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NEOPLASTIC DISEASE

A Case of Feline T-cell Lymphoma with Tropism for Striated Muscle and Peripheral Nerve

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Summary

An 11-year-old female American shorthair cat was presented with a 3-month history of hindlimb ataxia and knuckling of the left forelimb. Clinical abnormalities included weight loss, hyperaesthesia of the neck and back, cardiac murmur and systemic muscle atrophy. The cat died 10 days after the initial presentation and a necropsy examination was performed. Grossly, extensive pale lesions were seen in the wall of the left ventricle and the septum of the heart. There were no detectable masses in the heart, skeletal muscles or peripheral nerves. Histopathological examination revealed diffuse, extensive infiltration of atypical lymphoid cells in the heart; the cardiac muscles were markedly degenerate and atrophic and were replaced by the neoplastic cells. Neoplastic cells with similar morphology were seen in all specimens of the skeletal muscles and peripheral nerves. Clonality analysis of the paraffin wax-embedded heart tissue revealed a monoclonal rearrangement of the gene encoding the T-cell receptor γ chain. Based on these findings, the case was diagnosed as T-cell lymphoma with tropism for striated muscle and peripheral nerve.

Keywords: cat; peripheral nerve; striated muscle; T-cell lymphoma

Lymphomas are one of the most common malignant neoplasms in cats and commonly affect the gastrointestinal tract, lymph nodes, kidneys and nasal cavity (Taylor *et al.*, 2009; Valli *et al.*, 2016). Lymphomas rarely infiltrate peripheral nerve (Mandrioli *et al.*, 2012; Del Grande *et al.*, 2014) or striated muscle (Takeuchi *et al.*, 2010; Jonavicius *et al.*, 2015; Binici *et al.*, 2018). To our knowledge, there is no report of primary skeletal muscle lymphoma in cats. Here we present the first case of feline lymphoma involving multiple peripheral nerves and multiple striated muscles, including both the skeletal and cardiac muscles.

An 11-year-old female American shorthair cat was presented to the Osaka Prefecture University Veterinary Medical Center, Osaka, Japan, with a history of ataxia of the hindlimbs and knuckling of the left forelimb that had not responded to glucocorticoid therapy administered for the previous 3 months. Physical examination revealed weight loss, hyperaesthesia of the neck and back, cardiac murmur (Levine 1/6) and systemic muscle atrophy. Abnormal laboratory findings included leucocytosis (27.6×10^9/l; reference interval $6.3–19.6 \times 10^9$/l), anaemia (packed cell volume 22% [reference interval 28–47%]; haemoglobin 73 g/l [reference interval 81–142 g/l]) and elevated levels of blood urea nitrogen (BUN, 32.1 mmol/l; reference interval, 4.3–14.6 mmol/l), creatinine (229.8 μmol/l; reference interval, 61.9–221 μmol/l) and lactate dehydrogenase (264 U/l; normal <201 U/l). The level of serum creatinine kinase was elevated 1 day after the initial

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presentation (>2000 U/l; normal <469 U/l) and remained higher than normal for 4 days. Atypical cells were not observed in the peripheral blood. Serological tests for feline leukaemia and feline immunodeficiency viruses were negative. The antibody titre for feline coronavirus was 1,600, suggesting a subclinical infection. A right lateral thoracic radiograph revealed enlargement of the cardiac silhouette (vertebral heart scale, 9.0; normal 7.5 ± 0.3). B-mode echocardiography showed moderate hypertrophy of the left ventricular papillary muscle and left atrium enlargement (left atrium-to-aorta ratio, 1.61; normal <1.5). Although the cat received fluid therapy, the general physical condition and increased BUN and creatinine were not improved by the therapy. Computed tomography and magnetic resonance imaging could not be performed because of the poor condition. The cat died 10 days after the initial presentation. Necropsy examination was performed after the carcass had been frozen for 2 days and then thawed overnight.

At necropsy examination, severe atrophy of the musculature of the hindlimbs was observed. Extensive pale areas were seen in the wall of the left ventricle and the septum of the heart (Supplementary Fig. 1). The right kidney was atrophic and a dark red nodule, 1 cm in diameter, was observed in the adipose tissue around the left kidney. The articular surface of the left humeral head was discoloured (Supplementary Fig. 2). There were no detectable masses in the heart, skeletal muscles or peripheral nerves (Supplementary Fig. 3). No gross lesions were detected in the internal organs.

Tissue specimens were collected from the liver, spleen, kidneys, heart, lungs, stomach, small and large intestines, trachea, pancreas, adrenal glands, lymph nodes (mesenteric), bone marrow, eyes, skeletal muscles (femoral, back, lingual, anal sphincter and ocular muscles), humeral head, brain, spinal cord at the levels of L3, L5, C1 and C5 and peripheral nerves (brachial plexus, sciatic and spinal root nerves). These were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE). Sections were also subjected to immunohistochemistry (IHC) with primary antibodies specific for CD3 and CD20 (Supplementary Table 1). After dewaxing and pretreatment, tissue sections were processed in a Histostainer™ (Nichirei Biosciences, Tokyo, Japan). Briefly, sections were treated with 5% skimmed milk in phosphate buffered saline (PBS) for 10 min and reacted with each primary antibody for 1 h. After incubation in 3% H_2O_2 for 15 min, application of horseradish peroxidase-conjugated secondary antibody (Histofine Simple Stain MAX PO®; Nichirei Biosciences) for 30 min was performed. Positive reactions were 'visualized' with 3, 3′-diaminobenzidine (DAB Substrate Kit; Nichirei Biosciences). Sections were counterstained lightly with haematoxylin. Non-immune mouse or rabbit IgG was substituted for primary antibody as a negative control. Infiltrating lymphocytes in the same section served as an internal positive control.

Histopathological examination revealed diffuse, extensive infiltration of neoplastic round cells with round to oval nuclei and scant eosinophilic cytoplasm in the heart (Figs. 1 and 2). The cardiac muscles were markedly fragmented, atrophied and replaced by the neoplastic cells. Cellular and nuclear atypia were moderate (Supplementary Fig. 4). Mitotic figures were often seen (31 per 10 high-power [×400] fields). Some neoplastic cells were positive for CD3 (Supplementary Fig. 5), while they were negative

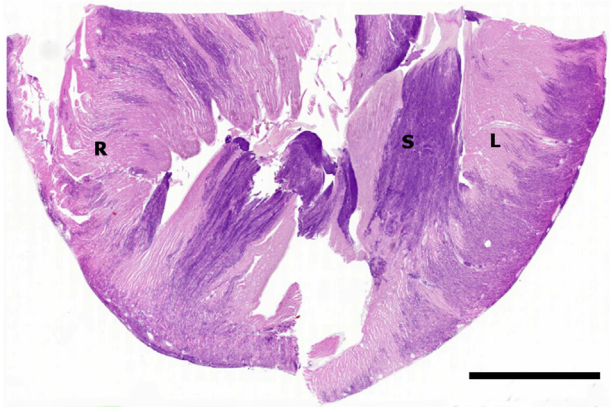

Fig. 1. Diffuse infiltration of basophilic neoplastic lymphoid cells into the heart. L, wall of left ventricle; R, wall of right ventricle; S, ventricular septum. HE. Bar, 5 mm.

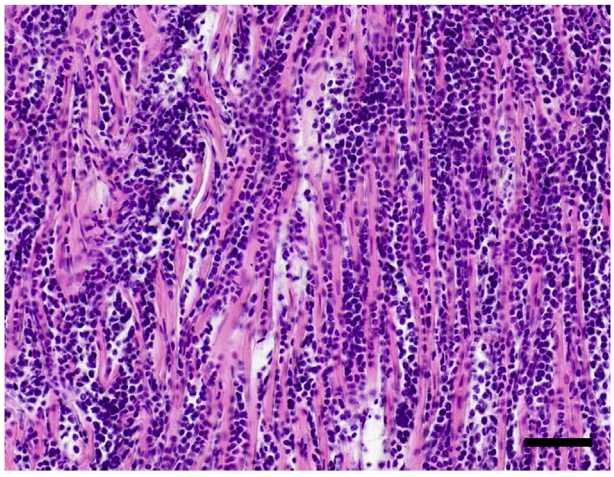

Fig. 2. Cardiac muscle is markedly degenerate and atrophic and is replaced by neoplastic lymphoid cells. HE. Bar, 50 μm.

for CD20 (Supplementary Fig. 6). Clonality analysis was performed from the paraffin wax-embedded tissue of the heart in which the neoplastic involvement was most extensive. The result showed a monoclonal rearrangement of the gene encoding the γ chain of the T-cell receptor (TCR; Supplementary Fig. 7). Therefore, the cardiac lesion was diagnosed as T-cell lymphoma.

Neoplastic cells with similar morphology were seen in all specimens of the skeletal muscles (Fig. 3 and Supplementary Fig. 8) and peripheral nerves (Fig. 4 and Supplementary Fig. 9). The neoplastic cells were also seen in the dark red nodule in the adipose tissue around the left kidney and in the gastric mucosa. However, neoplastic cells were not observed in the central nervous system, kidneys, intestines or lymph nodes. Histopathological findings in other organs included bile duct cystadenoma in the liver and chronic nephropathy. There was extensive loss and degeneration of the articular cartilage, a decrease in bone marrow cells and mild fibrosis in the medullary cavity of the left humeral head, while the right humeral head was intact.

Based on these findings, this case was diagnosed as T-cell lymphoma with tropism for striated muscle and peripheral nerve. Lymphomas primarily involving striated muscle or peripheral nerve are rare, but are reported in man, dogs and cats. Human lymphomas rarely involve both the peripheral nerve and skeletal muscle (Advani *et al.*, 2015). In man, peripheral nerve or skeletal muscle lymphomas tend to infiltrate along the anatomical structure and peripheral nerve lymphomas typically do not invade the central nervous system from the cranial or peripheral nerve roots (Oya, 2014). Some types of lymphomas are expected to have affinity for the peripheral nerves or skeletal muscles. In man, primary peripheral nerve lymphomas are mostly diffuse large B-cell lymphomas (Misdraji *et al.*, 2000). The patients have ataxia, hyperaesthesia, pain and muscle atrophy caused by the infiltration of lymphoma into the peripheral nerves. Skeletal muscle lymphomas are rarer than peripheral nerve lymphomas. In human skeletal muscle lymphomas, the B-cell phenotype is the most common, although natural killer cell lymphoma, T-cell lymphoma and Hodgkin's disease have been also described to involve the muscle (Surov, 2014). Clinical symptoms of such skeletal muscle lymphomas include pain, muscle atrophy, paraesthesia and ataxia. In the present case, the clinical signs such as ataxia of hindlimbs, knuckling of the left forelimb, hyperaesthesia of the neck and back and muscle atrophy were thought to be caused by the infiltration of the lymphoma into the peripheral nerves and skeletal muscles.

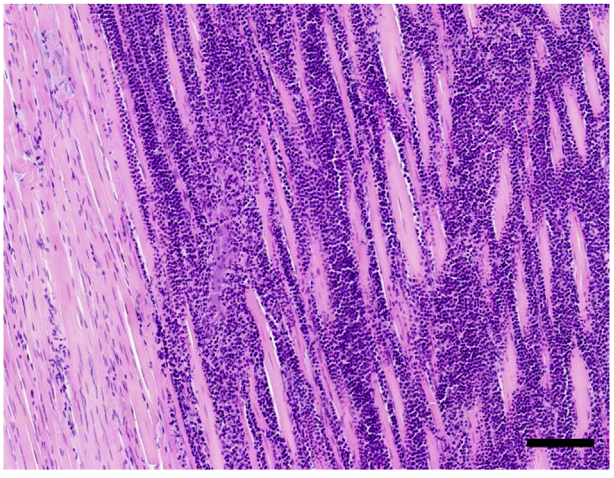

Fig. 3. Focal, dense infiltration of neoplastic lymphoid cells into the femoral muscle with marked muscle atrophy. HE. Bar, 100 μm.

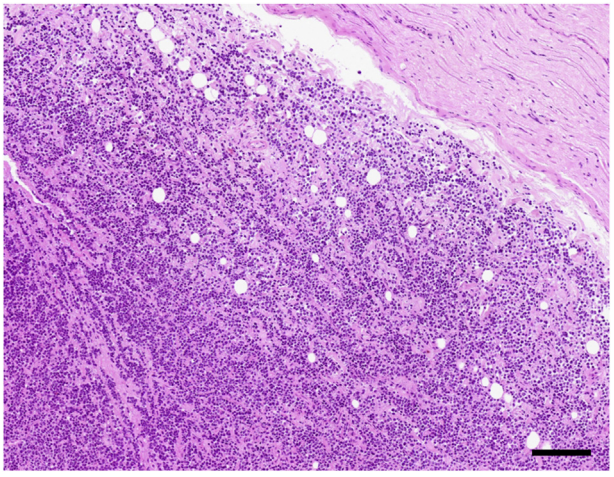

Fig. 4. Focal, dense infiltration of neoplastic lymphoid cells into the brachial plexus. Nerve fibres are fragmented and replaced by neoplastic cells. Note the presence of less affected nerve bundles (top right). HE. Bar, 100 μm.

Since the present lymphoma did not form a mass in any organ, it was difficult to diagnose this case as lymphoma before necropsy examination. Some human cases have masses or swelling of peripheral nerves (Agrawal *et al.*, 2013) and skeletal muscles (Matikas *et al.*, 2013), while others have no apparent masses and are detectable only after biopsy or necropsy examination (Asanome *et al.*, 2018). In cats, B-cell lymphoma in multiple peripheral nerves (e.g. sciatic nerve, multiple brachial plexus) without mass formation has been reported (Higgins *et al.*, 2008). Therefore, neurotropic lymphoma should be considered in the differential diagnosis when an animal presents with neurological signs such as ataxia and hyperaesthesia.

In this case, myocardial injury by the lymphoma was severe and extensive and was considered to be the major cause of death. Primary cardiac tumours are uncommon in dogs and cats and lymphomas infrequently involve the heart (Treggiari *et al.*, 2017). Cardiac neoplasms can cause severe, life-threatening clinical signs with short median survival times. Canine T-cell lymphoma with prominent cardiac and peripheral nerve involvement (Nakagun *et al.*, 2018) and feline primary cardiac lymphoma (Shinohara *et al.*, 2005) are reported. The canine case had enlargement of the cardiac silhouette and shared many similarities with the present case, including involvement of the heart and multiple peripheral nerves, clinical signs (e.g. hindlimb ataxia) and T-cell monoclonality; however, skeletal muscle involvement was not detected in the canine case.

The carcass was kept frozen for 2 days and then thawed overnight before the necropsy examination in this case. Immunoreactivity can be weakened in previously frozen tissue for certain antibodies (Edgerton *et al.*, 2000). The smaller portion of CD3-positive neoplastic cells in this case, compared with samples taken during necropsy examination of fresh carcasses, could be the result of the freezing and thawing. Given the result of the clonality analysis from the same paraffin wax-embedded heart tissue, genomic analyses are considered to be more tolerant to freezing and thawing than IHC.

In summary, to the best of our knowledge, this is the first report of feline lymphoma involving both striated muscle and peripheral nerve. The present case contributes to further understanding of the biological and pathological features of lymphomas in animals.

Conflict of Interest Statement

The authors declare no conflicts of interest with respect to the publication of this manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2019.02.002.

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