Metastasized Leydig cell tumor in a dog

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Summary

We present the clinical findings, diagnosis and treatment of an 11-year old intact male Fox Terrier with a malignant Leydig cell tumor of the right testicle, which metastasized to the skeletal musculature of the left hind limb. The primary tumor and the metastasis were resected with narrow margins. The dog was treated with metronomic chemotherapy using thalidomid and dyclophosphamide. Local recurrence at the site of the metastasis and a pulmonary metastasis were present 30 months after surgery. The dog was euthanized.

Keywords: canine, Leydig cell tumor, malignant, metastasis, dog

Metastasierender Leydig-Zwischenzelltumor beim Hund

In diesem Fallbericht werden die klinischen Symptome, Diagnose und Therapie eines 11-jährigen, unkastrierten Fox Terriers, mit einem malignen Leydigschen Zwischenzellentumor am rechten Hoden, der in die linke Oberschenkelmuskulatur metastasiert hat, beschrieben. Der Primärtumor sowie die Metastase wurden chirurgisch reseziert. Im Anschluss wurde der Hund einer metronomischen Chemotherapie mit Thalidomid und Dyclophosphamid unterzogen. Dreissig Monate nach dem chirurgischen Eingriff wurden im Operationsbereich eine rezidivierende Metastase sowie eine Lungenmasse diagnostiziert. Der Hund wurde euthanasiert.

Schlüsselwörter: Rind, Ultrasonographie, Haubenmotorik, Pansenmotorik, Primärzyklus, Sekundärzyklus

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Introduction

In dogs the prevalence of testicular tumors varies from 1 to 27% (Reif et al., 1979; MacLachlan and Kennedy, 2002; Grieco et al., 2008) depending on the study. Seminoma, Interstitial or Leydig cell tumor and Sertoli cell tumor are the most common types with a prevalence of 33.9%, 33% and 26,4% respectively (Nødtvedt et al., 2011). Interstitial cell tumors arise from Leydig cells. They are common in dogs and are also described in other species such as horses, cattle, cats, and man. Interstitial tumors usually develop in older animals. They can be uni- or bilateral, single or multiple in one testicle (MacLachlan and Kennedy, 2002). The occurrence of different tumor types in the same testicle has also been described (Grieco et al., 2008). Seminoma and Sertoli cell tumors are more common in cryptorchid testes than in scrotal testes (Reif et al., 1979; Nødtvedt et al., 2011). Leydig cell tumors are generally considered benign although malignant forms have been described. An irregular cell form and an increased mitotic rate histologically characterize these. The distinction between benign and malignant forms is based on histological criteria such as infiltrative growth and can be difficult to diagnose (MacLachlan and Kennedy, 2002). Metastasized Sertoli cell tumors and Seminoma have been described in dogs (Tennant and Kelly, 1992; Dhaliwal et al., 1999), but to our knowledge metastasis of Leydig cell tumor in the muscles has not yet been reported in a dog.

History and clinical examination

An 11-year old intact male Fox Terrier, 11.2 kg, was referred to the Small Animal Clinic, University of Zurich, with a rapidly growing mass in the left hind limb. The referring veterinarian had noted a small mass in the right testicle. A fine-needle aspiration (FNA) of the mass in the limb had been performed prior to referral. The mass consisted of spindeloid to oval cells with clearly demarcated intracytoplasmatic lipid vacuoles. Because the nuclei were round to oval with vesicular chromatin pattern, large irregularly formed nucleoli and moderate anisocytosis, anisokaryosis and anisonucleoliosis the diagnosis was spindle cell sarcoma, most likely a liposarcoma (Fig. 1). On physical examination a soft non-movable mass measuring 10 × 6 × 6 cm was palpated in the left hind limb. The right testicle contained a firm nodule of 1 × 1,5 cm. The dog was slightly lame (grade 1 out of 5) without showing any pain on orthopedic examination.

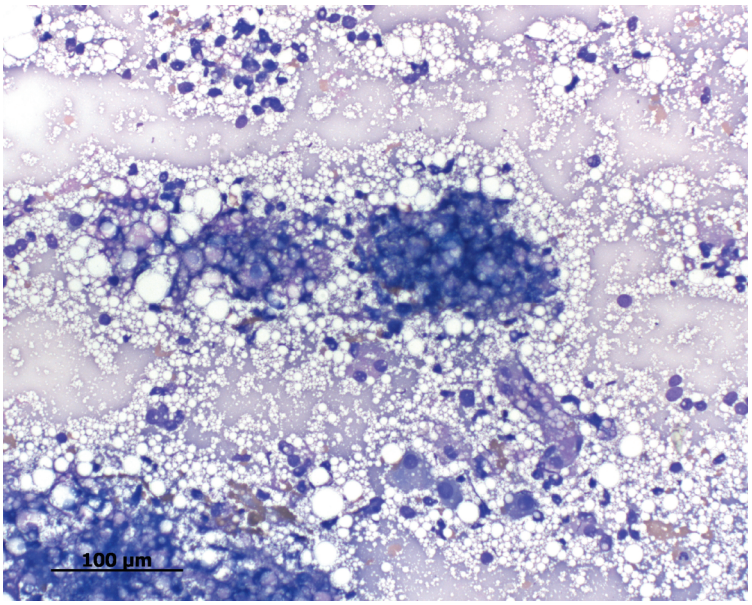

Figure 1: Fine-needle aspiration of the metastasis in the hind limb. Large round to spindeloid cells with bluish cytoplasm and round to oval nuclei with vesicular chromatin pattern. Neoplastic cells show often clear, well demarcated intracytoplasmatic vacuoles (modified wright staining, 20× objective).

Further examination

Blood chemistry and a complete cell count were within reference values. A CT-scan of the thorax, abdomen and both hind limbs revealed a poorly delineated space-occupying lesion caudal to the left femur that extended to the ischial tuberosity (Fig. 2). In transverse images, the area of the left limb measured 61.1 cm^2 at the point of major distension, which was 1.59 times larger than the contralateral side at the same level (38.35 cm^2). After contrast media application a discontinuing rim-enhancement was visible. The mass showed heterogeneous contrast uptake with small cavities. There was no involvement of bones or lymph nodes. The thorax and abdominal cavity were normal. Amputation of the left hind leg with caudal hemipelvectomy was advised but the owner requested less invasive treatment. Surgery was therefore limited to debulking of the mass and bilateral orchiectomy.

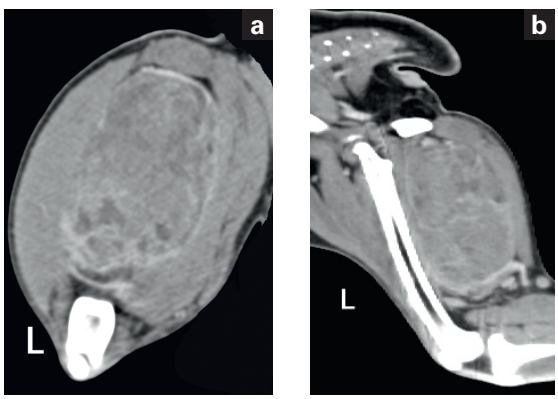

Figure 2: CT images of the left hind limb mass, (a) native transversal view of the mass, (b) post contrast sagittal view.

Surgery and histology

Firstly, bilateral orchiectomy and scrotal ablation was performed than the mass was debulked. At surgery the mass was partially enclosed in a pseudocapsule. The mass extended beyond the pseudocapsule and infiltrated the surrounding muscles. A marginal resection, debulking was performed. The sciatic nerve was dissected free from the tumor. The limb was weight bearing on the day after surgery. The patient was discharged 2 days after surgery.

Histologically the testicular mass was composed of solid cell nests supported by a fine fibrovascular stroma. In some areas neoplastic cells bordered directly on blood filled, vascular lacunae. The neoplastic cells were polyedric to ellipsoid with a moderate amount of eosinophilic cytoplasm, round to oval nuclei with vesicular chromatin pattern and one often clearly demarcated nucleolus. There was low to moderate anisocytosis and anisokaryosis. Only one mitotic figure could be found in the entire mass. Large areas of necrosis were scattered though the parenchyma of the tumor. Some neoplastic cells showed clear demarcated fatty vacuoles in their cytoplasm (Fig. 3a). Few tumor emboli were found in blood vessels of the plexus pampiniformis. The left testicle was free of disease. The histological appearance of the mass in the left hind limb was similar to that in the right testis but the mitotic rate was 6 mitotic figures in 10 fields taken with the 40×-objective.

A special staining with Prussian blue following a routine protocol was performed on both masses. Neoplastic cells in the right testicle as well in the left hind limb showed mild depositions of ferric oxide (Fe (3+)) (Gross et al., 2005).

Immunohistochemistry

Immunohistochemistry using a polyclonal anti-S100 antibody (Code No.H0066, Dako®) on both masses was performed to exclude a highly differentiated liposarcoma. Two 3 µm thin paraffin sections were mounted on positively charged glass slides and boiled in a microwave with citrate buffer (S2031, Dako®) for 30 min. For antigen retrieval and further processing endogenous peroxidase was blocked (Peroxidase Blocking Reagent, S2001, Dako®) for 5 min. at room temperature. Afterwards, the ChemMate kit was applied (ChemMateTM, Detection

Kit, rabbit, mouse, code No. K5003, Peroxidase, Dako®) as described by the manufacturer. AEC was used as chromogen. Both neoplasms showed no signal using S100 antibody. Therefore the diagnosis was Leydig cell tumor with metastasis to the left hind limb (Fig. 3b).

GnRH-stimulation test and preputial smear

A GnRH- stimulation test (de Gier et al., 2012) was performed 9 days after surgery, to detect hormonal activity of the remaining tumor cells. The dog received buserelin (Receptal®, MSD Animal Health GmbH, Luzern, Switzerland) in a dose of 0.4 µg/kg intravenously. Plasma concentrations of testosterone and estradiol were less than 0.1 ng/ml and between 5.2 and 7.3 pg/ml respectively. Estradiol concentration were slightly higher than the cut-off value (5.8 pg/ml) proposed by de Gier et al. (2012). No sings of cornification of preputial mucosal cells were detected in the preputial smear (Dreimanis et al., 2012).

Metronomic chemotherapy

Adjuvant treatment consisted of metronomic chemotherapy consisting of dyclophosphamide (10 mg/m^2 every second day) and thalidomid (4.46 mg/kg once daily) per os, for life (Burton et al., 2011; Marconato et al., 2011). The owner was instructed how to administer the chemotherapeutic agent cyclophosphamide as well as the thalidomide safely. After 26 months, the owner decided to discontinue chemotherapy without our knowledge. Thirty months after surgery the dog was presented to the referring veterinarian because of a growing mass in the scar measuring 3 × 3 × 1 cm. FNA, thoracic radiographs and abdominal ultrasound were performed. Cytology confirmed recurrence of the Leydig cell tumor. Chest x-ray revealed a single nodule in the lung and abdominal ultrasonography showed multiple nodules in the spleen. The owner requested euthanasia.

Discussion

In men malignant Leydig cell tumors are rare with 15–20% of patients having metastatic disease at the time of diagnosis. Retroperitoneal lymph nodes are most frequently involved (70%). Other metastatic sites are the liver (45%), the lungs (40%) or the bones (25%) (Bertram et al., 1991; Al-Agha and Axiotis, 2007; Vasilakaki et al., 2011) . Histological differentiation of benign from malignant Leydig cell tumor is challenging (Cheville et al., 1998; Al-Agha and Axiotis, 2007; Vasilakaki et al., 2011). Malignant Leydig cell tumors are treated by orchiectomy and retroperitoneal lymphadenectomy because this tumor does not respond favorably to chemotherapy and/or irradiation. The mean survival time ranges from 2 months to 17 years in men (Al-Agha and Axiotis, 2007).

In our canine patient the cytological diagnosis differed from the final histological diagnosis. This is not unusual as FNA gives an incorrect diagnosis in 15% of soft

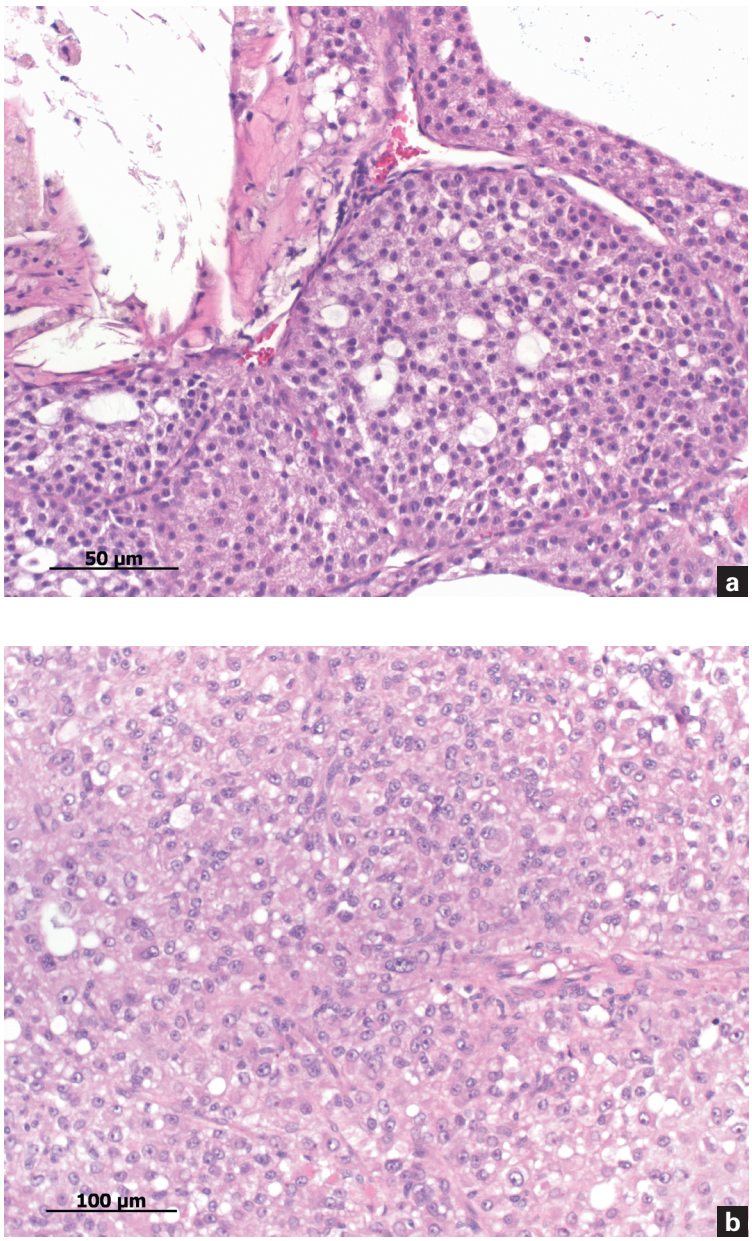

Figure 3: a) Histology of the neoplastic mass (right testicle) consisting of polygonal cells in the testicular parenchyma with moderate amount of pale eosinophilic cytoplasm and few clearly demarcated intracytoplasmatic lipidvacuoles (H&E, 20× objective). b) Histology of the metastatic neoplasm from the left hind leg excised from the skeletal musculature. Solid cell nests of polygonal cells with moderate amount of eosinophilic, sometimes vacuolated cytoplasm (lipid deposition) and round to oval nuclei with a more vesicular chromatin pattern (H&E, 20× objective).

tissue sarcomas (Liptak and Forrest, 2007). The special staining and immunohistochemistry that we applied is not specific for liposarcoma or Leydig cell tumor. Lipoma and highly differentiated liposarcoma give a positive signal with anti S100 antibody but do not store iron. Leydig cells in contrast store Fe (3+) bound on ferritin in their cytoplasm (Gross et al., 2005; Hilbe et al., 2006) which can be stained with Prussian blue. From the morphology of the neoplastic cells in combination with the antibody signals and the Iron staining we concluded that the tumors in the testis and the leg are both of Leydig cell origin. The tumor in the leg was considered a metastasis from the primary tumor in the testis. In men 16.6–30% of malignant Leydig cell tumors are hormonally active (Carmignani et al., 2006; Vasilakaki et al., 2011). They may produce testosterone and occasionally 17β-estradiol (E2) which may cause feminization (Bercovici et al., 1981). Neither nipple enlargement, pendulous preputium, attractiveness for other males or alopecia and bone marrow hypoplasia (Dreimanis et al., 2012) were seen in this case. However, due to the borderline elevated estradiol values in the GnRH-stimulation test we cannot completely exclude the presences of hormonal active cells. Metastases of seminoma and Sertoli cell tumors in dogs can be treated with chemotherapy or with radiation (McDonald et al., 1988; Tennant and Kelly, 1992; Dhaliwal et al., 1999; Takiguchi et al., 2001; Lucas et al., 2011). We used metronomic chemotherapy because encouraging results were obtained in incompletely resected or metastasized soft tissue sarcomas with little or no side effects (Elmslie et al., 2008; Marchetti et al., 2011). Thalidomid possesses anti-angiogenic effects, due to its anti-inflammatory and immune-modulatory proprieties. Furthermore, it can be used safely in dogs and cats (Teo et al., 2001; Leo et al., 2014). For these reasons thalidomid has been used in combination with dyclophosphamide. Whether the nodules in the lung and the spleen were metastases of the primary tumor of the testis could not be confirmed because the owner denied necropsy. The newly formed mass in the skeletal musculature of the left hind leg was considered a local recurrence. We conclude that a Leydig cell tumor is able to form distant metastasies that can be treated with surgery and adjuvant metronomic chemotherapy.

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References

Al-Agha O. M., Axiotis C. A.: An in-depth look at Leydig cell tumor of the testis. Arch. Pathol. Lab. Med. 2007, 131: 311–317.

Bercovici J. P., Tater D., Khoury S., Charles J. F., Floch J., Leroy J.P.: Leydig cell tumor with gynecomastia: hormonal effects of an estrogen-producing tumor. J. Clin. Endocrinol. Metab. 1981, 53: 1291–1296.

Bertram K. A., Bratloff B., Hodges G., Davidson H.: Treatment of malignant Leydig cell tumor. Cancer 1991, 68: 2324–2329.

Burton J. H., Mitchell L., Thamm D. H., Dow S. W., Biller B. J.: Low-dose cyclophosphamide selectively decreases regulatory T cells and inhibits angiogenesis in dogs with soft tissue sarcoma. J. Vet. Intern. Med. 2011, 25: 920–926.

Carmignani L., Salvioni R., Gadda F., Colecchia M., Gazzano G., Torelli T., Rocco F., Colpi G. M., Pizzocaro G.: Long-term followup and clinical characteristics of testicular Leydig cell tumor: experience with 24 cases. J. Urology 2006, 176: 2040–2043.

Cheville J. C., Sebo T. J., Lager D. J., Bostwick D. G., Farrow G. M.: Leydig cell tumor of the testis: a clinicopathologic, DNA content, and MIB-1 comparison of nonmetastasizing and metastasizing tumors. Am. J. Surg. Pathol. 1998, 22: 1361–1367.

De Gier J., Buijtels J. J. C. W. M., Albers-Wolthers C. H. J., Oei C. H. Y., Kooistra H. S., Okkens A. C.: Effects of gonadotropin-releasing hormone administration on the pituitary-gonadal axis in male and female dogs before and after gonadectomy. Theriogenology 2012, 77: 967–978.

Dhaliwal R. S., Kitchell B. E., Knight B. L., Schmid B. R.: Treatment of aggressive testicular tumors in four dogs. J. Am. Anim. Hosp. Assoc. 1999, 35: 311–318.

Dreimanis U., Vargmar K., Falk T., Cigut M., Toresson L.: Evaluation of preputial cytology in diagnosing oestrogen producing testicular tumours in dogs. Small Anim. Pract. 2012, 53: 536–541.

Elmslie R. E., Glawe P., Dow S. W.: Metronomic therapy with cyclophosphamide and piroxicam effectively delays tumor recurrence in dogs with incompletely resected soft tissue sarcomas. J. Vet. Intern. Med. 2008, 22: 1373–1379.

Grieco V., Riccardi E., Greppi G. F., Teruzzi F., Iermanò V., Finazzi M.: Canine testicular tumours: a Study on 232 Dogs. J. Comp. Path. 2008, 138: 86–89.

Gross T. L.: Liposarcoma. In: Skin Diseases of the Dog and Cat: Clinical and Histopathologic Diagnosis. 2nd Eds. T. Gross, P. Ihrke, E. Walder, V. Affolter, Blackwell Science Ltd, Oxford, 2005, 772-776.

Hilbe M., Jaros P., Ehrensperger F., Zlinsky K., Janett F., Hässig M., Thun R.: Histomorphological and immunohistochemical findings in testes, bulbourethral glands and brain of immunologically castrated male piglets. Schweiz. Arch. Tierheilk. 2006, 148: 599–608.

Leo C., Stell A., Borrego J., Martinez de Merlo E., Ruess-Melzer K., Lara-Garcia A.: Evaluation of low-dose metronomic (LDM) cyclophosphamide toxicity in cats with malignant neoplasia. J. Feline Med. Surg., Doi: 10.1177/1098612X13518938

Liptak J. M., Forrest L. J.: Soft tissue sarcoma. In: Small Animal Clinical Oncology. Eds. S. J. Withrow and D. M. Vail, Saunders, Missouri, 2007, 425–453.

Lucas X., Rodenas C., Cuello C., Gil M. A., Parilla I., Soler M., Belda E., Agut A.: Unusual systemic metastases of malignant seminoma in a dog. Reprod. Dom. Anim. 2011, 47: 59–61.

MacLachlan N. J., Kennedy P. C.: Tumours of the genital systems. In: Tumors in domestic animals. 4th edn. Ed. D. J. Meuten, Iowa State Press, Iowa, 2002, 563-564.

Marchetti V., Giorgi M., Fioravanti A., Finotello R., Citi S., Canu B., Orlandi P., Di Desidero T., Danesi R., Bocci G.: First-line metronomic chemotherapy in a metastatic model of spontaneous canine tumours: a pilot study. Invest. New Drugs 2011, 30: 1725–1730.

Marconato L., Ruess-Melzer K., Buchholz J., Kaser-Hotz B.: Neue Konzepte in der humanen Onkologie: Können sie in der Veterinärmedizin eingesetzt werden? Schweiz. Arch. Tierheilk. 2011, 8: 351–360.

McDonald R. K., Walker M., Legendre A. M., van Ee R. T., Gompf R. E.: Radiotherapy of metastatic seminoma in the dog. Case reports. J. Vet. Intern. Med. 1988, 2: 103–107.

Nødtvedt A., Gamlem H., Gunnes G., Grotmol T., Indrebø A., Moe L.: Breed differences in the proportional morbidity of testicular tumours and distribution of histopathologic types in a population-based canine cancer registry. Vet. Comp. Oncol. 2011, 9: 45–54.

Reif J. S., Maguire T. G., Kenny R. M., Brodey R. S.: A cohort study of canine testicular neoplasia. J. Am. Vet. Med. Assoc. 1979, 175: 719–723.

Takiguchi M., Iida T., Kudo T., Hashimoto A.: Malignant seminoma with systemic metastases in a dog. Small Anim. Pract. 2001, 42: 360–362.

Tennant B., Kelly D. F.: Malignant seminoma with gross metastases in a dog. J. Small Anim. Pract. 1992, 33: 242–246.

Teo S. K., Evans M. G., Brockman M. J., Ehrhart J., Morgan J. M., Stirling D. I., Thomas S. D.: Safety profile of thalidomide after 53 weeks of oral administration in beagle dogs. Toxicol. Sci. 2001, 59: 160–168.

Vasilakaki T., Michalis L., Skafida E., Arkoumni E., Delliou E., Grammatoglou X., Kontovourkis P., Papamichail V., Stamatiou K.: An unusual case of unilateral malignant leydig cell tumour of the testis. Case Rep. Oncol. 2011, 4: 132–135.

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