A five-year cohort study on testicular tumors from a population-based canine cancer registry in central Italy (Umbria)

A FIVE-YEAR EPIDEMIOLOGICAL SURVEY ON TESTICULAR TUMORS IN A POPULATION-BASED CANINE CANCER REGISTRY IN CENTRAL ITALY (UMBRIA)

Abstract

Canine testicular tumors account for about 90% of tumors affecting the male genitalia. Seminomas (SEM), Sertoli cell tumors (SCT), and interstitial cell tumors (ICT) are the most common histological diagnoses, but their incidence shows high variability among studies. The aim of this study is to report the results, acquired in five years from the population-based Umbria Canine Cancer Registry, on testicular tumors.

From 2014 to 2018, 388 testicular tumors (on 1969 total male tumors) were diagnosed. The median incidence was 35 cases/100,000 dogs, with a prevalence of 19,7%. Most tumors were diagnosed as ICT (50%), with fewer SEMs and SCTs (29% and 17%, respectively). Only 3% of tumors were mixed germ cell–sex cord-stromal tumors (MGC-SCST). Ten percent of cases had multiple tumors in the same testicle, with the combination SEM-ICT being prevalent (69.2%). Tumors in cryptorchid testes were 5.9% of the total, mostly SCT (60.9%). Mean age at diagnosis was 10.7±2.7, with similar values for different types of tumor. The most represented breeds were Golden Retriever, Bulldog, and West Highland White Terrier, whereas a decreased risk was observed in Pugs, Poodles, Italian segugio, Boxer and Italian cane corso breeds. A value of <0.3 cm (major diameter) of lesions at the moment of trimming was associated with a final histological diagnosis of tumor.

Canine Cancer Registries, if provided with reliable data on the total population, can offer solid information on incidence and prevalence of different types of tumors in specific territories, contributing to the supervision of its inhabitants' health.

Key words: Canine Cancer Registry; testicular neoplasms; incidence; risk factors; dogs.

Background

Testicular tumors are common in dogs, representing about 90% of all tumors diagnosed on male genitalia. (Liao et al., 2009; Nødtvedt et al., 2011) Different epidemiological studies revealed that testicular cancer in dogs is the fourth most common site, after cutaneous, mammary and oral tumors. (Vascellari et al., 2009), being the second most common anatomic site of tumor development in intact males (Liao et al., 2009; Merlo et al., 2008).

Canine testicular tumors can arise either from sex-cords stroma (Sertoli cell tumor and interstitial cells tumor, also known as "Leydig cell tumor"), or from germ cells (seminoma, embryonal carcinoma, and teratoma). Mixed tumors, such as mixed germ cell–sex stromal cell tumors (MGC-SCST) and gonadoblastomas, are instead characterized by a mixture of different types of tumor cells. (Meuten, n.d.)

Sertoli cell tumor (SCT) originates from Sertoli cells of seminiferous tubules. Macroscopically, these tumors are firm, whitish and usually well demarcated within the testicular parenchyma. Neoplastic cells are arranged in islands or tubules and supported by abundant mature fibrous tissue. These tumors can be utterly categorized into intratubular or diffuse form. (Kennedy P.C., 1998; Meuten, n.d.) These tumors are the most common in cryptorchid testes and are dogs with inguinal hernias are reported to bear a higher tumor risk. (Hayes et al., 1985; Hayes and Pendergrass, 1976). Interstitial cell tumors (ICT) derive from interstitial Leydig cells. Macroscopically, they usually protrude from the cut surface and are yellow/brown in color. Histologically, ICT occur in three main patterns: solid-diffuse, cystic-vascular and pseudoadenomatous. Seminoma (SEM), instead, originates from testicular germ cells. These tumors can be also further classified into the intratubular type, the early stage of tumor development, that can progress into the diffuse type, characterized by large, nodular aggregates, separated by fibrous septa and blood vessels. (Kennedy P.C., 1998; Maxie, 2016; Meuten, n.d.)

Different studies investigated on the frequency of the different types of testicular cancer in dogs, but often the inclusion/exclusion criteria and the evaluation of the numerator (the total canine population) are differently assessed, therefore providing non-comparable data. (D'Angelo et al., n.d.; Grieco et

al., 2008; Hayes and Pendergrass, 1976; Liao et al., 2009) Dated studies report a prevalence of testicular tumors ranging from 0.91% to 5.8% (Cotchin, 1960; Hahn et al., 1992; Reif et al., 1979), while, more recent studies indicated a higher prevalence range (5.4%-27%). (Grieco et al., 2008; Nødtvedt et al., 2011; Ortega-Pacheco et al., 2006; Svara et al., 2014)

In Italy, incidence data on canine tumors are still limited. A first study, published in 2008 by Merlo and colleagues, estimated the incidence of canine tumors in the city of Genoa. The incidence rate (IR) of tumors of the genitourinary tract was of 16.7/100.000 dogs, but no further specifications on testicular tumors were included in the study. (Merlo et al., 2008) In a more recent study from Baioni and colleagues, instead, the IR of SEM (66) and SCT (34) were calculated. (Baioni et al., 2017)

Testicular tumors are reported to occur most frequently in adult and old dogs, (Liao et al., 2009) differently from humans, where testicular cancer is the most common tumor among young men (15-40 years old). (Chia et al., 2010) Variability in tumor incidence has been described for several dog breeds as well as for ethnic groups in man, suggesting a genetic component to cancer susceptibility. (Maruthappu et al., 2015; Nødtvedt et al., 2011) Cryptorchidism is considered the principal risk factors for primary testicular tumors development in humans. (Cheng et al., 2018) This is true also in canine species, where also inguinal hernia represents a risk factor. (Hayes et al., 1985)

In the last decade, the number of Animal Cancer Registries increased all over the world and also in Italy. (Grüntzig et al., 2015; Manuali et al., 2019; Merlo et al., 2008; Tedardi et al., 2015; Vascellari et al., 2009) The Canine Cancer Registry (CCR) of Umbria (central Italy) was created in 2013. This registry includes information on the histological diagnosis of examined canine lesions, together with other data on breed, sex, age, general status, habitat, environment, diet, date of surgical excision, date of tumor diagnosis, tumor stage (TNM), type of sampling, topography (ICD-O codes), anamnesis and owner address with GIS coordinates.

The aim of this study is to report the results on canine testicular tumors acquired from the population-based Canine Cancer Registry of Umbria during a period of five year (2014-2018), including data on relative prevalence of each type of tumor

Materials and Methods

Data source

The data for this study were collected from the web-based platform of the Canine Cancer Registry (CCR) of Umbria (central Italy). This platform is an integral part of the regional canine demographic registry, which allows an affordable calculation of the whole canine population of the area. Histological diagnoses are all performed in a double-blind fashion; whether a discordance occurs among the two pathologists, a third one will intervene in defining the final diagnosis.

The evaluation and classification neoplastic lesions are performed according to the WHO's criteria for canine tumors and the topographical and morphological keys set by the International Classification of Diseases for Oncology (ICD-O). (Kennedy P.C., 1998)

Information on dogs demography, breed, sex, age, general status (good/preserved/poor), habitat (urban/rural), environment (apartment/garden/mixed/kennel), diet (wet/dry/mixed), date of surgical excision, date of tumor diagnosis, tumor stage (TNM), source (biopsy/surgery/necropsy), topography (ICD-O codes), anamnesis and owner address with GIS coordinates were obtained. (Manuali et al., 2019) For analytical purposes, dogs diagnosed with testicular tumor were divided in three age groups: young (<6 years old), adult (6 to 10 years old) and old dogs (>10 years old). In order to symbolize the geographical distribution of testicular tumors and to create an appropriate map, the Quantum GIS Geographic Information System (http://qgis.osgeo.org) was used. Our analysis started from municipalities in which testicular tumors represented ≥20% of the total tumors. They were categorized as follow: 20-39%, 40-60% and 100%.

Histological evaluation

Testicles were fixed in 10% neutral buffered formalin. Then, lesions were described (colour, consistency and shape). The major diameter of each parenchymal lesion was measured with a caliper and recorded at the moment of trimming after proper fixation in formalin. Representative samples of

the tumor were processed for histopathology. Four µm thick sections were cut and routinely stained with hematoxylin and eosin (HE). The diagnosis was determined in a blind fashion by two pathologists, randomly selected among the group that works for the Canine Cancer Registry. Whether the two pathologists do not agree on the diagnosis, a third one is involved in the final diagnosis.

Statistical analysis

Statistical analysis was performed with the One-way analysis of variance (ANOVA). The PMR of testicular tumors, compared to the total number of tumors found in male dogs, was calculated according to Dohoo. (Dohoo I, 2009) Only breeds with more than 25 tumors were selected. The PMR was computed by considering the number of testicular tumors in each breed (b1), the number of all tumors for the breed (n1), the number of testicular in each other breed (b0), and the number of tumors in all other breeds. Given these quantities, the PMR is defined as the ratio of (b1/n1) and (b0/n0). Only breeds with at least 25 tumors were selected and reported, but all breeds are used each time in the computation of the denominator (b0/n0). Confidence intervals at the 95% level are reported for each PMR, and standard deviations computed in the logarithmic scale and with a first-order approximation, yielding non-symmetrical limits in the original PMR scale.

To establish a trimming cut-off to allow the private practitioner to formulate with more sensitivity a clinical suspicion of testicular tumor, the analysis of the receiver operating characteristic (ROC) curve on the major diameter meastrimming dimension was performed. ROC analysis quantifies the accuracy of diagnostic tests or other evaluation modalities used to discriminate between two states or conditions, which are here referred to as normal and abnormal or control and case. The analysis uses the ROC curve, a graph of the sensitivity versus 1- specificity of the diagnostic test. The sensitivity is the fraction of positive cases that are correctly classified by the diagnostic test, whereas the specificity is the fraction of negative cases that are correctly classified. The cut-off choice typically falls to the value that identifies the highest correctly classified case proportion. The analysis was carried out for all intratubular cancers without distinction between sertoliomas and seminomas.

Results

Incidence and prevalence of testicular tumors in the sample population

Within the CCR, a total of 1969 tumors were diagnosed in male dogs from 2014 to 2018. Among these, 388 (17.16%) had testicular origin and were included in this study.

From 2014 to 2018 the annual incidence (IR) of testicular tumors (number of cases/100,000) in the dogs of Umbria was of 12/100,000, 34/100,000, 37/100,000, 46.9/100,000 and 45.8/100,000. Instead, considering all canine male tumors the annual prevalence was 1.7%, 7.3%, 6.7%, 7.3% and 7.9%. The 5 years prevalence was of 19.7% (388 out of a total of 1969) of all registered tumors in male dogs. General status (good/preserved/poor) of the dog, habitat (urban/rural), environment (apartment/garden/mixed/kennel), diet (wet/dry/mixed), and geographical distribution were examined but did not reveal any noteworthy result.

Histological diagnosis of testicular tumors

Of the 388 tumors diagnosed, 194 (50%) were ICT, 113 (29%) were SEM, 68 (17%) were SCT, and 13 (3%) MCG-SCST. Most of the diagnosed tumors were solitary; only 24 cases (6%) showed more than one testicular tumor, either in the same testis or bilaterally. Unilateral tumors were detected in 350 dogs (97.4%), occasionally exhibiting more than one tumor type within the testicular parenchyma (10%). When multiple tumors were diagnosed within the same testis, the combination SEM-ICT was the most frequently observed (69.2%) followed by SCT-ICT (23.1%) and SEM-SCT (10.2%). Tumors in cryptorchid testes were diagnosed in 23 dogs (5.9%); the most represented were SCT (60.9%) and SEM (17.4%).

Dogs with testicular tumors

The age of dogs at the moment of the histological diagnosis of a testicular tumor ranged from 1 to 17 years (mean age 10.7±2.7 years). Dogs diagnosed with testicular tumor were divided in three age groups: young (<6 years old), adult (6 to 10 years old) and old dogs (>10 years old). Older dogs were the most commonly affected (264/388; 68%) followed by adult dogs (104/388; 26.8%) and young dogs (20/388; 5.2%). The age-group stratification of testicular tumors is detailed in Table 1. Focusing on the histopathological types of tumor distribution and the age of dogs, graphical profiles were described (Figure 1). Specifically, dogs diagnosed with ICT ranged from 1 to 17 years. Most cases were diagnosed in 11-year-old animals (mode), whereas the median age was of 10.8±2.7. An ICT diagnosis was increasingly more frequent in dogs aged 7 to 11 years. SEMs were diagnosed in dogs aging from 4 to 17 years; this diagnosis was common in dogs of 11 years (mode), with a median of 10.6±2.7. Similarily, SCTs were diagnosed in dogs raging from 4 to 17 years of age, with a mode of 11 years and a median of 10.5±3.0. Finally, MGCSCT were diagnosed in dogs between 7 and 14 years of age, with a mode of 9 years and a median of 10.7±2.5. Breed-specific differences in risk for testicular tumors development were found in Golden Retriever, Bulldog, West Highland White Terrier, Mixed breed, German Dachshund, Epaneul Breton, German Shepherd, English setter and Jack Russel Terrier (Figure 2). Instead, a decreased risk was observed in Labrador Retriever, Boxer, Shih Tzu and Pugs. Labrador retriever and German Shepherd are the only breeds, among the one selected, to have a computed PMR below one, the threshold corresponding to no protective nor increased-risk effect. The highest observed PMR refers to the Golden Retriever, with a value of 2,21, thus suggesting an increased risk of testicular tumor for the specific breed. When confidence interval is considered, none of the selected breeds have significant difference with respect to the case of no effect PMR=1, potentially due to high variability in the data and the limited sample size for inferential purposes.

Major diameter of testicular tumors/lesions

The ROC curve analysis (Figure 3) was conducted on 80 ICT, 58 SEM and 22 SCT. Results showed that all tumors that were not detected during trimming were diagnosed as non-tumors (hyperplasia of interstitial cell, small foci of orchitis, etc.). Moreover, lesions detected during trimming but with a major diameter of 0.1 or 0.2 cm were more likely to have a final non-tumor diagnosis (Table 2). This proportion was inverted with lesions with a diameter >0.3 cm, which was assessed as the cutoff value to distinguish non-tumor testicular lesions from neoplastic ones. Table 2 shows the trimming values and the classification based on the histological diagnosis. The sensitivity values, specificity, the proportion of correctly classified cases, and two likelihood values (LR + for positive values and LR- for negative values) for each trimming value are reported in Table 3.

Discussion

- Trimming value could be helpful, but it must be remembered that formalin causes shrinking of tissue, therefore the value is, at now, valid only for the pathologist during trimming procedure.

- Unilateral tumors were detected in 97.4% of dogs while 10% had more than one testicular tumor either multiple (different tumors in the same testicle), or one type tumor in each testicle as previously reported. (Lipowitz et al., 1973)

- In multiple tumors the combination SEM-ICT accounted the 69.2% of cancer cases. Tumors cryptorchid testes represented the 5.9% of cancer cases with a highest frequency of SCT (60.9%).

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Fig.1. Graphical representation of ICT, MGC-SCST, SCT and SEM histological diagnosis distribution in different ages in dogs.

Fig. 2. Probability Morbidity Ratios for breeds with at least 25 tumor cases in the timeframe considered; error bar reported for confidence intervals at 95% level, with a vertical line at 1 denoting a PMR corresponding to absence of protective/increased-risk effect.

Figure 3: ROC curve

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