NOTE *Clinical Pathology*

Perivertebral B-Cell Lymphoma in a Queensland Koala (*Phascolarctos cinereus adustus*) with Paralytic Symptoms in the Hind Limbs

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ABSTRACT. A male Queensland koala (*Phascolarctos cinereus adustus*) at Kanazawa Zoological Gardens (Kanagawa, Japan) exhibited paralytic symptoms in the hind limbs. Computed tomography and magnetic resonance imaging revealed a mass on the left ventral side of the 11th to 13th thoracic vertebrae, and the presence of myelitis or edema in the spinal cord. The koala was under anesthesia during the examination and suddenly developed ventricular fibrillation and died. Necropsy revealed a firm flat ovoid hemorrhagic mass on the vertebrae. Following a microscopic examination including immunohistochemistry, the perivertebral mass was diagnosed as B cell lymphoma. Therefore, neoplastic cell infiltration into the spinal cord may cause paralytic symptoms in the hind limbs.

KEY WORDS: computed tomography (CT), immunohistochemical, lymphoma, magnetic resonance imaging (MRI), Queensland koala.

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At Kanazawa Zoological Gardens (Kanagawa, Japan), a male Queensland koala (*Phascolarctos cinereus adustus*) aged 10 years and 10 months and weighing 7.0 kg exhibited paralytic symptoms in both hind limbs, and the limb muscles were completely flaccid. Complete blood cell count and the serum biochemical values were within the reference range [2]. Neurological examination revealed absence of postural (proprioception, placing, and hopping) and sensation reactions (deep pain) in both hind limbs. Spinal reflexes, cranial nervous system, and neurological examination of the front limbs were normal. Radiographs were taken under manual restraint and revealed the normal skeletal structure.

The koala was transferred to Nihon University Animal Medical Center (Kanagawa, Japan) for CT (Aquilion 16; Toshiba Medical Systems Co., Tochigi, Japan) and MRI (EXCELART Vantage; Toshiba Medical Systems Co.) examinations because spinal cord disease was considered. Anesthesia was induced (by mask) and maintained by inhalation of 1.5% isoflurane (Doubutsuyou Isofururan; Mylan Pharmaceutical Co., Tokyo, Japan) in oxygen at 2.0 *l*/min. CT revealed the presence of a mass on the left ventral side, extending from the 11th to 13th thoracic vertebrae. The spinal cord lesion was not revealed by CT examination (Fig. 1a). T1-weighted (TR=600 msec, TE=15 msec), contrast T1-weighted (TR=600 msec, TE=15 msec) by gadoteridol (ProHance; Eisai Co., Ltd., Tokyo, Japan) at 0.2 m*l*/kg, T2-weighted (TR=4,000 msec, TE=120 msec), and fluid-attenuated inversion recovery (FLAIR) (TR=8,000 msec, TE=109 msec) images confirmed the CT finding of a perivertebral mass. The mass had a slightly heterogeneous intensity, with a partially hyperintense signal on transverse contrast T1-weighted, T2-weighted, and FLAIR images (Fig. 1b–e). Although the spinal cord exhibited semioval hyperintense areas on transverse contrast T1-weighted, T2-weighted, and FLAIR images, displacement of the spinal cord was not observed. The spinal cord also exhibited hyperintense areas on sagittal contrast T1-weighted, T2-weighted, and FLAIR images (Fig. 2). The koala suddenly developed ventricular fibrillation and died due to suppression of the cardiovascular system. Anesthetic depth was not maintained at a suitable level during the MRI examination.

A necropsy of the koala was conducted at the Laboratory of Veterinary Pathology at Nihon University. Gross examination revealed a firm flat ovoid hemorrhagic mass measuring 3.0 × 2.0 × 1.0 cm on the left ventral side of the 11th to 13th thoracic vertebrae (Fig. 1f). The lungs were dark red and edematous. No other significant gross findings were detected. A wide range of tissue samples was fixed, stained with hematoxylin and eosin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and examined by light microscopy. The neoplasm had infiltrated into the skeletal muscle, bone marrow, and dura mater of the spinal cord, and the spinal cord was slightly compressed (Fig. 3). Furthermore, hemorrhage and hyperemia in the spinal cord and remarkable vacuolation of nerve cells were observed. The perivertebral mass exhibited diffuse, sheet-like proliferation of neoplastic plasma cells and lymphocytes (Fig. 4). The neoplastic

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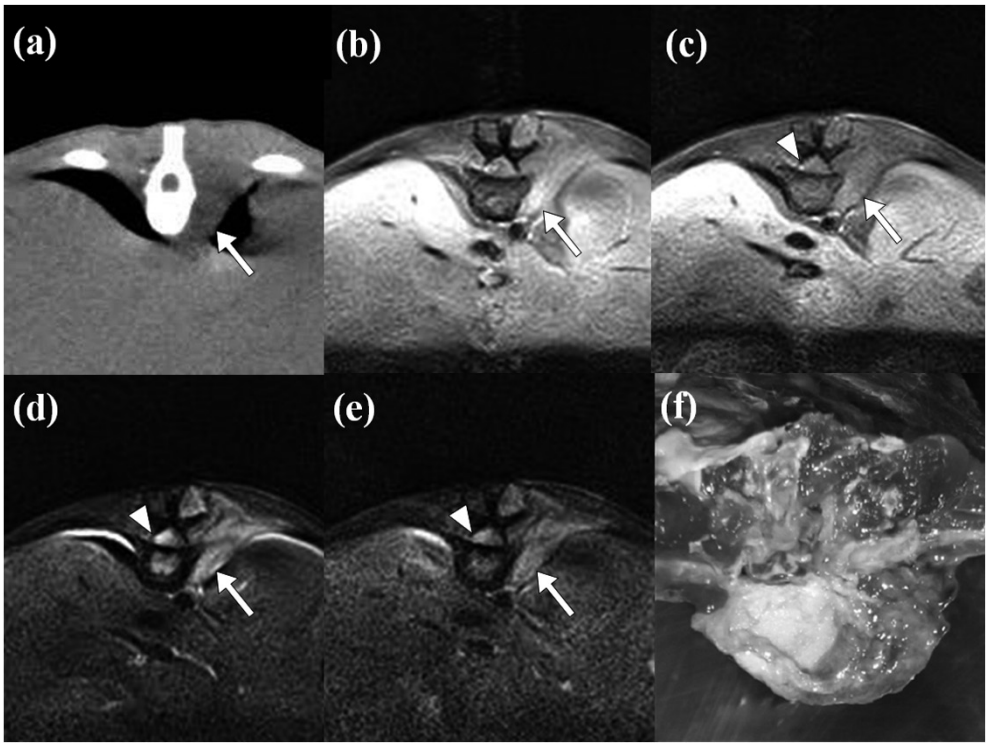

Fig. 1. Transverse images of a perivertebral mass in a Queensland koala. (a) Transverse computed tomography image showing a perivertebral mass (arrow) on the left ventral side of the 12th thoracic vertebra. (b) A T1-weighted image showing the perivertebral mass as an isointense signal. (c) Contrast T1-weighted, (d) T2-weighted, and (e) fluid-attenuated inversion recovery images showing the perivertebral mass (arrow) and spinal cord (arrowheads) as partially hyperintense signals. (f) A macroscopic view showing the perivertebral mass (arrow) on the left side of the thoracic vertebrae.

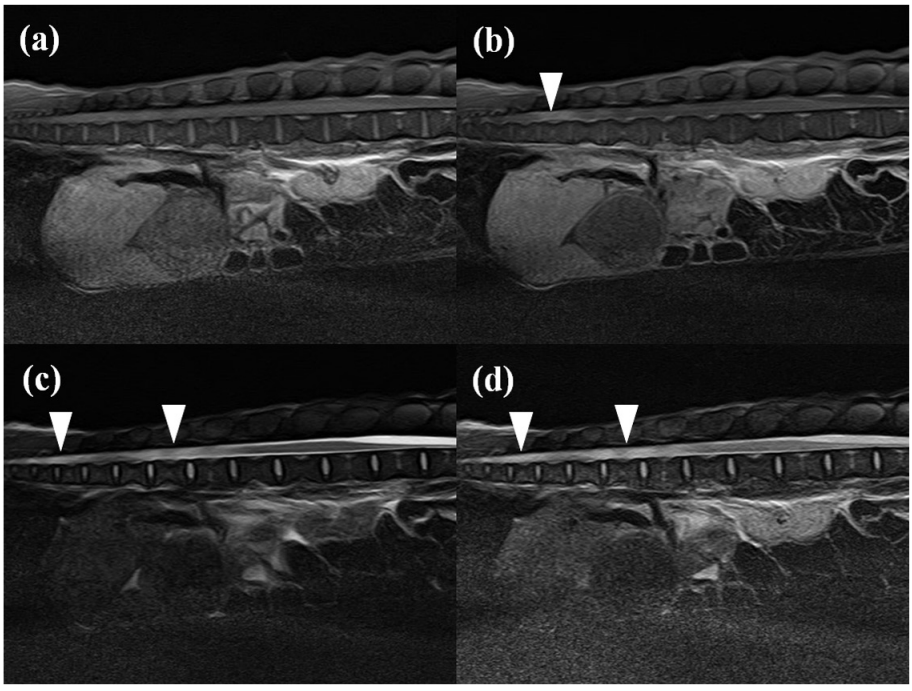

Fig. 2. Sagittal magnetic resonance images of vertebrae of the Queensland koala. (a) A T1-weighted image showed the spinal cord as isointense signal. (b) Contrast T1-weighted, (c) T2-weighted, and (d) fluid-attenuated inversion recovery images showed a hyperintense signal (arrowheads) in the spinal cord due to myelitis or edema, and the possibility of neoplastic cell infiltration was considered.

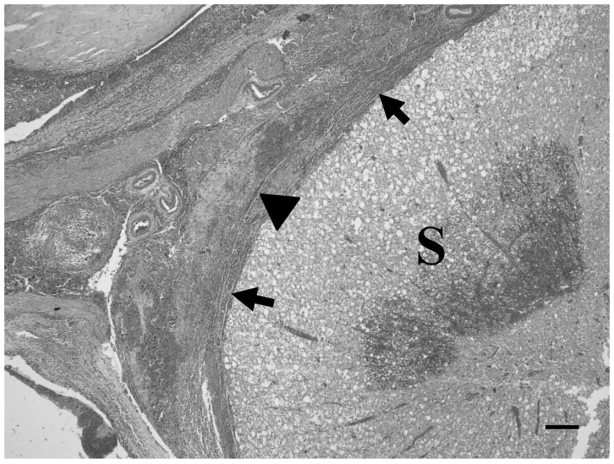

Fig. 3. Microscopic analysis of the spinal cord. The neoplasm (arrow) infiltrated into the dura mater (arrowhead) of the spinal cord (S), which was slightly compressed. (HE, bar=500 μm.)

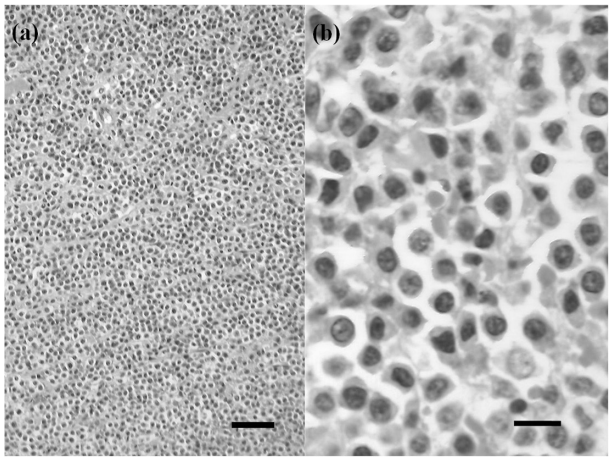

Fig. 4. Microscopic analysis of the perivertebral mass. (a) Diffuse, sheet-like proliferation of neoplastic cells was observed. (HE, bar=60 μm.) (b) Small- to medium-sized, round neoplastic cells with 1 or 2 clear nucleoli were seen. (HE, bar=10 μm.)

cells were small- to medium-sized (size range: 4–8 μm) and round, with 1 or 2 clear nucleoli and diffuse Russell bodies. The mitotic index of the neoplastic cells was 5.4 in 10 high-power fields. Metastases of the neoplastic lymphocytes were detected in the lungs, diaphragm, stomach, liver, spleen, and mesenteric and mandibular lymph nodes.

Immunohistochemical analysis of the perivertebral mass was performed using the EnVision^{TM}+ system by the standard peroxidase-labeled dextran-conjugated polymer method [9]. The following primary antibodies were used: rabbit polyclonal anti-human Oct-2 (Santa Cruz Biotechnology Inc., Santa Cruz , CA, U.S.A.; 1:50), rabbit polyclonal anti-human kappa light chains (Dako Japan, Tokyo, Japan; 1:1,000), monoclonal anti-mouse CD79b (Santa Cruz Biotechnology; 1:50), mouse monoclonal anti-human CD138 (Dako Japan; 1:25), and the antigenic determinant-specific rabbit anti-human PAX5 (Thermo Scientific; Kanagawa, Japan; 1:50). The neoplastic cells were positive for Oct-2, and weakly positive for kappa light chains, CD79b, and CD138. Because the section was negative for PAX5, we confirmed that the perivertebral mass was mainly composed of plasma cells.

In the present case, the morphology and immunolabeling of neoplastic cells in the perivertebral mass indicated that the neoplasm could be a solitary plasmacytoma or a multiple myeloma, according to the current diagnostic criteria of the International Myeloma Working Group [7]. However, the perivertebral mass comprised not only plasma cells but also neoplastic lymphocytes, and the neoplastic cells in the metastases were clearly identified as neoplastic lymphocytes. Furthermore, hypercalcemia, abnormal serum creatinine values, anemia, and bone lesion, which are usually observed in multiple myeloma, were not seen. Therefore, the final diagnosis of the neoplasm was B-cell lymphoma, in which the B cells were differentiating into plasma cells.

The primary antibodies may be effective on the sections of the koalas. CD3, CD5, and CD79b were used in the previous study [4, 5]. However, the effectiveness of the markers Oct-2, kappa light chains, CD138, and PAX5 has not been demonstrated in koalas. The positive staining for Oct-2, kappa light chains, and CD138 may indicate that the antibodies properly adhered to the antigens in the section. Moreover, we morphologically confirmed that the neoplastic lymphocytes and plasma cells in the lymph node of the koala were positive and negative, respectively, for PAX5. Therefore, the primary antibodies may be correctly expressed in the present study.

Lymphoid neoplasia is reported to be the most common form of neoplasia in koalas [3]. Since 1961, various authors have reported isolated cases of spontaneous lymphoid neoplasia in both captive and free-ranging koalas [1, 5, 6]. The previous study described that the 19 of the 51 lymphoma cases were extranodal lymphomas likely originated from stomach, intestine, spleen, liver, brain, lungs, and conjunctiva [5]. In our case, the tumor developed in the unique site and should be added a list of extranodal lymphomas in the koala.

CT and MRI were found to be effective for the clinical diagnosis of a perivertebral mass. Clinical signs of lymphoid neoplasia may be variable depending on the organs affected [2]. In the present study, lymphadenopathy, a specific clinical sign of lymphoid neoplasia, was not detected, and spinal cord disorders such as vertebral fracture and herniated disk disease were considered [8]. Transverse CT images of the vertebra were effective in detecting the perivertebral mass, but ineffective in revealing the spinal cord lesion. On the other hand, MRI can show abnormalities in the spinal cord. In conjunction with a histopathological examination, MRI may reveal the presence of myelitis or edema in the spinal cord and the possibility of neoplastic cell infiltration. Therefore, MRI was effective for visualizing the spinal cord lesion and ruling out other spinal cord diseases. To our knowledge, this is the first report to reveal the effectiveness of CT and MRI for the diagnosis of perivertebral lymphoma in koalas.

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