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Original Contribution

NUCLEAR MORPHOMETRY AS AN ADDITIONAL METHOD OF DIAGNOSIS IN SPONTANEOUS FELINE MAMMARY GLAND ADENOMAS AND CARCINOMAS

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ABSTRACT

Thirty-five spontaneous feline mammary gland tumours (4 adenomas, 11 tubulopapillary carcinomas, 13 solid carcinomas, and 7 cribriform carcinomas) were analysed by computer-assisted nuclear morphometry on Hemacolor® stained cytologic specimens. Computerized cytomorphometry was performed and the mean nuclear area (MNA), mean nuclear perimeter (MNP), mean nuclear diameter (MND) and nuclear roundness (NR) of studied tumours were assessed. A minimum of hundred nuclei per lesion was examined. The statistical analysis revealed significant differences between benign and malignant neoplasm. The results indicated that computer-assisted nuclear morphometry could be used as an additional method for differentiation of benign from malignant feline mammary gland tumours on cytologic specimens.

Key Words: cytology, computer-assisted morphometry, feline mammary gland epithelial tumours

INTRODUCTION

The first objective quantitative measurement of microscopic objects dates back to the 17 th century when Antoni van Leeuwenhoek developed a "system" (a prototype of the current microscope) to measure microscopic objects (1) Using sand grain and hairs from his head as a reference, he measured human erythrocytes to be approximately 25.000 times smaller than a small grain of sand (2). Since then, scientists have developed technologies to further improve the observational quality to further improve the observational quality of the human visual system by which we are able to observe and measure objects or object features that would otherwise remain hidden (1,3). The advent of computers and digital technology is such a development that fundamentally changed (microscopic analysis) visualisation of images. 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 (3, 4). 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 (4). The morphometric parameters are related to the size and shape (area, perimeter, diameter, roundness) or to the chromatin aspect of stained nuclei (5).

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 (6,7), feline squamous cell carcinomas (8), canine round cell tumours (9), canine and feline melanocytic tumours (10), canine acanthomatous ameloblastomas (11) and canine mammary gland tumours (12, 13, 14).

The aim of this study was to define

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whether the computer-assisted nuclear morphometry could be used as an additional method for differentiation of benign from malignant feline mammary gland tumours on cytologic specimens.

MATERIAL AND METHODS

Tumours

The study was performed on 35 spontaneous feline mammary gland epithelial tumours (4 adenomas, 11 tubulopapillary carcinomas, 13 solid carcinomas, and 7 cribriform carcinomas). The tumours 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 by fine-needle aspiration biopsy from four different areas of tumour formations, were fixed immediately with *Merckofix spray*® (Merck, Darmstadt, Germany) and stained with *Hemacolor* ® (*Merck, Darmstadt, Germany*). 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 and were stained with haematoxylin/Eosin (H/E). All diagnoses were confirmed histopathologically according to WHO International Histological Classification of Tumours of Domestic Animals (15).

Nuclear morphometric analysis

The material obtained for cytopathological processing was analysed with a Motic Professional B3 digital microscope (*Motic, China Group Co Ltd, Hong Kong, China*) coupled to a computer equipped with the Image Pro Plus® analysis system (*Media Cybernetics, Silver Spring, MD, USA, version 4.5.0.29 for Windows 98/NT/2000*). The measurements were calibrated with the aid of a micrometer ruler (Motic®). Fields containing neoplastic cells were randomly selected in the areas of highest cellularity, with x 40 objective lens. The images created by the computer system were stored in the system digital memory, formatted as .jpeg files and displayed on the monitor screen (**Figure 1**). At least 100 nuclei were analysed in each case. Precautions were taken to include only intact nuclei. After selection of the proper portion of the cytological specimens and taking the digital photos, the nuclei borders were outlined using the "Draw/Merge object" function with the aid of a computer mouse. The morphometric parameters evaluated in this study were mean nuclear area (MNA; μm^2), mean nuclear perimeter (MNP; μm), mean nuclear diameter (MND; μm) and nuclear roundness (NR).

Statistical analysis

Statistical analysis of the data was done using a one way analysis of variance (ANOVA) followed by the LSD post hoc test (*Statistica 6.0, StatSoft, Tulsa, OK, USA*) at $p < 0.001$ level of significance.

RESULTS

The data for the investigated nuclear parameters are presented for each of the 35 tumours examined on **Table 1.** The values for the groups are given on **Table 2.** The mean values of MNA, MNP and MND increased gradually in the following order: adenoma, tubulopapillary carcinoma, cribriform carcinoma and solid carcinoma. NR increased in order adenoma solid carcinoma, tubulopapillary carcinoma and cribriform carcinoma. The statistical analysis revealed significant differences between benign and malignant neoplastic cells.

DISCUSSION

Computer-assisted morphometry can be applied both in cytology and in histology, but cytological application is more convenient for practical purposes (12, 16). On cytologic smears the cells are arranged in one plane, thus their morphometric evaluation is easier. Also, by using cytologic specimens, the differentiation between benign and malignant tumours may be made preoperatively, improving the ability of veterinarians and owners to make decisions regarding the patient (12).

In this study, we found that nuclear morphometric parameters MNA, MNP, MND and NR differed significantly between adenomas and different histological type feline mammary gland carcinomas. These results suggest that investigated morphometric parameters may be useful in the preoperative evaluation of feline mammary epithelial tumours by cytology. In this way we confirm the diagnostic value of nuclear morphometric

analysis reported to different tumour in veterinary (7, 8, 9, 10) and human medicine (1,3,4,5). We did not determine the nucleus/cytoplasm ratio or cytoplasmic measurements because of the difficulty in delimiting the cellular margins in cytopathologic slides. The mean values of area, perimeter and diameter obtained from cytologic smears were significantly higher than measurements on histologic specimens reported by De Vico and Maiolino (16), but this could be explained by the different fixation and smear preparation used for cytology and histology (17).

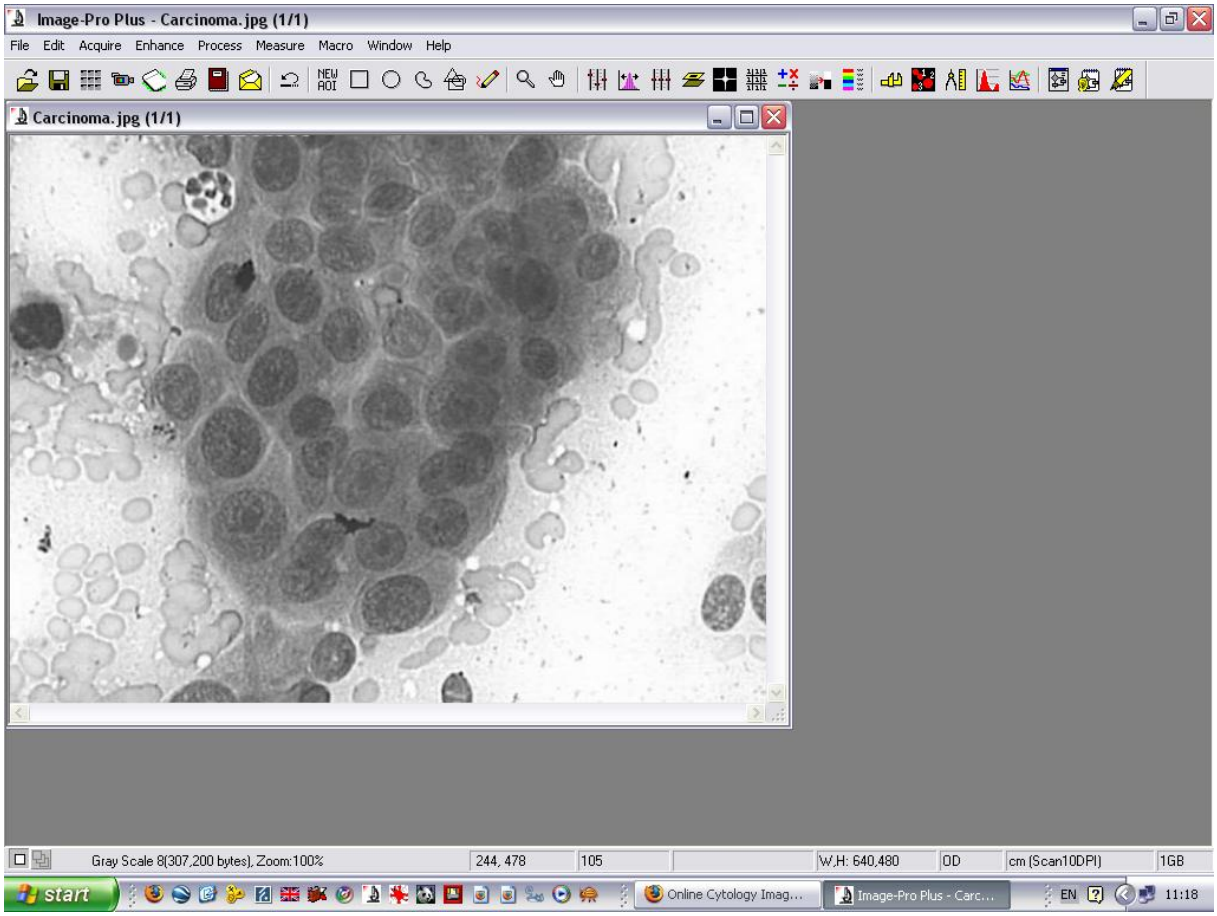

Figure 1. *A cytological picture of feline mammary gland solid carcinoma from the "Image Pro Plus" program*

In conclusion, our results suggest that computer-assisted morphometry could be used as an additional tool for differentiation between feline mammary gland adenomas and carcinomas.

Although we did not examine the prognostic value of investigated parameters, our results suggest that quantitative nuclear analysis could also provide prognostic information on the biological behaviour in feline mammary carcinomas, since the known metastatic potential of solid carcinomas is higher than that of other carcinomas (18). Apparently, additional studies are warranted not only to establish "fixed" minimum and maximum values for each morphometric parameter but also for application of nuclear morphometry as a prognostic method in feline mammary gland neoplasm. Therefore future investigations in this area are really necessary.

Table 1. *Values of the morphometric nuclear parameters in each of the examined tumours.*

MNA (μm^2)	MNP (μm)	MND (μm)	NR
Adenomas (n=4)			
72.70	30.68	9.43	1.06
73.28	30.34	9.51	1.03
71.24	29.29	9.35	1.00
71.47	29.91	9.39	1.04
Tubulopapillary carcinomas (n=11)	33.25	10.17	1.11
84.68	33.21	10.18	1.08
83.94	32.74	9.90	1.08
78.91	30.14	9.92	1.14
81.32	33.47	10.32	1.11
86.75	32.06	9.85	1.10
79.54	33.38	10.16	1.07
85.37	32.71	10.12	1.10
83.53	32.70	10.26	1.10
85.6	33.32	9.49	1.11
77.2	31.18	9.46	1.08
73.28			
Solid carcinomas (n=13)			
98.07	36.02	10.95	1.07
98.56	29.52	8.91	1.11
110.08	37.29	11.66	1.10
101.55	36.37	11.16	1.15
99.97	35.95	11.08	1.08
101.80	36.05	11.20	1.11
111.86	38.44	11.72	1.14
79.63	32.25	9.88	1.12
106.49	37.00	11.49	1.15
95.66	35.89	10.77	1.20
89.51	34.29	10.44	1.11
122.14	39.97	12.61	1.11
95.66	35.89	10.77	1.06
Cribriform carcinomas (n=7)			
91.06	33.84	10.60	1.17
97.98	35.29	10.99	1.18
91.29	34.23	10.60	1.09
89.08	33.91	10.45	1.12
80.52	32.26	9.94	1.07
100.66	36.31	11.12	1.31
102.18	35.67	11.23	1.09

MNA, mean nuclear area; MNP, mean nuclear perimeter; MND, mean nuclear diameter NR, nuclear roundness.

Table 2. *Values of nuclear morphometric parameters in different histological types.*

Histological type	*MNA (range) and mean value (μm²) ± SD*	*MNP (range) and mean value (μm) ± SD*	*MND (range) and mean value (μm) ± SD*	*NR (range) and mean value ± SD*
Adenoma (n=4)	(71.24 - 73.28) 72.17 ± 0.96	(29.96 - 30.38) 30.16 ± 0.23	(9.35 - 9.51) 9.42 ± 0.06	(1.00 - 1.06) 1.03 ± 0.02
Tubulopapillary carcinoma (n=11)	(73.82 - 86.75) 81.89 ± 4.09**	(31.18 - 33.47) 32.83 ± 0.68**	(9.46 - 10.32) 9.99 ± 0.29**	(1.07 - 1.14) 1.10 ± 0.01**
Solid carcinoma (n=13)	(79.63 - 122.14) 100.84 ± 10.56**, ΔΔ	(29.52 - 39.97) 35.76 ± 2.62**, ΔΔ	(8.91 - 12.61) 10.97 ± 0.9**, ΔΔ	(1.04 - 1.07) 1.06 ± 0.006**
Cribriform carcinoma (n=7)	(80.52 - 102.18) 93.25 ± 7.58**, ΔΔ	(32.26 - 36.31) 34.50 ± 1.36**, ΔΔ	(9.94 - 11.23) 10.71 ± 0.44**, ΔΔ	(1.07- 1.31) 1.15 ± 0.08*

MNA, mean nuclear area; MNP, mean nuclear perimeter; MND, mean nuclear diameter; NR, nuclear roundness
$^{*}P < 0.05$, $^{**}P < 0.01$ versus adenomas
$^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ versus tubulopapillary carcinomas

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