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Original article

The animal-dependent risk factors in canine osteosarcomas

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Abstract

Canine osteosarcoma (OSA) is a malignant neoplastic tumor, which develops from the primitive mesenchymal stem cell, that has or can acquire the capacity to produce neoplastic osteoid with possible neoplastic bone formation. Predisposition of some dog breeds to OSA indicates genetic background of oncogenesis. The aim of the study was to characterize animal-dependent risk factors for canine osteosarcoma development in Poland. The study was conducted on canine patients diagnosed cytologically or histopathologically as having OSA, and data on age, breed, sex, as well as tumor location and character were recorded. No sex predisposition to OSA was observed, mongrels were significantly underrepresented. Large and giant dogs accounted for 47% and 35% of all pedigree dogs, respectively, and both proved predisposed to OSA. A vast majority of OSA developed in the skeleton (appendicular skeleton was more commonly affected than axial skeleton), soft tissues were affected less often. Rottweiler dogs are strongly predisposed to OSA, suggesting that the genetic background is involved in the tumor development, and indicates that dogs of this breed are a promising object for further studies on OSA pathogenesis.

Key words: dog, epidemiology, osteosarcoma, risk factor, Rottweiler

Introduction

Canine osteosarcoma (OSA) is a malignant neoplastic tumor, which develops from the primitive mesenchymal stem cell that has or can acquire the capacity to produce neoplastic osteoid with possible neoplastic bone formation (Fanger et al. 2014). This highly malignant tumor is a suitable model for OSA in humans due to similarities in biological behaviour, common molecular features, sharing the same environment, and last but not least, relatively high incidence in dogs (Morello et al. 2011, Fenger et al. 2014). Etiology of osteosarcoma is still unknown, however canine OSA is the most common tumor diagnosed at the site of the metallic implant placement. Other possible causative factors include ionizing radiation, minor chronic trauma, post-orthopedic surgery bone infracts, and bone infections (Burton et al. 2015). Predisposition of some breeds to OSA indicates the genetic background of oncogenesis. Among numerous breeds considered to be predisposed to OSA Rottweilers, Great Danes, Saint Bernards, Irish Wolfhounds, Doberman Pinchers, Iris setters, Boxers, Scottish Deerhounds, Golden Retrievers, German

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Shepherds, Borzoi, Leonberger, and Greyhounds are consistently mentioned (Thompson and Pool 2002, Morello et al. 2011, Dobson 2013, Culp et al. 2014, Fanger et al. 2014, Romano et al. 2016).

The most common location for canine OSA is the appendicular skeleton, whereas axial bones are less often affected. Extra-skeletal (soft tissues) tumors are also recognized (Morello et al. 2011). Appendicular OSA is locally aggressive and producing bone destruction. Moreover, metastatic potential in such cases is very high, with pulmonary micrometastases present at the time of diagnosis in 80% of dogs. It was shown that appendicular OSA carries worse prognosis than OSA situated in the axial skeleton (Nagamine et al. 2015). The less common form of canine OSA are extra-skeletal (or soft tissue) tumors that show aggressive biological behaviour with high rate of recurrences (Langenbach et al. 1998, Duffy et al. 2015, Rebhun et al. 2016). Histological morphology of canine OSA is heterogeneous with numerous subtypes possible to diagnose in the microscopic examination, however, histologic subtyping of these neoplasms seems not to have any prognostic value (Nagamine et al. 2015). Young age, elevated serum ALP activities, high histologic grade, systemic dissemination, and high tumor volume have been revealed as negative prognostic indicators.

Despite practical importance of canine osteosarcomas in veterinary medicine, studies concerning this problem in Poland have not been published so far. The aim of the study was to characterize animal-dependent risk factors in canine osteosarcoma in Poland.

Materials and Methods

Cases collection and eligibility criteria

The study was conducted on canine patients of the Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine Warsaw University of Live Sciences (SGGW), from 2000 to 2015. In this period all cases diagnosed cytologically or histopathologically (including surgical specimen submissions and samples collected during necropsy) with OSA were provisionally collected and data on age, breed, sex, as well as tumor location and character (bony osteosarcoma or soft-tissue osteosarcoma) were recorded. Samples for cytological examination were obtained by a fine-needle biopsy of bony masses and soft tissues lesions. For routine examination at least 2 smears of good quality were dried, fixed in 70% methanol, stained with Giemsa solution and examined by light microscopy. Samples for histopathology were fixed in 4% formalin, and routine hematoxylin-eosin staining method was applied on wax embedded and 4 μm thick slides were examined under a light microscope. Eventually, only cases in which diagnosis of OSA was unequivocally confirmed with cytological or histopathological methods twice by the same cytologist (RS) were included in the study. Diagnosis of OSA was established on the basis of widely accepted microscopic criteria (Baker and Lumsden 2000, Thomson and Pool 2002).

Statistics. Numerical variables were given as an arithmetic mean, standard deviation (SD) and range, and compared between groups using the unpaired-sample Student's t-test. Categorical variables were presented as counts and percentages, and compared between the groups with the Mann-Whitney U test (ordinal variables) and the Pearson chi-square test or Fisher exact test (nominal variables), depending on the expected distribution of the variable in a contingency table. A two-sided significance level (α) of 0.05 was assumed in all analyses. Hypothesized risk factors were investigated by calculating crude odds ratios (OR) with their 95% confidence intervals (95% CI). The theoretical distribution of breeds and body weights of Polish dogs was developed using databases of two Polish veterinary general practices located in the capital city (data from 2009-2013), and served as a control group (n=10.000) in a case-control analysis. Breed predisposition to OSA was calculated for 12 breeds represented by at least three individuals. ORs were produced by comparing each breed with mixed breed dogs, which served as a reference category. Moreover, pedigree dogs were classed with respect to the body weight achieved by adult individuals into four categories: small (<15 kg), medium (15-25 kg), large (25-45 kg) and giant (>45 kg). ORs for each category were calculated by comparing a given category with all categories of heavier dogs (in the case of small and medium dogs) or all categories of lighter dogs (in the case of large and giant dogs) merged together. Statistical and epidemiological analyses were performed in Statistica 12 (StatSoft Inc.) and EpiTools (Sergeant 2016), respectively.

Results

In the study period 120 dogs (67 females and 53 males) met eligibility criteria. Their age ranged from 9 months to 19 years with the mean (SD) of 8.7 (3.4) years, without the difference between sexes (p=0.441) or pedigree and mixed breed dogs (p=0.901). No sex predisposition to OSA was observed: OR 1.1 (CI 95%: 0.8, 1.6), p=0.482. Data on dog breed were available for 112 patients, of which only 12 were mon-

Table 1. Breed predisposition to osteosarcoma.

Breed	Polish theoretical distribution of 10 000 dogs No. (%)	Affected with osteosarcoma (n=112) No. (%)	Odds ratio (OR)	95% confidence interval (95% CI)	p-value
Mongrel	3096 (31.0)	12 (10.7)			
Rottweiler*	146 (1.5)	22 (19.6)	16.5	10.1, 27.0	<0.001
German Sheppard	727 (7.3)	8 (7.1)	1.0	0.5, 2.0	0.986
American Staffordshire	270 (2.7)	5 (4.5)	1.7	0.7, 4.2	0.234
Golden retriever*	152 (1.5)	5 (4.5)	3.0	1.2, 7.5	0.030
Pointers*	119 (1.2)	5 (4.5)	3.9	1.5, 9.7	0.010
Boxer	293 (2.9)	5 (4.5)	1.5	0.6, 3.8	0.387
Doberman*	106 (1.1)	4 (4.5)	3.3	1.3, 9.5	0.030
Giant schnauzer*	63 (0.6)	4 (4.5)	5.8	2.1, 16.3	0.010
Labrador retriever	295 (2.9)	3 (2.7)	0.9	0.3, 2.9	0.999
Cane corso*	28 (0.3)	3 (2.7)	9.8	2.9, 32.7	0.003
Caucasian shepherd*	33 (0.3)	3 (2.7)	8.3	2.5, 27.5	0.007
Collie*	51 (0.5)	3 (2.7)	5.4	1.7, 17.5	0.022
Miniature schnauzer	248 (2.5)	3 (2.7)	1.1	0.3, 3.4	0.758

* Breeds significantly over-represented among the diseased dogs. Included are only breeds represented by at least three individuals.

Table 2. Odds ratio (OR) for particular groups of pedigree dogs in regard of body mass achieved by adult individuals.

Body mass of adult individuals	Polish theoretical distribution of 6904 pedigree dogs No. (%)	Pedigree dogs affected with osteosarcoma (n=100) No. (%)	OR	95% CI	p-value
Small (<15 kg)**	3121 (45.2)	8 (8.0)	0.1	0.05, 0.2	<0.001
Medium (15-25 kg)**	776 (11.2)	10 (10.0)	0.5	0.2, 0.9	0.023
Large (25-45 kg)*	2398 (34.7)	47 (47.0)	4.2	2.5, 7.3	<0.001
Giant (>45 kg)*	609 (8.8)	35 (35.0)	5.6	3.7, 8.5	<0.001

* Significantly over-represented among the diseased dogs. ** Significantly under-represented among the diseased dogs

grels (10.7%), and the remaining 100 were pedigree dogs of 35 breeds. Out of 12 breeds represented by at least three individuals seven proved predisposed to OSA (Table 1). Mongrels were significantly underrepresented compared to pedigree dogs: OR=0.3 (CI 95%: 0.2, 0.5), p<0.001. Large and giant dogs accounted for 47% and 35% of all pedigree dogs, respectively, and both proved predisposed to OSA (Table 2). When merged together and compared to the combined class of small and medium dogs, OR for large and giant pedigree dogs turned out to be 5.9 (CI 95%: 3.5, 9.9), p<0.001. Osteosarcomas affected all parts of the body; the vast majority of OSA developed in the skeleton (79.0%), soft tissues were affected less often (21.0%). Skeletal OSA were more common in the appendicular skeleton (75.5%), while the axial skeleton was affected in 24.5% of cases: cranial bones in 15 cases, scapula – 4 cases, ribs – 2 cases, vertebra and pelvis 1 case each. Appendicular OSA were mostly bony tumors (87.2%). Even though, more than a half of osteosarcomas situated on the head, neck and body were also bony tumors (57.6%). Location (axial vs. appendicular skeleton) and character (skeletal vs. soft tissue) of OSA were not linked to dogs' age (p=0.066 and p=0.280, respectively), sex (p=0.464 and p=0.607, respectively), body size (p=0.392 and p=0.356, respectively) or being a pedigree dog (p=0.999 and p=0.700, respectively).

Discussion

Our study presents an extensive epidemiological analysis of animal-dependent risk factors for canine OSA, carried out on a large sample of 120 dogs. The microscopic diagnosis of osteosarcoma can be challenging, especially when a small sample has been sent for the histopathological examination or cytology is the only diagnostic method used (Wehrle-Martinez et al. 2016). Nevertheless, cytology is considered an accurate method of presurgical diagnosis of canine osteosarcoma (Baker and Lumsden 2000). To avoid equivocal or false results we included in this study only these cases in which identification of areas with osteogenic activity of neoplastic cells was possible or cytological microscopic picture was typical for osteosarcoma and OSA was recognized twice by the same cytologist.

In our study pure breed dogs were fourfold more likely to have OSA than mongrels. However, body weight of mongrels was unknown, and lesser body weight rather than other genetic characteristics may have been responsible for this observation. Breeds which proved predisposed to OSA in the present study generally correspond to predilected breeds reported elsewhere (Thompson and Pool 2002, Morello et al. 2011, Kruse et. Al. 2012, Dobson 2013, Culp et al. 2014, Fanger et al. 2014, Romano et al. 2016). An important discrepancy is lack of predilection of German shepherds and Labradors in the present study. It seems that this discrepancy is not a result of low popularity of these breeds, as they account for roughly 7% and 3% of dogs presented to Polish veterinary clinics, respectively. It is probably a result of differences in genetic diversity of dogs in various countries, however the influence of environmental factors and the attitude of the dogs' owners to performing cytological or histopathological tests should also be included. Compared to other studies we enrolled fewer mongrels – 11% vs. 20-30% (Culp et al. 2014, Nagamine et al. 2015, Romano et al. 2016). Even though, genetic diversity in dogs' population in various geographic regions could play its role, the owners of mongrels might be less committed in diagnostic process and economic aspect might affect the study population.

It has been shown that Rottweilers are predisposed to development of a few types of neoplastic tumors, including histiocytic sarcoma, lymphoma and osteosarcoma (Dobson 2013). Numerical and structural alterations in genome are believed to be strongly involved in tumor etiopathogenesis in this breed. Genetic analysis revealed specific recurrent cytogenetic aberrations in pure-breed dogs, including Rottweilers, such as loss of *WT1, TP53, CDKN2A, PTEN, RB1* (Thomas et al. 2009). The first two abnormalities occurred exclusively or more frequently in Rottweilers than in other breeds, and as many as 48% and 24% of Rottweilers with osteosarcoma had deletion of WT1 gene or TP53 gene, respectively (Dobson 2013, Fenger et al. 2014). Moreover, germline mutation in the receptor tyrosine kinase MET involved in pathogenesis of canine OSA was identified mainly in Rottweilers (Liao et al. 2006). It was shown that besides numerous similarities in tumor biological behavior, parallel genetic features of human and canine osteosarcoma cells also exist (Mueller et al. 2007, Rowell et al. 2011). So, Rottweilers can be an excellent oncologic animal model for researches on human OSA (Fenger et al. 2014, Schiffman and Breen 2015).

It is well known that high body weight of dogs predisposes them to develop appendicular OSA. In our study large and giant pedigree dogs were 6 times more prone to OSA in comparison to medium and small pedigree dogs. However, it has also been postulated that large size of adult animals and tall shoulder height is a more reliable predictive factor for the development of OSA than dog's breed (Rosenberg et al. 2007, Eberle et al. 2010, Kruse et al. 2012, Rehbun et al. 2016). In most studies male dogs are slightly more often affected with OSA than females (Spodnick et al. 1992, Romano et al. 2016), although there are publications which contradict this statement (Eberle et al. 2010, Anfinsen et al. 2011, Coyle et al. 2013, Fenger et al. 2014, Nagamine et al. 2015, Rebhun et al. 2016). It was also suggested that in Rottweilers, Saint Bernards and Great Danes females suffer more commonly than males (Morello et al. 2011). However, we did not find sex predisposition to OSA. Moreover, we were unable to disclose any differences in sex predisposition in pedigree vs. mongrel dogs and between dogs with appendicular tumors, soft tissue tumors and axial skeleton tumors, as well as in 22 Rottweilers with OSA regardless of tumor location.

The mean age of dogs with OSA has been shown to range from 6 to 9 years (Anfinsen et al. 2011, Romano et al. 2016, Rebhun et al. 2016). Some studies have also implied that the age distribution of dogs with OSA has two peaks: the first between 18-24 months, and the second between 7-10 years, however, the majority of skeletal OSAs in dogs occur in the latter period (Dernall et al. 2007, Culp et al. 2014, Fanger et al. 2014, Nagamine et al. 2015). The mean age was similar in our study, however, we did not observe bimodal distribution of the disease. Some studies have suggested that dogs with appendicular OSA are younger than animals with non-appendicular tumors, whereas other have not (Eberle et al. 2010, Coyle et al. 2013, Culp et al. 2014, Rebhun et al. 2016). Analogically to the results of the study conducted on the population of large breed dogs with primary bone

tumors (Anfinsen et al. 2011), we did not find differences in mean age between any subclasses of dogs enrolled in our study population.

Our results regarding location of OSA are consistent with so far conducted studies, with OSA most commonly arising from the appendicular skeleton, in particular, of forelimbs. Appendicular form of the disease accounts for roughly three fourths of all cases of OSA, affecting mainly the metaphyses of long bones (Anfinsen et al. 2011, Morello et al. 2011, Szigetvari et al. 2013, Rebhun et al. 2016). Forelimbs seem to be affected more often than hind limbs, and the distal radius and proximal humerus appear to be the most common locations of canine OSA (Eberle et al. 2010, Morello et al. 2011, Nagamine et al. 2015, Szigetvari et al. 2013, Rebhun et al. 2016). A non-appendicular canine OSA tends to be recognized much less often (Anfinsen et al. 2011, Rebhun et al. 2016). Among dogs with osteosarcomas affecting the axial skeleton, the cranial, especially the mandibular bone are most common, accounting for 7% to 15% of cases (Kruze et al. 2012, Coyle et al. 2013). Except for the scull bones the most common axial locations are ribs, scapula, and pelvis, whereas vertebrae and sternum are rarely involved (Kruse et al. 2012, Szigetvari et al. 2013).

The epidemiological data on canine osteosarcoma in Poland are generally similar to those published by other authors, however, they contradict the existence of sex predisposition. Rottweiler dogs are strongly predisposed to OSA, suggesting that the genetic background is involved in the tumor development, and indicates that dogs of this breed are a promising object for further studies on OSA pathogenesis.

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