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Primary bilateral renal diffuse large B-cell lymphoma with central nervous system metastases in a captive brown bear (*Ursus arctos*) - a case report

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ABSTRACT

We present the first report of primary bilateral renal diffuse large B-cell lymphoma with central nervous system metastases, in a 7 year-old, captive female brown bear. After 7 months of progressive neurological disease, the animal was euthanized. On necropsy, neoplastic nodules with infiltrative growth in the kidneys were found. Neoplastic proliferation of a similar pattern was found compressing the cerebellum and brain stem, and growing through the nervus maxillaris and nervus ophtalmicus. Histology revealed a highly cellular round cell neoplasm, with an infiltrative diffuse growth pattern through the kidneys and nervous parenchyma. On immunohistochemistry, the neoplastic cells stained for the B-cell marker Pax-5. Our report suggests that lymphoma should be considered as a differential diagnosis in bears with a chronic progressive neurological disease.

Key words: brown bear, *Ursus arctos*, primary renal large B-cell lymphoma, central nervous system metastases, pathology

Introduction

Clinical, haematological and biochemical parameters in animals suffering from lymphoma are nonspecific, and reflect impairment of the affected organ function

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(TRUONG et al., 2001; MODIANO, 2010; VILLA et al., 2011). Detection of changes in the biochemical profile of a functionally impaired organ is the first step toward identification of the affected organ/organs. The last step in clinical confirmation of a lymphoma requires fine-needle aspiration, supported by ultrasound, magnetic resonance imaging (MRI) or computed tomography (CT), biopsy or organonectomy (TRUONG et al., 2001; MODIANO, 2010; VILLA et al., 2011). Clinical management in captive wild animals is restricted in comparison with companion animals because of the mandatory physical restraint and the use of anesthesia prior to every single examination protocol (CAULKETT, 2007). Specialized and customized diagnostic facilities, supported by hematological and biochemical laboratories, adjusted for different wild species, are not available for the majority of institutions that care for captive wild animals. This raises the possibility that a progressive chronic disease may be poorly treated and often falsely diagnosed in captive, wild individuals. So far, only three cases of lymphomas in brown bears (*Ursus arctos*) have been documented. All of them presented as alimentary lymphoma, with an non-specific alimentary clinical presentation, and pathology confirmation of primary intestinal involvement and metastatic spread to the mesenteric lymph nodes and liver (ZWART et al., 1974; BLANCQUAERT et al., 1984; YOON et al., 2001).

The availability of postmortem description of etiology connected with neurological diseases in captive brown bears is also restricted to a few cases of cerebrovascular atherosclerosis, meningoencephalitis caused by Equine Herpesvirus 9, nonsuppurative meningoencephalomyelitis caused by West Nile virus and internal hydrocephalus (MILLER and McDONOUGH, 2008; DONOVAN et al., 2009; DUTTON et al., 2009; KÜBBER-HEISS et al., 2009). Additionally, urological impairment in *Ursus arctos* remains poorly described, with only one case of uraemia due to urinary bladder rupture (HUBENOV et al., 1999). In order to expand the list of diseases described in captive *Ursus arctos*, we present the first case of primary renal lymphoma of the diffuse large B-cell immunoblastic phenotype with fatal clinically manifested central nervous system (CNS) involvement.

Case history

A seven year-old, captive female brown bear from the Kuterevo Bear Sanctuary, Kuterevo, Croatia, was reported to have been suffering from chronic food intake difficulties for seven months. Clinical signs developed, in the form of slowly progressive swallowing and coordination problems, depression, followed by apathy, head swinging, and ataxia. The neurological deficits increased in frequency and severity, showing constant disorientation, gait disturbances and balance problems. An inflammatory or neoplastic disease, with localization in the brain stem, was suspected. Due to the grave prognosis, the animal was euthanized.

Necropsy findings

A full necropsy showed good body condition with the panniculus measuring up to 5 cm in the lumbosacral region. Examination results were negative for the presence of abnormalities in the oral cavity, jaw, chewing muscles or esophagus. The cortical tissue elements of both kidneys protruded as whitish to light-yellowish nodules (1 to 3 cm in diameter) from the thick layer of perirenal fat. Bilateral nodular renomegaly and complete loss of specific multilobar kidney architecture was evident after the fibrose capsules were removed. On the longitudinally sections cut through the kidneys, a massive replacement of the functional renal lobes was observed, with coalescing light-yellowish, homogenous, plump nodules (Fig. 1). Parallel sections of the kidney parenchyma showed that less than 20% of the right and 10% of the left renal original tissue had been preserved. Examination of the skull and brain revealed bilateral, occipito-rostral orientated subdural, whitish-grey nodules, measuring approximately 25 mm in length and 15 mm in width, extending in parallel with and compressing the medulla oblongata, cerebellum and brain stem (Fig. 2). Both the nervus (n.) maxillaris and n. ophtalmicus were swollen, with fine nodular matter present on the surface. All other organs and tissues examined appeared normal macroscopically, aprt from the lungs, which were oedematous as a consequence of euthanasia. Despite the fact that the animal suffered from end-stage kidney disease, no morphological presentation of uraemia or anaemia was found on the carcass.

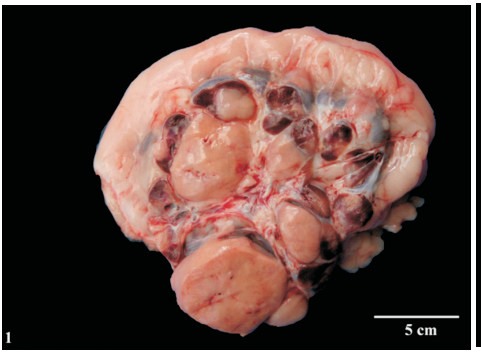

Fig. 1. Renal ymphoma, one half of the right kidney, transverse section from pole to pole. Coalescing yellowish-neoplastic nodules within cortex, medulla and pelvis replacing and compressing of the compound kidney units remnants.

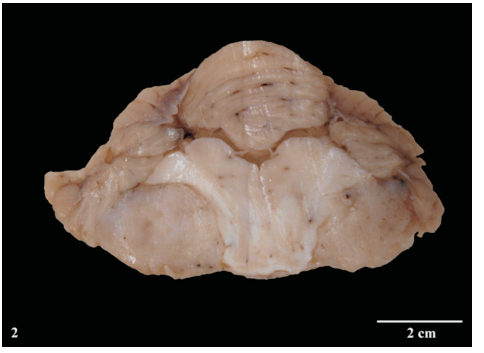

Fig. 2. Transversal section thru the cerebellum and brain stem. Bilateral symmetrical metastases (whitish-gray proliferations in the cerebellum with compression of the brain stem).

Histology

Representative samples of all organs and tissues were fixed in 10% neutral buffered formalin. The fixed tissues were processed routinely and stained with haematoxylin and eosin (HE).

Histopathological examination of the kidneys, brain and affected brain nerves showed massive, densely populated, unencapsulated, round cell type, malignant growth. The neoplastic cells were of a large lymphocytic type, with cell diameter of 2 to 3 red blood cells. The cytoplasm of these cells was scant to moderate, usually apparent as a narrow basophilic perinuclear ring. The nuclei were centrally located, round, oval or cleaved, of a vesicular pattern, with mostly one, or occasionally two, large round nucleoli (Fig. 3 inset). The mitotic index was high, up to 12 per HPF. Necrotic areas were observed within sheets of anaplastic lymphocytes. Microscopically, the complete loss of renal architecture was evident within the neoplastic nodules examined. Fusions of former kidney lobes were observed due to anaplastic lymphocyte growth and invasion of all structures except the arteries, veins and the renal capsule (Fig. 3). Remnants of original kidney tissue suffered from compressive atrophy.

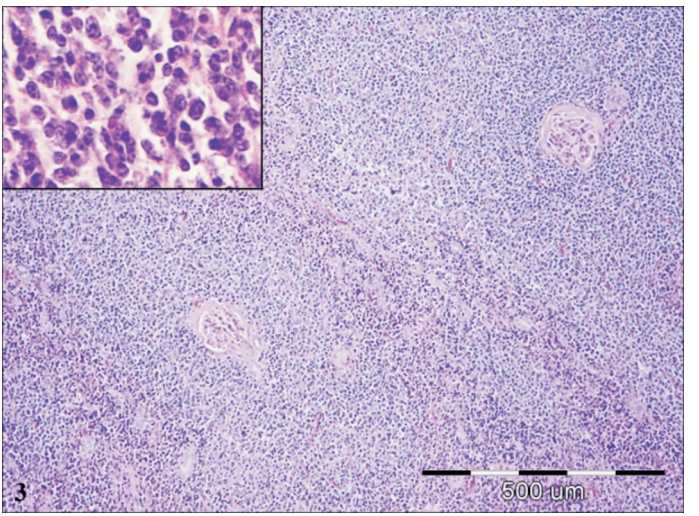

Fig. 3. Lymphoma, kidney, Hematoxylin and eosin (H&E) staining, ×100. Inset: Higher magnification of neoplastic lymphocytes, H&E, ×400.

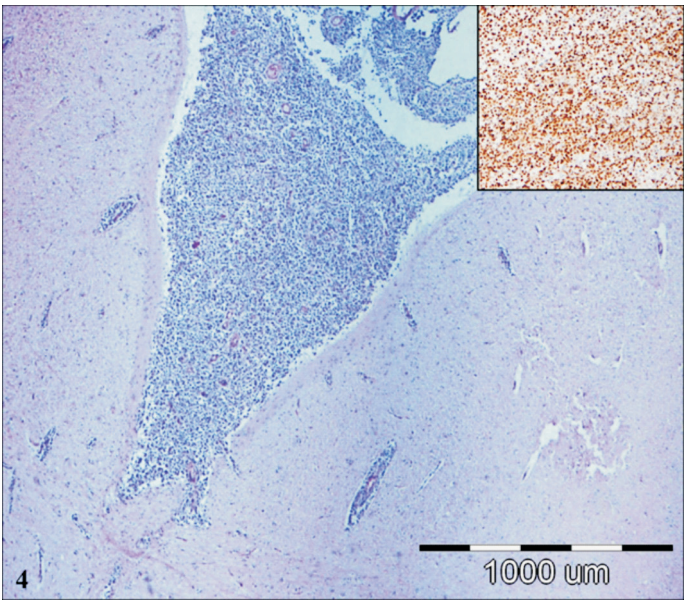

Fig. 4. Lymphoma, brain stem. Diffuse spread of neoplastic cells through the subarahnoidal and Virchow-Robin space with formation of perivascular cuffs. H&E, ×40. Inset: Immunohistochemic labeling with Pax-5 antibodies, ×100.

Affection of the brain tissue with metastases showed a specific microscopic pattern of dissemination. Anaplastic lymphocytes used the subarachnoid and Virchow-Robin space to spread through the CNS and reach deeper layers of neuropil or white matter, where they formed perivascular cuffs in the medulla oblongata, cerebellum and brain stem (Fig. 4). Advanced lesions, consisting of large sheets of anaplastic cells, replaced layers of the white matter within the midbrain. Lymphoma extended through the complete intracranial length of the n. trigeminus and n. opticus in a diffuse manner, up to the level of the canalis opticus.

Immunophenotyping of the lymphoma detected within the kidneys and CNS was provided on the formalin fixed, paraffin embedded tissue sections, with monoclonal mouse-anti human CD3 (DAKO) and Pax-5 (Novacastra™) antibodies. All neoplastic cells showed strong labeling with Pax-5, confirming the B-lymphocytic origin of all the neoplastic infiltrations (Fig. 4 inset). Based on its morphological, histological and IHC appearance, as well as its anatomic distribution, the detected neoplasia was diagnosed, according to the revised WHO Classification (FRITZ et al., 2013), as a high grade, primary bilateral renal diffuse large B-cell lymphoma, with CNS involvement. The homogenous

population of large, uniform, anaplastic, aggressive, rapidly growing lymphocytic neoplasms strongly suggested the immunoblastic cell type of large B-cell lymphoma.

Discussion

Extranodal lymphomas with primary renal involvement have been commonly described in cats (*Felis catus*) in association with host genetic factors, retrovirus infections, chronic inflammation and immunosuppression (MOONEY et al., 1987; SCHMIEDT et al., 2009; DURHAM et al., 2014). Spontaneous mutations, as a cause of primary renal lymphoma (PRL), with or without CNS dissemination, have so far only been described in three dogs (*Canis familiaris*) (ZHAO et al., 1993; LANE and LOBETTI, 2002; DURNO et al., 2011). In wild carnivores, a single case of extranodal lymphoma with renal brain and meningeal involvement with large lymphoblastic cells was reported in a raccoon (*Procyon lotor*) (ROHER and NIELSEN, 1984). Despite the fact that PRL is also a rare lymphoma form in human medicine, comprehensive retrospective studies showed the high independent risk factor of PRL for CNS relapse (VILLA et al., 2011). Further, within the low number of PRL reported in veterinary medicine, it seems possible that carnivorans, such as dogs and raccoons, are likely to develop CNS metastases as well (ROHER and NIELSEN, 1984; LANE and LOBETTI, 2002).

Although the mechanism of PRL dissemination into the CNS remains unclear, in human medicine it has been statistically correlated with the B-cell lymphoma immunophenotype, while in dogs a single PRL with CNS involvement was of T-cell lymphocyte origin (LANE and LOBETTI, 2002; VILLA et al., 2011). In cats, dissemination to the CNS seems to be connected with a multicentric form of lymphoma that also spread to the kidneys, as described in humans (TRUONG et al., 2001; VILLA et al., 2011). In the current case, we showed that in a brown bear, PRL can metastasise to the CNS, causing advanced symptoms of neurological disturbance prior to any clinical and pathomorphological manifestation of end-stage kidney disease.

Necropsy revealed that the kidneys were too damaged to be able to excrete creatinine and urea effectively, yet no signs of chronic uremia, as would be excepted, were found. Perhaps the 30% of functional parenchyma in a condition of a state of lower perfusion maintained the excretion of metabolic waste products in this case. Since bears have the unique ability to recycle urea by hydrolyzation in the gut by urease-expressing bacteria, elevation of the serum urea may have been avoided by this elimination mechanism (HISSA et al., 1998; STENVINKEL et al., 2013).

Since no urinalysis or precise biochemical values were obtained from serum tests, this phenomenon will remain unclear in the present bear. Anaemia is also a common consequence in end-stage kidney disease, since the cells that produce erythropoietin are damaged as the consequence of the general parenchymal loss (NELSON et al., 1983;

ECKARDT, 2001). In the current case, surprisingly, anaemia was not found on necropsy. We assume that brown bears harbor pathways of extra-renal erythropoietin synthesis, as shown in rats, where low levels of erythropoietin mRNA were found in the liver (SCHUSTER et al., 1992).

In conclusion, this case report presents the first finding of PRL of the diffuse B-cell immunoblastic phenotype, with clinically evident CNS metastases, in a brown bear. Neoplasia in wildlife is rarely reported due to the difficulties in obtaining research material and the short lifespan of free ranging animals. Captive individuals are more available for investigation and especially pathological evaluation, so information from these investigations could be used for all individuals of a species. Our report suggests that lymphoma should be taken into account as a differential diagnosis when a bear with progressive neurological disease presents with no evidence of an infectious disease.

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BECK, A., S. RELJIĆ, I.-C. ŠOŠTARIĆ-ZUCKERMANN, M. WRZOSEK, D. HUBER: Primarni obostrani difuzni B-velikostanični limfom bubrega s metastazama u središnjem živčanom sustavu u smeđe medvjedice (*Ursus arctos*) držane u zatočeništvu. Vet. arhiv 86, 857-865, 2016.

SAŽETAK

Opisan je prvi slučaj bilateralnoga primarnog difuznog B-velikostaničnog limfoma bubrega s metastazama u tkivo središnjega živčanog sustava u sedmogodišnje smeđe medvjedice držane u zatočeništvu. Životinja je eutanizirana zbog sedmomjesečne progresivne neurološke bolesti. Obdukcija je pokazala postojanje nodularne infiltrativne neoplazme u oba bubrega. Također je utvrđeno neoplastično nodularno bujanje u području malog mozga i moždanog debla koje je dovelo do značajne kompresivne atrofije navedenih struktura te zahvatilo vidne i maksilarne živce. Histološka pretraga pokazala je da su bubrezi i mozak zahvaćeni difuznim infiltrativnim rastom neoplastičnih pojedinačnih okruglih stanica. Visoka imunohistokemijska pozitivnost neoplastičnih stanica na Pax-5 marker dokazala je da je riječ o B-staničnom limfomu. Ovaj nalaz trebao bi doprinijeti uvrštavanju limfoma na listu diferencijalnih dijagnoza u slučaju pojave kronične progresivne bolesti centralnog živčanog sustava u medvjeda.

Ključne riječi: smeđa medvjedica, *Ursus arctos*, primarni difuzni B-velikostanični limfom, bubreg, metastaze, središnji živčani sustav, patologija