

## Standard Article

*J Vet Intern Med* 2017;31:864–871**Comparative Assessment of the Accuracy of Cytological and Histologic Biopsies in the Diagnosis of Canine Bone Lesions**S. Sabattini, A. Renzi, P. Buracco, S. Defourny, M. Garnier-Moiroux, O. Capitani, and G. Bettini 

**Background:** Osteosarcoma (OSA) should be differentiated from other less frequent primary bone neoplasms, metastatic disease, and tumor-like lesions, as treatment and prognosis can vary accordingly. Hence, a preoperative histologic diagnosis is generally preferred. This requires collection of multiple biopsies under general anesthesia, with possible complications, including pathological fractures. Fine-needle aspiration cytology would allow an earlier diagnosis with a significant reduction of discomfort and morbidity.

**Hypothesis/Objectives:** The aim of this study was to compare the accuracy of cytological and histologic biopsies in the diagnosis of canine osteodestructive lesions.

**Animals:** Sixty-eight dogs with bone lesions.

**Methods:** Retrospective study. Accuracy was assessed by comparing the former diagnosis with the final histologic diagnosis on surgical or post-mortem samples or, in the case of non-neoplastic lesions, with follow-up information.

**Results:** The study included 50 primary malignant bone tumors (40 OSAs, 5 chondrosarcomas, 2 fibrosarcomas, and 3 poorly differentiated sarcomas), 6 carcinoma metastases, and 12 non-neoplastic lesions. Accuracy was 83% for cytology (sensitivity, 83.3%; specificity, 80%) and 82.1% for histology (sensitivity, 72.2%; specificity, 100%). Tumor type was correctly identified cytologically and histologically in 50 and 55.5% of cases, respectively.

**Conclusions and Clinical Importance:** The accuracy of cytology was similar to histology, even in the determination of tumor type. In no case was a benign lesion diagnosed as malignant on cytology. This is the most important error to prevent, as treatment for malignant bone tumors includes aggressive surgery. Being a reliable diagnostic method, cytology should be further considered to aid decisions in the preoperative setting of canine bone lesions.

**Key words:** Bone tumors; Cytology; Dog; Osteosarcoma.

The majority of destructive bone lesions in dogs are neoplastic in origin, and almost all primary bone tumors are malignant.<sup>1</sup> Osteosarcoma (OSA) accounts for up to 85% of primary skeletal malignancies, followed by chondrosarcoma (CSA), hemangiosarcoma (HSA), fibrosarcoma (FSA), myeloma, and lymphoma. Additionally, the skeleton can be affected by metastatic lesions. A presumptive diagnosis of bone malignancy can be based on signalment, history, physical

**Abbreviations:**

|      |                                 |
|------|---------------------------------|
| CSA  | chondrosarcoma                  |
| FNAC | fine-needle aspiration cytology |
| FSA  | fibrosarcoma                    |
| HB   | histologic biopsy               |
| HSA  | hemangiosarcoma                 |
| OSA  | osteosarcoma                    |

examination, and radiographic changes, including severe osteolysis and periosteal reaction.<sup>1</sup> However, several benign diseases might resemble malignant tumors both clinically and radiographically, such as osteomyelitis, traumatic, and dysplastic lesions, thus needing to be included in the differential diagnosis.<sup>2,3</sup>

Although aggressive treatment is required for all primary malignant bone neoplasms, therapeutic decisions, and prognosis might differ considerably according to tumor type. The median survival time for dogs with appendicular OSA treated with limb amputation and chemotherapy ranges from 8 to 18 months.<sup>4–7</sup> Primary HSA of bone is an equally aggressive tumor, with median survival times <1 year.<sup>1</sup> Conversely, CSA and FSA share a lower metastatic potential and surgery alone might be curative.<sup>1,8</sup>

Histologic interpretation of bone biopsies is usually recommended to obtain a preoperative diagnosis. Nevertheless, this procedure requires general anesthesia and complications might occur, including pathological fractures, increased pain, hematoma, and local seeding of tumor cells;<sup>9–11</sup> the latter might be a serious concern if a limb-sparing procedure has been planned. In addition, histologic results are not always conclusive, because biopsies might be of inadequate size or quality or not sufficiently representative.<sup>3</sup>

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*Cytological and histological samples derived from the clinical activity of the veterinary teaching hospitals of the universities of Bologna and Turin. The microscopical evaluation was performed at the Department of Veterinary Medical Sciences, University of Bologna.*

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Fine-needle aspiration cytology (FNAC) offers several advantages over histologic biopsy (HB), including minimal invasiveness, lower risk of complications, ease of sample collection, and rapid results.<sup>12</sup> However, it also has several limitations, including the inability to evaluate tissue architecture; this might prompt a generic diagnosis of malignancy without further classification of tumor type.<sup>13–15</sup> In addition, clinicians can be concerned about the difficulty in performing an adequate cytological sampling from a bone lesion, because of the challenge of penetrating the bone cortex.

To determine the reliability of FNAC as a diagnostic procedure for canine bone lesions, we reviewed this experience over the past 15 years at our institutions. The accuracy of cytology was compared with that of HB. In particular, we evaluated the ability of both methods to discriminate between benign and malignant lesions and, among the latter, to correctly identify tumor type. Additionally, we evaluated the potential effects of multiple clinicopathological variables on FNAC accuracy, to determine the most appropriate use for this procedure.

## Materials and Methods

### Case Selection

A retrospective study was performed on canine osteodestructive lesions diagnosed from 2000 to 2016 at the Department of Veterinary Medical Sciences (University of Bologna) and the Department of Veterinary Sciences (University of Turin).

Dogs receiving a FNAC, a HB or both for diagnostic purposes were considered for inclusion. The cases with a final diagnosis on a surgical or post-mortem histologic sample were selected for inclusion.

If the first diagnosis was consistent with a benign or a non-neoplastic process, for which surgery was not indicated, the correctness of diagnosis was assessed by evaluating the long-term outcome.

Primary cutaneous or oral tumors with local bone infiltration (eg, squamous cell carcinoma, melanoma) were not included.

The radiographic images were retrospectively evaluated by 2 of the authors (PB, OC) to assess the amount of bone lysis and periosteal reaction. The amount of bone lysis was estimated in proportion to the entire lesion and classified as mild (<20%), moderate (20–50%), or severe (>50%). Periosteal reaction was assessed by comparing the thickness of the reaction (Rt) with the thickness of unaffected cortex (Ct) and classified as absent, mild ( $Rt < Ct$ ), moderate ( $Rt = Ct$ ), or severe ( $Rt > Ct$ ).

The cytological samples were collected under local anesthesia (if necessary) by fine-needle aspiration with 21–22 Gauge needles and 2.5–5-mL syringes. The sampling site was selected by radiographic examination and palpation, in order to collect cells from lesser-mineralized areas and to introduce the needle where the periosteal reaction was minimal. The collected material was then deposited and smeared on glass slides, which were allowed to air-dry, stained with May–Grünwald–Giemsa, and cover slipped.

The histologic samples were collected by means of an 8–11 gauge Jamshidi needle. The biopsy site was planned based on radiographic findings. Dogs underwent general anesthesia and were surgically prepared. After practicing a 2–3 mm skin incision with a scalpel, the cannula with the stylet locked in place was advanced through the soft tissue until bone was reached. Then the stylet was removed, and the bone cortex was penetrated with the

cannula with the aid of rotation movements, being careful not to penetrate the cortex on the opposite side, and then withdrawn. The obtained specimens were expelled from the cannula with a probe. When feasible, the procedure was repeated through the same incision with a different redirection of the instrument. Histologic slides were obtained after formalin-fixation, decalcification (if necessary), and paraffin embedding. Sections were cut at 4  $\mu$ m and stained with hematoxylin and eosin.

### Microscopic Evaluation

All FNAC and HB preparations were re-examined (SS, AR, SD) without knowledge of the previous and final diagnoses. Diagnosis was by consensus. The pathologists were not blinded to imaging studies and clinical findings.

The evaluated cytological variables included cellularity (classified as absent, low, moderate, or abundant), blood contamination (classified as absent, low, moderate, or abundant), prevalent cell population and its characteristics, secondary cell populations and noncellular material.

The histologic assessment of HBs (first histologic diagnosis) and of surgical or post-mortem samples (final diagnosis) was carried out according to the schemes of the World Health Organization (WHO).<sup>16</sup> For sarcomas, the histologic grade of malignancy was assessed according to previously published criteria.<sup>17–19</sup> Osteosarcomas were further divided into 2 categories on the basis of osteoid production (productive and poorly productive).

### Statistical Analysis

Accuracy, sensitivity, specificity, positive, and negative predictive values of FNAC and HB were assessed by comparing the first diagnosis (cytological or histologic) with the final histologic diagnosis on surgical or post-mortem samples, or with the clinical outcome if surgery was not performed. In particular, we evaluated the diagnostic accuracy of both methods in correctly identifying malignant neoplastic lesions and, within these, in diagnosing the specific tumor type. The results were presented in a confusion matrix. The confidence intervals of sensitivity and specificity were analyzed by the receiver operating characteristics (ROC) method. The effects of lesion site, tumor diameter, bone lysis, periosteal reaction, cellularity, blood contamination, tumor grade, and osteoid production on the possibility to obtain a correct cytological diagnosis were further evaluated with Fisher's exact test. Data were analyzed by SPSS statistical software<sup>a</sup>. *P* values < .05 were considered significant.

### Statement of Animal Care

This study is a retrospective investigation carried out on archived tissue samples from canine bone lesions. As the research did not influence any therapeutic decision, approval by an Ethics Committee was not required. All the examined samples were collected for diagnostic purposes as part of routine standard care. Owners gave informed consent to the use of clinical data and stored biological samples for teaching and research purposes.

## Results

### Study Population

Review of medical records identified 68 cases of canine bone lesions that were sampled by FNAC (*n* = 53) or HB (*n* = 28). Thirteen cases were sampled by both methods. Eighteen dogs were mixed-breed;

among purebred dogs, the most represented were Boxer (n = 7), Rottweiler (n = 7), German shepherd (n = 6), and Labrador retriever (n = 4). There were 35 males (51.5%; 4 castrated) and 33 females (48.5%; 17 spayed). The median age was 8.6 years (range, 1.5–14.3), and the median weight was 30.5 kg (range, 4–68). Lesions involved the appendicular skeleton in 58 cases (85.3%) and the axial skeleton in 10 (14.7%).

Radiographs were available for review in 50 cases (73.5%). Bone lysis was graded as mild in 8 cases (16%), moderate in 15 cases (30%), and severe in 27 cases (54%). Periosteal reaction was classified as absent in 11 cases (22%), mild in 13 cases (26%), moderate in 12 cases (24%), and severe in 14 cases (28%). The median diameter of lesions was 5 cm (range, 1–25 cm).

**Final Diagnoses**

The final diagnosis was obtained by the histologic examination of surgical or post-mortem samples in 58 cases. Benign lesions consisted of 1 granulomatous mycotic osteomyelitis and 1 foreign body (vegetal material) osteomyelitis. Malignant lesions included 40 OSAs, 5 CSAs, 2 FSAs, 3 poorly differentiated sarcomas, and 6 carcinoma metastases (mammary carcinoma, n = 5; tonsillar squamous cell carcinoma, n = 1). Seven sarcomas (14%) were grade I, 21 (42%) were grade II, and 22 (44%) were grade III. There were 28 osteoblastic, 5 chondroblastic, 3 fibroblastic, 2 giant cell, and 2 poorly differentiated OSAs. Twenty-four (60%) were classified as osteoproliferative and 16 (40%) as poorly osteoproliferative.

In the remaining 10 cases, surgical or post-mortem samples were not available, but a malignant process was excluded based on the evidence of no clinical progression and long-term survival with no surgery or chemotherapy (median, 4 years; range, 1–6 years). Two of these cases (20%) were sampled by FNAC, 6 (60%) by HB and 2 (20%) by both methods. Among FNACs, 1 case was nondiagnostic, 2 were diagnosed as osteomyelitis and one as reactive bone. Among HBs, 1 case was diagnosed as osteochondroma, 1 as osteomyelitis, 4 as reactive bone and 2 as normal bone.

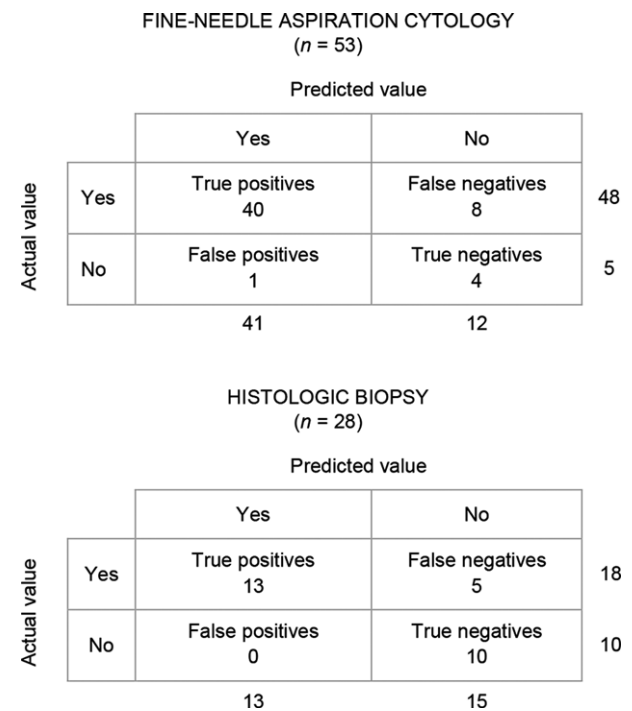
**Histologic Biopsy Diagnoses**

Twenty-eight lesions were sampled by HB. The mean number of biopsy samples per case was 3 (range, 1–7). The accuracy of HB compared with the final diagnoses was 82.1%. In particular, 10 of 10 benign lesions (specificity: 100%; 95% CI: 69.2–100%) and 13 of 18 malignant lesions (sensitivity: 72.2%; 95% CI: 46.5–90.3%) were correctly identified. Positive and negative predictive values were 100 and 66.7%, respectively. The area under the curve was 0.861 (95% CI: 0.723–0.999; P = .002) (Fig 1). The 5 unidentified cases (27.8%) included 1 OSA diagnosed as osteomyelitis, 2 OSAs, and 1 FSA diagnosed as reactive bone and 1 CSA diagnosed as chondroma. Among malignant lesions, HB correctly identified tumor type in 10 cases (55.5%), whereas in 3 OSA cases (16.7%), a generic diagnosis of

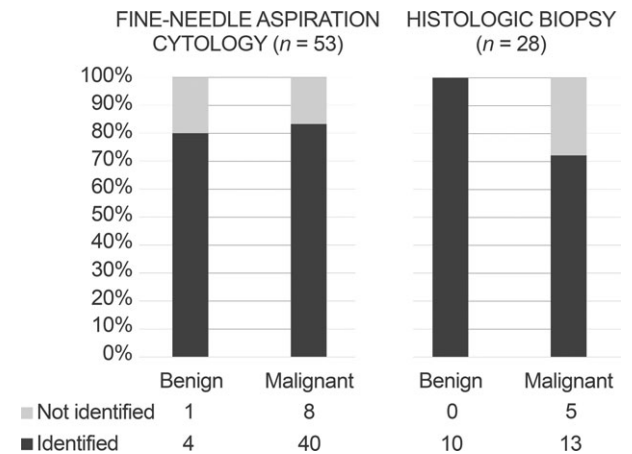
sarcoma was made. All metastatic lesions (n = 3) were correctly identified (Figs 2, 3; Table 1).

**Fine-Needle Aspiration Cytology Diagnoses**

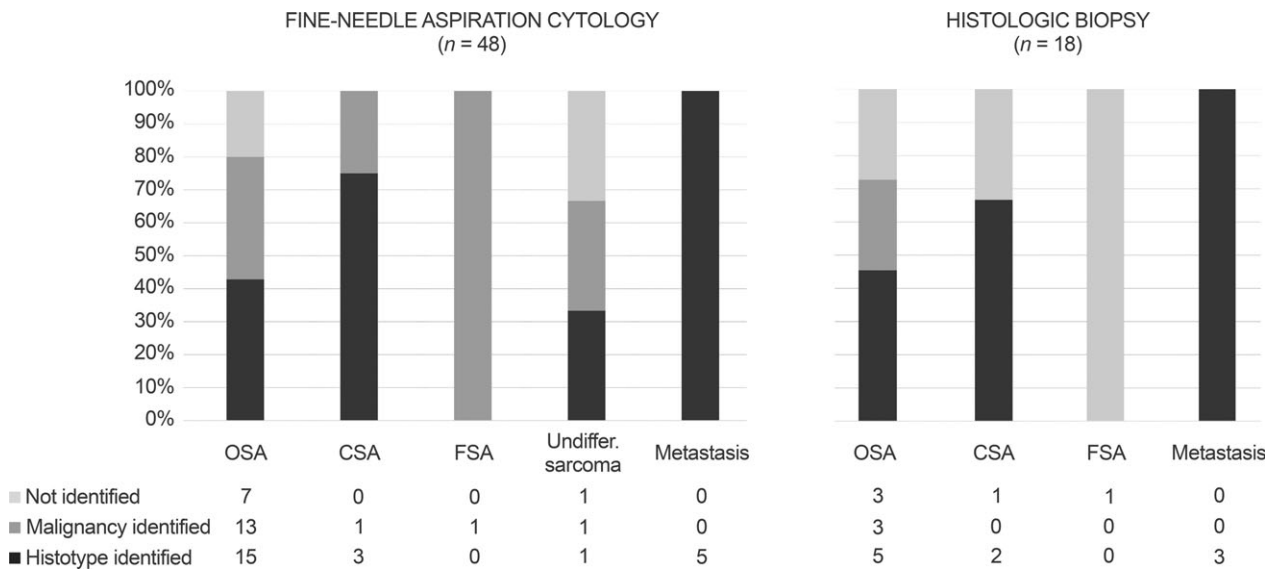
Fifty-three lesions were sampled by FNAC. Representative examples of cytological specimens are provided in Figure 4. The mean number of slides per case was 5 (range, 1–17). Cellularity was poor in 16 cases (30.2%) and moderate to high in the remaining 37 cases



**Fig 1.** Confusion matrix of actual and predicted malignancy in a series of canine bone lesions sampled by fine-needle aspiration cytology or histologic biopsy.



**Fig 2.** Diagnostic accuracy of fine-needle aspiration cytology and histologic biopsy observed in 68 cases of canine benign and malignant osteodestructive lesions.



**Fig 3.** Diagnostic accuracy of fine-needle aspiration cytology and histologic biopsy observed in 56 cases of canine malignant osteodestructive lesions grouped according to tumor type.

**Table 1.** Comparative assessment of the accuracy of fine-needle aspiration cytology (FNAC) and histologic biopsies (HB) in the diagnosis of canine bone lesions.

|  | FNAC (%)     | HB (%)       |
|--|--------------|--------------|
| Total number of cases  | 53           | 28           |
| Proportion of cases correctly identified as malignant or benign (accuracy) | 44/53 (83)   | 23/28 (82.1) |
| Nondiagnostic cases  | 9/53 (17)    | –            |
| Malignant lesions  | 48           | 18           |
| Proportion of malignant lesions correctly identified (sensitivity)         | 40/48 (83.3) | 13/18 (72.2) |
| Tumor type correctly diagnosed   | 24/48 (50)   | 10/18 (55.5) |
| Osteosarcomas  | 15/35 (42.8) | 5/11 (45.4)  |
| Chondrosarcomas  | 3/4 (75)     | 2/3 (66.7)   |
| Fibrosarcomas  | 0/1 (0)      | 0/1 (0)      |
| Poorly differentiated sarcomas   | 1/3 (33.3)   | –            |
| Bone metastasis  | 5/5 (100)    | 3/3 (100)    |
| Benign lesions   | 5            | 10           |
| Proportion of benign lesions correctly identified (specificity)            | 4/5 (80)     | 10/10 (100)  |

(69.8%). Blood contamination was high in 25 cases (47.2%) and low to moderate in 28 (52.8%). Overall accuracy for FNAC was 83%, with 4 of 5 benign lesions (specificity: 80%; 95% CI: 28.4–99.5%) and 40 of 48 malignant lesions (sensitivity: 83.3%; 95% CI: 69.8–92.5%) correctly identified. Positive and negative predictive values were 97.6 and 33.3%, respectively. The area under the curve was 0.817 (95% CI: 0.603–1.030;  $P = .002$ ) (Fig 1). All the 9 unidentifiable cases (17%; 7 OSAs, 1 poorly differentiated sarcoma and 1 benign lesion) had been considered inadequate for a cytological diagnosis because of insufficient cellularity. Among malignant lesions, cytology correctly identified tumor

type in 24 cases (50%). In other 16 cases, a diagnosis of malignancy was reached, but tumor type was not identified, or it was incorrect. In particular, 11 cases (10 OSAs and 1 FSA) were generically diagnosed as sarcomas, 2 chondroblastic OSAs were diagnosed as CSAs, 1 poorly differentiated sarcoma and 1 CSA were diagnosed as OSA, and 1 OSA was diagnosed as giant cell tumor of bone. All the 5 cases of bone metastasis from epithelial tumors were correctly diagnosed on cytology (Figs 2, 3; Table 1).

The proportion of correct diagnoses did not differ significantly according to tumor location (appendicular or axial), tumor diameter ( $\leq 0$  > than the median value), the amount of osteolysis or periosteal reaction, and tumor grade. Considering the characteristics of the smear, poor cellularity was significantly associated with a lower accuracy ( $P < .001$ ). When OSAs were divided according to the production of osteoid matrix evaluated on surgical or post-mortem samples, poorlyproductive tumors were correctly diagnosed in 35.7% of cases, whereas productive OSAs were recognized in 52.4% of cases; however, this difference was not statistically significant (Table 2).

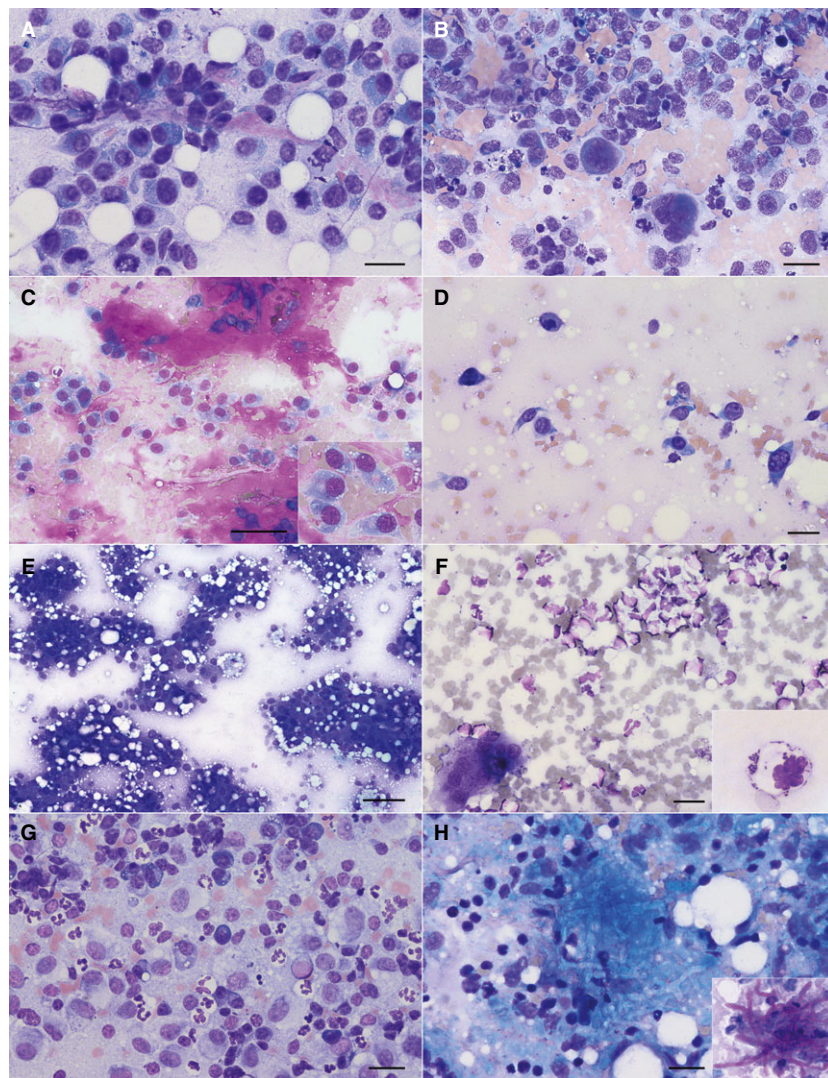
### Concurrent FNAC and HB Diagnoses

Thirteen cases (3 benign and 10 malignant lesions) were sampled with both FNAC and HB. Concordance between the 2 methods was observed in 9 cases (69.2%), 2 benign, and 7 malignant lesions. In all the 13 cases, at least 1 of the 2 techniques provided the correct diagnosis.

### Discussion

The aim of this study was to compare the diagnostic accuracy of bone cytology with that of HB, using the





**Fig 4.** Fine-needle aspirates of canine osteodestructive lesions. (A) Osteosarcoma (OSA). Pleomorphic population of malignant osteoblasts associated with pink strands of osteoid matrix. Cells are oval to elongated, with peripheral nuclei and prominent nucleoli. Few mitoses are observed. (B) OSA, giant cell type. Giant multinucleated and binucleated cells admixed with atypical mononuclear cells. (C) Chondrosarcoma. Neoplastic chondroblasts interspersed in a large amount of magenta extracellular matrix. Cell cytoplasm contains few clear vacuoles and fine pink granulation. Nuclei are large, round, with coarse chromatin. (D) Poorly differentiated sarcoma. Scattered spindle-shaped cells with prominent anisokaryosis and nuclear atypia. (E) Carcinoma metastasis. Clusters of disorderly arranged epithelial cells with variably sized cytoplasmic vacuoles and moderate anisokaryosis. (F) Suppurative, septic osteomyelitis. Numerous degenerated neutrophils with both intracellular and extracellular bacterial cocci. (G) Pyogranulomatous osteomyelitis. A mixed population of inflammatory cells including large epithelioid macrophages, moderately degenerate neutrophils, plasma cells, and few lymphocytes. (H) Granulomatous, mycotic osteomyelitis. Several negative images of poorly staining fungal hyphae, along with multinucleated giant cells, epithelioid macrophages, lymphocytes, and cellular debris. Hyphae stain pink with Periodic Acid-Schiff (inset). Fungal culture was positive for *Aspergillus terreus*. May-Grünwald-Giemsa and Periodic Acid-Schiff (H, inset). Bars, 25  $\mu$ m (A, B, D, F-H) and 50  $\mu$ m (C, E).

histologic diagnosis on surgical or post-mortem samples as the gold standard. Results demonstrated that both FNAC and HB are equally reliable diagnostic techniques in the preoperative diagnosis of canine osteodestructive lesions. Sampling errors were determinant in affecting the accuracy of both techniques. The main limits were related to the lack of recognition of malignant lesions, ie, false negatives. Conversely, in no case a benign lesion was diagnosed as malignant. This type of error is the most important to prevent, as the treatment

of choice for malignant bone tumors is radical surgery. Quite surprisingly, the 2 methods were equivalent also in the determination of tumor type. Thus, the advantages of FNAC, namely, timeliness, ease of performance, and decreased cost and discomfort, make attempting a diagnosis by this technique worthwhile. In those cases where cytology fails to yield a diagnostic sample, a traditional biopsy can be performed, because the greatest chance of success can be achieved by a combined use of both procedures.

**Table 2.** Influence of clinicopathological parameters on the accuracy of fine-needle aspiration cytology (FNAC) in the diagnosis of canine bone lesions.

| Variable                           | Evaluation                               | Proportion of Pathologic Processes Identified (Accuracy) (%) | P     |
|------------------------------------|--|--|-------|
| Lesion site                        |  |  |       |
| Appendicular                       | Clinical                                 | 35/43 (81.4)   | .672  |
| Axial                              |  | 9/10 (90)  |       |
| Tumor diameter                     |  |  |       |
| ≤5 cm                              | Radiographic                             | 19/21 (90.5)   | .260  |
| >5 cm                              |  | 19/25 (76)   |       |
| Bone lysis                         |  |  |       |
| Mild                               | Radiographic                             | 5/6 (83.3)   | .720  |
| Moderate to severe                 |  | 27/33 (81.8)   |       |
| Periosteal reaction                |  |  |       |
| Absent to mild                     | Radiographic                             | 14/19 (73.3)   | .235  |
| Moderate to severe                 |  | 18/20 (90)   |       |
| Cellularity                        |  |  |       |
| Poor                               | Cytological                              | 7/16 (43.8)  | <.001 |
| Moderate to high                   |  | 37/37 (100)  |       |
| Blood contamination                |  |  |       |
| High                               | Cytological                              | 19/25 (76)   | .278  |
| Low to moderate                    |  | 25/28 (89.3)   |       |
| Tumor grade (sarcomas)             |  |  |       |
| I/II                               | Histologic (surgical/post-mortem sample) | 18/23 (78.3)   | .704  |
| III                                |  | 17/20 (85)   |       |
| Osteoid production (osteosarcomas) |  |  |       |
| Productive                         | Histologic (surgical/post-mortem sample) | 11/21 (52.4)   | .491  |
| Poorly productive                  |  | 5/14 (35.7)  |       |

Understanding the diagnostic performance of cytology and histopathology for specific tissues and lesions can help choosing between FNAC and biopsy in a given clinical situation.<sup>12</sup> For bone lesions, the accuracy of preoperative diagnosis is particularly important. Thus, they require a diagnostic procedure allowing not only the differentiation between benign and malignant processes, but also between OSA, other primary bone tumors with a less aggressive biologic behavior and metastatic lesions.<sup>3,19</sup>

Histologic examination is presently considered the gold standard method. The assessment of tissue architecture and relationships with surrounding tissues should allow a better identification of tumor type. Potential limitations of incisional biopsies are the small sample size, with limited tissue available for examination, and a high frequency of crush artifacts or morphologic artifacts because of tissue decalcification. Additionally, the sampled material might not be representative of the primary pathologic process, because of necrotic areas, hematic lacunae, or reactive bone.

The advantages of cytology over histology can be, in addition to a lower morbidity, the possibility to carry out sampling at multiple points, increasing the likelihood of collecting neoplastic cells, and the possibility to

observe the obtained preparations extemporarily, and repeat sampling at need. Moreover, the morphology of collected material is usually good, as it does not require decalcification. The intrinsic limitation of cytological diagnosis resides in the impossibility to appraise tissue architecture. Thus, bone cytology might yield a generic diagnosis of sarcoma and not allow for a further classification of tumor type. Another possible limitation is the fact that certain bone lesions exfoliate with difficulty, thus providing preparations with poor cellularity.<sup>13,14</sup>

In this study, the accuracy of cytology (83%) in discriminating between benign and malignant lesions was similar to that of histologic biopsies (82.1%). With both techniques, in no case was a benign lesion diagnosed as malignant, although the specificity of cytology was decreased because of 1 nondiagnostic case. In comparison, sensitivity was lower, with 16.7% of malignant lesions not identified cytologically and 27.8% histologically. The presented accuracy of bone cytology is comparable to the data obtained in previous studies. In human medicine, cytology shows a sensitivity and specificity of 86 and 94.7%, respectively, and an accuracy of 83% in identifying a primary malignant bone tumor.<sup>20,21</sup>

Similar studies in veterinary medicine reports accuracies between 97 and 69% in differentiating benign and malignant lesions.<sup>11,15,22,23</sup> Several elements partially limit the interpretation and comparison of the results with these studies. In some of those, the histologic diagnosis being compared with cytological diagnosis was indifferently obtained from surgical/post-mortem samples or from small incisional biopsies. This might obviously affect the reliability of results, since, as we observed in this study, the preoperative biopsy does not always correspond to the definitive histologic diagnosis. Additionally, most authors report a generic diagnosis of cancer, but it is not clearly specified whether the tumor type was identified. Finally, some authors have elected to exclude nondiagnostic cases from the assessment of accuracy, whereas others have included them. In this study, we considered appropriate to maintain nondiagnostic samples in data analysis, because the possibility of obtaining adequate cytological preparations from a bone lesion was among the hypotheses of the study and, indeed, sample inadequacy plays an important role in limiting the diagnostic accuracy of cytology. According to this results, the percentage of cases in which tumor type was correctly identified was limited and, quite surprisingly, similar between histology (55.5%) and cytology (50%). With both methods, most CSAs and all epithelial metastatic lesions were correctly identified. Conversely, more than half of the OSA cases were generically diagnosed as "sarcomas", both cytologically and histologically. A generic preoperative diagnosis of malignant primary bone tumor might not affect the type of surgical approach; however, it might limit the possibility to formulate a prognosis and impair clinical decisions.

Both the histologic and cytological diagnoses of OSA are based on the detection of mesenchymal cells with malignant features in combination with osteoid. The amount of osteoid seemed to impact the likelihood of

identifying OSAs on cytology, but not systematically. The finding of osteoid is a reliable proof of the origin of the neoplasm, but it not always possible, even in the case of productive tumors, presumably because of the large variability among different areas of the same tumor. Indeed, OSAs can present a very heterogeneous histologic appearance, resulting in areas with variable differentiation, which might resemble other mesenchymal tumors (CSA, FSA, HSA). Additionally, in some cases it might be difficult to distinguish osteoid from a fibrous or chondroid matrix.<sup>3,24</sup> Additional staining methods can be applied to allow the differentiation of OSA from other mesenchymal tumors, that is, cytochemical staining for alkaline phosphatase or immunohistochemical staining for specific bone matrix proteins like osteonectin and osteocalcin. The main limitation of these methods is that reactive osteoblasts will stain positive as well, so criteria of malignancy must be assessed.<sup>15,25–27</sup>

Overall, the concordance of cytology and histology with the final diagnosis was not completely satisfactory.

Depending on the employed technique, this can be attributed to different causes. Four of 5 histologic diagnostic errors were due to a diagnosis of reactive bone tissue instead of a neoplastic process. In these cases, the pathologist correctly identified the process occurring in the observed preparations; however, the correct diagnosis was not reached because the sampling missed the neoplastic lesion. Indeed, the tissue surrounding a bone lesion is frequently involved in severe reactive processes, which might mislead the diagnostic judgment in case of superficial sampling.<sup>2,3,28</sup> As previously demonstrated, sampling the central areas provides the greatest accuracy rate in the case of destructive bone lesions.<sup>28</sup> Cytological mistakes accounted for 17% of the total and were in all cases due to inconclusive diagnoses because of hypocellular aspirates. In these cases, an extemporaneous evaluation of the cellularity of the samples either by macroscopic examination or by rapid stains could have helped to recognize the inadequacy of aspirates, and highlight the opportunity to obtain more samples at different sites. It has been reported that the cellularity of samples can affect not only the adequacy of preparations but also the level of accuracy in diagnostic cases.<sup>23</sup> However, the judgment of adequacy is subjective and often influenced by the experience of the pathologist and by the availability of clinical and radiographic data supporting the diagnostic evidence.

Notably, over 90% of the diagnostic errors in this study, both histologic and cytological, were attributable to sampling rather than interpretation. This demonstrates that a correct sampling technique, an adequate number of samples and the choice of sampling sites are at least as relevant as the pathologist's experience. Most importantly, in the cases where both cytological and histological samples were available, at least 1 of the 2 methods allowed to reach the correct diagnosis, suggesting that the greatest chance of success can be obtained by combining these techniques.

There are several limitations to the interpretation of these data. Because this was a retrospective study that

required cytology and histopathology, it was biased toward neoplastic lesions, because they are more likely to have biopsy or surgery performed. Consequently, we had a proportionally lower number of cases with a diagnosis of inflammation or non-neoplastic proliferation. These included 10 cases with no final confirmation on surgical or post-mortem samples, in which the lack of malignancy was only hypothesized based on long-term survival and no progression of clinical and radiographic signs. Nevertheless, it must be stated that follow-up alone cannot completely rule out a malignant process. Finally, the number of cases in which cytology and HB were both performed was limited, thereby reducing the possibility to compare the utility of these techniques on the same lesions.

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### Footnote

<sup>a</sup> SPSS, Inc, IBM Company, Chicago, IL

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*Conflict of Interest Declaration:* Authors declare no conflict of interest.

*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

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