SEMINOMA, SERTOLIOMA, AND LEYDIGOMA IN DOGS: CLINICAL AND MORPHOLOGICAL CORRELATIONS

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Received: April 27, 2012 Accepted: September 7, 2012

Abstract

The study aimed at presenting the most frequent male gonadal tumours in dogs, their clinical and histopathological aspects, at outlining aetiology and differential diagnosis of the tumours. As examples of the most frequently manifested testicular tumours, three clinical cases were presented, involving tumour of interstitial (Leydig) cells, tumour of Sertoli cells, and seminoma. Respective clinical diagnosis employed USG, X-ray patterns, and morphological and biochemical tests. The surgically sampled material was stained with H+E and an attempt was made to establish expression of E-cadherin, calretinin, and Ki-67. It was shown that histopathological diagnosis of testicular tumours in dogs is frequently very difficult and complex and requires multidirectional studies.

Key words: dogs, seminoma, sertolioma, leydigoma, calretinin, E-cadherin.

Testicular tumours used to be encountered relatively frequently in household animals and the lesions are most frequently noted in dogs (14, 19, 22, 23, 25, 35). On grounds of multi-year studies of Nielsen et al. (21), involving over 340 cases of testicular tumours, the World Health Organisation presented classification of such lesions. In line with results of the studies, it was accepted that the most common tumour type in testes involved tumour of interstitial cells (leydigoma), followed by tumours of supportive cells (sertoloma) and seminoma. Moreover, prevalence of such tumours in canine population was similar, although seminomas manifested a slightly higher frequency, which was later confirmed on several occasions (13, 16, 18, 33). One of principal factors, which increase frequency of manifestation in testicular tumours is cryptorchidism (27, 28). Most commonly, cryptorchidism is manifested unilaterally and the right testis used to be stopped in descent more frequently than the left one. If cryptorchidism affects only one testis, it may be accompanied by development of a tumour in the other, normally located testis. Abdominal cryptorchidism predisposes to the development of sertolioma since the temperature higher than that in the scrotal sac results in wasting of most cells except of Sertoli cells. In cases of inguinal cryptorchidism, the temperature is higher than that in the scrotal sac but lower than that in abdominal cavity, which, according to several authors, predisposes to the development of seminomas (4, 9). On the other hand, it was demonstrated that temperature exerts no significant effect on the development of leydigomas (5, 34).

An increased risk of testicular tumour development can be noted in morbid syndromes linked to testicular dysgenesis, such as testicular feminisation syndrome of Klinefelter syndrome in men (4, 27). The risk of a testicular tumour is also clearly augmented in cases of their insensitivity to androgens, due to mutation in the gene responsible for structure of androgen receptor (7).

Embryonal tumours of the testes develop from primitive cells, which may continue their differentiation along the gonadal line (to seminomas) or they may transform into a population of pluripotential cells (to non-seminomas). The pluripotential cells may remain at the stage of non-differentiated cells (carcinoma embryonale), they may differentiate into extraembryonal tissues (yolk sac tumour, choriocarcinoma), or toward somatic cells (teratoma) (4, 6).

Evaluation of malignancy grade in canine testicular tumours is based on analysis of morphology manifested by the primary tumour, condition of draining lymph nodes, and presence of distant metastases. The first grade encompasses tumours restricted to the testes; tumours of the second grade produce metastases restricted to retroperitoneal lymph nodes and to the diaphragm, while tumours of the third grade are additionally accompanied by distant metastases (1, 11). In cases of seminomas, the tumours spread mainly by lymphatic vessels and the metastases are located most...
frequently in retroperitoneal lymph nodes (6, 26). Non-seminomas yield metastases both through lymphatic and blood vessels, mainly to the lungs, liver, bones, and brain (6). Malignant forms of sertoliomas and leydigomas develop metastases mainly to inguinal and sublumbar lymph nodes (1, 17, 18, 31).

At the preliminary phase of development, testicular tumours are most frequently manifested by painless augmentation of the organ, asymmetry or a small nodule. Pain develops in cases of a rapid growth, bleeding to the tumour or upon cryptorchism. Azoospermia and/or oligospermia are frequently present (24). The tumour may be accompanied by infertility and dermatoses (acanthosis, seborrhoea) (24). Moreover, sertoliomas and seminomas may be hormonally active (secreting androgens or oestrogens) (20).

The study aimed at presenting the most common male gonadal tumours in dogs, their clinical and histopathological aspects, at outlining aetiopathogenesis and differential diagnosis of the tumours.

**Material and Methods**

**Case 1 – seminoma.** The case involved a dog of golden retriever breed with 40 kg of body weight, 10 years of age. The visit was provoked by an evident asymmetry and altered consistency of the testes. Palpation permitted to detect a soft lesion in the left testis. Rectal control of the prostate gland demonstrated its augmentation. Ultrasonography demonstrated increased volume of the prostate and a tumour in the testis. In the USG examination, the structure of the affected testis was non-homogenous, pointing to possible haemorrhagic or necrotic lesions (Fig. 1).

The prostate gland manifested a heterogeneous echo-structure, an increased echogenicity, and presence of hypoechogetic cysts. Chest radiogram demonstrated no metastases. Blood morphology and biochemistry fitted reference values.

**Case 2 – sertolioma.** Another case involved an eight-year-old dog of the small bulldog breed, weighing 37 kg. The dog was referred to the Department due to markedly enlarged left testis. Upon palpation a tough, compact lesion was detected, with an irregular, nodular surface. Ultrasonography detected a hyperechogenic tumour of a non-uniform structure, 21x12.7 mm in size (Fig. 2).

The right testis preserved a normal outlook. Chest radiogram detected no metastatic lesions. Blood morphology and blood biochemistry fitted the reference values.

**Case 3 – leydigoma.** The subsequent case involved a Dalmatian dog of 13 years of age and body mass 26 kg. Clinical examination of the dog detected a clear disproportion in consistency of the testes. Ultrasonography permitted to detect numerous small hypoechogetic cysts in both testes. In addition, in the right testis, a tumour was detected of 45.9x31.9 mm in size, containing a hypoechogetic structure of 16 mm in size (Fig. 3).

![Fig. 1](image1.png) Ultrasonographic pattern of canine testis in sagittal projection. Note a non-homogenous structure of parenchyma.

![Fig. 2](image2.png) Hyperechogenic lesion, 21.0x12.7 mm in size, within a normal parenchyma in left testis.

![Fig. 3](image3.png) Ultrasonographic pattern of a canine testis with a visible, demarcated lesion of 45.9x31.9 mm in size and a hypoechogetic structure of 16 mm in length.
Fig. 4. Cross-section of canine testis containing seminoma.

Fig. 5. Cross-section of canine testis containing sertolioma.

Fig. 6. Cross-section of canine testis containing leydigoma.

Prostate gland showed a uniform echogenicity but was enlarged, around 4 cm in length. Chest radiogram documented an accentuated pattern of bronchi, most probably due to senile calcification of bronchial walls. A marked dilation of pulmonary blood vessels and an increased shadow of the myocardium was noted. No metastases were detected. Except of a slightly increased level of lymphocytes in blood, no abnormalities were detected in biochemical or morphological tests.

Section of the excised testes detected:
- **case 1**: grey-yellowish-creamy parenchyma, occasionally divided by delicate lamellae of connective tissue, slightly bulging above the plane of cross-section (Fig. 4);
- **case 2**: parenchyma or a nodular structure, grey-beige in colour, with fine light-yellowish spots, also bulging above the plane of the cross-section (Fig. 5);
- **case 3**: a round, well enveloped tumour, with grey-brownish parenchyma of a soft consistency, with evident cysts (Fig. 6).

The isolated fragments of altered testes were fixed for 24 h in 7% buffered formalin, routinely processed to paraffin blocks, and cut to 4 µm thick sections. In histopathological examination of haematoxylin and cosin stained microscope preparations, WHO classification of canine testicular tumours was applied. Immunohistochemical investigations were conducted on 4 µm paraffin sections, placed on silanised microscope slides (DAKO). Subsequently, the sections were deparaffinised in xylene and passed through decreasing concentrations of alcohol to water. Calretinin and E-cadherin antigens were detected using EnVision™ FLEX Target Retrieval Solution, High pH (50x) (DAKO), heating the preparations in a water bath at 96°C for 20 min, while Ki-67 antigens were detected in EnVision™ FLEX Target Retrieval Solution, Low pH (50x) (DAKO), heating the preparations in a water bath at 96°C for 20 min. Endogenous peroxidase was blocked in EnVision™ FLEX Peroxidase-Blocking Reagent for 10 min. Then, the sections were overlaid with solutions of primary antibodies: Monoclonal Mouse Anti-Human Calretinin Clone DAK-Calret 1 (DAKO), dilution of 1/100, Monoclonal Mouse Anti-Human E-Cadherin, Clone NCH-38 (DAKO), dilution of 1/50, Monoclonal Mouse Anti-Human Ki-67 Antigen Clone MIB-1 (DAKO), dilution of 1/100. All sections were incubated for 20 min at room temperature. Subsequently, the preparations were washed in EnVision™ FLEX Wash Buffer (20x), overlaid with EnVision™ FLEX /HR SM802 visualisation system, and incubated at room temperature for 20 min. The immunohistochemical reaction was developed with solution of 3,3 diaminobenzidine tetrahydrochloride (DAB), EnVision™ FLEX DAB+ Chromogen (DAKO). Then, the sections were washed in distilled water, counterstained with haematoxylin, and dehydrated in a row of alcohols. The material was cleared in xylene and closed under cover slips in a balsam.

Microphotographs of all studied tumours were subjected to computer-assisted image analysis using a computer coupled to Olympus BX53 optical microscope (Olympus, Japan). The system was capable of storing images and performing their digital analysis. The measurements were done using cell2A software (Olympus Soft Imaging Solution, Germany).
Results and Discussion

In microscope examination of the first case, neoplastic hyperplasia of a diffuse seminoma type was detected. Cells with oval or round nuclei and relatively scanty cytoplasm prevailed. Neoplastic cells tightly filled seminiferous tubules and in several sites disrupted their walls and by infiltrating the sublayer formed a uniform neoplastic texture. Moreover, presence of small and focal lymphocyte infiltrates was demonstrated (Fig. 7).

Seminoma represents an embryonal tumour, derived from primary genital cells. It is a relatively common testicular tumour in dogs, particularly in older dogs (mean age of 10 years). Its development is predisposed by cryptorchidism. Seminoma can be uni- or bilateral, single or multiple. It develops more frequently in the right, as compared to the left testis. Its size varies and the increase in size may cause enlargement of the testis (13, 18, 33). Seminomas are relatively soft although not as soft as sertoliomas. On a cross-section they manifest a homogenious, grey or creamy outlook. Large tumours may contain foci of coagulative necrosis, usually free of haemorrhages. In line with WHO categories, seminomas include an intratubular type or type free of infiltration and a diffuse type. The intratubular type is thought to represent the early stage of seminoma development. It consists of aggregates of embryonal seminiferous cells, replacing normal lining of seminiferous tubules. Outlook of the cells is typical: they are large, clearly demarcated, they contain a large, most frequently vesicular cell nucleus with evident nucleolus and a scanty, basophilic cytoplasm. Abnormal figures of mitotic division are frequently noted (13, 15, 21). Many of the tumours contain focal aggregates of lymphocytes. In the diffuse type, the most frequent form of seminoma, tumour cells transgress seminiferous tubuli and form bands or islands of a relatively uniform texture. The cells are tightly packed and well outlined. They contain a large cell nuclei with one or two nucleoli (13, 21). In the structure of neoplastic cells, individual vacuolised histiocytes are dispersed (21). Normal and abnormal mitoses are frequent. Focal and/or perivascular aggregates of mature lymphocytes can be noted (13, 21). The tumour manifests a tendency to infiltrate lymphatic and blood vessels of spermatic cord. Metastases of seminomas in dogs are seldom encountered, in contrast to men, in whom the tumours comprise as many as 40%-50% embryonal tumours and show a much more pronounced tendency to develop metastases (10, 13). Even if very sporadic in dogs, a malignant form of the tumour may be encountered, e.g., the described by Takiguchi et al. (32) case of seminoma with metastases to scrotal skin, liver, kidneys, and peritoneum.

In the other case, microscope examination disclosed neoplastic proliferation of Sertoli cells (sertolioma) of a diffuse type. The histopathological pattern contained spindle-shaped cells with oval, less frequently round hyperchromatic nuclei and loci of a clearly vacuolised cytoplasm. Figures of mitotic division were infrequent (Fig. 9).
The tumour develops from Sertoli cells of seminiferous tubuli. Around half of sertolomas are disclosed in the testes with atrophy of seminiferous tubules due to cryptorchism (3, 18). The risk of sertoloma development clearly increases when the testes do not descend to the scrotal sac (18). Most frequently, the tumour is unilateral, solid on its cross-section, white or grey, demarcated from the surrounding tissue. It may be sufficiently large to evidently deform the testis. Large malignant tumours infiltrate tunica alba, epididymis, and spermatic cord (18). In view of their histological structure, sertolomas are categorised into their intratubular types: type free of infiltration or diffuse type. The former type is formed by neoplastic cells with indistinct outlines, round, or oval cell nucleus and a vacuolised, acidophilic cytoplasm, frequently containing grains of lipochromic pigments (15). The cells are arranged in a palisade manner on basement membrane of seminiferous tubules and they may penetrate the membrane. Mitotic figures are very rare (2, 3). The diffuse type cells have an irregular size and shape. They form large aggregates with no evident canalicular structures, separated by the blood vessel-supplied parenchyma and fibrous septa (18, 21). Spaces filled with erythrocytes are also encountered (25).

Around 20%-30% dogs with sertoloma demonstrate signs of hyperoestrogenism, exhibiting a combination of feminisation, gynecomastia, atrophy of the other testis, metaplasia of prostatic epithelium (with frequent its supplicative inflammation), alopecia, and atrophy of bone marrow (20, 25, 29).

In the subsequent studied case, neoplastic proliferation of Leydig cells (leydigoma) was diagnosed. The histopathology included large oval or multiangular cells, with rich cytoplasm, containing numerous fine vacuoles. The cells manifested a small to an average size and hyperchromatic nuclei. Mitotic figures were infrequent and tumour structure contained focal haemorrhages (Fig. 10).

Leydigoma is a tumour originating from an uncontrolled proliferation of interstitial Leydig cells. Principal function of the cells involves secretion of male hormones of the androgen group. Macroscopically, the tumours used to be described as small, individual or multiple foci of yellow to brown colour, soft in palpation, clearly demarcated from the surrounding tissue, frequently containing haemorrhagic foci (8). They may be located in a single, or both testes (30, 12).

The histological pattern includes neoplastic cells of a round or multiangular shape (8), less frequently elongated, spindle-shaped (25). Cytoplasm of neoplastically transformed Leydig cells may contain vacuoles of various size. Cell nuclei are usually small, round, with individual nucleoli (25). Similarly to sertoloma tumours, histopathology of leydigoma may include erythrocytes-filled cysts (8).

The cases of testicular tumours described in our paper represent the most frequently encountered tumours of male gonads encountered in veterinary practice. This has inspired the authors to describe them in detail both in their histopathological and clinical aspects, attempting to clarify their aetiopathogenesis and to facilitate differential diagnosis of testicular tumours in dogs.

Pathomorphologists describing testicular tumours frequently encounter difficulties in establishing the correct diagnosis. This may reflect, on one side, frequently insignificant differences between healthy as compared to neoplastically transformed tissue (e.g., some cases of leydigoma) and, on the other side, diagnostic problems may be posed by certain forms of leydigoma and sertoloma, which may resemble each other very much. Finally, the so-called mixed tumours should be mentioned, the correct diagnosis of which requires highly experienced histopathologists. In such cases, the continuously developing immunohistochemical techniques may be helpful. Using appropriate cellular markers, a given type of a tumour may be confirmed or excluded and in some cases even prognosis can be specified. These methods are routinely used in doubtful cases of testicular tumours in men. In veterinary medicine, unfortunately, routine histopathological diagnosis does not include immunohistopathological methods, which reflects high prices of the antibodies and much more restricted knowledge on behaviour of detected proteins in animals, and their significance for carcinogenesis. For the purpose of this study, we have selected such markers as calretinin, E-cadherin, and Ki-67 proliferation antigen. It should be added that calretinin belongs to the newest markers applied in diagnosis of testicular tumours in dogs.

Our attempts to estimate the localisation and level of chosen proteins brought satisfactory results. Estimations of calretinin in leydigoma resulted in a strong cytoplasmic and nuclear reaction in neoplastically transformed supportive Leydig cells (Fig. 11).

In seminoma expression of calretinin was scanty and in seminoma no expression of the marker was detected. Possibly, the presence of the marker positively correlates with hormonal function of the tumour.
In sertolioma, in turn, the demonstrated expression of E-cadherin, the clinically important protein, linked with metastatic potential of the tumour, and was detected in the spindle-shaped interstitial cells typical for the tumour (Fig. 12).

In this case, cellular reaction manifested the typical for the protein membranous character and a less pronounced cytoplasmic character. In seminoma and leydigoma expression of E-cadherin proved to be very weak. In microscope patterns of seminoma, in which we attempted to demonstrate expression of Ki-67 proliferation antigen, a pronounced nuclear reaction was detected in stimulated cells, ready for a mitotic division. This pointed to a high proliferative potential of the tumour and manifested positive correlation with an unfavourable course of the disease (Fig. 13). In sertolioma and leydigoma expression of Ki-67 was weak (+).

Summing up, histopathological diagnosis of testicular tumours in dogs is frequently very difficult and complex. Nevertheless, an experienced histopathologist using clinical anamnesis, macroscopic pattern of the tumour, its histological analysis and, at present, also immunohistochemical characteristics can establish a correct diagnosis. This is of a high practical significance since a reliable histopathological diagnosis allows for a precise prognosis and for selection of appropriate methods of therapeutic management.

References
