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Original Contribution

USE OF NUCLEAR ROUNDNESS AS A COMPLEMENTARY METHOD FOR DIFFERENTIATION OF BENIGN FROM MALIGNANT CANINE MAMMARY GLAND EPITHELIAL TUMOUR ON CYTOLOGIC SMEARS

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ABSTRACT

A fine-needle aspiration biopsy (FNAB) was performed on thirty-eight spontaneous canine mammary gland epithelial tumours (adenomas (n=8), fibroadenomas (n=8) tubulopapillary carcinomas (n=9), solid carcinomas (n=6) and anaplastic carcinomas, (n=7). The neoplastic cells were fixed immediately with Merckofix® spray (Merck®, Darmstadt, Germany) and stained with Hemacolor® (Merck®, Darmstadt, Germany). The digitalized images of each cytologic finding were captured for image analysis. Computer-assisted morphometry of randomly selected nuclei was performed and the values of the morphometric parameter nuclear roundness were assessed. The analysis of data revealed significant differences between benign and malignant tumours ($p < .001$, ANOVA/LSD test). The results indicate that morphometric parameter nuclear roundness could be used as a complementary method for differentiation of benign from malignant canine mammary epithelial tumours on cytologic smears.

Key words: image analysis, computer-assisted morphometry, canine mammary gland epithelial tumours

INTRODUCTION

Image analysis is the measuring and counting of microscopic images in order to obtain information of diagnostic importance (1) Image analysis could be divided into cellular morphometry, or measuring cellular components and cytometry, counting the whole cells (2). Nowadays, computerized image analysis is not only a well-established and highly developed methodology but it is becoming widely used and more and more applied in various diagnostic fields in clinical pathology. As a part of image analysis the morphometry is a quantitative description of geometric figures of cellular structures in any dimension. It has several advantages over conditional visual assessment: objectivity, reproducibility, and the ability to detect changes not immediately apparent to the naked eye (3). The morphometric parameters are related to the size and shape (area, perimeter, diameter, roundness) or to the chromatin aspect of stained nuclei (4). In veterinary medicine, especially in oncology, the interest in image analysis has increased progressively. There are several reports indicating the usefulness of computer-assisted nuclear morphometry in diagnosis of canine mast cell tumours (5, 6), feline squamous cell carcinomas (7), canine round cell tumours (8), canine and feline melanocytic tumours (9), feline mammary carcinomas (10) and canine mammary gland tumours (11, 12).

The aim of this study was to evaluate whether morphometric parameter nuclear roundness could be used as a complementary method for differentiation of benign from malignant canine mammary gland epithelial tumours on cytologic smears.

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MATERIALS AND METHODS

Tumours

The study was performed on 38 spontaneous canine mammary gland epithelial tumours (8 adenomas, 8 fibroadenomas, 9 tubulopapillary carcinomas, 6 solid carcinomas and 7 anaplastic carcinomas). The tumour samples were collected at the time of surgical removal from dogs presented to the Department of Veterinary Surgery, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria.

Cytologic and histopathologic processing

The neoplastic cells, sampled preoperatively from four different areas of tumour formations were fixed immediately with Merckofix® spray and stained with Hemacolor®. A material for histopathology analysis was obtained at the time of surgical removal of tumours. It was fixed in 10% formaldehyde and routinely processed. Eight 4-µm sections were obtained from each tumour sample and were stained with hematoxyllin/eosin (H/E). All diagnoses were confirmed histopathologically according to WHO International Histological Classification of Tumours of Domestic Animals (13).

Nuclear morphometric analysis

The material obtained by cytopathologic processing was analysed with a Motic Professional B3 digital microscope (*Motic Inc. Ltd, Hong Kong, China*) equipped with Image Pro Plus® image analysis software (*Image Pro Plus 4.5.0.29. for Windows 98/NT/2000, Media Cybernetics Inc., Silver Spring, MD, USA*). The computer used was equipped with 2.00 GHz Celeron® Intel® processor with 256 Megabytes of RAM and 17-inch monitor (*Samsung Electronics, Slovakia Ltd, Galanta, Slovakia*). The images created by the computer system were formatted as jpeg files. The magnification was x1000 and the resolution was 1024/768 pixels. Computer-assisted morphometry of randomly selected nuclei was automatically performed. A minimum of 100 nuclei were analysed in each case. Overlapped and fragmented nuclei were not measured. In mixed mammary gland tumours (fibroadenomas) the nuclei of the epithelial cells were measured. The investigated morphometric parameter was nuclear roundness (a perfectly circular structure has a roundness value of 1.0 and values >1 indicate irregular shapes).

Statistical analysis

Statistical analysis of the data was done using an one way analysis of variance (ANOVA) followed by the LSD post hoc test (Statistica 6.0, StatSoft, Tulsa, OK, USA) at a $p<001$ level of significance.

RESULTS

The data are presented on the **Table 1**. We found reliable differences in nuclear roundness between benign and malignant tumours. No significant difference was found in nuclear roundness between adenomas/fibroadenomas and tubulopapillary carcinomas/solid carcinomas.

Table 1. *Values of morphometric parameter nuclear roundness in adenomas, fibroadenomas, tubulopapillary carcinomas, solid carcinomas and anaplastic carcinomas (***p < .001, ANOVA/LSD test)*

Group	***Nuclear roundness***		***Significance of differences (p)***				
	N	*Mean ± SD*	*A*	*FA*	*TC*	*SC*	*AC*
Adenoma (A)	8	1.10 ± 0.01	-	-	***	***	***
Fibroadenoma (FA)	8	1.10 ± 0.03	-	-	***	***	***
Tubulopapillary carcinoma (TC)	9	1.19 ± 0.09	***	***	-	-	***
Solid carcinoma (SC)	6	1.20 ± 0.14	***	***	-	-	***
Anaplastic carcinoma (AC)	7	1.33 ± 0.34	***	***	***	***	-

DISCUSSION

There are several reports indicating the usefulness of the nuclear roundness for differentiation of benign from malignant breast tumours in humans (14, 15, 16, 17). Moreover, some investigators have used the data from morphometric analysis for grading and predicting the biological behaviour of breast neoplasms (14, 17-25). Although the image analysis technique is relatively well known in veterinary oncology there are only several reports of computer-assisted morphometric investigations of canine mammary gland tumours. All of them have been performed on histologic slides. Destexhe

et al. (12) studied ploidy, S-phase fraction, and nuclear area of 90 canine mammary tumours (30 benign and 60 malignant) to discriminate benign and malignant lesions. Their analysis indicated marked differences in the mean nuclear area between benign and malignant tumours. Juntes and Pogacnik (26) performed computer-assisted morphometric analysis to evaluate nuclear area, number of area of AgNORs per nuclear area, ratio of nuclei with five or more AgNORs, nuclear perimeter, area fraction between nuclear area and area of AgNOR. These researchers detected significant differences between normal and malignant mammary tumours for all of the measured parameters. Ciurea et al. (11) attempted to define quantitative objective criteria for diagnosis of mammary adenoma and adenocarcinoma in dogs using several morphometric parameters - nuclear area, nuclear perimeter, roundness, nuclei per millimeter of basement membrane and minimal distance from cells to basement membrane. They examined 11 specimens from normal canine mammary tissue, 17 specimens from mammary adenomas and 33 specimens from mammary adenocarcinomas by computerized morphometric analysis. The analysis showed that these parameters gradually increased from normal to high-grade malignancy, similar to the findings in our study.

According to De Vico et al. (27) computerized morphometry can be applied in cytology and histology, but cytological application is easier to perform and more convenient for practical purposes. The measurement procedure is easier on cytologic than on histologic specimens, because of the more homogeneous background of the cytologic smears. Moreover, on cytologic smears the cells are arranged in one plane, thus their morphometric evaluation is easier (3, 28).

The results from our analysis indicated that nuclear roundness could be used for differentiation of benign from malignant canine mammary gland epithelial tumours on cytologic smears. In the present study the malignant cells had more irregular nuclear shapes than cells in benign tumour. The values of nuclear roundness were the lowest in adenomas and fibroadenomas and the highest in anaplastic carcinomas. The mean values of nuclear roundness were increased gradually as follows - adenomas, fibroadenomas, tubulopapillary carcinomas, solid carcinomas and anaplastic carcinomas. This indicated that computerized morphometric analysis would be helpful in automated grading of canine mammary gland carcinomas on cytologic material.

Sampling, fixing and staining of the cells are the most important factors determining the quality of cytologic findings for image analysis. In our study, the cells were sampled from four different areas of neoplastic formations. In this way from one neoplastic formation, we prepared several cytologic specimens and subsequently chose the best ones for morphometric analysis. Protection of cells from environmental conditions could be performed by using of cells fixatives. Each fixative changes the shape of the cells in different way, so if we use different fixatives to protect them in the same study the data from morphometric evaluation would not be objective. For this reason it is necessary to perform future standardisation of fixative procedure and we recommend using Merckofix spray®, which protects cells with polyglycol film for several weeks.

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