

QUANTITATIVE MORPHOLOGY AS A PROGNOSTIC FACTOR IN FELINE SPONTANEOUS CUTANEOUS SQUAMOUS CELL CARCINOMAS

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In a study on cytological specimens from 30 cats with histologically confirmed cutaneous squamous cell carcinoma, the morphometric variables mean nuclear area (MNA, μm^2), mean nuclear perimeter (MNP, μm), mean nuclear diameter (D mean, μm), minimum nuclear diameter (D min, μm) and maximum nuclear diameter (D max, μm) were studied and compared to metastases in regional lymph nodes. The mean values of these parameters were significantly greater in cats with lymph node metastases compared to parameters of tumour cells from cats which were lymph node-negative. A significant positive correlation was observed between all studied morphometric parameters and metastases in the regional lymph nodes. In conclusion, computer -assisted nuclear morphometry could be used as a prognostic method in the diagnosis of spontaneous feline cutaneous squamous cell carcinomas.

Key words: nuclear morphometry, feline cutaneous squamous cell carcinomas, metastases, prognosis.

INTRODUCTION

Squamous cell carcinomas represent about 15% of skin neoplasms in cats [1-3]. The most frequent localization of tumours is the nasal planum, the eyelids, and the ears. Approximately in 30% of affected patients lesions are multiple. Usually, animals between 9 and 14 years of age are affected [2-4]. The occurrence of the tumours are associated with prolonged exposure to ultraviolet light, lack of pigment in the epidermis and sparse hair coat at the sites of development. Papillomavirus DNA has been detected in the tumor, indicating that this virus could be potential causative factor [4]. Squamous cell carcinomas are locally invasive, but can metastasize to regional lymph nodes. Metastasis usually affects mandibular, retropharyngeal lymph nodes and lungs [5].

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The purpose of this study is to determine whether some quantitative nuclear morphometric parameters could be used as an objective prognostic factors in feline spontaneous cutaneous squamous cell carcinomas.

MATERIALS AND METHODS

Animals

The study was performed on 30 cats with spontaneous cutaneous squamous cell carcinomas. In thirteen animals metastases were detected in the regional lymph nodes during the diagnosis. Neoplastic cells obtained preoperatively by fine needle aspiration biopsy were fixed immediately using Merckofix spray[®] (Merck, Darmstadt, Germany) and stained with Hemacolor[®] (Merck, Darmstadt, Germany). After surgical removal, all neoplasms were classified pathohistologically according to WHO International Histological Classification of Tumors of Domestic Animals [1].

Quantitative analysis

The computer cytomorphometric analysis of the neoplastic cell nuclei was performed using a microscope & software^a. The system was calibrated in advance using the micrometer ruler. In each case at least 100 nuclei were examined. From the measurement were excluded all overlapped, deformed or ruptured cell nuclei. The morphometric parameters examined were mean nuclear area (MNA, μm^2), mean nuclear perimeter (MNP, μm), maximum, minimum and mean nuclear diameter (D max, D min, D mean, μm).

Statistical analysis

Association of investigated nuclear parameters in non-metastatic and metastatic neoplasms were assessed by a Mann-Whitney U test (Statistica 6.0, StatSoft, Tulsa, OK, USA) at a level of significance $P < 0.05$.

RESULTS

The data from the studied morphometric parameters MNA, MNP, D max, D min, D mean for each of the investigated tumors are presented in Table 1. The mean values of the indicators were significantly higher in cats with metastases in the lymph nodes compared to non-metastatic neoplasms (Table 2). In addition, a significant positive correlation was observed between all studied morphometric parameters and metastases in the regional lymph nodes (Table 3).

^aMotic Professional B3 digital microscope (Motic, China Group Co. Ltd, Hong Kong, China). Image Pro Plus[®] analysis system (Media Cybernetics, Silver Spring, MD, USA, version 4.5.0.29 for Windows 98 / NT / 2000).

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

Feline squamous cell carcinomas	MNA (μm^2)	MNP (μm)	D max (μm)	D min (μm)	D mean (μm)
1	70.18	29.85	10.3	8.56	9.28
2	73.42	30.54	10.54	8.74	9.55
3	79.63	32.25	11.82	8.42	9.88
4	77.27	33.32	12.99	6.81	9.49
5	78.91	32.74	12.39	7.67	9.90
6	75.84	30.79	10.18	8.87	9.66
7	77.53	33.38	13.15	6.96	9.58
8	80.72	35.74	14.66	6.81	9.64
9	71.10	33.99	14.22	5.76	9.04
10	83.53	32.71	11.41	8.94	10.12
11	98.56	35.96	12.21	10.17	11.01
12	95.66	35.89	12.97	8.79	10.77
13	85.37	33.38	12.13	8.27	10.16
14	85.23	34.11	12.68	7.90	10.12
15	94.36	36.02	12.11	8.67	10.69
16	88.45	33.54	11.43	9.77	10.45
17	98.07	36.02	12.58	9.68	10.95
18*	96.58	37.36	8.41	14.74	11.20
19*	94.42	35.02	9.17	12.52	10.74
20*	86.75	33.47	9.55	11.50	10.32
21*	110.08	32.29	10.61	12.70	11.66
22*	101.55	36.37	9.95	12.89	11.16
23*	103.10	36.65	10.01	12.71	11.25
24*	111.86	38.47	10.30	13.35	11.72
25*	102.75	38.09	8.31	14.75	11.56
26*	106.49	37.00	10.81	12.74	11.49
27*	122.14	39.97	10.15	14.57	12.61
28*	102.72	37.01	8.85	13.29	11.46
29*	118.32	38.73	10.61	13.49	12.02
30*	115.28	39.88	9.56	14.99	12.56

MNA, mean nuclear area; MNP, mean nuclear perimeter; D mean, mean nuclear diameter; D min, minimum nuclear diameter; D max, maximum nuclear diameter. * Metastasizing squamous cell carcinomas.

Table 2. Number of cases (n), mean (m) and standard deviation (Δ m) of the measured parameters for the non-metastasizing (NM) and metastasizing (M) feline cutaneous squamous cell carcinomas

Group Parameter	NM (n=17) m \pm Δ m (range)	M (n=13) m \pm Δ m (range)	Significance p
MNA (μm^2)	83.16 \pm 9.16 (70.18-98.56)	105.54 \pm 9.95 (86.75-122.14)	p=0.0001
MNP (μm)	33.54 \pm 1.97 (29.85-36.02)	36.94 \pm 2.28 (32.29-39.97)	p=0.0001
D max (μm)	12.22 \pm 1.23 (10.18-14.66)	13.40 \pm 1.06 (11.50-14.99)	p=0.0001
D min (μm)	8.28 \pm 1.17 (5.76-10.17)	9.71 \pm 0.82 (8.31-10.81)	p=0.0001
D mean (μm)	10.01 \pm 0.59 (9.04-11.01)	11.51 \pm 0.64 (10.32-12.61)	p=0.0001

Table 3. Correlation coefficients (r) between the nuclear morphometric parameters and metastases in the regional lymph nodes ($p < 0.05$)

	MNA (μm)	MNP (μm)	D max (μm)	D min (μm)	D mean (μm)
Metastases in the regional lymph nodes	0.78	0.65	0.50	0.57	0.79

MNA, mean nuclear area; MNP, mean nuclear perimeter; D mean, mean nuclear diameter; D min, minimal nuclear diameter; D max, maximal nuclear diameter.

DISCUSSION

In the veterinary literature there are several publications related to the prognostic significance of computer morphometry in tumor pathology. In canine perianal adenocarcinomas a statistically significant correlation between survival and morphometric parameters MNA, MNP, D max and D mean were found, which literally means that animals with high values of the morphometric parameters have a poor prognosis [6]. Nuclear cytomorphometric analysis is able to differentiate recurrent from non-recurrent canine and feline basal cell carcinomas [7,8]. Similarly, in another study in eight dogs with sebaceous carcinomas, statistically significant differences were found between MNA, MNP, D min and D mean in dogs with metastases during tumour diagnosis compared to animals without metastases [9]. In feline mammary gland neoplasms, MNA, MNP, MND and NR (nuclear roundness) have prognostic significance based on their correlation with patient survival [10]. Interesting results have been reported in the study of the prognostic significance of computer morphometry in canine cutaneous mastocytomas [11]. The authors compared two groups of patients: one group with MNA $< 62.39 \mu\text{m}$ (classified pathohistologically as low differentiated - 100% survival over 2334 days) and a second group of patients with MNA $> 62.39 \mu\text{m}$ (classified pathohistologically as highly differentiated - 0% survival after 752 days). In another study of thirty skin canine mastocytomas, the morphometric parameters MNA and NR were not statistically significant in tumors with varying degrees of neoplastic growth. Meanwhile, between the groups was revealed credibility between MNA, MNP, nuclear cytoplasmic ratio, and percentage of multinuclear cells [12].

At the same time, there is no similar studies in cats with skin squamous cell carcinomas. In a previous study of cytological preparations of seventeen spontaneous canine squamous cell carcinomas we compared the morphometric parameters MNA, MNP, D min, D max, D mean with metastases in regional lymph nodes [13]. We found statistically significant differences in MNA, MNP and D mean between metastatic and non-metastatic tumours and concluded that computer morphometry could be used to determine metastatic potential in these tumours. In another study, we also found statistically significant differences in the same morphometric parameters between canine squamous cell carcinomas with different degrees of neoplastic growth [14].

In conclusion, computer-assisted nuclear morphometry could be used as a prognostic method in the diagnosis of spontaneous feline cutaneous squamous cell carcinomas. The methodology is relatively easy to perform and the future will show whether it will go beyond the sphere of research and will come into practice.

Authors' contributions

SR carried out the morphometric studies, participated in the design of the study and performed the statistical analysis.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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KVANTITATIVNA MORFOLOGIJA KAO PROGNOСТИČKI FAKTOR KOD SPONTANIH KUTANIH SKVAMOZNO-ĆELIJSKIH KARCINOMA MAČAKA

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Pregledom citoloških uzoraka poreklom od 30 mačaka kod kojih je histološkim metodama potvrđen kutani skvamozno-ćelijski karcinom, obavljeno je ispitivanje morfometrijskih promenljivih veličina srednje vrednosti površine jedra ($MNA, \mu m^2$), srednje vrednosti perimetra jedra ($MNP, \mu m$), srednje vrednosti prečnika jedra (D -srednja vrednost, μm), minimalna vrednost prečnika jedra (D -min, μm) kao i maksimalni prečnik jedra (D max, μm). Ove su veličine poređene sa vrednostima koje su nađene u okviru metastaza u regionalnim limfnim čvorovima. Srednje vrednosti ovih parametara bile su značajno veće kod mačaka sa metastazama u limfnim čvorovima u poređenju sa tumorskim ćelijama poreklom od mačaka kod kojih nije bilo metastaza u regionalnim limfnim čvorovima. Značajna pozitivna korelacija je uočena između svih morfometrijskih parametara koji su ispitivani i metastaza u regionalnim limfnim čvorovima. Na osnovu rezultata može da se zaključi da se morfometrija jedra pomoću kompjutera, može da koristi kao prognostički metod u postavljanju dijagnoze spontanih kutanih skvamozno-ćelijskih karcinoma mačaka.