Slov Vet Res 2016; 53 (1): 37 - 44
UDC 636.7.09:616-006.04:616-091.8:57.086

Original Research Article

QUANTITATIVE ANALYSIS IN CANINE CUTANEOUS SOFT TISSUE SARCOMAS AND REACTIVE SPINDLE CELL PROLIFERATIONS ON CYTOLOGICAL SMEARS

Radostin Simeonov

Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Corresponding author, E-mail: rsimeonov@uni-sz.bg

Abstract: Stained cytological specimens from twenty-four canine cutaneous soft tissue sarcomas and twenty-four canine spindle cell reactive proliferations were analyzed by computer-assisted nuclear morphometry. In each case, the nuclei of at least 100 neoplastic and reactive spindle cells were measured. The studied morphometric parameter was nuclear roundness (NR). The aim of the present study was to evaluate the possibility of quantitative differentiation between canine cutaneous soft tissue sarcomas and reactive spindle cell proliferations. The mean values of NR were statistically significantly higher in soft tissue sarcomas than in non-neoplastic lesions. Statistical differences in NR were found between liposarcoma and haemangiopericytoma. Cytomorphometric differences between soft tissue sarcomas divided by histologic grade were not revealed. Differences, related to age, sex, breed or location of the lesions between non-neoplastic and neoplastic lesions were also not observed. The results indicated that the morphometric parameter NR could be used as an additional tool for differentiation between canine cutaneous soft tissue sarcomas and reactive spindle cell proliferations.

Key words: computer-assisted morphometry; nuclear roundness; soft tissue sarcomas; spindle cell proliferations; dog

Introduction

Soft tissue sarcomas are a population of mesenchymal neoplasms that account for 15-20% of all cutaneous and subcutaneous tumours in dogs (1, 2). Most of the neoplasms are solitary and affect predominantly middle-aged and senior dogs. There is no sex and breed predilection with the exception of synovial cell sarcomas in dogs (2). Despite their heterogeneity, soft tissue sarcomas have a similar biological behavior. They are locally invasive and post-operative recurrences are reported in 7-30 % of cases (1, 2). Metastases are observed in about 20 % of the patients (3-5). The most important outcome predictor of this type of neoplasms is their grade determined histopathologically (1). Cytological diagnosis of soft tissue sarcomas is a challenge to pathologists. Pathological samples usually contain few cells as neoplastic cells are hardly exfoliated. In addition, the differentiation of sarcoma cells from reactive connective tissue processes by cytology is difficult to reliably differentiate (3-5). Computer-assisted nuclear morphometry is an objective image analysis for estimation of chosen parameters in individual cell (6, 7). The resulting data are objective and the technique is quickly performed using conventional microscopic analysis. The last investigations in

Received: 10 April 2016
Accepted for publication: 7 December 2016

this area showed that morphometry could be used as an additional tool for diagnosis and prognosis in some neoplastic diseases in small animals (8-10). Moreover, some researchers have used this non-traditional diagnostic method for detailed grading of malignant tumours (11, 12).

The aim of the present study was to evaluate the applicability of quantitative differentiation between canine cutaneous soft tissue sarcomas and reactive spindle cell proliferations.

Materials and methods

Animals

The survey was performed in 24 dogs with spontaneous soft tissue sarcomas -fibrosarcoma, (n=8), liposarcoma, (n=8), haemangiopericytoma, (n=8) and 24 dogs with reactive spindle cell proliferations: granulation tissue, (n=12) and dermal fibrosis, (n=12). All investigated formations were localized on skin epidermis and dermis. Patients' records were obtained from the database of the Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, Bulgaria (Table 1). Age, sex, breed of the dogs, location of the lesion, tumour grade and histologic diagnoses were recorded. In difficult cases an immunohistochemically investigation of neoplastic tissues was also performed (Fig. 1).

Cytological and histopathological processing

The material was obtained preoperatively by fine-needle aspiration biopsy, fixed immediately with Merckofix spray (Merck®, Darmstadt, Germany) and stained with Hemacolor (Merck®, Darmstadt, Germany). The cells were taken from four different areas of the formations. Later, material for histopathological examination was obtained from all patients. Samples were fixed in 10% neutral formalin and routinely processed. The final diagnoses were confirmed histopathologically (13). Histologic sections from all tumours were subsequently graded according to a system adopted from human medicine (14). The system was first applied for canine soft tissue sarcomas in 1980 year (14-16). It is based on tissue type and differentiation, mitotic rate, and percent necrosis - Table 1. (5,14).

Computer-assisted morphometry

The material for cytology was morphometrically analysed via trinocular digital microscope [Motic Professional B3 digital microscope (Motic, China Group Co Ltd, Hong Kong, China], and microscope image analysis software [Image Pro Plus® analysis system (Media Cybernetics, Silver Spring, MD, USA, version 4.5.0.29 for Windows 98/NT/2000)], Fig. 2. The measurements were calibrated with the aid of a micrometer ruler. Microscopic fields were randomly selected in the areas of highest cellularity, using x 40 objective lens. Images created by the computer system were stored in the system's digital memory, formatted as .jpeg files and displayed on the monitor screen (Fig. 1). At least 100 nuclei were analyzed in each case. Precautions were taken to include only intact cells. 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 parameter evaluated in this study was nuclear nuclear roundness (NR). A perfectly circular structure has a roundness value of 1.0 and values > 1 indicate irregular shapes.

Statistical analysis

Data from the morphometric analysis were statistically analyzed by the Mann-Whitney U test (Statistica 6.0, StatSoft, Tulsa, OK, USA) at a level of significance $P < 0.05$.

Results

The mean values of the studied parameter were statistically significantly higher in soft tissue sarcomas than in reactive spindle cell proliferations. The statistical analysis revealed significant difference between liposarcoma and haemangiopericytoma (Table 2). Cytomorphometric differences between soft tissue sarcomas divided by histologic grade were not revealed. Differences, related to age, sex, breed or location of the lesions between non-neoplastic and neoplastic lesions were also not observed.

Table 1: Grading system for soft tissue sarcomas in the dog (Kuntz et al., 1997)

Degree of differentiation	
Score 1	Resembles normal adult mesenchymal tissue
Score 2	Specific histologic type
Score 3	Undifferentiated
Mitotic index (no. of figures per 10 high power field)	
Score 1	0-9
Score 2	10-19
Score 3	> 20
Tumour necrosis	
Score 1	None
Score 2	< 50 % of tissue is necrotic
Score 3	> 50 % of tissue is necrotic
Histologic grade (cumulative score)	
Grade I	≤ 4
Grade II	5-6
Grade III	≥ 7

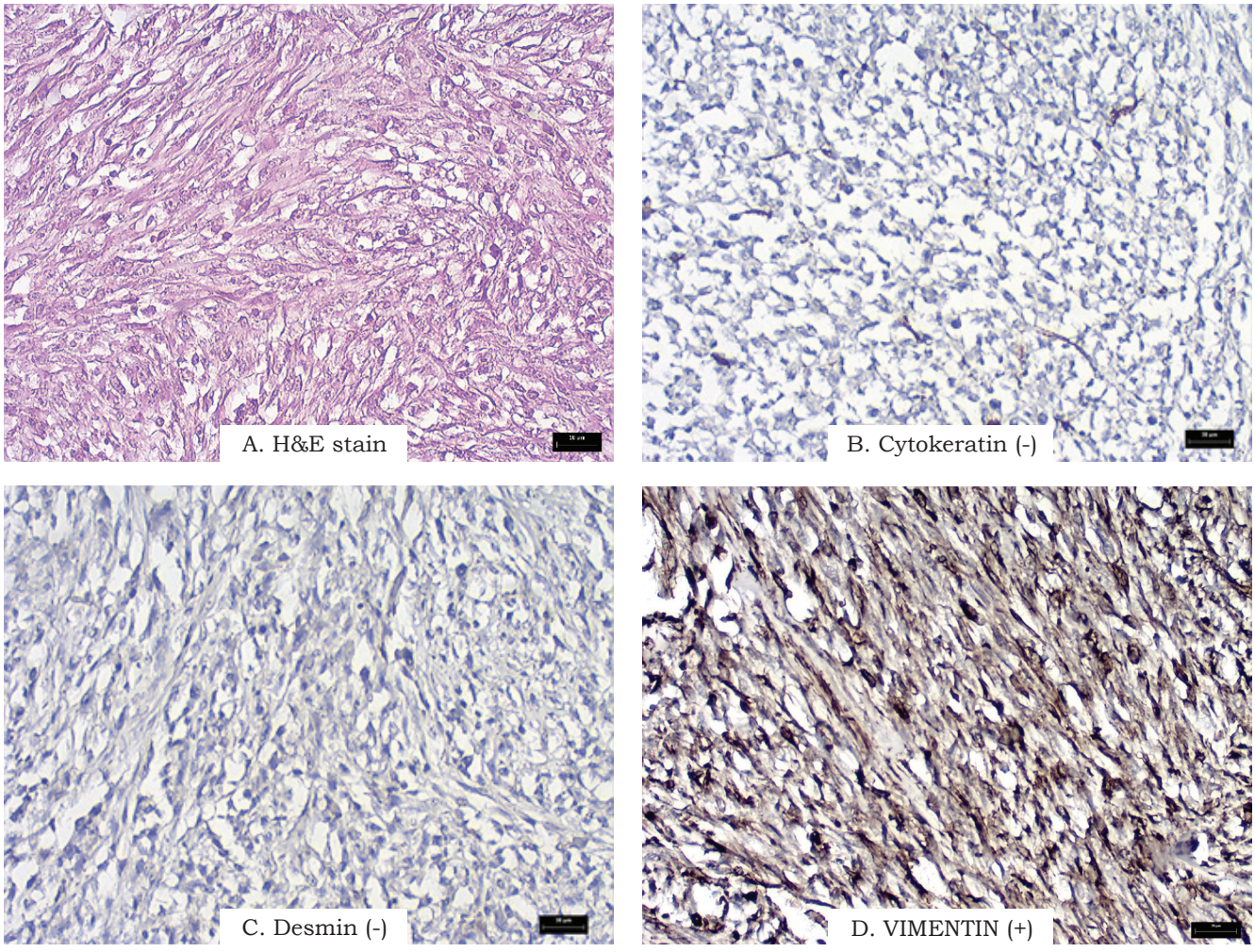

Figure 1: Fibrosarcoma. Immunohistochemistry. Neoplastic cells are Cytokeratin (-), Desmin (-) and Vimentin (+)

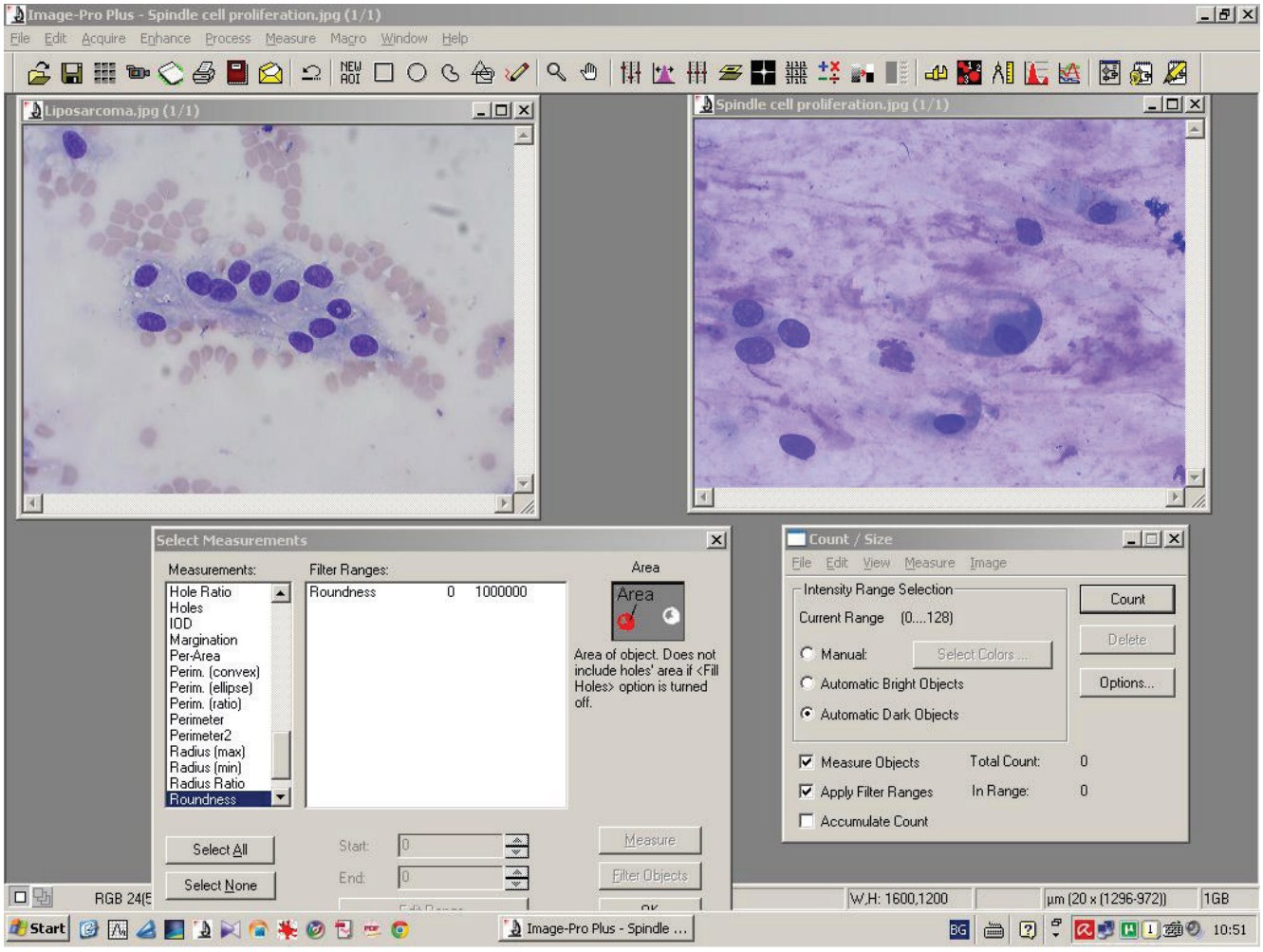

Figure 2: Main window of used software (Image Pro Plus®). Image of cytologic specimens from liposarcoma (left) and spindle cell proliferation (right)

Discussion

Bittinger et al. (17) investigated the diagnostic significance of different morphometric parameters in the differential diagnostics of human soft tissue tumours. The authors concluded that assessment of nuclear parameters may be helpful in the correct diagnosis and differential diagnosis of soft tissue tumours and tumour-like lesions of fibrous origin. Although the image analysis technique is relatively well known in veterinary pathology, there are only few reports of computer-assisted morphometry of NR in neoplastic and non-neoplastic formations. Results from one study (18) showed that NR could be used in the preoperative differentiation of benign from malignant canine mammary gland tumours. In this investigation, the malignant cells had more irregular nuclear shapes than cells in benign tumours. The values of NR were the lowest in adenomas and fibroadenomas and the highest in anaplastic carcinomas. The mean values of NR were as followed in ascending order: adenomas, fibroadenomas, tubulopapillary, solid and anaplastic carcinomas. This indicated that computerized morphometric analysis could be helpful for automated grading of canine mammary gland carcinomas on cytologic specimens. Meachem et al.[5] investigated quantitatively forty-four spontaneous canine soft tissue sarcomas and five reactive connective tissue processes. They found out that in reactive processes the nuclei were bigger and of considerably variable shape and size compared to sarcoma cells. Statistically significant differences ($P<0.05$) between reactive connective tissue processes and neoplasms were established for studied morphometric

Table 2: Clinico-morphological details of the dogs, included in this study

Case number	Breeds	Age (years)	Gender	Localization of lesions	Histologic	Tumour grade
Soft tissue sarcomas						
1	Mixed	11	M	Limbs	Fibrosarcoma	Grade II
2	Mixed	10	M	Head	Fibrosarcoma	Grade I
3	Mixed	9	M	Neck	Fibrosarcoma	Grade II
4	German shepherd	12	F	Limbs	Fibrosarcoma	Grade I
5	Rottweiler	13	F	Trunk	Fibrosarcoma	Grade II
6	Mixed	8	M	Trunk	Fibrosarcoma	Grade I
7	Mixed	7	F	Limbs	Fibrosarcoma	Grade I
8	Mixed	9	F	Limbs	Fibrosarcoma	Grade I
9	Labrador retriever	10	M	Trunk	Liposarcoma	Grade I
10	Doberman pincher	12	F	Trunk	Liposarcoma	Grade II
11	Boxer	11	M	Trunk	Liposarcoma	Grade I
12	Rottweiler	8	M	Neck	Liposarcoma	Grade I
13	Mixed	12	F	Limbs	Liposarcoma	Grade II
14	Mixed	11	F	Trunk	Liposarcoma	Grade I
15	Labrador retriever	10	M	Trunk	Liposarcoma	Grade I
16	Collie	10	M	Neck	Liposarcoma	Grade I
17	Mixed	7	F	Limbs	Haemangiopericytoma	Grade I
18	Boxer	12	F	Limbs	Haemangiopericytoma	Grade II
19	German shepherd	13	M	Trunk	Haemangiopericytoma	Grade I
20	German shepherd	11	F	Trunk	Haemangiopericytoma	Grade I
21	Mixed	9	M	Trunk	Haemangiopericytoma	Grade I
22	Poodle	8	F	Trunk	Haemangiopericytoma	Grade I
23	Mixed	9	M	Trunk	Haemangiopericytoma	Grade I
24	Mixed	9	M	Limbs	Haemangiopericytoma	Grade I
Reactive spindle cell proliferations						
1	Rottweiler	5	M	Trunk		
2	Mixed	4	M	Trunk		
3	Mixed	9	F	Trunk		
4	Collie	10	F	Trunk		
5	Poodle	8	M	Limbs		
6	Poodle	9	F	Limbs		
7	Boxer	8	M	Limbs		
8	Rottweiler	7	F	Limbs		
9	German shepherd	5	M	Trunk		
10	German shepherd	3	F	Trunk		
11	English cocker spaniel	8	M	Trunk		
12	English cocker spaniel	7	M	Neck		
13	Mixed	5	F	Neck		
14	Mixed	8	F	Limbs		
15	Rottweiler	7	F	Limbs		
16	Labrador retriever	9	M	Trunk		
17	Mixed	7	M	Trunk		
18	Mixed	8	F	Trunk		
19	Bulldog	9	F	Neck		
20	Poodle	5	M	Limbs		
21	Collie	5	M	Limbs		
22	Mixed	6	F	Trunk		
23	German shepherd	7	F	Trunk		
24	Mixed	8	M	Trunk		

Table 3. Nuclear roundness in canine cutaneous soft tissue sarcomas and reactive spindle cell proliferations.

Nuclear parameter	Canine soft tissue sarcomas (n = 24)	Reactive spindle cell proliferations (n = 24)	P value
Nuclear roundness	1.38 ± 0.37 [1.05 ± 2.09] Fibrosarcoma (n=8) 1.27 ± 0.31 [1.05 – 1.96] Liposarcoma (n=8) 1.25 ± 0.31* [1.07 – 2.00] Haemangiopericytoma (n=8) 1.63 ± 0.38* [1.07 – 2.09]	1.19 ± 0.09 [1.09 ± 1.44]	P = 0.01

* Means differ significantly, $P < 0.05$.

[ ] – minimum and maximum values

parameters. At the same time, however, they did not find cytomorphometric differences between different type of soft tissue sarcomas divided by histologic grade, mitotic index, or tumour necrosis score. The authors concluded that computer cytomorphometry could distinguish soft tissue sarcomas from reactive connective tissue processes in the dog. The last investigation in this area (19), demonstrated that the quantitative differentiation of reactive connective tissue processes from soft tissue sarcomas in dog is possible, but the same is not true for the different canine soft tissue sarcomas.

In this study for each tumour we used grading system which is relatively simple, reproducible and easy to perform. A total of 24 tumours were graded. Of these 18 (75 %) were classified as grade I, and 6 as grade II (25 %). The sarcoma cells of grade I neoplasms most closely resembled normal adult mesenchymal cells by type. The histologic type of sarcomas grade II tumours could also be determined, although the differentiation was not so clear. In these cases, we used immunohistochemistry markers for detail morphological identification. In our study we did not find any significant differences in NR between grade I and grade II canine soft tissue sarcomas.

The grading system of soft tissue sarcoma is not accepted by some veterinary pathologists mainly to the fact that they applied it for the group rather than to each neoplastic type (20). Moreover, before grading a soft tissue tumour, it is important to confirm the neoplastic disease and this way to eliminate reactive spindle cell proliferations and amelanotic melanomas (21).

Computer-assisted morphometric analysis could be applied both in cytology and histology, but according to us and other investigators (22) the cytological application is more convenient for practical purposes. Moreover, the utilization of kits for rapid fixation and staining of cytological smears allows for standardization of cytomorphometric procedures. Apart the rapid staining, they provide sufficient information for cells and cellular structures (23).

In summary, the results from our study demonstrated that morphometric parameter nuclear roundness could be used as an effective auxiliary tool for differentiation between canine soft tissue sarcomas and reactive spindle cell proliferations. Due to the relative small number of the studied formations, it is obviously necessary to perform further studies in the field of canine soft tissue sarcoma quantitative analysis. This would be of practical value for both pathologists and clinicians.

References

1. Priester W. Skin tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine. J Natl Cancer Inst 1973; 50: 457–66.

2. MacEwen E, Powers B, Macy D. Soft tissue sarcomas. In: Withrow S, MacEwen E, eds. Small

animal clinical oncology. 3rd ed. Philadelphia: WB Saunders, 2000: 283–304.

3. Ehrhart N. Soft tissue sarcomas in dogs: a review. J Am Anim Hosp Assoc 2005; 41: 241–6.

4. Ettinger S. Principles of treatment for soft tissue sarcomas in dog. Clin Tech Small Anim Pract 2003; 18: 118–22.

5. Meachem M, Burgess H, Davies J, et al. Utility of nuclear morphometry in the cytologic evaluation of canine cutaneous soft tissue sarcomas. J Vet Diagn Invest 2012; 24(3): 525–30.

6. Baak J, Ort J. A manual of morphometry in diagnostic pathology. New York: Springer-Verlag, 1983.

7. Baak, J, Ort J. Manual of quantitative pathology of cancer diagnosis and prognosis. Berlin: Springer-Verlag, 1991.

8. De Vico G, Maiolino P. Prognostic value of nuclear morphometry in feline mammary carcinomas. J Comp Pathol 1997; 117: 99–105.

9. Maiolino P, Restucci B, Papparella S, et al. Nuclear morphometry in squamous cell carcinomas of canine skin. J Comp Pathol 2002; 127(2-3): 114–7.

10. Marconato L, Marchetti V, Francione D, et al. Morphometrical approach for predicting regional lymph node micrometastatic load in canine mast cell tumours: preliminary results. Vet Comp Oncol 2008; 6(3): 162–70.

11. Strefezzi R, Xavier J, Catao-Dias L. Morphometry of canine cutaneous mast cell tumors. Vet Pathol 2003; 40: 268–275.

12. Maiolino P, Cataldi M, Paciello O, et al. Nucleomorphometric analysis of canine cutaneous mast cell tumours. J Comp Pathol 2005; 133 (2/3): 209–11.

13. Goldschmidt M, Dunstan R, Stannard A, et al. Histologic classification of tumors of the skin of domestic animals, World Health Organization International classification of tumors of domestic animals. 2nd ser. Vol III. Washington: Armed Forces Institute of Pathology, American Registry of Pathology, 1998.

14. Kuntz C, Dernell S, Powers E, et al. Prognostic factor for surgical treatment of soft tissue sarcomas in dogs: 75 cases (1986–1996). J Am Vet Med Assoc 1997; 211: 1147–51.

15. McChesney G, Dewhirst M, Gilette E, et al. Response of canine soft tissue sarcomas to radiation or radiation plus hyperthermia: a randomized phase II study. Int J Hyperthermia 1992; 8: 309–20.

16. Dennis M, McSporran K, Bacon N, et al.Prognostic factor for cutaneous and subcutaneous soft tissue sarcomas in dogs. Vet Pathol 2013; 48: 73–84.

17. Bittinger A, Barth P, Nerreter V. Nuclear morphometry of soft tissue tumours of fibrous origin. Zentralbl Pathol 1994; 140(4/5): 351–6.

18. Simeonov R, Simeonova G. Computerized morphometry of mean nuclear diameter and nuclear roundness in canine mammary gland tumours on cytologic smears. Vet Clin Pathol 2006; 35(1): 88–90.

19. Simeonov R, Ananiev A, Gulubova, M. Quantitative morphology in canine cutaneous soft tissue sarcomas. Vet Clin Oncol 2015;13(4): 481–4.

20. Dobson J, Samuel S, Milstein H, et al. Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. J Small Anim Pract 2002; 43: 240–6.

21. McSporran K. Histologic grade predicts recurrence for marginally excised canine subcutaneous soft tissue sarcomas. Vet Pathol 2009; 46: 928–33.

22. De Vico J, Sfacteria A, Maiolino P, Mazullo G. Comparison of nuclear morphometric parameters in cytologic smears and histologic sections of spontaneous tumours. Vet Clin Pathol 2002; 31:16–8.

23. Caruntu I. Microscopic morphometry: a modern approach. Rev Med Chir Soc Med Natl Iasi 2003; 107: 9–18.

KVANTITATIVNE ANALIZE KOŽNIH MEHKOTKIVNIH SARKOMOV PRI PSIH IN PROLIFERACIJE REAKTIVNIH VRETENASTIH CELIC V CITOLOŠKIH BRISIH

R. Simeonov

Povzetek: Namen predstavljene raziskave je bil kvantitativno ovrednotiti razliko med kožnimi mehkotkivnimi sarkomi in proliferacijo reaktivnih vretenastih celic pri psih. Z računalniško podprto analizo smo analizirali morfološke značilnosti jeder v 24 obarvanih prepratih kožnih mehkotkivnih sarkomov in 24 proliferacij reaktivnih vretenastih celic. Pri vsakem primeru smo analizirali vsaj 100 jeder in morfometrijsko določili okroglost jedra (OJ). Povprečna vrednost OJ je bila statistično značilno višja pri mehkotkivnih sarkomih kot pri neoplastičnih lezijah. Ugotovili smo tudi statistično značilne razlike v okroglosti jeder med liposarkomi in haemangiopericitomi. Med različnimi histološkimi tipi mehkotkivnih sarkomov, starostjo, spolom, pasmo in lokacijo poškodbe pa ni bilo citomorfometričnih razlik. Rezultati nakazujejo, da je morfometrični parameter OJ dodaten pokazatelj za razlikovanje med pasjimi mehkotkivnimi sarkomi in proliferacijami reaktivnih vretenastih celic.

Ključne beside: računalniško podprta morfometrija; okroglost jedra; mehkotkivni sarkomi; proliferacija vretenastih celic; pes