EFFICACY OF FINE NEEDLE ASPIRATION IN THE DIAGNOSIS OF SPONTANEOUS MAMMARY TUMORS

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ABSTRACT

The cytopathology exam has been utilized to diagnose mammary disorders in female dogs. The success of cytopathology results depends on, among other factors, the diagnostic accuracy of the exam. The present study aimed to compare the cytopathological and histopathological findings from mammary disorder cases in female dogs and evaluate the lesion cytopathology exam accuracy. The concordance degree was evaluated between the cytopathology and histopathology exams of 67 female dogs, carriers of palpable breast lumps, cared for at the university hospital from which the following indicators were calculated: sensitivity, specificity, positive predictive value, negative predictive value, false positives, false negatives, and exam accuracy. The sensitivity was 95.23%, the specificity 75%, positive predictive value 98.36%, negative predictive value 60%, and accuracy 94.02%. There were 2 false negatives and 1 false positive. In conclusion the cytopathology exam can be amply utilized as a means of diagnosis with high accuracy and sensitivity in the first examination of female dogs with mammary tumors.

Keywords: cytopathological diagnosis, accuracy, mammary tumor, female dogs.

EFICÁCIA DA CITOLOGIA ASPIRATIVA NO DIAGNÓSTICO DE TUMORES MAMÁRIOS ESPÔNTANEOS

RESUMO

O exame citológico tem sido utilizado no diagnóstico das afecções mamárias das cadelas. O êxito no resultado da citologia dependerá, além de outros fatores, da acurácia diagnóstica deste exame. O objetivo deste estudo foi comparar os achados citológicos e histológicos das afecções mamárias de cadelas e avaliar a acurácia do exame citológico nestas lesões. Avaliou-se o grau de concordância entre os exames citológico e histológico de 67 cadelas, portadoras de nódulo mamário palpável, atendidas em um hospital universitário e foram calculados os indicadores: sensibilidade, especificidade, valor preditivo positivo, valor preditivo negativo, falso positivo, falso negativo e acurácia do exame. A sensibilidade foi de 95,23%, a especificidade de 75%, o valor preditivo positivo de 98,36%, o valor preditivo negativo de 60% e a acurácia de 94,02%. Ocorreu 1 resultado falso positivo e 2 falso negativos. Conclui-se que o exame citológico pode ser amplamente utilizado como meio de diagnóstico com elevada acurácia e sensibilidade no atendimento primário das cadelas com tumor de mama.

Palavras-chave: diagnóstico citológico, acurácia, tumor de mama, cadela.

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LA EFICACIA DE LA ASPIRACIÓN CON AGUJA FINA EN EL DIAGNÓSTICO DE TUMORES MAMARIOS ESPONTÁNEOS

RESUMEN

El examen citológico se ha usado en el diagnóstico de cáncer de mama en perras. El éxito de la citología depende, entre otros factores, la precisión diagnóstica de este examen. El objetivo de este estudio fue comparar los resultados de citología y examen histológico de las enfermedades de mama en las perras y evaluar la exactitud de la citología de estas lesiones. Se evaluó el grado de acuerdo entre los exámenes citológicos e histológicos de 67 perros, que sufren de bulto palpable en la mama atendidos en un hospital universitario y se calcularon los indicadores: sensibilidad, especificidad, valor predictivo positivo, valor predictivo negativo, falsos positivos, falsos negativo y exactitud de la prueba. La sensibilidad fue del 95,23%, especificidad 75%, valor predictivo positivo del 98,36%, valor predictivo negativo del 60% y el 94,02% de precisión. Hubo un resultado positivo falso y dos falsos negativos. Se concluye que el examen citológico puede ser ampliamente utilizado como una herramienta de diagnóstico de alta precisión y sensibilidad en la atención primaria de perros con cáncer de mama.

Palabras clave: diagnóstico citológico, la precisión, el tumor de mama, perra

INTRODUCTION

When a mammary disorder is discovered, the analysis of the patient is based on the clinical history and the physical and imaging exams; most of the time these analyses are not sufficient to characterize the tumor as benign or malignant. In these cases, the cytopathological exam has been used to clear the primary diagnosis from the mammary lesion. The cytopathology exam consists of the microscopic analysis of the morphological alterations of individual cells spread onto histological slides, fixed and stained. The cells can be obtained by exfoliation or by puncture (1, 2).

Cytopathology by puncture, as a diagnosis instrument, was used initially in the mid 1920s (3, 4). The use of cytopathology by puncture in the diagnosis of the mammary disorders was not divulged in the beginning because it was believed that neoplastic cells may disseminate along the trajectory of the needle and thus affect the pathologists' ability to establish a conclusive diagnosis from the obtained material. Since the late 60's, the studies published by Franzen and Zajicek (5) have served as a basis to reveal and reintroduce this method, on a worldwide scale, as a means of diagnosis in mastology, which became more important with the passing of the years. At the present time it is widely accepted as one of the screening exams for women's mammary lesions; and has been used for diagnosis of mammary lesions in female dogs (6-10).

Cytopathology by puncture is a low-cost minimally invasive method of diagnosis utilized to differentiate between neoplastic and non-neoplastic lesions and between benign and malignant mammary tumors. The results obtained from the appropriate use of this method enable reduction in the number of invasive biopsies and lead to a faster therapeutic procedure that would reveal a more appropriate prognosis, thus avoiding many unnecessary surgical interventions (2, 10-14).

The most important causes of failure in the use of the cytopathological exam to diagnose mammary disorders are the frequencies of unsatisfactory material, the number of false-negative results and the number of false-positive results (8, 12, 13, 15). Therefore, appropriate cytopathology training and nuclear malignancy criteria (for example, Papanicolaou staining) as well as good samples of representative areas of the lesion – without

necrosis or inflammation and stained by methods that show ample evidence of cytoplasmic malignancy criteria (for example, Giemsa staining) –are all essential to obtaining clinically important cytopathological results (5, 9, 14).

Additionally, the success of the cytopathological diagnosis of mammary tumors depends on the incisiveness of the diagnosis, in other words, one's precision in diagnosing the mammary lesion cases correctly. For that reason, studies have been performed to compare the preoperative cytopathological exam with the histopathological study of the corresponding surgical specimen in order to determine the efficiency, sensitivity and specificity of this method for diagnosing mammary tumors in female dogs, criteria that varied, respectively from 63 to 92.9%, 65 to 88.6% and 83 to 100% (9-14).

Therefore, given that the cytopathological exam as an important procedure in the investigation of the mammary lesions and that there is considerable variation in the accuracy of the method, which must always be well established so that the results of a test can be reliable, the present study aimed to compare the cytopathological and histopathological findings in mammary disorders of female dogs cared for at the School of Veterinary Medicine and Animal Husbandry (FMVZ) at the São Paulo State University (UNESP), Botucatu, São Paulo, and to evaluate the accuracy of the cytological exam in these lesions.

MATERIAL AND METHODS

From the animals cared for at the FMVZ hospital, UNESP, Botucatu, São Paulo, 67 female dogs were selected, all of which were bearers of a tangible nodule on the inguinal or abdomen caudal mammary gland.

The tumors from the selected female dogs were submitted to the cytopathological exam. For cytopathological analysis, samples of different points from the affected mammary glands were obtained by cyto-puncture with or without suction using hypodermic needle 22 G1 1/4 `` (Injex®, São Paulo, Brazil) and a 10 ml syringe (Injex®, São Paulo, Brazil). The collected material was spread onto histological slides, fixed with methanol p.a. (Merck®, Darmstadt, Germany) and ethanol 95% (Merck®, Darmstadt, Germany) and stained by May-Grünwald Giemsa and Papanicolaou techniques, respectively. The samples were examined via light microscopy (ZEISS - I Model AXIO Imager A1) with at 10x magnification to verify the quality control of the material, 20x to evaluate the morphological expression of the cells, and finally 40x to conclude the diagnosis. According to the malignancy criteria adopted by Allen, Prasse and Mahaffey (11) the samples were classified into 3 groups: benign lesion (mammary hyperplasia, benign epithelial tumor, benign mixed tumor), malignant (malignant epithelial tumor, malignant complex tumor, malignant mixed tumor) and inconclusive or insufficient (a smear with less than 5 cell groups, with each group containing 5 to 10 cells).

After routine procedures of anesthesia and local antiseptic the animals were submitted to mastectomy. The tumor fragments were removed and fixed in 10% buffered formalin solution and the specimens of each surgical part were processed routinely and stained with *hematoxylin* and eosin. The reading of the slides was accomplished with a light microscope (ZEISS - I Model AXIO Imager A1) and enabled the lesions to be classified as benign (adenoma and benign mixed tumor) or malignant (simple and complex carcinoma).

The only female dogs considered were those that presented an interval of less than two months between the cytopathological exam and the confirmative histological exam.

The cytopathological results for malignancy (benign or malignant) were compared with their respective histopathological diagnoses and the degree of consistency was evaluated among the exams calculating the sensitivity, specificity, positive predictive value, negative predictive value, false positive number, false negative number and accuracy.

In order to calculate the sensitivity, a portion of patients with malignant mammary tumor whose cytopathology results indicated malignancy were considered malignant by the

cytopathological exam when they presented at least three of the malignancy criteria adopted by Allen, Prasse and Mahaffey (11), confirmed by the histopathology. The mammary tumors recognized by histopathology as malignant that were not diagnosed as such in the cytopathology were considered false negatives.

To calculate the specificity, the portion of patients with benign mammary lesions whose cytopathology results were benign were those who presented the absence of at least three malignant criteria according Allen, Prasse and Mahaffey (11) in the cytopathological evaluation, confirmed by histopathology. The mammary tumors recognized by the histopathology as benign that were diagnosed as malignant in the cytology were considered false positives.

The positive predictive value was calculated based on the probability of the mammary lesion being malignant via histology when the cytology result was malignant whereas the negative predictive value was the probability that the lesion was histopathologically benign when the cytological diagnosis was benign.

The accuracy of the cytological exam corresponds to the proportion of cases correctly diagnosed by cytopathology.

RESULTS

Among the 67 tumors tested by cytopathology (Table 1), the distribution showed that most of the female dogs (n=61; 91.04%) presented malignant tumors; 5 female dogs (7.46%) were bearers of benign tumors and in only 1 case (1.50%) the material was considered unsatisfactory for analysis (which was diagnosed as a malignant tumor via histopathology). In what is refered to as the histopathological study, the following results were observed: 63 female dogs (94.03%) presented malignant tumors while 4 (5.97%) bore benign tumors.

The cytopathological analysis identified 1 hyperplastic nodule (1.49%), 2 benign epithelial tumors (2.98%), 2 benign mixed tumors (2.98%), 11 compound malignant tumors (16.42%), 25 malignant epithelial tumors (37.32%), 25 malignant mixed tumors (37.32%) and 1 case of insufficient material (1.49%), totaling 67 cases. The histological study verified 2 adenomas (2.98%), 2 mixed benign tumors (2.98%), 26 simple carcinomas (38.81%) and 37 complex carcinomas (55.23%). In some samples observed there was an inflammatory component associated with the neoplasm process.

Table 1. Distribution of the cytopathological results by histopathological malignancy diagnosis of the 67 studied mammary tumors, selected at the FMVZ hospital, UNESP, Botucatu, São Paulo.

Histopathological Diagnosis	Cytopathological Diagnosis			Total
	Benign	Malignant	Insufficient	
Benign*	3	1	0	4
Malignant**	2	60	1	63
Total	5	61	1	67

* Adenoma and benign mixed tumor

* *Simple carcinoma and complex carcinoma

The distribution of the cytopathology results according to the histopathological diagnosis of the mammary tumors studied is displayed in Table 1. The cytopathological method presented a sensitivity of 95.23%, specificity of 75%, positive predictive value of

98.36%, negative predictive value of 60% and accuracy of 94.02%. In addition to 2 false negatives, there was 1 false positive result.

DISCUSSION

Cytopathology by puncture was introduced in medical practice after modifications of the standard methodology of exfoliation cytology, developed by Doctor Georgios Papanicolaou in the middle of the 19th century (1). With the success of the technique it increased in use and there was significant improvement in the preparation and interpretation of the punctured material in several disorders, besides is used in the clinical setting for mammary lesions in female dogs (6, 7, 9, 10).

In order to obtain such selectivity, the cytopathological evaluation in combination with, the clinical evaluation of the female dogs carriers of mammary tumors, has been used as a diagnosis instrument by the Service of Veterinary Pathology of FMVZ, UNESP, Botucatu since 1994. Such collected data provided us a validated basis for a better filing of the information obtained in this study.

In the population studied, the correlation between the cytopathological and histopathological exams showed that the sensitivity of the cytopathological exam is high (95.23%), while defining positivity as the presence of malignant mammary tumors. However, its specificity (75%) is below the value considered appropriate, indicating that some female dogs would be diagnosed falsely as bearers of malignant mammary tumors; however, its high sensitivity means that practically all the malignant tumors would be detected.

Since the high sensitivity of the cytopathological exam for the malignancy diagnosis was influenced by the low false negative rate (4.77%), it can be inferred that the cytopathology result will rarely be negative in the presence of a malignant mammary tumor and that, in these cases, a therapeutic intervention and prognosis will be more precise. The false negative results indicate omission of malignant cases and can be attributed mainly to inadequate sampling but also to failure in the interpretation of the material (10, 14). In this study, several samples of the same nodule were collected and submitted to both staining techniques, Giemsa and Papanicolaou, which provided appropriate material, good visualization and interpretation of the cellular components and possibly contributed to the low percentage of false negatives in the cytopathological diagnosis.

The method's specificity may present a different percentage if the number of benign tumor samples were larger. However, it could be said that its sensitivity depends on the occurrence of false positive results which are almost exclusively attributable to mistakes in interpreting the sample, commonly related to the lack of experience, negligence and use of low quality staining techniques (8, 15). This can also be related to the presence of cellular atypia caused by active inflammation (2, 12). Therefore when present, it is suggested to reduce the inflammatory process and, if necessary, to acquire a new puncture (11, 13). So as to avoid the tendency of overestimation of the results that can be responsible for more invasive or unnecessary interventions (5). The inflammatory component present in the smear of this study was certainly shown to be a negative factor in the accurate diagnosis of the benign lesion falsely diagnosed as malignant.

The presence of unsatisfactory material can be attributed partly to the cellular inadequacy or technical mistakes during the obtainment and processing of the sample. Inexperience of the professional performing the examination, previous treatments or lipomatous, fibrocystic or hemorrhagic lesions can, in the cyto-puncture, result in scarce and thus insufficient material (2, 11, 12). In all the analyzed cases puncturing the center of the tumors was avoided, in order to reduce the risk of collecting necrotic material, in favor of puncturing several areas of tumor formation. Therefore, the possibility of obtaining low quality material (red blood cells, necrosis) was minimized.

The positive and negative predictive values found in the studied population were 98.36% and 60%, respectively, which represents a chance of approximately 99% that female dogs with a positive diagnosis of malignancy will actually present a malignant mammary tumor and a 60% probability that female dogs diagnosed as negative for malignancy will actually not present mammary cancer. The predictive values of the test refer only to the studied population (n=67), in other words, the patients assisted in FMVZ, UNESP at Botucatu, whose cytopathological exam was confirmed by the histopathological exam according to the established criterion. Nevertheless, the sensitivity and specificity of a test are exclusive properties of the tests' diagnosis and do not vary considerably when establishing other study criteria, unless there are changes in the technique or mistakes in application (16). Thus, in spite of possibly overestimating benign cases, the 94.02% accuracy signifies a high capacity for correct diagnosis and demonstrates that the cytopathological exam, when conducted in an appropriate manner, can be indicated as a fast, early and effective method for the diagnosis of mammary lesions in female dogs.

The results of this and of other studies (9-14) allow to characterize the cytopathological method as being easily executed, faster than histopathology, and having sensitivity equal or superior to the biopsy methods.

CONCLUSIONS

Therefore, cytopathology can be widely utilized as a method of diagnosis with high accuracy and sensitivity in the first examination of female dogs with mammary tumors, mainly for the diagnosis and surgical planning in relation to malignant mammary tumors, and thus enables treatment through only a single intervention without the necessity of additional interventions to obtain safe margins. However, its result should not be the only diagnosis instrument in the mammary lesion cases. Instead, the clinical, imaging and, subsequently, histopathology findings should also be taken into account.

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