Evaluation of sensitivity and specificity of cytologic examination: 269 cases (1999–2000)

Michele Cohen, DVM; Mark W. Bohling, DVM; James C. Wright, DVM, PhD, DACVPM; Elizabeth A. Welles, DVM, PhD, DACVP; Joseph S. Spano, DVM, PhD, DACVP

Objective—To determine sensitivity and specificity of cytologic examination used in a clinical setting.

Design—Retrospective study.

Animals—216 dogs, 44 cats, 4 horses, 2 ferrets, 1 llama, 1 rat, and 1 mouse.

Procedure—Records were reviewed of cases in which a cytologic diagnosis was followed by a surgical biopsy or postmortem examination within 3 days with subsequent histopathologic diagnosis. Diagnoses were compared for agreement at various levels, including complete agreement, partial agreement, no agreement, or no comparison possible because of insufficient or incorrect cytologic specimen. Levels of agreement were compared for different categories of lesions, including neoplastic, inflammatory, dysplastic-hyperplastic-other, and normal tissue. Additionally, levels of agreement for neoplastic lesions were categorized with regard to cell type, degree of malignancy, and location. Sensitivity and specificity of cytologic examination were calculated.

Results—At the level of general agreement (complete and partial agreement), the sensitivity of cytologic examination ranged from 33.3 to 66.1%, depending on the location of the lesion. Cytologic examination was most accurate when used to diagnose cutaneous and subcutaneous lesions and least accurate for diagnosis of liver lesions. Cytologic examination was most effective in diagnosis of neoplastic disease and least effective in diagnosis of dysplastic or hyperplastic conditions.

Conclusions and Clinical Relevance—Cytologic examination is a valuable diagnostic tool, although our results indicated lower accuracy than previously reported. False-negative results (missing a diagnosis) were far more common than false-positive results (categorizing a healthy animal as diseased); therefore, if the clinical index of suspicion is high, cytologic examination should be repeated or another technique should be selected to rule out the suspected condition. (*J Am Vet Med Assoc* 2003;222:964–967)

Cytology, first introduced to veterinary medicine in the 1960s, is regarded as an indispensable diagnostic tool.[1,2] Cytology provides a rapid, minimally invasive, and inexpensive means of obtaining a preliminary diagnosis.[3] In addition, fine-needle aspiration cytology allows specimens to be obtained from sites that are not easily accessible to surgical biopsy.[3] Limitations of cytology include inferior ability to evaluate tissue architecture[4,5] and difficulty in obtaining a diagnostic specimen in lesions that exfoliate poorly. Despite these limitations, fine-needle aspiration cytology is highly regarded as a valuable tool in human oncology. Accuracy rates > 90% have been reported[3,6-8] in humans for results of fine-needle aspiration cytology, compared with those obtained by histologic examination. Few reports[4,9-11] exist in the veterinary literature regarding accuracy of cytology. Moreover, in all previous veterinary studies, to the authors' knowledge, specimens for cytologic examination were obtained from the lesion after excision or necropsy, rather than before surgery.

The purpose of this retrospective study was to examine a large number and variety of cases in which a diagnosis was made via cytologic and histopathologic examination in order to evaluate the accuracy of cytology used in a clinical setting.

Criteria for Selection of Cases

The medical records of the Department of Pathobiology at the **Auburn University Veterinary Medical Teaching Hospital (AUVMTH)** were reviewed to select animals from which biopsy and cytologic specimens were obtained between September 1999 and August 2000. Inclusion in the study required that cytologic examination of the lesion was performed within 3 days of surgical biopsy or necropsy and histopathologic examination.

Procedures

Information regarding the method of collection of the cytologic specimen (eg, fine-needle aspiration vs impression smear, size of needle and syringe, technique utilized) was unavailable. Biopsy specimens were fixed in neutral-buffered 10% formalin for 24 hours to several days, depending on tissue thickness. The tissues were processed routinely and stained with H&E. Special stains were ordered, if deemed necessary, for interpretation by the pathologist following initial review. Cytologic specimens were stained with 2 commercially available stains.[a,b]

Cytologic specimens were read by 1 of 2 clinical pathologists, and biopsy specimens were read by 1 of 5 histopathologists. Previously reported results of both examinations were retrospectively reviewed and compared.

Using the histopathologic diagnosis as the gold standard, results were reviewed on the basis of overall agreement. The sampled tissue was categorized by means of location as epidermal, dermal or subcutaneous, lymph node, spleen, liver, bone, nasal, and

From the Departments of Clinical Sciences, Small Animal Medicine and Surgery (Cohen, Bohling), and Pathobiology (Wright, Welles, Spano), College of Veterinary Medicine, Auburn University, AL 36849.

Presented in part at the Veterinary Cancer Society Meeting, Baton Rouge, La, October, 2002.

Address correspondence to Dr. Cohen.

other. In addition, specimens were categorized by use of histopathologic diagnoses as inflammatory, neoplastic, dysplastic-hyperplastic-other, normal, and insufficient specimen. Neoplastic specimens were classified further as benign round cell, benign epithelial, benign mesenchymal, malignant round cell, malignant epithelial, and malignant mesenchymal tumors. The cytologic diagnosis was compared with the histopathologic diagnosis to assess accuracy; results were grouped into 4 categories as complete agreement, partial agreement, disagreement, and insufficient specimen. Criteria for inclusion in the complete agreement category required that the cytologic diagnosis be exactly or almost exactly the same as the histopathologic diagnosis (eg, a diagnosis of conjunctivitis by histopathologic examination and a diagnosis of conjunctivitis or purulent inflammation of the conjunctiva by cytologic examination). Diagnoses in the partial agreement category had less than exact agreement but were, in part, correct (eg, a histopathologic diagnosis of hemangiopericytoma and a cytologic diagnosis of spindle cell tumor). The disagreement category included diagnoses that did not match, such as histopathologic diagnosis of fibrosarcoma and cytologic diagnosis of fibroplasia. The category of insufficient specimen was assigned if either the cytology or histopathology report indicated that finding or if the authors deemed the diagnoses to be in disagreement from insufficient sampling or inappropriate sampling of the lesion (eg, a diagnosis of lymphosarcoma from a lymph node biopsy specimen and a diagnosis of fat and blood from attempted aspirate of a lymph node).

Results obtained from specimens determined to be neoplastic on the basis of histopathologic examination were further analyzed. Complete agreement between diagnoses for a neoplastic lesion required a precise diagnosis by use of cytologic examination (eg, a histopathologic diagnosis of fibrosarcoma and a cytologic diagnosis of fibrosarcoma). The partial agreement category included agreement at the level of malignancy and cell type (eg, leiomyoma and benign spindle cell tumor), agreement on cell type and disagreement on degree of malignancy (eg, fibrosarcoma and fibroma), agreement on degree of malignancy and disagreement on cell type (eg, sarcoma and carcinoma), and agreement on the diagnosis of neoplasia but different cell type and degree of malignancy (eg, benign epulis and fibrosarcoma). The other categories included no agreement and insufficient specimen.

For this study, sensitivity was defined as the probability that use of cytology would detect disease and was calculated as the number of cases with agreement between cytologic and histopathologic diagnoses for a disease, divided by the total number of cases in which animals had that disease. Sensitivities were calculated for accuracy of cytologic diagnoses in categories that included overall histopathologic diagnosis, overall histopathologic diagnosis by location, histopathologic diagnosis by neoplastic description, and neoplasia by location. Specificity was defined as the probability that use of cytology would detect the absence of disease and was calculated as the number of cases with agreement between cytologic and histopathologic diagnosis of no disease, divided by total number of cases with no disease.

Specificities were calculated for neoplastic and non-neoplastic lesions. χ^2 Analysis was used to assess statistical differences within each of these groups. A value of $P \leq 0.05$ was considered significant.

Results

Two-hundred sixty-nine cases met criteria for inclusion into our study. Specimens were submitted from AUVMTH patients (n = 248) and non-AUVMTH patients (21). Species distribution included 216 dogs, 44 cats, 4 horses, 2 ferrets, 1 llama, 1 rat, and 1 mouse. Specimens were obtained from various locations and included 65 epidermal, 50 dermal or subcutaneous, 30 nasal, 24 liver, 15 bone, 10 lymph node, 5 spleen, and 70 other (gastrointestinal tract, brain, eye, and site unknown) specimens.

Histopathologic diagnoses included 132 neoplasms (benign and malignant), 77 inflammatory lesions, 8 normal tissues, and 52 other diagnoses (eg, hyperplastic-dysplastic). Histopathologic diagnoses of neoplastic specimens included 41 malignant mesenchymal tumors, 40 malignant epithelial tumors, 20 malignant round cell tumors, 15 benign mesenchymal tumors, 11 benign epithelial tumors, 3 benign round cell tumors, and 2 unspecified malignancies.

Cytologic diagnoses included 107 neoplasms (benign and malignant), 63 inflammatory lesions, 37 normal tissues, 24 insufficient specimens, and 38 other diagnoses. Cytologic diagnoses of neoplastic lesions included 32 malignant epithelial tumors, 26 malignant mesenchymal tumors, 15 malignant round cell tumors, 14 benign mesenchymal tumors, 9 benign epithelial tumors, 2 benign round cell tumors, and 9 unspecified malignancies.

Complete agreement between cytologic and histopathologic diagnoses was found in 102 (37.9%) cases. Using histopathologic findings as the gold standard, distribution of the cases in the complete agreement category included 42 inflammatory lesions, 42 neoplastic lesions, 2 normal tissues, and 16 other diagnoses. Partial agreement was found for 49 (18.2%) cases. The majority of these lesions were neoplastic (n = 42); 1 was normal tissue, and 6 were classified as other diagnoses. Disagreement between histopathologic and cytologic diagnoses was found for 88 (32.7%) cases. Distribution of these cases included 31 inflammatory lesions, 26 neoplastic lesions, 3 normal tissues, and 28 other diagnoses. Insufficiency of the submitted specimens was reported in 30 (11.2%) cytologic specimens. Of the 30 insufficient cytologic specimens, 4 were inflammatory lesions, 22 were neoplastic lesions, 2 were normal tissues, and 2 were other lesions.

Analyzed by location, there was complete agreement for 28 of the 65 (43%) cutaneous lesions, 18 of 50 (36%) subcutaneous lesions, 5 of 10 lymph nodes, 3 of 5 splenic lesions, 7 of 24 (29%) hepatic lesions, 14 of 30 (47%) nasal lesions, 0 of 15 osseous lesions, and 27 of 70 (39%) other sites. Partial agreement was evident in specimens from 15 cutaneous, 11 subcutaneous, 0 lymph node, 0 spleen, 1 liver, 9 bone, 3 nasal, and 10 other sites. No agreement was evident in specimens from 18 cutaneous, 8 subcutaneous, 4 lymph node, 2 spleen, 16 liver, 4 bone, 12 nasal, and 24 other sites. Insufficient specimens were obtained in 4 cuta-

neous, 13 subcutaneous, 1 lymph node, 2 bone, 1 nasal, and 9 other sites.

Overall sensitivity of cytologic examination at the level of complete agreement with results of histologic examination was 37.9% when insufficient specimens were included in the calculation and 42.7% without inclusion of insufficient specimens. When complete and partial agreement were considered together in the analysis, sensitivity of cytologic examination increased to 56.1% with inclusion of insufficient specimens and 63.2% without inclusion of insufficient specimens. Sensitivity for detecting inflammatory lesions was 54.5% at the level of complete agreement (there were no instances of partial agreement in the category of inflammatory lesions) with inclusion of insufficient specimens and 57.5% without inclusion of insufficient specimens. Sensitivity for detecting hyperplastic and dysplastic lesions at the level of complete agreement was 30.8% with insufficient specimens and 32% without inclusion of insufficient specimens. When complete and partial agreement results were combined, sensitivity increased to 42.3% with and 44% without insufficient samples. For normal tissue, sensitivity at the level of complete agreement was 25% with and 33% without insufficient samples. Sensitivity increased to 37.5% with and 50% without insufficient samples when complete and partial agreements were combined for normal tissue.

Of the neoplastic lesions (n = 132), complete agreement between cytologic and histopathologic evaluation was evident in 42 cases (**Table 1**). Partial agreement was evident in 42 cases, and disagreement was evident in 26 cases. For 22 cases, insufficient specimens were submitted. Sensitivity of cytologic examination for detecting neoplastic lesions at the level of complete agreement with histologic examination was 31.8% with inclusion of insufficient specimens and 38.2% without inclusion of insufficient specimens. When complete and partial agreement results were combined, sensitivity increased to 63.6% with inclusion of insufficient specimens and 76.4% without inclusion of insufficient specimens. Comparisons of the sensitivities of cytologic diagnosis among histopathologic categories (inflammation, hyperplasia-dysplasia-other, normal, and neoplasia) did not reveal significant differences (P = 0.054; power, 0.626). Within the category of neoplasia, there were no significant differences in sensitivity of cytologic diagnosis (P = 0.496; power, 0.353) among any of the tumor subcategories.

Table 1—Level of agreement between cytologic and histopathologic diagnoses for 132 neoplasms classified by tumor type

Tumor type	Complete agreement	Partial agreement	No agreement	Insufficient specimen
Benign round cell	0	2	1	0
Benign mesenchymal cell	5	3	1	6
Benign epithelial cell	4	4	2	1
Malignant round cell	10	4	3	3
Malignant mesenchymal cell	6	20	7	8
Malignant epithelial cell	17	9	11	3
Unclassified malignancy	0	0	1	1

Values indicate number of cases.

Categorized by location, sensitivity of cytologic examination of cutaneous lesions was 43.1% at the level of complete agreement including insufficient specimens and 45.9% without insufficient specimens. When complete and partial agreement results were combined and considered a positive result, sensitivity increased to 66.1% with inclusion of insufficient specimens and 70.5% without inclusion of insufficient specimens. Sensitivity at the level of complete agreement was lowest for bone specimens, with 0% complete agreement with and without insufficient specimens. When complete and partial agreement results were combined, sensitivity for cytologic detection of bone lesions increased to 60% with inclusion of insufficient specimens and 69.2% without inclusion of insufficient specimens. Overall, sensitivity for detection of liver lesions was lowest with 29.2% sensitivity at the level of complete agreement and 33.3% when complete and partial agreement were considered as positive results. There were no insufficient specimens in this category. Sensitivity of cytology for detection of liver lesions was significantly (P = 0.007; power, 0.774) lower than for lesions in other locations. No significant differences in sensitivity were detected among other locations.

Neoplasms were further classified according to location. Sensitivity of cytologic detection of nasal tumors was highest with sensitivity of 64.3% at the level of complete agreement with histologic results and 78.6% when complete and partial agreement results were combined. Sensitivity for detection of bone tumors was 0% when considered at the complete agreement level. There were no significant differences in the sensitivities of cytologic diagnosis among tumors classified by location (P = 0.08; power, 0.702).

Specificity of cytology for neoplastic lesions was 82.5%. Positive predictive value of cytology in the diagnosis of a neoplastic lesion was 77.8%. Positive predictive value of cytology in the diagnosis of non-neoplastic lesions was 70.2.%.

Discussion

Our calculated measures of diagnostic accuracy of cytologic examination were lower than anticipated and lower than those reported in most studies in the veterinary and human literature. We believe that several factors had an influence on the accuracy of cytologic diagnosis. These factors involved either methodologic differences or characteristics of the tissue or lesion that was sampled. Examination of these factors may be useful for others who use cytology as a diagnostic tool.

The first factor that affects accuracy is the timing and method of sampling. For example, in a review of 100 masses, Eich et al[8] examined the accuracy of cytology, compared with histology, in the diagnosis of neoplastic versus non-neoplastic conditions. They reported an overall sensitivity of 89% and specificity of 100%, with a positive predictive value of a positive test for neoplasia of 100%. Similarly, Vos et al[4] examined results from 322 specimens and reported a sensitivity of 95.6%, specificity of 65.4%, and positive predictive value of 93.5% for cytology. Specimens were obtained

either during surgery or at postmortem examination in both of these studies. Intraoperative sampling would be expected to improve accuracy in at least 2 important ways. First, the risk of sampling error is reduced to virtually zero. Second, the clinical pathologist would have the advantage of more data (eg, exact location of the lesion or gross appearance) to help in making the diagnosis. In contrast, most of the cytologic specimens in our clinical pathology service were obtained before surgery as part of the initial diagnostic evaluation.

Another possible factor in our study concerned the technical aspects of obtaining and preparing the specimen. Although fine-needle aspiration generally is considered to be technically simple to perform, there is still a learning curve involved in aspiration technique and in making a high-quality smear. A substantial number of the specimens at our institution are taken by fourth-year veterinary students whose relative lack of experience may contribute to sampling error.

Additionally, in another study, tissue scrapings as well as fine-needle aspirates were used to make smears for examination.[4] Scrapings should provide a larger specimen than fine-needle aspirates, potentially resulting in improved sensitivity. This may be of particular importance with poorly exfoliative lesions such as many mesenchymal neoplasms. Impression smears of a surgical biopsy specimen or postmortem organ examination also are expected to yield a higher degree of accuracy, because the lesion can be seen directly and sampled. In our study, cytologic evaluation of nasal tumors had the highest level of sensitivity at 78.6%, possibly because nearly all of these evaluations were made on impression smears of biopsy specimens. Similarly, Kristensen[12] reported a higher sensitivity and specificity for cytologic determination of liver lesions when specimens were obtained with impression smears rather than fine-needle aspirates. Furthermore, Long[13] reported on the higher accuracy with touch and smear preparations, compared with medium-pressure impressions in the cytologic diagnosis of intracranial lesions.

Location and size of the lesion also appeared to affect sensitivity. For example, when categorized by location, sensitivity for liver lesions was only 33.3%, which was significantly lower than for other locations. This result was not unexpected, as many of the liver conditions were small focal or multifocal lesions, making it likely that fine-needle aspiration would obtain a nonrepresentative specimen and thus yield a diagnosis of normal hepatocytes. These findings are similar to previous reports[11,12] of the poor accuracy of cytology for liver lesions and further supports the theory of confounding factors in sampling.

Tissue characteristics, such as degree of exfoliation, were also important for the accuracy of a cytologic diagnosis. When round cell, epithelial, and mesenchymal neoplasms were compared, we found that the percentages of exactly matched diagnoses were 43.5, 41.2, and 19.6%, respectively. When complete and partial agreement results were combined, sensitivities increased to 69.6, 66.7, and 60.7%, respectively. Griffiths et al,[9] in a report on 147 skin tumors in dogs, also noted higher sensitivity for detection of round cell and epithelial tumors (100 and 75%, respectively) than for mesenchymal neoplasms (50%). These results may be obtained because round cell and epithelial neoplasms typically exfoliate cells much more readily than do mesenchymal neoplasms, resulting in a more representative specimen.[14] In addition, evaluation of lymph nodes by fine-needle aspiration reportedly yields a sensitivity of 100% and specificity of 96% for the evaluation of metastatic disease.[15]

Another factor that influenced comparisons between cytologic and histologic specimens was the effect of insufficient or incorrect sampling for cytologic diagnosis. Some researchers who have evaluated sensitivity of cytologic results have dealt with this problem by censoring all specimens from the data set if the cytologic specimen was inadequate. We chose to include such specimens in our data because our goal was to determine sensitivity of cytologic diagnosis in a clinical setting, and we believed that sampling error might constitute an important source of the overall error. When inadequate specimens were removed and sensitivities recalculated, we found minor effects on all categories with the exception of neoplastic diseases for which sensitivity of cytologic findings improved from 63.6 to 76.4%. This may have been due to the focal nature of most neoplastic lesions, which results in a greater opportunity for technical error in sampling. This finding underscores the importance of obtaining and submitting multiple aspirates of any suspected neoplastic lesion in order to increase diagnostic accuracy.

[a]Harleco red stain, EMD Chemicals Inc, Gibbstown, NJ.
[b]QuickDip blue stain, Mercedes Medical Inc, Sarasota, Fla.

References

1. Roszel JF. Exfoliative cytology in diagnosis in malignant canine neoplasms. *Vet Scope* 1967;12:14–20.
2. Kristensen A, Feldman BF. Cytology in veterinary practice. *Nord Vet Med* 1986;38:321–332.
3. Morrice WB, DeNicola DB. Advantages and disadvantages of cytology and histopathology for the diagnosis of cancer. *Semin Vet Med Surg (Small Anim)* 1993;8:222–227.
4. Vos JH, van den Ingh TS, van Mil FN. Non-exfoliative canine cytology: the value of fine needle aspiration and scraping cytology. *Vet Q* 1989;11:222–236.
5. Kline TS, Neal HS. Needle aspiration biopsy: a critical appraisal. *JAMA* 1978;239:36–39.
6. Rosa G, Boschi R, Boscaino A, et al. Intraoperative cytology in breast cancer diagnosis. *Diagn Cytopathol* 1993;9:623–631.
7. Chonmaitri IS. Imprint cytologic examination as an adjunct to frozen section. *J Med Assoc Thai* 1993;76:458–464.
8. Eich CS, Whitehair JG, Moroff SD, et al. The accuracy of intraoperative cytopathological diagnosis compared with conventional histopathological diagnosis. *J Am Anim Hosp Assoc* 2000;36:16–18.
9. Griffiths GL, Lumsden JH, Valli VEO. Fine-needle aspiration cytology and histologic correlation in canine tumors. *Vet Clin Pathol* 1984;13:13–17.
10. Wallon CJ, Gilbert S, Snaps S, et al. Imprint and brush cytology in the diagnosis of canine intranasal tumors. *J Small Anim Pract* 1996;37:423–427.
11. Roth L. Comparison of liver cytology and biopsy diagnosis in dogs and cats: 56 cases. *Vet Clin Pathol* 2001;30:35–38.
12. Kristensen AT. Liver cytology in cases of canine and feline hepatic disease. *Compend Contin Educ Pract Vet* 1990;12:797–808.
13. Long SN. Evaluation of rapid staining techniques for cytologic diagnosis of intracranial lesions. *Am J Vet Res* 2002;63:381–386.
14. Ogilvie GK, Moore AS. *Managing the veterinary cancer patient*. Yardley, Pa: Veterinary Learning Systems, 1995; 47–50.
15. Langenbach A, McManus PM, Hendrick MJ. Sensitivity and specificity of methods of assessing the regional lymph nodes for evidence of metastasis in dogs and cats with solid tumors. *J Am Vet Med Assoc* 2001;218:1424–1428.