NOTE *Pathology*

Neurolymphomatosis in a cat

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ABSTRACT. A 9-year-old male mixed breed cat showed chronic progressive neurological symptoms, which are represented by ataxia and seizures. At necropsy, spinal roots and spinal ganglions at the level of sixth cervical nerve to second thoracic nerve were bilaterally swollen and replaced by white mass lesions. Right brachial plexus and cranial nerves (III, V and VII) were also swollen. A mass lesion was found in the right frontal lobe of the cerebrum. Histologically, neoplastic lymphocytes extensively involved the peripheral nerves, and they infiltrated into the cerebral and spinal parenchyma according to the peripheral nerve tract. Immunohistochemically, most neoplastic lymphocytes were positive for CD20. The clinical and histological features in this case resemble those of neurolymphomatosis in humans.
KEY WORDS: cat, lymphoma, neurolymphomatosis, peripheral nerve

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Neurolymphomatosis is a rare condition defined as infiltration of neurotropic lymphoma or leukemia in the peripheral nervous system [1, 4]. It is characterized by following four clinical presentations; painful involvement of nerves or nerve roots; cranial neuropathy with or without pain; painless involvement of peripheral nerves; and painful or painless involvement of a single peripheral nerve [1]. Most human neurolymphomatoses have been reported to be caused by B-cell lymphomas when classified by the Revised European American Lymphoma or World Health Organization system [4, 5]. In animals, neurolymphomatosis is also very rare [6, 8–11, 13]. To our knowledge, only three cats have been reported as neurolymphomastosis [6, 9, 10].

The central nervous system (CNS) is sometimes affected by neoplastic lymphocytes in human neurolymphomatosis [1]. In animals, a cat with neurolymphomatosis has been reported to have CNS lesions, without obvious clinical CNS signs [10]. This paper describes clinical and pathological findings of a feline neurolymphomatosis case having clinical CNS signs due to lymphoma with extensive involvement of CNS.

A 9-year-old male mixed breed cat was presented with ataxia of right forelimb. At the first visit, neurological examination revealed the upper motor neuron sign of the right forelimb. At the second visit after seven months, the cat showed seizures. The light reflex of right eye, the eyelid reflex, corneal reflex and nictitating membrane reflex of left eye were lost. The lower motor neuron sign of the right forelimb was detected. After that, the cat occasionally showed vomiting and seizures, and was dead at 10 months from the first visit. A complete necropsy was performed immediately after death. During his life, dexamethasone and sulfamethoxazole-trimethoprim therapy for 1 to 14 days improved the condition of the case. Mannitol, furosemide and diazepam were administered to prevent seizures. Magnetic resonance imaging (MRI) examination revealed a mass lesion in the right frontal lobe of the cerebrum (Fig. 1). Serologically, feline immunodeficiency virus was detected, and feline leukemia virus was negative.

At necropsy, spinal ganglions and spinal nerves appeared bilaterally swollen at the level of sixth cervical nerve (C6) to second thoracic nerve (Th2) (maximum diameter 7 mm at C8; Fig. 2). Also, right brachial plexus was swollen compared to the opposite side. A white solid mass lesion (10 mm in diameter) was present in the right frontal lobe of the cerebrum, and the border between the mass lesion and cerebral parenchyma was indistinct. The right cerebrum was edematously swollen. Cranial nerves (III, V and VII) were bilaterally swollen. In addition to the lesions in the nervous systems, adrenal glands were bilaterally swollen (30 mm in diameter). Diff Quik-stained (International Reagents, Kobe, Japan) stamp smear of the cerebral mass showed a single population of immunoblasts with the size of 2–3 times as large as red blood cells. The immunobasts had scant pale basophilic cytoplasms and round to pleomorphic nuclei with prominent nucleoli (Fig. 3).

Samples of the representative lesion and routine organs were collected. Tissues were fixed in 10% neutral phosphate-buffered formalin and processed routinely. Paraffin wax-embedded tissues were sectioned at 4 μm thickness for hematoxylin and eosin stain, Bodian stain, Luxol fast blue stain and immunohistochemistry using primary antibodies

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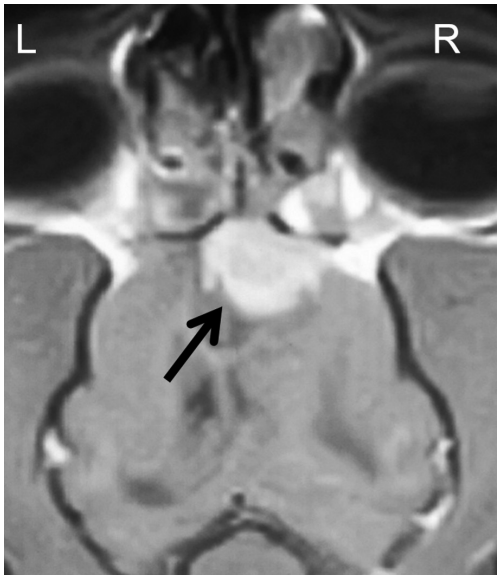

Fig. 1. Cranial T1-weighted magnetic resonance imaging (transverse plane). A mass lesion is present in the right frontal lobe of the cerebrum (arrow).

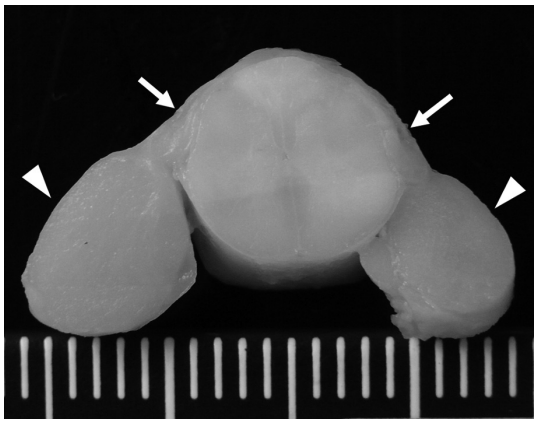

Fig. 2. Transversal section of the spinal cord at the level of eighth cervical nerve. The spinal nerves are bilaterally swollen (arrows), and the spinal ganglions are bilaterally replaced by a whitish mass lesion (arrowheads). Scale bars=1 mm intervals.

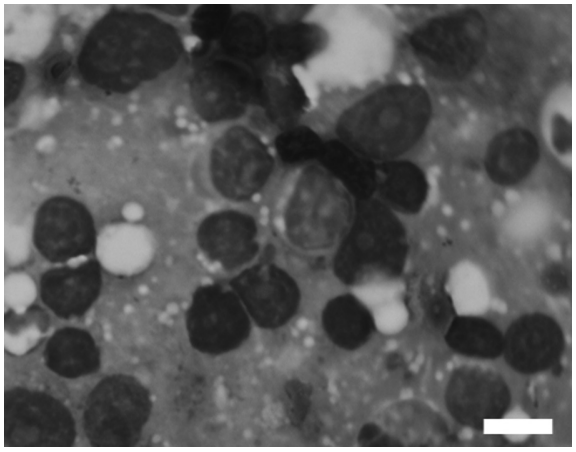

Fig. 3. Cytology of cerebral mass stamp smear. Lymphoblasts with scant pale basophilic cytoplasms and round to pleomorphic nuclei with prominent nucleoli. Diff Quik. Bar=20 μm.

for CD20 (Dako, Glostrup, Denmark; diluted 1:400), CD3 (Dako; diluted 1:20) and proliferation cell nuclear antigen (Dako; diluted 1:100). Immunohistochemistry was performed with Envision-labeled polymer reagent (Dako) and 3,3′-diaminobenzidine tetrahydrochloride as a chromogen (Dako). All immunohistochemistry preparations were counterstained with Mayer's hematoxylin. Feline lymph node was used as a positive control. A specificity control was carried out by replacing the primary antibody with phosphate-buffered saline. Paraffin-embedded samples of cerebral mass and spinal ganglion were submitted to polymerase chain reaction (PCR)-based clonality analysis (Kahotechno, Fukuoka, Japan).

Microscopical examination revealed an infiltrative proliferation of neoplastic lymphocytes in various peripheral nerves, such as the nerve roots and ganglions of the spinal cord, right brachial plexus and the cranial nerves (III, V and VII). In the spinal cord at the level of C6 to Th2, the neoplastic lymphocytes infiltrated into the endoneurium and perineurium of the nerve roots (Fig. 4A). The swollen spinal ganglions were mostly replaced by neoplastic lymphocytes, and remaining gangliocytes became atrophic (Fig. 4B). The swollen cranial nerves were infiltrated by neoplastic lymphocytes and showed the same histological findings as the nerve roots of the spinal cord. Bodian stain and Luxol fast blue stain revealed marked axonal loss with the formation of spheroids and demyelination in the neoplastic lesion. Remaining axons were separated by the neoplastic cells, and myelin sheaths were dilated. The neoplastic cells had discrete cell borders, scant amounts of amphophilic cytoplasms and round to oval nuclei with one to three nucleoli. Moderate anisocytosis and anisokaryosis were observed in the neoplastic cells. Mitotic figures were 25.8 ± 6.6 per 5 fields (400×) of each spinal ganglion (C2 to Th2). Immunohistochemically, the neoplastic cells were diffusely positive for CD20 (Fig. 5A), and a few number of CD3-positive cells were present (Fig. 5B). Approximately 50% of neoplastic cells were positive for proliferative cell nuclear antigen. Along the nerve tract of the dorsal nerve root, CD20-positive neoplastic cells infiltrated into the dorsal horn of the spinal cord at the level of C6 to Th2 (Fig. 6). The spinal parenchyma where neoplastic cells infiltrated into showed spongy changes. The mass lesion in the frontal lobe of cerebrum also consisted of neoplastic lymphocytes. Neoplastic lymphocytes spread in the subarachnoid spaces of the frontal lobe. From the surface of the cerebrum, some neopalastic cells infiltrated into the cerebral cortex. Perivascular cuffing was observed. Severe neuronal loss, edema and gliosis were observed in the cerebral cortex where neoplastic cells infiltrated. No other neoplastic lymphocytes were found in the other lobe of the cerebrum,

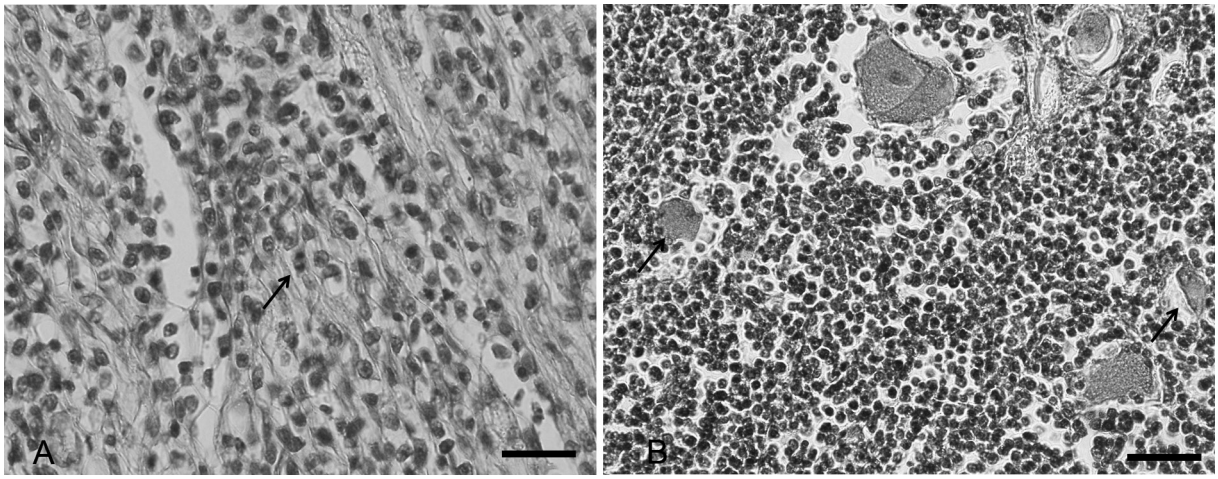

Fig. 4. Neoplastic lymphocytes with mitotic figures (arrow) infiltrate in the endoneurium and perineurium of dorsal root of eighth cervical nerve (A). Spinal ganglion of eighth cervical nerve is mostly replaced by neoplastic lymphocytes, and remaining gangliocytes become atrophic (B, arrows). Hematoxylin and eosin. Bars=40 μm.

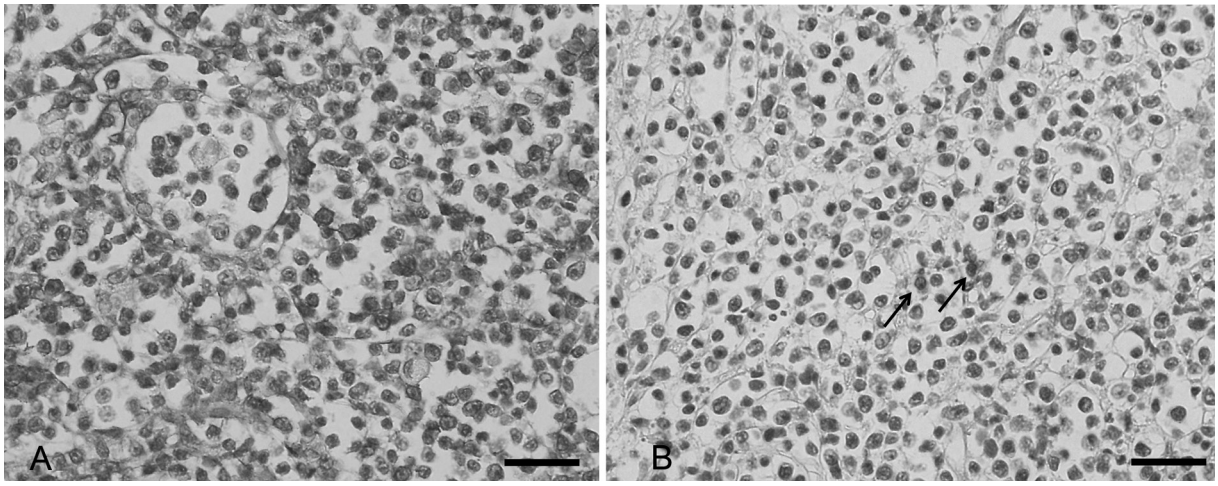

Fig. 5. Most neoplastic lymphocytes are immunohistochemically positive for CD20 (A), and a small number of CD3-positive T-lymphocytes are present (B, arrows). Immunohistochemistry counterstained with Mayer's hematoxylin. Bars=30 μm.

midbrain, cerebellum and medulla oblongata. Additionally, neoplastic lymphocytes were observed in the medulla of the adrenal glands and heart. The adrenal medulla was almost completely replaced by the neoplastic lymphocytes. The focal lymphoma lesion in the heart was restricted just under the endocardium of the left ventricle. PCR-based clonality analysis was negative. Based on these clinical, gross, histological and immunohistological findings, the present case was finally diagnosed as neurotropic lymphoma.

Neurolymphomatosis with neurotropic lymphoma is rare in veterinary literature, however, it has been reported in following three cats; case 1, which had neurotropic lymphoma lesions in the sciatic nerves and multiple nerves of the brachial plexus [6]; case 2, which had neurotropic lymphoma lesions in the eighth spinal ganglions and spinal roots, and right brachial plexus [8]; and case 3, which had neurotropic lymphoma lesions in all cranial nerves, except optic nerves, semilunar ganglia, brain stem and cervival spinal cord [9]. Compared with previous feline cases, the severe involvement of the CNS is a character of the present case. Tissue damage, represented by neuronal loss, in the CNS seems to be responsible for the severe neurological symptoms including seizures.

Immunohistochemistry for CD20 implied that the lymphoma in this case was B-cell origin. It is consistent with previous two feline neurolymphomatosis cases [6, 9]. Also, B-cell lymphoma has been reported in a dog [9] and three horses with neurolymphomatosis [8]. On the other hand, a dog and a cat with T-cell lymphoma have been reported as neurolymphomatosis [10, 13]. As well as human medicines [1], B-cell lymphoma seems to have a tendency to cause neurolymphomatosis in animals. Although a small

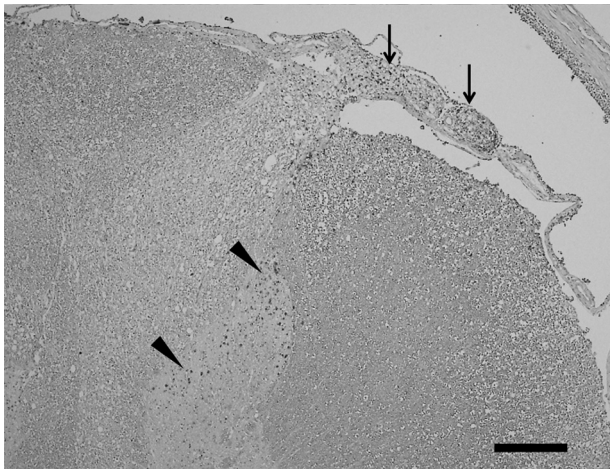

Fig. 6. From the dorsal root (arrows) to dorsal horn (arrowheads), CD20-positive neoplastic B-cells distribute along with the nerve tract in the spinal cord at the level of eighth cervical nerve. Immunohistochemistry counterstained with Mayer's hematoxylin. Bar=300 μm.

number of CD3-positive T-lymphocytes were found in the lymphoma lesion of this case, feline B-cell lymphoma has been reported to be associated with reactive proliferation of T-lymphocytes [2, 12]. On the other hand, we should take attention to CD20-positive T cell lymphoma recently reported in humans [7]. PCR-based clonality analysis was negative in this case, however, it was reported that monoclonality was detected in 63% of human lymphoma cases by PCR–based clonality analysis from formalin-fixed paraffin-embedded samples [3].

The primary lesion of the lymphoma in this case is not identified, because lymphoma in this case was detected at postmortem examination and was already spread through the body. The largest mass lesion was present in the frontal lobe of the cerebrum. Observing spinal cord specimens, neoplastic lymphocytes seemed to infiltrate into the spinal cord parenchyma according to the peripheral nerve tracts. However, it is unlikely that cerebral lymphoma spread through the peripheral nerves according to the nerve tracts, because brain lesion was not found in other regions including the brain stem.

Neurolymphomatosis is rare, however, it should be considered in the differential diagnosis for cats exhibiting progressive neurological symptoms, with or without a history of lymphoma. In the present case, the chronic progressive neurological symptoms severely threatened the quality of life, and the high malignancy of this lymphoma was suggested by its broad distribution, invasive growth towards the central nervous system and high proliferative activity. This case could not be diagnosed as neurolymphomatosis during his life, and an appropriate treatment could not be performed. As in human medicine, MRI and biopsy of affected nerves may be helpful for antemortem diagnosis. Clinically and pathologically, further cases should be investigated to identify the features of neurolymphomatosis in this species.

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