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Original article

Epidemiological study of canine mast cell tumours according to the histological malignancy grade

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Abstract

The aim of the study was to identify significant relationships between the tumour malignancy grade and dogs' age, breed, sex, size, and location of mast cell tumours (MCTs). MCTs accounted for 13.27% of all diagnosed canine skin tumours. The highest incidence was recorded among Boxers, Labrador Retrievers, American Staffordshire Terriers, and Golden Retrievers. Statistical analysis revealed significantly higher probability of occurrence of the grade I mast cell tumour in the French Bulldog in the head, neck, torso, and limb regions, the grade-II mast cell tumour in Boxer, Doberman, Dachshund, shepherds, and setters in the scrotal region, and the grade III mast cell tumour in Shar-Pei in the axilla region. In the group of the oldest dogs aged 11-16, there was higher risk of development of MCTs grade II and III. Young dogs (aged 2-3 and 4-6) were found to be more prone to development of MCTs grade I. There was no correlation between MCTs grade and dogs' sex and size. To the authors' knowledge this is the first report on statistical relationships between the degree of mast cell tumour malignancy and dogs' phenotypic traits, age and tumour location. This analysis indicate predilections for development of the particular mast cell tumour malignancy degrees in certain dog breeds, age, and anatomical location.

Key words: dog, mast cell tumours, malignancy grade, epidemiological study

Introduction

Mast cell tumours (MCTs) are the most frequent canine skin tumours as they account for 7-21% of all diagnosed neoplastic lesions of this organ (Welle et al. 2008, Villamil et al. 2011). Depending on the degree of tumour cell differentiation, these tumours have diverse clinical pictures. They may have a form of small, well-demarcated single or multiple tumours, infiltrate the surrounding tissues, or metastasise to lymph nodes and internal organs (Murphy et al. 2004). Being the most important prognostic factor, the degree of

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differentiation of MCTs determines not only the diverse morphology and metastatic potential of tumours but also the response to therapy and prognosis (Dobson et al. 2002, Murphy et al. 2004, Dobson et al. 2007, Brznden et al. 2010). Furthermore, as reported in the literature, the development and behaviour of MCTs is closely associated with dogs' breed, age, and sex as well as the location of the neoplasm (Dobson et al. 2002, White et al. 2011, Villamil et al. 2011, Warland and Dobson 2013, Zinc et al. 2014). Boxers are at the greatest risk of the disease, but Shar-Peis, Rhodesian Ridgebacks, Pugs, Weimaraners, Labrador Retrievers, Beagles, and Golden Retrievers are frequently mentioned in this context as well (Dobson 2013). It should be noted that the prevalence of MCTs in the different dog breeds varies between countries and is often dependent on the geographical region (Villamil et al. 2011, Warland and Dobson 2013, Leidinger et al. 2014). Moreover, there are only single reports presenting epidemiological analysis of MCTs in relation to the malignancy grade, which is the basic prognostic factor determining the course of the neoplastic disease (Grabarević et al. 2009, Artuković et al. 2014). To date, no reports of the incidence of MCTs in the dog population have been published in Poland. Epidemiological oncological examinations are aimed at not only acquisition of data on the prevalence and development of the analysed malignancy type but also identification of factors that may induce the development and influence the biological behavior of tumours. Such investigations facilitate accurate prediction of the course of the neoplastic disease and implementation of appropriate methods for treatment of MCTs.

The aim of the study was to identify the relationships between the tumour malignancy grade according to Patnaik's 3-grade scale and dogs' age, breed, size, and the location of the neoplastic lesion. The paper presents data on the incidence and distribution of this type of canine tumours in the south-eastern Poland.

Materials and Methods

The analysis involved 263 dogs of various breeds and mixed dogs diagnosed with skin mast cell tumour and selected based on the histopathological examination of 2046 cases of canine skin tumours recorded in 2003-2013. These tumours originated from dogs undergoing surgery at the Veterinary Clinic of the University of Life Science in Lublin and in private veterinary practices from the area of south-eastern Poland. The clinical data on the dogs' breed, age, sex, and tumour location were taken from referrals for examinations delivered together with tissue material destined for histopathological analyses. No information regarding castration or sterilisation of the examined dogs was included in the statistical analysis due to lack of data.

Prior to the microscopic evaluation, the slides were routinely stained with hematoxylin and eosin as well as toluidine blue. The histopathological analysis was performed according to the WHO classification and was based on the 3-grade malignancy scale proposed by Patnaik et al. (1984) and Hendrick et al. (1998), taking into account depth of infiltration, cellularity, cellular and nuclear pleomorphism, presence of giant cells, cytoplasmic granules, number of nucleoli, and number of mitotic figures. Grade I mast cell tumours usually develop in the dermis and less frequently in subcutaneous tissue and they are distinctly delimited from adjacent tissues. The cells resemble normal mast cells; they are monomorphic, round or oval, and have a centrally located cell nucleus. The nucleoli are hardly visible. Mitotic figures are very seldom found. There are distinct, large, and abundant cytoplasmic granularities. Grade II tumour cells arranged in strands or large clusters often infiltrate subcutaneous tissue. They are large, slightly pleomorphic, and have fewer granularities than cells of the previous malignancy grade. The cell nuclei are slightly enlarged and the nucleolus is clearly discernible. The mitotic activity is negligible or moderate and the number of cells exhibiting mitotic figures is 0-2/hpf. Grade III mast cell tumours (poorly differentiated) have the greatest sizes, infiltrate subcutaneous tissue, and their epithelium often exhibits ulceration. The cells and cell nuclei are characterised by substantial pleomorphism. The granularities are often invisible and have to be stained with Giemsa stain or toluidine blue to be visualised. The cell nuclei are large, round, doubled, or sometimes numerous. The nucleoli are usually single and clearly visible, and the high mitotic cell activity has a value of 3-6/hpf. Additionally, atypical mitotic figures are often observed.

The dogs were divided into three size groups as follows: small (S, estimate of the wither height – ewh: 30-45cm), medium (M, ewh: 45-60 cm), and large (L, ewh> 60 cm) (Pionnier-Capitan et al. 2011). Four age groups were distinguished: (1) dogs aged 2-3 years, (2) 4-6 years, (3) 7-10 years, and (4) 11-16 years. According to the location of the tumour, the dogs were divided into nine groups: (1) head, (2) pelvic limb, (3) pectoral limb, (4) scrotum (5) tail, (6) axilla, (7) inguinal region, (8) neck, and (9) torso.

Frequencies were determined using the SAS 9.4 procedure PROCFREQ (SAS Institute, Cary, NC, USA). The probability of the malignancy grade (I,II,III) occurrence, depending on the size, age, breed, sex, and tumour location, was estimated using

Table 1. The mean age and the number of dogs by breed.

Breed	No. of dogs	(%)	Age	
			lsm*	se**
Boxer	74	28.14	7.14[b]	0.28
Cross breed dog	71	27.00	8.86[b]	0.28
Labrador	20	7.60	7.55[b]	0.53
American StaffordshireTerrier	14	5.32	8.07[b]	0.63
Golden Retriever	11	4.18	4.91[c]	0.72
Different type of Shepherds (German Shepherd, Tatra Sheepdog, Caucasian Shepherd, Polish Lowland Sheepdog)	10 (6, 2, 1, 1)	3.80	8.60[b]	0.75
Shar-pei	10	3.80	5.50[c]	0.75
Dachshund	7	2.66	9.57[a]	0.90
Bernese Mountain Dog	5	1.90	6.80[bc]	1.06
Miniature and Standard Schnauzer	5	1.90	11.46[a]	1.06
French Bulldog	4	1.52	7.25[bc]	1.19
Doberman	4	1.52	9.50[a]	1.19
Irish Setter	4	1.52	9.50[a]	1.19
Weimaraner	3		9.33	
Cocker Spaniel	2		6.50	
Jack Russel Terrier	2		6.00	
Syberian Husky	2		7.50	
Border Collie	1		2.00	
Briard	1		9.00	
Bull Terrier	1		5.00	
Chinese Crested Dog	1		5.00	
Dog de Bordeaux	1		6.00	
Fox Terrier	1		16.00	
Maltese	1		6.00	
Manchester Terrier	1		9.00	
English Mastiff	1		5.00	
Poodle	1		6.00	
Rottweiler	1		11.00	
Tibetan Spaniel	1		13.40	
Welsh Terrier	1		6.00	
West Highland White Terrier	1		9.00	
Yorkshire Terrier	1		11.00	

a,b,c – The values in the columns marked with various letters differ significantly at $p \leq 0.05$.
lsm* – least-squares means
se** – standard error

Table 2. The mean age of the studied dogs by gender.

Sex	No. of dogs	(%)	lsm*	se**
Male	153	58.17	7.82	0.22
Female	110	41.83	7.92	0.26

lsm* – least-squares means
se** – standard error

Table 3. The mean age of the dogs with mast cell tumours by the tumour grade (according to the Patnaik grading system).

Grading	No. of dogs	(%)	Age	
			lsm*	se**
Grade I	103	39.16	6.680[b]	0.248
Grade II	140	53.23	8.509[a]	0.213
Grade III	20	7.60	9.400[a]	0.563

a,b – The values in the columns marked with various letters differ significantly at $p \leq 0.05$.
lsm* – least-squares means
se** – standard error

Table 4. The probability (lsm) of an occurrence of the particular grade of mast cell tumour in different age groups.

Age group (years)	No. of dogs	(%)	Tumour grade					
			Grade I		Grade II		Grade III	
			lsm*	se**	lsm*	se**	lsm*	se**
2-3	11	4.18	0.727[a]	0.140	0.182[b]	0.147	0.091	0.079
4-6	69	26.24	0.594[a]	0.056	0.377[b]	0.059	0.029[b]	0.031
7-10	143	54.37	0.336[b]	0.039	0.601[a]	0.041	0.063[b]	0.022
11-16	40	15.21	0.150[b]	0.073	0.650[a]	0.077	0.200[a]	0.041

a,b – The values in the columns marked with various letters differ significantly at p≤0.05.
lsm* – least-squares means
se** – standard error

Table 5. The probability (lsm) of an occurrence of the particular grade of mast cell tumour depending on the gender.

Sex	No. of dogs	(%)	Tumour grade					
			Grade I		Grade II		Grade III	
			lsm*	se**	lsm*	se**	lsm*	se**
Male	153	58.17	0.379	0.040	0.543	0.041	0.078	0.022
Female	110	41.83	0.409	0.047	0.518	0.048	0.073	0.025

lsm* – least-squares means
se** – standard error

Table 6. The probability (lsm) of an occurrence of the particular grade of mast cell tumour depending on the breed.

Breed	No. of dogs	(%)	Tumour grade					
			Grade I		Grade II		Grade III	
			lsm*	se**	lsm*	se**	lsm*	se**
Boxer	74	28.14	0.459[bc]	0.055	0.527[ab]	0.058	0.014[b]	0.030
Crossbreed Dog	71	27.00	0.338[bc]	0.056	0.577[ab]	0.059	0.085[b]	0.030
Labrador	20	7.60	0.500[b]	0.105	0.500	0.111	0.000[b]	0.057
American Staffordshire Terrier	14	5.32	0.357[bc]	0.125	0.500	0.132	0.143	0.068
Golden Retriever	11	4.18	0.636[b]	0.142	0.273[bc]	0.149	0.091	0.077
Different type of Shepherds	10	3.80	0.300[bc]	0.148	0.600[ab]	0.156	0.100	0.080
Shar-pei	10	3.80	0.000[c]	0.148	0.700[b]	0.156	0.300[a]	0.080
Dachshund	7	2.66	0.000[c]	0.177	0.857[a]	0.187	0.143	0.096
Bernese Mountain Dog	5	1.90	0.400	0.210	0.400	0.221	0.200	0.114
Miniature and Standard Schnauzer	5	1.90	0.000[c]	0.210	0.800[b]	0.221	0.200	0.114
French Bulldog	4	1.52	1.000[a]	0.235	0.000[c]	0.247	0.000[b]	0.127
Doberman	4	1.52	0.250[bc]	0.235	0.750[ab]	0.247	0.000[b]	0.127
IrishSetter	4	1.52	0.250[bc]	0.235	0.750[ab]	0.247	0.000[b]	0.127

a,b,c – The values in the columns marked with various letters differ significantly at p≤0.05.
lsm* – least-squares means
se** – standard error

Table 7. The probability (lsm) of an occurrence of the particular grade of mast cell tumour depending on the size of dogs.

Size	No. of dogs	(%)	Tumour grade					
			Grade I		Grade II		Grade III	
			lsm*	se**	lsm*	se**	lsm*	se**
Small (including crossbreed dog)	96	36.50	0.354	0.050	0.552	0.051	0.094	0.027
Medium	132	50.19	0.409	0.043	0.530	0.044	0.061	0.023
Big	35	13.31	0.429	0.083	0.486	0.085	0.086	0.045

lsm* – least-squares means
se** – standard error

Table 8. The probability (lsm) of an occurrence of the particular grade of mast cell tumour depending on the location of the tumours.

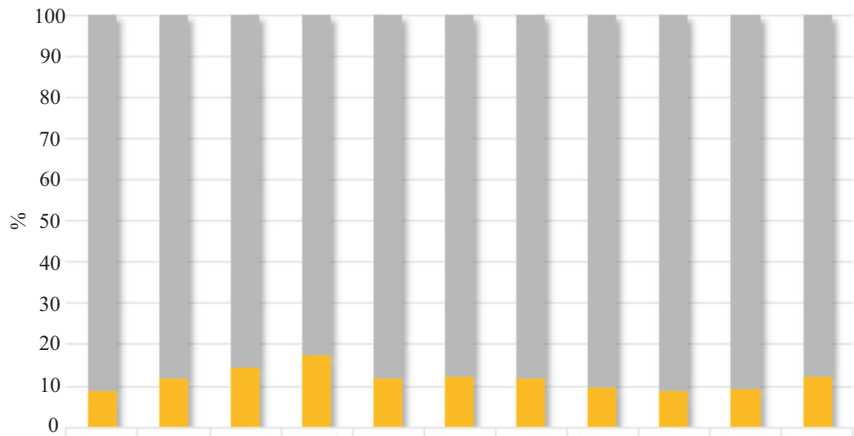
Location	No. of dogs	(%)	Tumour grade					
			Grade I		Grade II		Grade III	
			lsm*	se**	lsm*	se**	lsm*	se**
Trunk	97	36.88	0.423[a]	0.049	0.495[b]	0.050	0.082[b]	0.027
Pelvic limb	51	19.39	0.431[a]	0.067	0.471[b]	0.069	0.098[b]	0.037
Thoracic limb	34	12.93	0.529[a]	0.082	0.471[b]	0.085	0.000[b]	0.045
Head	31	11.79	0.387[a]	0.086	0.581	0.089	0.032[b]	0.047
Scrotum	15	5.70	0.067[b]	0.124	0.867[a]	0.128	0.067[b]	0.068
Axillary area	14	5.32	0.071[b]	0.128	0.643	0.132	0.286[a]	0.070
Neck	10	3.80	0.500[a]	0.152	0.400[b]	0.157	0.100	0.083
Inguinal area	6	2.28	0.167	0.196	0.833	0.202	0.000[b]	0.107
Tail	5	1.90	0.400	0.214	0.600	0.222	0.000[b]	0.117

a,b – The values in the columnsmarked with various letters differ significantly at $p \leq 0.05$.
lsm* – least-squares means
se** – standard error

the method of least-squares means (lsm+se (standard error). The association between the data was analysed using the SAS 9.4 procedure PROC GLM (SAS Institute, Cary, NC, USA). Associations with $p \leq 0.05$ were considered significant. Dog breeds represented by 4 and more individuals were included in the analysis of the probability (lsm) of occurrence of the mast cell tumour malignancy grades.

Results

The 263 cases of MCTs accounted for 13.27% of all the diagnosed canine skin tumours. The percentage of MCTs in each year is presented in Fig. 1. The highest value, 21.09%, was noted in 2006 and the lowest index of 9.7% was recorded in 2003. Mast cell tumours were diagnosed in 35 dog breeds and in mixed dogs (Table 1). In 22 breeds MCTs were noted sporadically (from 1 to 3 cases per breed). The highest number was recorded among Boxers (28.1% of all the dogs examined), Labrador Retrievers, American Staffordshire Terriers, and Golden Retrievers (from 4.2 to 7.60%). Mixed-breed dogs constituted a large group as well (27%). Statistical analysis revealed that the Miniature and Standard Schnauzer, Doberman, Dachshund, and Irish Setter dogs developed the disease at a significantly older age than the dogs from the other breeds. The malignancies mostly affected male individuals, and the age was not significantly different between the males and females (Table 2). Assessment of the malignancy grade of the analysed tumours performed in accordance with Patnaik's classification revealed predominance of intermediate grade-II differentiation 53.23%. Well-differentiated grade-I was represented by 39.16% and poorly-differentiated grade-III by 7.60% of cases (Table 3, Figs. 2-5). It was found that dogs diagnosed with grade-I malignancy were significantly younger than dogs with grade-II and grade-III MCTs, with no significant age differences (Table 3). Significant differences were noted in the probability of occurrence of the respective tumour malignancy grades among the age groups (Table 4). The probability of occurrence of grade I was significantly higher in the case of dogs from the two youngest groups, compared with the other age groups. A reverse correlation was found in the case of grade II, for which the probability of occurrence was significantly highest in the two groups of the oldest dogs. The significantly highest probability of diagnosis of grade III was observed for the oldest dog group (Table 4). The sex had no significant effect on the probability of occurrence of the respective malignancy grades (Table 5). The statistical analysis of the probability of the malignancy grades occurrence in the investigated breeds showed the highest probability of grade-I mast cell tumour in the French Bulldog and the significantly lowest one in Dachshund, Shar-Pei, and schnauzer dogs, in which grade I had not been diagnosed (Table 6). Grade-II mast cell tumour was reported to occur with a significantly higher probability in the Boxer, Doberman, Dachshund, shepherd, and setter breeds, compared with the other dogs. The significantly highest probability of grade III was reported in the case of Shar-Pei and the significantly lowest probability was noted in the Boxer, French bulldog, Doberman, Labrador Retriever, Irish Setter, and mixed-breed, in which grade III was either not found or occurred marginally (Table 6). Noteworthy, grade I was the only malignancy degree noted in the



		2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
skin tumors	n=2046	92	98	106	128	166	168	170	219	227	288	384
mast cell tumors	n=263	9	13	18	27	22	23	23	23	22	29	54

Fig. 1. The percentage of mast cell tumours in dogs in relation to canine skin tumours in the years 2003-2013.

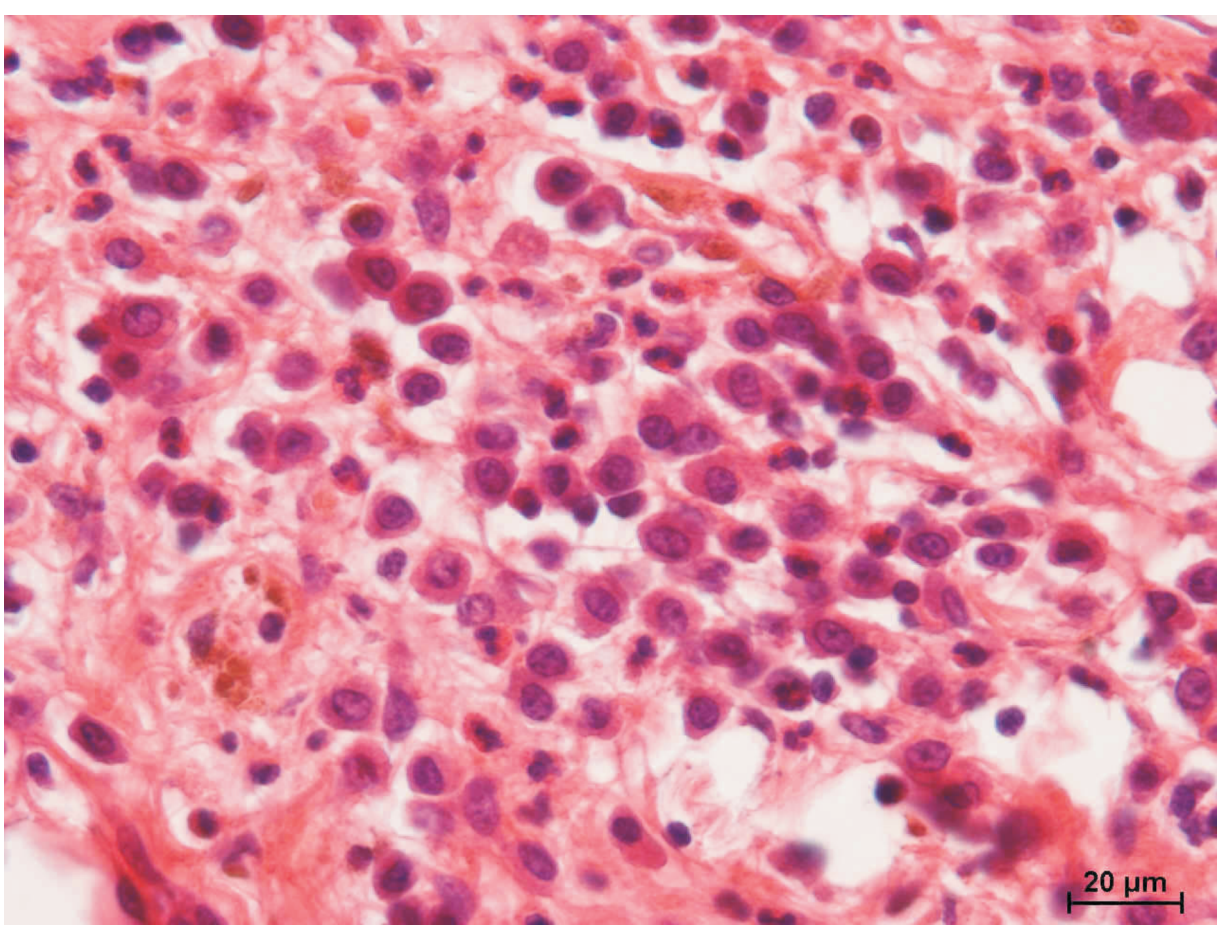


Fig. 2. Photomicrograph of well-differentiated mast cell tumour (grade I). Round monomorphic cells with intracytoplasmic granules. HE, x400.

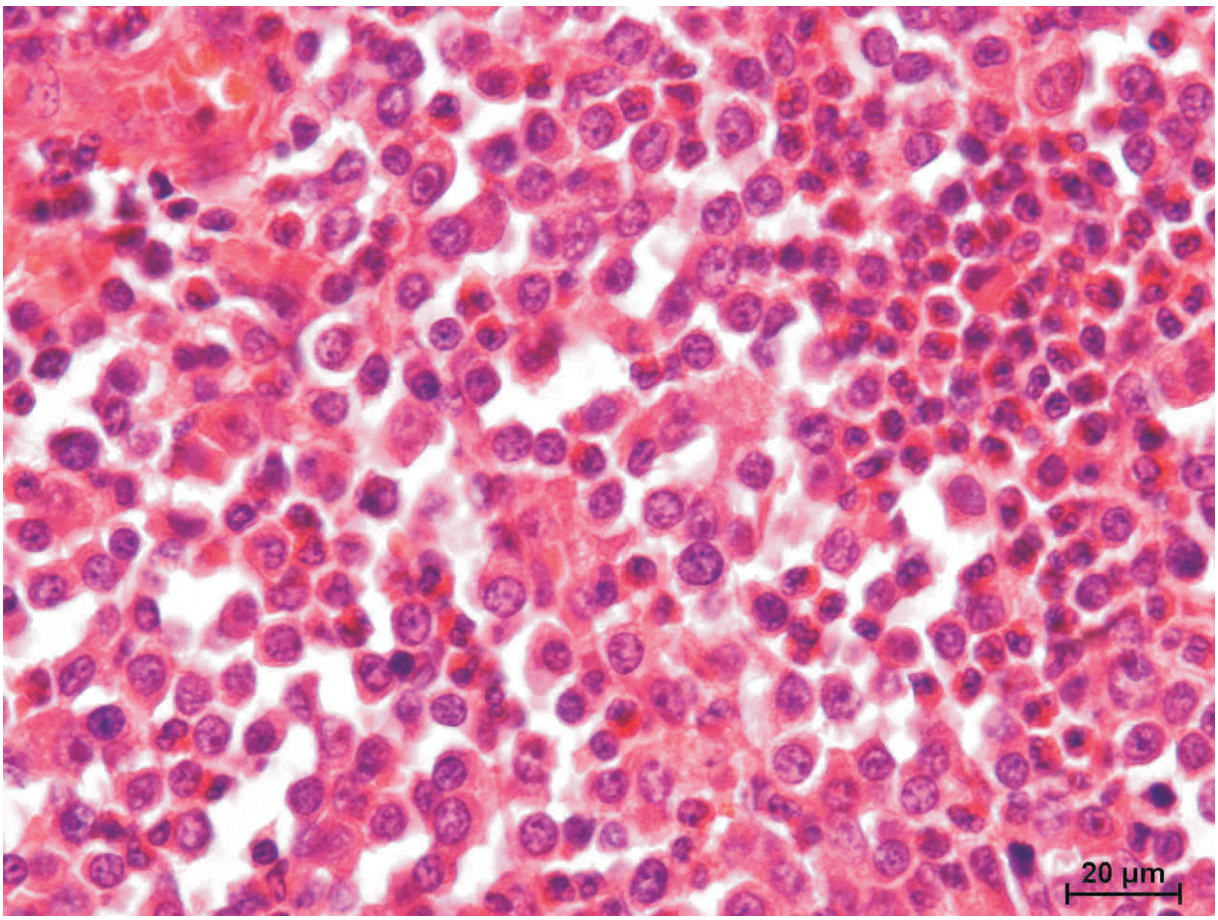


Fig. 3. Photomicrograph of intermediate-differentiated mast cell tumour (grade II). Round to ovoid neoplastic cells with large nuclei and scattered chromatin. Intermingled with the tumour cells are numerous eosinophilic granulocytes. HE, x400.

French Bulldog, whereas Dachshunds, Shar-Peis, and Schnauzers were diagnosed only with grade II and grade III tumours (Table 6). In almost all breeds (with the exception of the French Bulldog and Golden Retriever), the probability of occurrence of grade II was the highest, in comparison with the other malignancy grades (Table 6). The body size did not exert a significant effect on the probability of occurrence of the particular malignancy grades (Table 7). The greatest number of MCTs was found on the torso (37% of all body regions) and extremities (32%) (Table 8). The probability of the particular malignancy grade occurrence exhibited significant differences depending on the location of the tumour (Table 8). The significantly highest probability of occurrence of grade I was noted in five out of nine body regions (head, neck, torso, pelvic limb, and pectoral limb). Grade II was most probable to be diagnosed in the case of scrotum tumours, compared with the malignancies of the neck, torso, and limb regions (Table 8). In comparison with the other sites, the highest probability of occurrence of grade III was reported for the axilla region.

Discussion

The analysed MCTs accounted for 13.27% of all skin tumours. Similar results were reported from Croatia – 13.47% (Artuković et al. 2014) and Greece – 13.8% (Kaldrymidou et al. 2002). Lower values were obtained in investigations conducted in Austria – 9.7% (Leidinger et al. 2014) and the USA – 10.98% (Villamil et al. 2011). Higher proportions than the values reported in this study were found in epidemiological studies from Australia – 16.1% (Rothwell et al. 1987), the UK – 19.2% (Bostock 1986), and in other research from Poland – 15.2% (Jasik and Reichert 2009). It should be underlined that the proportion of MCTs among other skin tumours in 2003-2013 varied (Fig. 1), but corresponded to the range of 7-21% reported in the literature (Welle et al. 2008, Villamil et al. 2011).

A majority of mast cell tumour cases in Poland were reported in Boxers (28.14%), Labrador Retrievers, American Staffordshire Terriers, Golden Retrievers, and Shar-Peis (from 4.2 to 7.60%) (Table 1). As shown by the literature, all the breeds presented

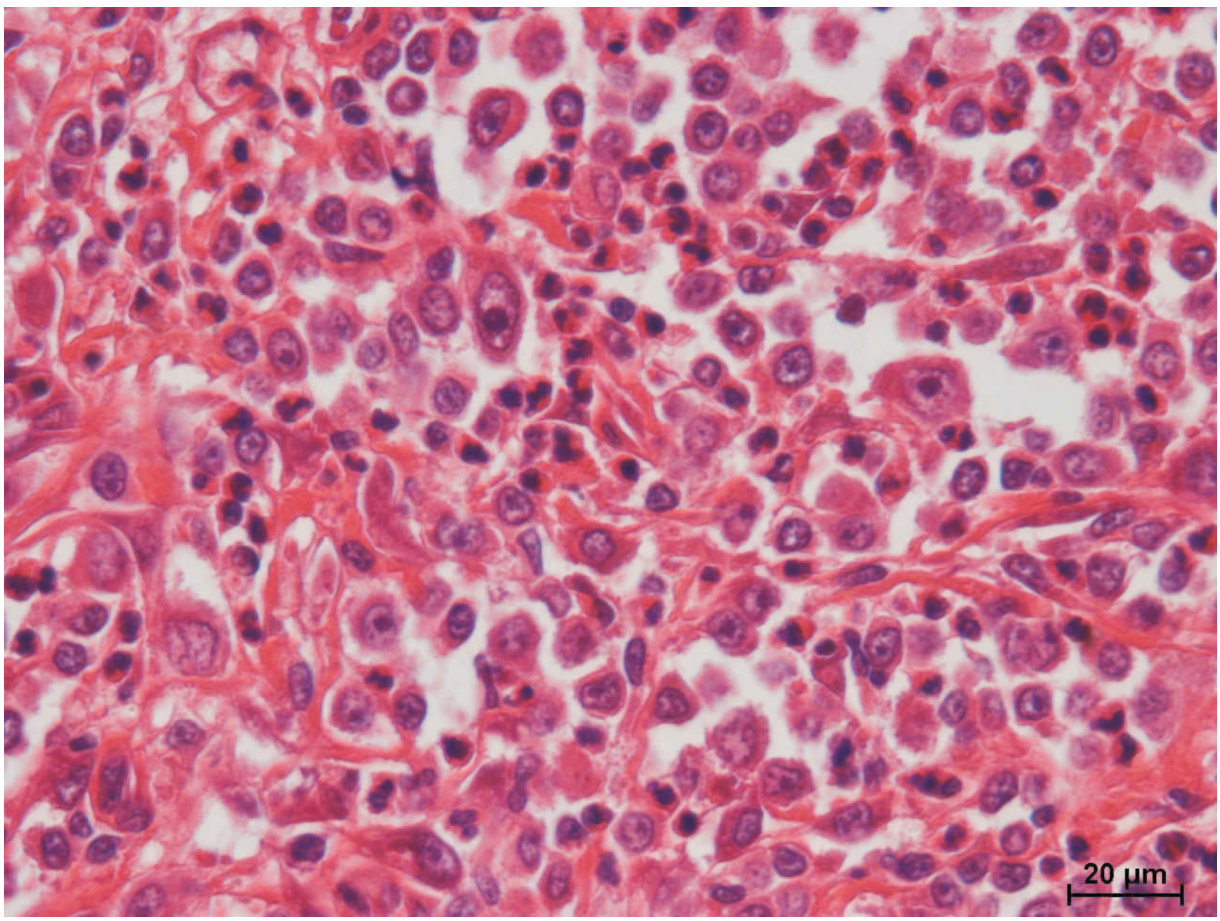


Fig. 4. Photomicrograph of poorly-differentiated mast cell tumour (grade III). Note pleomorphic non-granulated cells with variably sized and shaped nuclei and prominent nucleoli. HE, x400.

in this study are characterised by an increased incidence of mast cell tumours (Welle et al. 2008, Dobson 2013). In other regions of Europe, MCTs were most frequently diagnosed in Boxer dogs as well (Kessler et al. 1997, Grabarević et al. 2009). In many reports, the risk of malignancy development in a given breed is assessed with the odds ratio (OR) specifying the risk of disease development (Villamil et al. 2011, Warland and Dobson 2013, Leidinger et al. 2014). No such analyses were performed in the dog population studied due to the difficulty in selection of the control group. Literature reports show various breeds that are at higher risk of mast cell tumour development. Investigations conducted by Leidinger et al. (2014) in Austria showed a high risk of the disease in the Boxer, Dogo Argentino, Tibetan Spaniel, Beauceron, and Pyrenean Dog breeds. In turn, in a study conducted in the UK, Warland et al. (2013) reported a predisposition for development of MCTs in Boxers, Labrador Retrievers, Golden Retrievers, and Staffordshire Bull Terriers. Data reported from the USA indicate that, besides boxer dogs, also vizslas, Rhodesian Ridgebacks, Boston Terriers, Weimaraners, and Chinese Shar-Peis exhibit higher incidence of the disease (Villamil et al. 2011). The breed diversity presented may be associated with the geographical region studied and the selection of the control group, which comprised an insured population (Warland and Dobson 2013), registered in the cynological union (Kennel Club registrations) or hospitalised dogs (Warland and Dobson 2013, Leidinger et al. 2014). The present results mostly agree with findings reported by Warland et al. (2013). Recent investigations conducted on Labrador Retrievers suggest that low levels of 25(OH)D3 might be a risk factor for MCT in this breed (Wakshlag et al. 2011). In turn, the American Staffordshire Terrier is one of the bull-dog type breeds. There is a hypothesis that Boxers and bull-dog type breeds may be related and have a common ancestor in their phylogenetic history (Peters 1969). MCTs were more frequent in males (58.17%) than in female dogs (41.83%) (Table 2), which is in agreement with reports provided by other authors (Grabarević et al. 2009, Artuković et al. 2014, Leidinger et al. 2014). However, a majority of researchers believe that there is no sex predilection for the development of this type of neoplasia (Peters 1969, Miller 1995, Shoop et al. 2015). Similarly, the present results have confirmed

that the sex has no significant effect on the probability of occurrence of MCTs with the respective malignancy grades. In turn, some literature reports indicate that castration and sterilisation increase the risk of mast cell tumour development (White et al. 2011, Zinc et al. 2014). However, since there were no complete data concerning these treatments in the studied dog group, we did not analyse this relationship. The histological evaluation of the tumours was based on the 3-grade classification proposed by Patnaik (1984). Although the scale has been used since 1984, there are no epidemiological studies addressing the malignancy grades in veterinary literature. Available data confirm the clear correlation between the mastocytoma differentiation degree and the length of dogs' post-surgery survival period. In investigations conducted by Patnaik et al. (1984), 93%, 44%, and 6% of animals with well, intermediate, and poorly differentiated tumours, respectively, survived for 1500 post-surgery days. In turn, another study has shown that 100%, 92%, and 46% of dogs with grade I, grade II, and grade III mastocytoma survived for a year after surgery (Murphy et al. 2004). Assessment of the malignancy degree of the analysed tumours in accordance with Patnaik' scale has shown predominance (53.23%) of tumours with intermediate grade-II differentiation (Table 3). Grade-II tumours in Patnaik's scale are the most frequently diagnosed MCTs (Murphy et al. 2004, Grabarević et al. 2009, Br(nden et al. 2010, Artuković et al. 2014, Leidinger et al. 2014); however, assessment thereof arouses considerable controversy (Bostock 1986, Kiupel et al. 2011). Given the discrepancies in the assessment of the mast cell tumour grade, a new simplified classification system was proposed by Kiupel et al. (2011), in which MCTs were divided into low-grade MCTs and high-grade MCTs. The 3-grade classification developed by Patnaik (1984) is still widely used by pathologists (Artuković et al. 2014, Leidinger et al. 2014), the two-grade system devised by Kiupel et al. (2011) is commonly applied as well (Camus et al. 2016) or both scales are used simultaneously (Śmiech et al. 2016). The present study was based on the standard Patnaik scale, as a majority of the collected material originated from the period before the introduction of Kiupel's two-grade scale.

MCTs can develop at any age, but the peak of their incidence is noted between 7.5 and 9 years of dog's age (Patnaik et al. 1984, Dobson and Scase 2007, Welle et al. 2008, O'Connell and Thomson 2013). In the present study, the average age at which the disease developed was similar for females and males (Table 1). A similar average age (7.5) of dogs affected by MCTs was observed in investigations conducted in Austria (Leidinger et al. 2014) and Croatia (Dobson et al. 2002). A 2-year-old Border Colli was the youngest dog in the population examined in this study (Table 1). In turn, there is a literature report about a 3-month-old Shar-Pei dog diagnosed with mast cell tumour (Miller 1995, Welle et al. 2008). In the present study, significant correlations were noted in the probability of the occurrence of the mast cell tumour malignancy grades between the four age groups (Table 3). In the group of the oldest dogs, aged 11-16 years, there was a higher risk of development of higher- malignancy tumours assessed as grade II and III; in turn, young dogs (aged 2-3 and 4-6) were found to be more prone to development of grade-I MCTs, which are a milder type (Table 4). The risk of development of malignancies increases in older dogs, aged over 10 years (Dobson et al. 2002). This was confirmed also in the case of MCTs. Shoop et al. (2015) have found that the risk of mast cell tumour development is 41-fold higher in 10-year-old dogs than in 2-year-old individuals. The present study has shown a greater probability of higher malignancy-grade tumours along with dogs' age.

Mast cell tumours show very variable clinical behaviour, which may be influenced by the breed. Although boxer dogs, bulldog breeds, and pugs are at higher risk of MCT development, these breeds tend to have less aggressive low-grade tumours. In contrast, Labrador Retrievers and Shar-Pei tend to develop aggressive tumours, while Golden Retrievers are at risk of developing multiple neoplasms (Dobson 2002).

In the present study, the highest probability of development of mast cell tumour grade I was noted in French Bulldogs and grade III in Shar-Peis (Table 6), which agrees with literature data (Miller 1995, McNiel et al. 2006). It should also be noted that the poorly differentiated grade-III tumours often develop in the Shar-Pei breed at an early age (Miller 1995). The average age of these dogs in the present study was 5.5 years (Table 1), as in the reports from the UK (Shoop et al. 2015). In Boxers, in the present study there was the highest probability of the occurrence of grade-II tumours. According to the literature data, grade I (Bostock et al. 1986, Thamm and Vail 2001, Sapierzyński 2010) and grade II (Blackwood et al. 2012) tumours have been diagnosed in boxers most frequently. In other studies, malignancy grade III tumours were the largest group (42,86%) (Artuković et al. 2014). The prevalence of tumours characterised by varying degrees of malignancy and different biological behaviour may be influenced by climatic factors associated with different geographical regions. The impact of genetic factors cannot be excluded either. The presence of a *c-kit* gene mutation in the nuclear genome in mast cell tumours with a higher malignancy grade has been reported (Zemke et al. 2002, Sailasuta et al. 2014). Moreover, as demonstrated by Zemke et al. 2002, there was no relationship between the presence of the mutation and the

dog's breed. In recent years, mutations have also been found in the mitochondrial DNA genome in many human and animal cancer diseases. Somatic mutations in the D-loop region of mitochondrial DNA have been identified in mast cell tumours, which may be associated with neoplastic transformation (Śmiech et al. 2016).

These investigations are the first to demonstrate the possibility of the development of grade-II mast cell tumour in Dobermans, Dachshunds, Shepherds, and Setters (Table 6). No such relationships have been described in the available veterinary literature so far. Our study has shown that the dog's body size did not have a significant effect on the probability of the occurrence of the analysed malignancy grades of MCTs (Table 7). In contrast, White et al. (2011) have demonstrated several times greater risk of mast cell tumour development in large and giant breeds than that in small breeds.

The greatest number of MCTs was observed on the torso (37%), extremities (32%), and head (11.79%) (Table 8). As demonstrated in the literature, MCTs can be located in any part of the body, but they develop most frequently on the torso (50-60%), limbs (25-40%), and head and neck (10%) (Welle et al. 2008); this was also confirmed in our study. A different distribution is presented in investigations reported by other authors (Grabarević et al. 2009, Br(nden et al. 2010, Leidinger et al. 2014). Examination of dogs from Denmark has revealed the greatest number of MCTs located in the inguinal region (40%) and on extremities (23%) (Brznden et al. 2010). In other studies, MCTs were most frequently localized on limbs (Grabarević et al. 2009, Leidinger et al. 2014). These differences may be associated with the different anatomical division of the body regions or with certain simplification involving the use of colloquial names or combining some body areas into one region (Jasik and Reichert 2009).

The statistical analysis has shown correlations between the malignancy grade and the anatomical location of the tumour, which have not been reported in the veterinary literature so far. The probability of occurrence of poorly differentiated grade-III tumours has been reported for the axilla region. As described in veterinary publications, the perineal, inguinal, and perianal regions are the unfavourable locations (Misdorp 2004, Welle et al. 2008). Other reports mention the inguinal area and the mucocutaneous junctions (Blackwood et al. 1986, Dobson and Scase 2003). Some of the authors claim that the worse prognosis of tumours located in certain regions, e.g. the inguinal or perineal-preputial areas, is often associated with the development of poorly differentiated MCTs therein (Govier 2003, Sfiligoi et al. 2005). Similar to the inguinal region, the axilla is exposed to mechanical stimulation and chronic inflammation, which may not only contribute to tumour development (Govier 2003) but also worsen the course of the disease. A completely different distribution was presented in the investigations conducted by Grabarević et al. (2009). Grade-III tumours diagnosed in dogs in Croatia were located mainly on the pelvic limb and neck. In contrast, our study has shown a significantly highest probability of occurrence of grade-I malignant tumours (Table 8). In turn, other investigations from that region revealed the greatest number of grade-III tumours in the scrotum and perineal areas (Artuković et al. 2014).

The present epidemiological study is the only report taking into account the statistical relationships between dogs' phenotypic traits, age, and tumour location, and the degree of mast cell tumour malignancy. It has been confirmed that the biological behaviour of MCTs is strongly correlated with their differentiation degree. The statistical analysis has revealed predilections for development of the respective mast cell tumour malignancy degrees in some dog breeds, age, and anatomical location. The study has proved the higher risk of development of grade-I MCTs in the French Bulldog in the head, neck, torso, and limb regions at an early age of 2-6 years. Mast cell tumours with an intermediate differentiation degree (grade II) have been shown to affect boxers, Dobermans, Dachshunds, Shepherds, and Setters aged 7-16 years and to be located in the scrotum region. In turn, poorly differentiated grade-III tumours most frequently develop in the axilla region in the oldest, 11-16-year-old Shar-Pei dogs. There was no correlation between the tumour grade and dogs' sex and size.

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