



Original Contribution

**NUCLEAR MORPHOMETRIC PARAMETERS IN RELATION TO
TUMOUR DIAMETER AND SURVIVAL IN CATS
WITH SPONTANEOUS MAMMARY GLAND CARCINOMAS**

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ABSTRACT

The study was performed on thirty-one spontaneous feline mammary gland carcinomas. Neoplastic cells were preoperatively obtained by fine-needle aspiration biopsy, fixed immediately with Merckofix spray[®] (Merck, Darmstadt, Germany) and stained with Hemacolor[®] (Merck Darmstadt, Germany). After surgical removal, tumour diagnoses were histopathologically confirmed. The tumour diameters were determined as > 3 cm or < 3 cm after surgical removal of neoplastic formations. Postoperative follow-up information was available for 25 (80.64 %) cats. The material obtained for cytopathological processing was analysed with image analysis system. Computerized cytometry was performed and the mean nuclear area (MNA), mean nuclear perimeter (MNP), mean nuclear diameter (MND) and nuclear roundness (NR) of investigated tumours were assessed. Data from nuclear morphometry were correlated with tumour diameters and survival using Pearson's correlation test (Statistica 6.0, StatSoft, Tulsa, OK, USA) at the level of significance $p < 0.05$. The results indicated that nuclear parameters MNA, MNP, MND and NR are not reliable prognostic indicators in feline mammary gland carcinomas based on the correlation analysis between them and tumour diameters and survival period.

Key words: nuclear morphometry, tumour diameter, survival, mammary tumours, cats

INTRODUCTION

Feline mammary gland tumours rank third in frequency, following lymphoid and cutaneous neoplasm and account for 12 % of all feline tumours and 17 % in female cats (1, 2) The incidence of mammary tumours in cats is reduced by 91 % in cats spayed prior to six months of age and by 86 % in cats spayed prior to one year, according to one study (3). Between 80 and 85% of the feline mammary tumours are malignant (1, 4). Many of the tumours, especially the large, more invasive neoplasm, adhere to the skin and are ulcerated. Lymphatic and lymph node invasion is frequently present and visible at necropsy. In several studies, more than 80% of the cats with a mammary malignancy had metastases to one or more of the following organs at the time of euthanasia: lymph nodes,

lungs, pleura, liver, diaphragm, adrenal glands, and kidneys (4, 5).

Human and veterinary pathologists are constantly faced with determining prognoses with different types of tumours. Despite the fact that nuclear morphometry has been widely studied in human breast cancer, little is known about this field of investigation in veterinary medicine. This report describes an investigation of quantitative nuclear parameters in relation to metastases and survival in cats with spontaneous mammary gland carcinomas.

MATERIALS AND METHODS

The study was performed on thirty-one spontaneous feline mammary gland carcinomas. Neoplastic cells were preoperatively obtained by fine-needle aspiration biopsy, fixed immediately with Merckofix spray[®] (Merck, Darmstadt, Germany) and stained with Hemacolor[®] (Merck Darmstadt, Germany). The fine-needle aspiration biopsy was performed by sampling cells from four different areas of

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tumours. After surgical removal, tumour diagnoses were histopathologically confirmed according to WHO International Histological Classification of Tumours of Domestic Animals (6). They were classified as follows: tubulopapillary carcinomas, n=11, solid carcinomas, n=13 and cribriform carcinomas, n=7.

The tumour diameters were determined as > 3 cm or < 3 cm after surgical removal of neoplastic formations. Postoperative follow-up information was available for 25 (80.64 %) cats. Survival period was defined as the time from first detection of the tumour to either the time of death or the date on which the cat was known to be alive. 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). Fields containing neoplastic

cells were randomly selected in the areas of highest cellularity, with x40 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). A correlation between the morphometric parameters and variables tested (metastases and survival) was evaluated using Pearson’s correlation test ($p < 0.05$) at a level of significance $p < 0.05$ (Statistica 6.0, StatSoft, Tulsa, OK, USA).

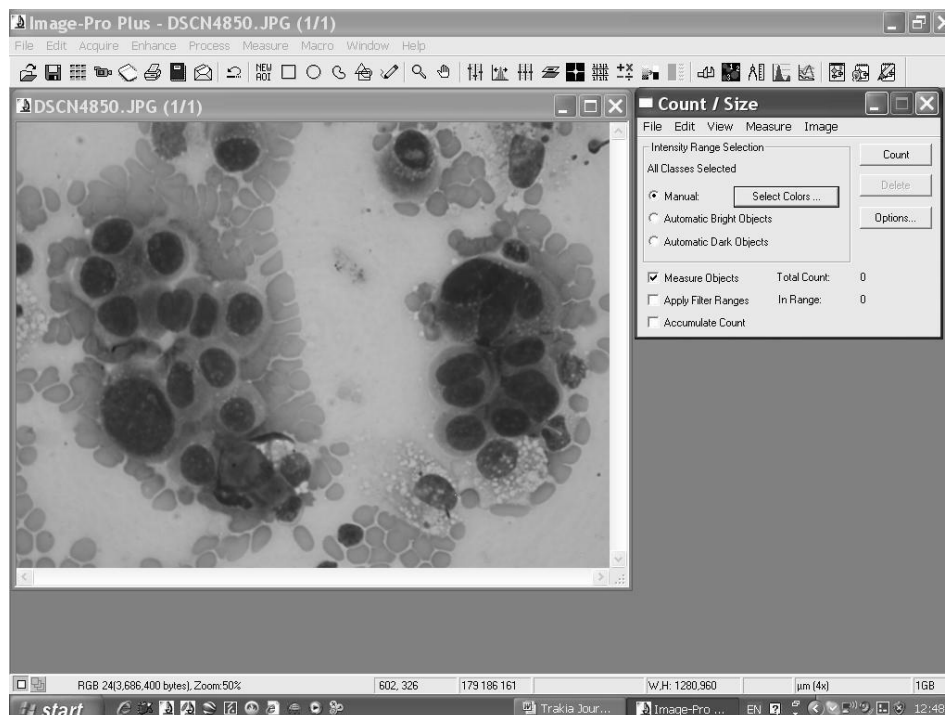


Figure 1. The menu “count/size” from “Image Pro Plus”. Preparation for quantitative nuclear analysis

RESULTS

The diameters of tumours and survival periods for animals included in this study are presented on **Table 1**. Data for the investigated nuclear parameters are presented on **Table 2**. A correlation between investigated morphometric parameters and variables tested (metastases and survival) was not found.

DISCUSSION

The diagnostic and prognostic value of nuclear morphometry in breast cancer is well known in human medicine (7, 8, 9 10). There are several reports about the diagnostic (11, 12, 13) and prognostic value of computerized morphometric analysis in canine mammary gland tumours (14). The most recent investigations in this area have emphasized

the diagnostic value of computer-derived nuclear features for staging canine mammary carcinomas and supported the hypothesis that nuclear morphometry could be very useful in assessing lymph node status in canine mammary gland cancer, particularly in the absence of lymph node tissue (14).

Table 1. Tumour diameters and survival of cats with mammary gland carcinomas included in this study

Tumours	Tumours diameter	Survival in months*
Tubulopapillary carcinomas (n=11)	<3 cm	-
	>3 cm	6
	>3 cm	-
	<3 cm	33
	<3 cm	22
	<3 cm	18
	<3 cm	30
	>3 cm	4
	<3 cm	-
	>3 cm	7
	>3 cm	3
Solid carcinomas (n=13)	<3 cm	-
	>3 cm	6
	<3 cm	22
	<3 cm	18
	<3 cm	21
	>3 cm	6
	>3 cm	4
	<3 cm	32
	<3 cm	27
	>3 cm	4
	>3 cm	5
	<3 cm	-
	<3 cm	22

Cribriform carcinomas (n=7)	>3 cm	-
	<3 cm	22
	<3 cm	17
	>3 cm	4
	>3 cm	3
	<3 cm	19
	>3 cm	6

*Survival period was defined as the time from first detection of the tumour to either the time of death or the date on which the cat was known to be alive.

In veterinary medicine there is only one study that has investigated prognostic value of nuclear morphometry in feline mammary carcinomas. De Vico and Maiolino (15) studied the relationship between six morphometric parameters in cats with mammary carcinomas of different histological type (tubular, papillary-cystic and solid) and survival time. They concluded that MNA and NR are not related to survival. The authors studied also some parameters that were not the object of our investigation. They claimed that the standard deviation of nuclear form factor and the coefficient of variation of nuclear form factor were reliable prognostic parameters in feline mammary carcinomas. No statistical differences were observed in the other morphometric parameters, although the mean values were higher in solid carcinomas than in tubular or papillary-cystic adenocarcinomas, as we found in our study.

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

Histological type	MNA (range) and mean value (μm^2) \pm SD	MNP (range) and mean value (μm) \pm SD	MND (range) and mean value (μm) \pm SD	NR (range) and mean value \pm SD
Tubulopapillary carcinoma (n=11)	(73.82 - 86.75) 81.89 \pm 4.09	(31.18 - 33.47) 32.83 \pm 0.68	(9.46 - 10.32) 9.99 \pm 0.29	(1.07 - 1.14) 1.10 \pm 0.01
Solid carcinoma (n=13)	(79.63 - 122.14) 100.84 \pm 10.56	(29.52 - 39.97) 35.76 \pm 2.62	(8.91 - 12.61) 10.97 \pm 0.9	(1.04 - 1.07) 1.06 \pm 0.006
Cribriform carcinoma (n=7)	(80.52 - 102.18) 93.25 \pm 7.58	(32.26 - 36.31) 34.50 \pm 1.36	(9.94 - 11.23) 10.71 \pm 0.44	(1.07- 1.31) 1.15 \pm 0.08

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

In conclusion, according to our results, the morphometric parameters MNA, MNP, MND and NR are not reliable prognostic indicators in feline mammary gland carcinoma based on the correlation analysis between them and metastases and survival period. Due to the

small number of samples studied, our results should however be regarded as tentative and further work is needed to confirm or not practical value of quantitative analysis in feline mammary gland carcinomas.

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