Mast Cell Tumor in Dogs – Incidence and Histopathological Characterization

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A B S T R A C T

Incidence of mast cell tumors, their distribution according to sex, breed, age and localisation in Croatia is not established yet. Also, the statistical significance of the various histopathological parameters according to Patnaik's scheme, in the diagnostics of the tumor grade was not performed. Investigation analysed mast cell tumors histopathologicaly characterized at the Department of General Pathology and Pathological Morphology of the Veterinary Faculty Zagreb from January 1ˢᵗ 2002 to Dezember 31ˢᵗ 2006. Sex, age, breed, localisation and tumor grade of each animal with tumor was recorded and statisticaly evaluated. Each histopathological variable was scored and compared with tumor grade. In the analyzed period, totally 1630 tumors were recorded, and mast cell tumors were found in 106 animals or in 6.5% of all cases. With statistically significant difference, this tumor was found in more cases in male dogs, and average age was 6.96 years. Boxers and retrievers were most frequent breeds, and localization was mostly on legs. Grade I tumor was found in 15.09%, grade II in 44.34% and grade III in 28.3% of animals. There were no significant correlations between tumor grade and age, breed, sex or localization. Considering the lesion scores compared with grade, statistically significant differences were found in cell shape, number of nucleoli, anisocytosis, anisokariosis, karyomegaly, mitoses, necroses, hemorrhages, cellularity, cell borders and collagenolysis.

Key words: mast cell tumor, skin, incidence, histopathological characterization, dogs, Croatia

Introduction

Cancer is the most frequent cause of death in dogs and cats more than 10 years old. It should be noted that 45% of dogs older than 10 years dies because of the malignant tumor consequences and 23% of all dogs dies because of tumor[1]. Among all tumors, the incidence of mast cell tumors in dogs is high and represents 11–27% of all skin tumors[2]. Mast cells are a heterogeneous cell population that originates in the bone marrow and matures in connective tissue. There are mucosal and connective tissue type of mastocytes. These cells contain granules which contain histamine, heparin, chondroitin sulphate, PAF, TNFα, PgD2, leukotriene C4, cathepsin G1, tryptase and/or chymase. The mostly affected organs with this tumor are skin, intestine, liver and spleen. Clinically, on the skin these tumors appear as erythematous, alopecic, edematous masses or plaques with common ulceration in larger tumors[3]. Luckily, most neoplastic cells resemble normal mastocytes which facilitate diagnosis. However, the problem is in establishing the degree of differentiation i.e. the grade of tumor. Namely, the widely accepted classification is Patnaik scheme[4] in which there are three different tumor grades with grade III as the most malignant type. There are as much as 22 different tumor features which should be estimated to obtain relatively objective diagnosis[5]. Of course, there is a lot of subjectivity and because of that there is 50–60% discordance between pathologists in establishing tumor grade[6]. Thus, there are very different data concerning the incidence of mast cell tumors and especially the grade incidence.

The purpose of this work is to compare tumor malignancy score of each feature with tumor overall grade and to find out statistically significant features which contributes to the tumor grade. Also, the work will present data concerning the incidence and distribution of this tumor in dogs in Croatia.

Materials and Methods

Tumor sampling

All tumors of dogs presented for histopathological analysis at the Department of General Pathology and Pathological Morphology during the period from January 1st, 2002 to December 31, 2006 were enclosed in this investigation.

Mast cell tumor analysis

In the analyzed period all identified skin mast cell tumors were recorded with breed, age, sex and tumor localization of each affected animal where ever it was possible. Namely, in some cases of mast cell tumors there were no data concerning the age, breed, sex or localization. All tumor samples were fixed in buffered formalin, embedded in paraffin, and cut in 5 μm thick slices. They were stained using hematoxilline-eosine (HE) routine method and special stain for establishing metacromasia of the

TABLE 1
PATNAIK SCHEME FOR GRADING OF MAST CELL TUMORS

Histopathological feature	Grade I.	Grade II.	Grade III.
Cellularity	Low	Medium	High
Cell size	Uniform	Medium anisocytosis	Severe anisocytosis
Giant and binuclear cells	None	Few	Often
Pleomorphism	None	Medium	High
Cytoplasmic granules	Distinct	Visible	Unidentifiable or very low visibility
Nuclei	Ovoid, uniform	Anisokariosis	Anisokariosis
Mitoses	None	Medium degree	Numerous
Mitotic index (x40)	<2	2–8	>8

TABLE 2
SCORING OF THE MAST CELL TUMORS HISTOPATHOLOGICAL FEATURES

Histopathological feature	Score		
	1	2	3
1. Localization	Dermis	Dermis and subcutis	Satellite tumors
2. Growth	Rows and/or small nests	Cords	Sheets
3. Cellularity	Low	Medium	High
4. Cell size	Small	Medium	Large
5. Cell shape	Uniform, round	Ovoid, binuclear	Pleomorphic
6. Cell borders	Distinct	Visible	Not visible
7. Cytoplasm	Ample	Distinct	Variable
8. Granules	Distinct	Visible	Unidentifiable (HE)
9. Shape of nuclei	Round	Ovoid	Pleomorphic
10. Localization of the nuclei	Paracentral	Central	Eccentric
11. Hyperchromasia	Visible	Marginal	Vesicular, scattered chromatin
12. Nucleolar status	Inconspicuous	Single	>2
13. Anisocytosis	Low	Medium	High
14. Kariomegaly	Low	Medium	High
15. Anisokariosis	Low	Medium	High
16. Mitotic Index	1–2	3–8	>8
17. Eosinophils	High	Medium	Low
18. Collagenolysis	Low	Medium	High
19. Necrosis	Low	Medium	High
20. Hemorrhages	Low	Medium	High
21. Mineralization	Low	Medium	High
22. Ulceration	Not present	Medium	High

mastocyte granules, toluidine blue method. The grade of each tumor was determined using the both basic Patnaik scheme (Table 1) and updated scheme with all scored features important for establishing tumor grade (Table 2) on randomly selected 44 mast cell tumors.

Statistics

The results obtained by scoring of the each histopathological feature (mean score) were compared and correlated with the grades obtained by histopathological examination. Data were analyzed for normality by the Kolmogorov-Smirnov test followed with one-way ANOVA or Kruskal-Wallis with the level of significance set at p<0.05 using STATISTICA (data analysis software system), StatSoft, Inc. (2005)., version 7.1. www.statsoft. com. Significance of distribution between the breed, age,

sex, localization and tumor grade was determined using the same software but the χ^2 test for distribution.

Results and Discussion

During the investigated period totally 1630 different tumors in dogs were analyzed among which the 106 (6.5%) mastocytomas were found. The mean age of the affected animals was 6.96 years and grade I tumor was found in 15.09%, grade II in 44.34% and grade III in 28.3% of all animals with skin mast cell tumor. Males (67 cases or 63.2%) were statistically significant more frequently affected in comparison to females (39 cases or 36.8%). This is interesting finding because in literature[7] there are no data which reports such different sex distribution. Also the incidence of this tumor is relatively low

TABLE 3
FREQUENCY OF TUMOR GRADES DEPENDING ON THE SEX AND AGE

	Grade I	Grade II	Grade III	Total
Male	13 (21.66%)	30 (50%)	17 (28.33%)	60
Female	3 (9.09%)	17 (51.51%)	13 (39.39%)	33
Age	6.87	6.42	7.12	

TABLE 4
FREQUENCY OF TUMOR GRADES DEPENDING ON THE LOCALIZATION OF THE TUMOR

	Grade I.	Grade II.	Grade III.	Total
Head	2	2	2	6
Neck	–	1	7	8
Forelimb	–	6	4	10
Hind limb	2	13	6	21
Thorax	2	3	1	6
Back	2	1	–	3
Abdomen	1	4	3	8
Perineum	3	–	1	4
Tail	1	–	–	1
Scrotum	1	7	1	9
Multiple	–	1	1	2

TABLE 5
FREQUENCY OF TUMOR GRADES DEPENDING ON THE BREED OF THE DOGS

	Grade I.	Grade II.	Grade III.	%
Golden retriever	3	4	–	7.5
German shepard	2	2	2	6.45
Boxer	–	9	3	12.9
Labrador	–	3	2	5.38
Terrier	–	2	–	2.1
Collie	–	2	–	2.1
Mixed		5	6	11.83
Not known	6	7	8	

TABLE 6
MEAN VALUES AND STANDARD DEVIATIONS OF THE SCORED HISTOPATHOLOGICAL FEATURES

Histopathological feature	Grade I		Grade II		Grade III	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
1. Localization	1.30	0.48	1.29	0.72	1.64	1.08
2. Cell size	1.30	0.48	1.29	0.72	1.64	1.08
3. Cell shape	1.80	0.79	2.86	0.48	3.00	0.00
4. Cytoplasm	1.80	0.63	1.90	0.83	2.14	0.77
5. Granules	1.30	0.67	1.29	0.72	1.21	0.43
6. Shape of nuclei	1.50	0.97	1.52	0.87	1.71	0.61
7. Nucleolus	1.00	0.82	1.67	0.58	1.71	0.91
8. Anisocytosis	0.10	0.32	1.05	1.02	1.36	1.01
9. Kariomegaly	0.50	1.08	1.00	1.14	2.14	1.03
10. Anisokariosis	0.70	0.82	1.52	1.17	1.86	0.66
11. Mitotic index	0.30	0.48	1.05	0.38	1.50	0.76
12. Eosinophils	1.50	0.97	1.29	1.01	1.29	0.61
13. Necrosis	0.20	0.63	1.00	1.22	2.43	1.16
14. Hemorrhage	0.20	0.63	0.86	0.96	1.21	1.19
15. Growth	1.80	0.79	1.90	0.30	1.93	0.62
16. Celullarity	1.70	0.95	2.10	0.54	2.36	0.50
17. Cell borders	1.80	0.63	1.24	0.44	1.43	0.51
18. Localization of the nuclei	2.10	0.57	1.67	0.91	1.64	0.93
19. Hyperchromasia	0.20	0.42	1.48	1.36	1.86	1.41
20. Collagenolysis	1.40	0.84	1.43	0.81	1.93	0.27
21. Mineralization	0.20	0.42	0.38	0.59	0.21	0.43
22. Ulceration	0.30	0.67	0.52	0.81	0.64	0.93

compared to literature[8]. The reason for this remains unclear but the insufficient routine histopathological analysis of the extirpated tumors can be involved. Namely, in some cases the owners don't want this analysis because of its cost.

Considering the breed distribution and tumor localization this data are in concordance with the literature[9,10]. Namely, susceptible breeds were boxers and retrievers and localization was predominately on the legs.

The comparison between tumor grade and breed, sex, age and localization revealed some interesting data which did not reach the level of significance probably because of too small number of cases. However, there are some interesting tendencies. Firstly, males tends to have more frequent tumor of grade I compared to females (Table 3), grade II is almost identical in both sexes, and grade III is more common in female animals. Concerning the localization (Table 4), grade III tumor was mostly localized on the neck and hind limbs, grade II on the hind limbs and grade I on the perineum. The most frequent localization of all tumors was hind legs. Mixed breed dogs had the most frequently grade III mastocytomas, while grade II was frequently found in boxers (Table 5). Based on this data, it seems that the animal most sensitive to develop grade III mast cell tumor should be female, mixed breed, and localization should be the neck.

Forty four different mast cell tumors were randomly chosen from the archive and additionally histopathologically analyzed. Their histopathological characteristics were estimated (scores 0–3), mean scores (Table 6) and statistical significance (Table 7) determined in relation to the grade of malignancy and statistically significant histopathological features for establishing correct tumor grade were: shape of the cells, presence of the nucleolus, anisocytosis, kariomegaly, anisokariosis, mitoses, necroses, hemorrhage, cellularity, cell borders and collagenolysis. Although there are similar attempts which are using computerized morphometric techniques[11], applying this methodology offers a more objective approach in grading not only the mast cell tumors but also any other neoplasm which has numerous histopathological features which has to be evaluated in the process of grading. This kind of analysis of the mast cell tumor histopathological characteristics in this research reduced tumor features needed to be scored on half, which could be very helpful especially for less experienced pathologists.

Acknowledgments

TABLE 7
STATISTICAL SIGNIFICANCE BETWEEN HISTOPATHOLOGICAL FEATURES AND TUMOR GRADE

Histopathological feature	Grade I vs. grade II	Grade II vs. grade III	Grade I vs. grade III
1. Localization	n.s.	n.s.	n.s.
2. Cell size	n.s.	n.s.	n.s.
3. Cell shape	**	n.s.	**
4. Cytoplasm	n.s.	n.s.	n.s.
5. Granules	n.s.	n.s.	n.s.
6. Cell shape	n.s.	n.s.	n.s.
7. Nucleolus	**	n.s.	n.s.
8. Anisocytosis	**	n.s.	**
9. Kariomegaly	n.s.	**	**
10. Anisokariosis	n.s.	n.s.	**
11. Mitotic index	**	**	**
12. Eosinophils	n.s.	n.s.	n.s.
13. Necrosis	n.s.	**	**
14. Hemorrhage	n.s.	n.s.	**
15. Growth	n.s.	n.s.	n.s.
16. Cellularity	n.s.	n.s.	**
17. Cell borders	**	n.s.	n.s.
18. Localization of the nuclei	n.s.	n.s.	n.s.
19. Hyperchromasia	**	n.s.	**
20. Collagenolysis	n.s.	**	n.s.
21. Mineralization	n.s.	n.s.	n.s.
22. Ulceration	n.s.	n.s.	n.s.

** – $p<0.05$; n.s. – not significant; vs. – versus

R E F E R E N C E S

1. WITHROW SJ, Why worry about cancer in pet animals? In: WITHROW SJ, MACEWEN EG (Eds) Small animal clinical oncology (W.B. Saunders Company, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1996). — 2. FOX LE, Mast cell tumors. In: MORRISON WB (Ed) Cancer in dogs and cats (Williams and Wilkins. Baltimore, Philadelphia, London, Paris, Bangkok, Buenos Aires, Hong Kong, Munich, Sydney, Tokyo, Wroclaw, 1998). — 3. GRABAREVIĆ Ž, Biologija i morfologija tumora. In: GRABAREVIĆ Ž (Ed) Veterinarska onkologija (DSK-FALCO, Zagreb, 2002). — 4. PATNAIK AK, EHLER WJ, MACEWEN EG, Vet Pathol, 21 (1984) 469. — 5. GOLDSCHMIDT MH, SHOFER FS, Skin tumors of the dog and cat (Butterworth, Heinemann, Oxford, Boston, Johannesburg, Melbourne, New Delhi, Singapore, 1998). — 6. SIMOES JPC, SCHONING P, BUTINE M, Vet Pathol, 31 (1994) 345. — 7. TYLER RD, COWELL RL, BALDWIN CJ, MORTON RJ, Introduction. In: COWELL RL, TYLER RD (Eds) Diagnostic Cytology and Hematology of the Dog and Cat (Mosby Inc, St. Louis, 1999). — 8. BRODEY RS, Adv Vet Sci Comp Med, 14 (1970) 309. — 9. MACY DW, Vet Clin North Am Small Anim Pract, 15 (1985) 783. — 10. GOLDSCHMIDT MH, HENDRICK MJ, Tumors of the skin and soft tissues. In: MEUTEN DJ (Ed) Tumors in domestic animals (Iowa State Press, Ames, 2002). — 11. STREFEZZI RF, XAVIER JG, CATAO-DIAS JL, Vet Pathol,40 (2003) 268.

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MASTOCITOMI PASA – UČESTALOST I HISTOPATOLOŠKA KARAKTERIZACIJA

S A Ž E T A K

Učestalost i distribucija mastocitoma pasa ovisno o spolu, pasmini, dobi i lokalizaciji ui Hrvatskoj još nije utvrđena. Isto tako, statistička značajnost pojedinih histopatoloških značajki u odnosu na stupanj malignosti tumora još nije istraživana. Istraživanje je provedeno na mastocitomima pasa utvrđenih u Zavodu za opću patologiju i patološku morfologiju Veterinarskog fakulteta u Zagrebu u razdoblju od 1.1. 2002. do 31.12. 2006. godine. Kod svake životinje određen je spol, pasmina, dob i lokalizacija tumora i određena je statistička značajnost u odnosu na stupanj malignosti. Dodatno su evaluirane histopatološke značajke koje su se statistički usporedile sa stupnjem malignosti tumora. U proučavanom razdoblju utvrđeno je ukupno 1630 tumora pasa, a mastocitomi su uočeni u 106 životinja (6,5%). Na razini značajnosti tumori su češće utvrđeni u muških životinja, a prosječna dob svih životinja s ovim tumorom je iznosila 6,96 godina. Najčešće zahvaćene pasmine su bile bokseri i retriveri, a najčešća lokalizacija je bila na nogama. Stupanj malignosti I je utvrđen u 15,09%, II u 44,34% i III u 28,3% tumora. Nije utvrđena statistički značajna korelacija između stupnja malignosti i spola, pasmine, lokalizacije i dobi životinja. Što se tiče pojedinačnih histopatoloških značajki važnih za određivanje stupnja malignosti, statistička značajnost je utvrđena kod oblika stanice, broja jezgrica, anizocitoze, anizokarioze, kariomegalije, mitotskog indeksa, nekroza, krvarenja, staničnosti, uočljivosti staničnih granica i kolagenolize.