VETERINARY MEDICINE. LITERATURE REVIEW

George Laurențiu NICOLAE^{1*}, Cătălina GEORGESCU¹, Teodoru SOARE¹,
Manuella MILITARU¹

¹University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei, 5th District, 050097, Bucharest, Romania

*Corresponding author email: george_nicolae_2006@yahoo.com

Abstract

Bone marrow (BM) evaluation is critical in diagnosing haematological disorders and staging different types of cancer. However, recent studies suggest its role in detecting disseminated tumour cells. This literature review aims to highlight the importance of BM evaluation and aid practitioners in collecting high-quality samples, focusing on domestic carnivores, horses, and laboratory animals, emphasising indications, types of specimens, and sampling techniques. Various indications for BM evaluation include peripheral blood abnormalities, lymphoma staging, myeloproliferative disorders and toxicological analysis. The main types of specimens are BM aspirate and BM core, each requiring a different type of needle. For domestic carnivores, the most accessible sites for collection are the proximal humerus and the iliac crest, while for horses, collection is performed from the sternum. In laboratory animals, BM aspiration is typically performed using the femur, tibia or the iliac crest as the collection site. BM sampling differs between species and can be challenging to obtain in some cases. Choosing the proper sampling technique and corroborating results with clinical and haematology data is important to maximise BM evaluation.

Key words: bone marrow, collection techniques, domestic carnivores, horse, laboratory animals.

INTRODUCTION

Bone marrow (BM) evaluation is a routine diagnostic tool in human medicine, but less used in veterinary practice. The difficulty of obtaining bone marrow samples can impact the quality of the specimens. BM samples include aspiration and core biopsy: each method offers its own advantages, and using both techniques for a comprehensive diagnosis is recommended (Das et al., 2023). It is also important to note that the instruments used for collecting samples from animals are often adapted from those used in human medicine. This fact can make it challenging to apply these techniques effectively in very small animals.

Bone marrow examination in animals is crucial for diagnosing and managing various conditions, especially haematological malignancies and disorders. These examinations provide important insights that guide therapeutic approach and, in laboratory settings, enhance our understanding of drug toxicity, highlighting their significance in both clinical and research fields.

It is important to emphasize that studies indicate that this procedure, although seemingly invasive

and labour-intensive, can be performed under suitable conditions with minimal trauma to the animal. Recovery is typically swift, and complications are rare (Woods et al., 2021).

This literature review highlights the significance of BM evaluation and assists practitioners in collecting high-quality samples, particularly focusing on domestic carnivores, horses, and laboratory animals. It emphasizes the indications, types of specimens, and sampling techniques.

MATERIALS AND METHODS

In order to review the literature on bone marrow sampling, relevant materials were gathered from the current database using the following keywords: bone marrow, collection techniques, domestic carnivores, horses, laboratory animals, literature review, and indications for BM examination. Selected articles were chosen to address the most pertinent aspects of bone marrow collection. The information obtained was compared with data available in reference books on veterinary pathology, clinical pathology, haematology, and oncology. The

following sections outline key aspects of bone marrow collection in veterinary practice.

Indications for BM sampling

Bone marrow evaluation is primarily indicated when changes in peripheral blood are noted. Several such abnormalities include: multilineage changes observed in peripheral blood combined with morphological atypia, most cases of pancytopenia (Mylonakis & Hatzis, 2017), persistent neutropenia, unexplained thrombocytopenia, and non-regenerative or poorly regenerative anaemia (Harvey, 2012). Another important role of bone marrow examination is lymphoma staging (Harvey, 2001). Pinto et al., in 2024, found that some dogs classified as stage V revealed peripheral blood abnormalities in the absence of bone marrow infiltration. Other authors report similar findings: lymphoma-related blood abnormalities suggest bone marrow infiltrates, but this is not certain (Graff et al., 2014) highlighting the importance of bone marrow evaluation as a tool for staging this neoplasm. Bone marrow aspirates are also valuable for identifying infectious or parasitic agents like *Leishmania infantum*, *Cytauxzoon felis*, and *Histoplasma capsulatum* (Wellman & Radin, 2004). Paparcone et al. (2013) concluded that bone marrow aspirate from the sternum is a practical method for the diagnosis of leishmaniasis in dogs. Also, BM evaluation can aid in identifying the cause of hyperproteinaemia associated with multiple myeloma, lymphoma, leishmaniasis, or systemic fungal infections (Harvey, 2001; Harvey, 2012). Similarly, hypercalcemia can be explained in the context of multiple myeloma or lymphoma (Roodman, 1997; Kohart et al., 2017). In dogs, iron stores can also be evaluated by examining the bone marrow (Phiri et al., 2009; Pawsat et al, 2021; Pawsat et al, 2023). In horses, bone marrow is a common source for harvesting mesenchymal stromal cells (MSC), which are used to treat various musculoskeletal diseases (Taylor & Cleg, 2011). Other studies show the utility of bone marrow in detecting disseminated tumour cells. Research in human medicine highlights the significance of bone marrow examination for detecting disseminated tumour cells (DTC) in mammary cancer, as it serves as an independent prognostic factor

(Braun et al., 2000; Siddappa et al., 2013). Bone marrow is considered the most accessible tissue for DTC analysis, as it can be collected during primary surgery (Vincent-Salomon et al., 2008). Marconato et al. (2019) suggest that disseminated tumour cells from the blood and bone marrow could serve as a potential biomarker for canine mammary cancer. The study found that dogs with detectable tumour cells in their bone marrow had a shorter survival time compared to those without detectable tumour cells. Additionally, bone marrow can be useful in identifying the cause of fever of unknown origin (Das et al., 2023). According to a case study, the authors concluded that bone marrow can be used to detect toxic chemicals in forensic veterinary medicine, particularly for investigating exposure to organophosphates and carbamates (Marcelino et al., 2020). Furthermore, bone marrow fat analysis can provide a post-mortem diagnostic tool that can give insights regarding antemortem starvation in emaciated animals (Raglus, 2019).

Types of bone marrow specimens

Bone marrow samples can be represented by either an aspirate or a core biopsy. For aspirates, the Illinois needle is usually used, while for core biopsy, the Jamshidi type needle is needed (Figure 10). Different types of needles are summarised in Table 2. In the field of veterinary medicine, bone marrow aspiration is preferred over bone marrow biopsy, primarily due to its cost-effectiveness and faster processing time (Harvey, 2012). The choice between aspiration and biopsy depends on the clinician's differential diagnoses and the specific information sought from the examination of the bone marrow. In many cases, aspiration and biopsy complement each other, and both may be necessary (Reeder et al., 2013). Cytology yields detailed information about the morphology of bone marrow cells that can be difficult to observe in histological preparations (Wellman & Radin, 2004). Aspirate samples are primarily used for cytology evaluation, but they can also be applied for flow cytometry or the preparation of paraffin section of bone-marrow aspirate, referred to as a clot section (Ong et al., 2015). Core samples, on the other hand, are utilized for histopathology (Figure 1) and immunohistochemistry.

Additionally, a rolling imprint can be performed from a core biopsy, which can then be used for cytology evaluation (Harvey, 2012). Imprints can also be obtained from post-mortem samples (King et al., 2014).

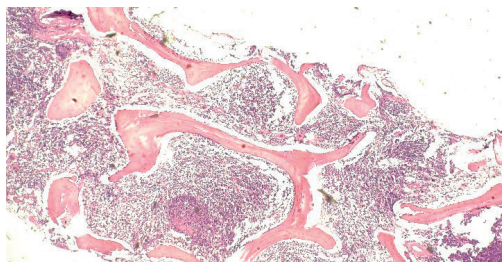


Figure 1. Microscopic aspects of a histological section of a core biopsy, haematoxylin and eosin stain, 50x magnification (Original)

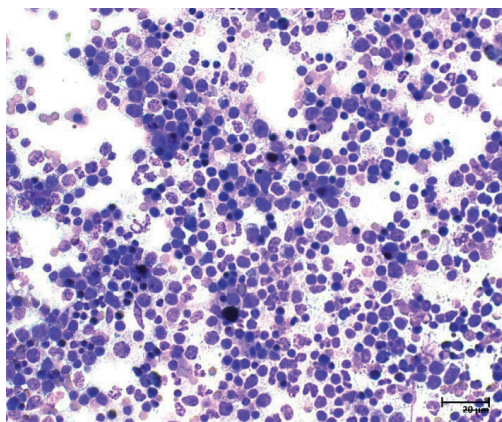


Figure 2. Microscopic aspects of a smear from an aspirate, May-Grünwald stain, 400x magnification (Original)

Cytology (Figure 2) effectively assesses the M:E ratio (myeloid to erythroid ratio) and cell morphology (Reeder et al., 2013), including dysplastic changes (Mori et al., 2020). Examples of pathologies that cytology aids in diagnosis include myelodysplastic syndrome, different types of leukaemia, lymphoma staging, multiple myeloma, precursor-targeted immune-mediated anaemia and other immune-mediated cytopenia.

Cytology can also be helpful for detecting microorganisms (Paparcone et al., 2013; Harvey, 2012). Multiple smears should be done from the aspirate, and they must be done rapidly

after sampling. Otherwise, the aspirate should be placed in an EDTA tube (Ayala-Trejo et al., 2015).

Histopathology is very useful for assessing bone marrow cellularity, localized changes such as inflammation, focal neoplasia or necrosis (Grindem et al., 2002), vascular lesions or examining bone tissue (Harvey, 2012). Such lessons include osteomyelitis, vascular amyloidosis, myelofibrosis or metastatic neoplasia (Grindem et al., 2002).

Another strong indicator for core biopsy is when an unsuccessful aspirate is encountered, a situation referred to as “dry taps” (Harvey, 2012). A core specimen should be at least 0.5 cm long (Weiss & Smith, 2002) and have enough active marrow. Grossly, cortical bone is white, while active marrow is red (Byers, 2017).

After performing a rolling imprint, samples submitted for histopathology or immunohistochemistry should be submitted in 10% buffered formalin.

Further sample processing implies decalcification, paraffin embedding and sectioning, which results in a longer turnaround time for these specimens.

Molecular tests are helpful for the determination of clonality in lymphoma or leukaemia (Avery, 2009). Immunohistochemistry is typically done in combination with histological assessment and is helpful in differentiating reactive from neoplastic lesions, characterisation of metastatic neoplasm and characterisation and classification of lymphoid neoplasms (Kremer et al., 2005).

Flow cytometry can be done in combination with cytology, or it can replace the cytological examination (Reagan et al., 2011). It is performed mainly in cases of myeloproliferative or lymphoproliferative disorders. In human medicine, it has been reported that flow cytometry can detect early myeloma or atypical cases and plays an important role in the differential diagnosis of different types of lymphomas (Yuan & Stetler-Stevenson, 2011). Samples submitted for flow cytometry should be placed in a sodium heparin tube (Lee et al., 2008).

Different types of bone marrow samples are summarised in Table 1 and different types of slides are shown in Figure 3.

Table 1. Sample types, advantages, disadvantages and uses

Sample Type	Advantages	Disadvantages	Used for
Core samples	Overall architecture Sections can be used for immunohistochemistry Accurate cellularity	More expensive Longer sampling time Longer processing time (decalcification) More invasive	Histopathology
			Immunohistochemistry
			Cytology (Rolling Imprints)
			Cytology (Smear)
Aspirate	Cost effective Faster sampling time Faster processing time Easier to assess cell morphology and detect dysplastic changes Easier to assess myeloid to erythroid ratio and cell count	Difficult to assess overall cellularity Focal lesions may be missed	Cloth section
			Flow cytometry

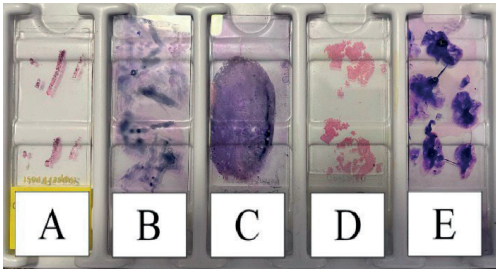


Figure 3. Different slides for bone marrow evaluation: A. Histological section from a core biopsy; B. Roll Imprint from a core sample; C. Smear from a BM aspirate; D. Clot section; E. Imprint from BM harvested from the femur, post-mortem (Original)

Election sites

The main sites for bone marrow collection in animals include the iliac crest, sternum, and proximal humerus, each presenting specific advantages and considerations for veterinary practitioners.

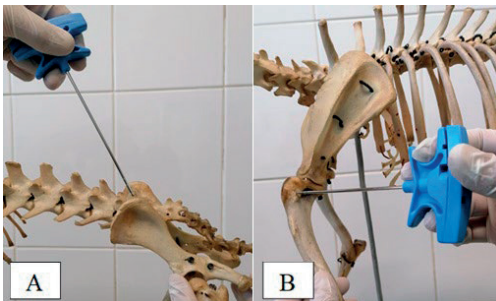


Figure 4. Election sites for BM samples in dogs - A. Iliac crest; B. Proximal humerus

The iliac crest (Figure 4A) is a common site in normal-weight dogs because it is easily accessible and the lateral approach is preferred in small dogs and cats with a thin iliac crest

(Raskin and Messik, 2012). The proximal humerus (Figure 4B) is frequent used in domestic carnivores, especially in obese dogs (Grindem, 1989). In small dogs and cats, the femur can also be used for sampling, including both aspirate and core biopsy; however, core samples are more challenging to obtain (Raskin & Messick, 2012). The sternum can be used in large breed dogs and horses (Harvey, 2012). One study suggests that, in dogs, the sternum is easier to aspirate than the proximal humerus or the iliac crest, with no notable differences in specimen quality among the three (Defarges et al., 2013). Additionally, it has been indicated that ilium samples tend to provide higher quality core specimens (Nicolae et al., 2023). Furthermore, in small dogs, using a 15 g needle for sampling the humerus is more practical than using a 13 g needle (Abrams Ogg et al., 2012). Studies have shown that appropriately sized needles enhance the quality of specimens obtained from small dog breeds (Abrams-Ogg et al., 2012; Raskin & Messick, 2012). Additionally, the use of rotary-powered devices can increase the efficiency and quality of aspiration, resulting in better cellular integrity and reduced pain (Tappin et al., 2014; Swords et al., 2010).

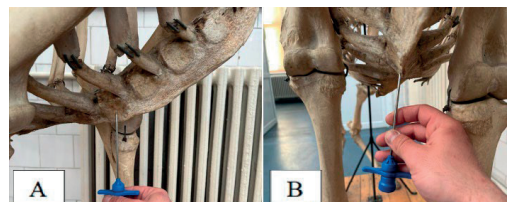


Figure 5. Sternum of a horse, showing the fourth vertebra: A. Lateral view; B. Frontal view

The coxal tuberosity can also be used as a sampling site in young horses and foals (Harvey, 2012). It has been concluded that aspirate samples from the sternum are of higher quality than those sampled from the coxal tuberosity in horses (Delling et al., 2012).

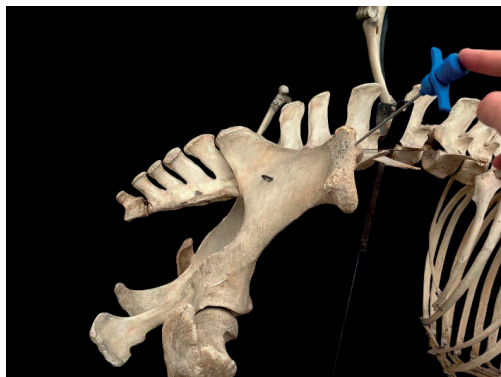


Figure 6. Coxal tuberosity from a horse (Original)

Sampling bone marrow from horses, whether from the sternum (Figure 5) or the coxal tuberosity (Figure 6), implies some risk for practitioners, mainly because of the position of the person collecting the sample (Ribitsch et al., 2010; Delling et al., 2012). In rats, samples usually are harvested from the iliac crest, trochanteric fossa or tibial crest (Brooks et al., 2022).

Sampling technique

Bone marrow sampling is typically performed under sedation. Local anaesthesia through infiltration of the subcutis and periosteum is also required, and may suffice for the aspiration of bone marrow if the animal is cooperative (Mylonakis & Hatzis, 2017).

The procedure must be performed in a sterile manner, which involves clipping hair and surgically scrubbing the selected site (Raskin & Messick, 2012).

When selecting the iliac crest as a collection site, the animal is positioned in sternal recumbency with the pelvic limbs fully flexed and the pelvic acropodium moved cranially under the axilla. The optimal puncture site is located just caudal to the most dorsal point of the dorso-cranial iliac crest (Townsend, 2008). Ensure that the needle is aligned parallel to the long axis of the iliac crest (Harvey, 2012). For the proximal humerus approach, the animals are placed in lateral

decubitus, on the side of the animal that matches the predominant hand of the practitioner, then, the shoulder joint is flexed and rotated to expose the site of collection (Villiers et al., 2016). After palpating the greater tubercle of the humerus, the needle is placed into the craniolateral aspect of the proximal humerus (Harvey, 2001) (Figures 7 and 8).

For the femur procedures, the animal is positioned in lateral decubitus, and the needle is aligned parallel to the axis of the bone (Raskin & Messick, 2012). For aspirating the sternum in dogs, Paparcone et al. (2013) placed the animals in right lateral right lateral decubitus with the right thoracic limb pulled cranially and the left thoracic limb pulled caudally, both being parallel to the animal's body.

When collecting samples from the sternum of a horse, the animal should be sedated and restrained appropriately. A stab incision is made in the skin over the sternum, and a bone marrow aspirate needle is introduced through this incision until it contacts the sternebrae, slightly lateral to midline (Sellon, 2006).

Ultrasonography can aid in identifying the right sternebrae (Eydt et al., 2016). An 11-gauge, 10 cm Jamshidi needle can be advanced through the stab incision and advanced to touch the ventral surface of the 5th sternebrae in the midline (Kasashima et al., 2010). Rowland et al. (2018) found that during sternal bone marrow aspiration in horses, there was no significant difference in pain response or reaction compared to a SHAM procedure, when assessed using salivary cortisol, heart rate variability, and sedation parameters. This was true both during the procedure and for two hours afterward.

In rats, a 26-gauge needle was initially used in a study by Vlahovic (1964) to access the femur's marrow cavity through the knee joint, with its position confirmed by extending a wire stylet.

A more recent technique from Ordodi et al. (2006) improves upon this method. In this technique, the rat is placed in dorsal recumbency, and the investigator stabilizes the leg. The needle is inserted perpendicularly through the skin and muscle above the knee joint until it contacts the femur. A rotating motion creates a hole at the junction of the epiphysis and diaphysis, then the needle is repositioned to enter the diaphysis channel.

In mice, Chung (2014) describes the technique for bone marrow aspirate from the femur. The authors describe the method as follows: first, the tibia is bent; the syringe is held with the thumb and index finger for stability; the needle is inserted through the patellar tendon, positioning it between the femoral condyles and into the femur's shaft, then it is rotated to be aligned with the shaft; the needle is slowly advanced and rotated while checking its placement by gently moving the syringe laterally.



Figure 7. BM aspiration from the proximal humerus of a dog (Original)



Figure 8. BM core sampling from the proximal humerus of a dog (Original)

Both the Illinois and Jamshidi needles are suitable for aspiration procedures. According to Villiers et al. (2016), in dogs, the procedure is described as follows: the needle is inserted into the sampling site, using alternating rotational movements and firm pressure to penetrate the bone cortex; once in the medullary cavity, the needle should remain stable; after removing the stylet, a 20 ml syringe is attached, and the syringe plunger is pulled sharply 2-3 times to collect the bone marrow; the syringe is removed, and the fluid is quickly transferred to microscope slides before it coagulates. To collect a core sample, the Jamshidi needle is advanced 2-3 cm into the medullary cavity

without a stylet and performed rapid rotational movements to section the specimen. The core sample is extracted by introducing the blunt probe of the needle pack in the tip of the needle (Harvey, 2012) (Figure 9).

For post-mortem collection of BM samples, according to King et al. (2014), any large bone can be broken obliquely or sawed to extract the bone marrow; touch imprint is made first; the remaining tissue can be submitted for histopathology.

Reeder et al. (2013) compared bone marrow samples obtained using two different techniques: the combined technique, which involves collecting an aspirate and a biopsy from the same site consecutively, and the direct technique, which involves obtaining a biopsy only, without prior aspirate. The study concluded that the histological specimens obtained from the combined technique were shorter in length and more frequently diluted with peripheral blood compared to those obtained from the direct technique. The appropriate size of the needle according to the animal weight is shown in Table 3.

Table 2. Different types of needles used for BM sampling (data from Veterinary Instrumentation UK Catalogue 2020)

Needle type	Specifications
Jamshidi	Aspirate/Core Biopsy Disposable
Klima	Aspirate Autoclavable
Rosenthal	Aspirate Autoclavable Similar to Klima
Illinois	Aspirate Disposable

Table 3. Choosing the size of the needle according to the weight of the animal (data from Veterinary Instrumentation UK Catalogue 2020)

Animal weight	Needle size
<5 kg	18G
5-15 kg	15-16G
15-30 kg	13G
30-50 kg	11G
>50 kg	8G

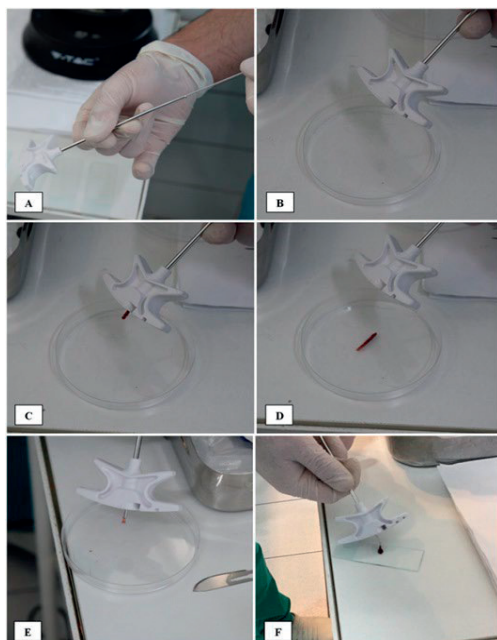


Figure 9. Bone marrow core samples: A-D. Evacuation of the BM core from the needle; E-F. Inappropriate samples: specimen too small (E), specimen missing, only BM liquid is present (F) (Original)



Figure 10. Bone marrow samples and needles. From top to bottom: Jamshidi type needle, Illinois type needle, Syringe with BM aspirate, rolling imprint smears and core sample (on the second smear) (Original)

CONCLUSIONS

The evaluation of bone marrow in veterinary practice is a critical diagnostic tool with significant implications for both clinical and research fields. Although less commonly utilized compared to human medicine, its importance cannot be overstated. Choosing the right specimen is crucial for effective BM evaluation. To achieve an accurate diagnosis, it is essential to correlate BM results with clinical and haematological data.

Through this literature review, it is evident that indications for bone marrow sampling extend beyond identifying blood dyscrasias, as it plays a pivotal role in lymphoma staging, detection of infectious agents, and understanding conditions such as hyperproteinaemia and hypercalcaemia. The examination of bone marrow also opens avenues for advanced research, particularly in understanding the behaviour of disseminated tumour cells, which may serve as significant prognostic markers in companion animals. For practitioners working with domestic carnivores, horses, and laboratory animals, prioritizing high-quality sample collection methods and adhering to established protocols can lead to improved diagnostic outcomes. Finally, bone marrow evaluation in veterinary medicine can enhance our capacity to manage complex health conditions effectively and contribute to advancements in veterinary pathology and oncology.

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