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A T-cell type multicentric lymphoma affecting central nervous system in a Cocker Spaniel dog

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Abstract : A 4-year-old female Cocker spaniel was presented with respiratory distress and abdominal distension. Pleural effusion, ascites, hepatosplenomegaly, and superficial lymphadenopathy were observed and multicentric lymphoma was diagnosed by cytological examination. Immunophenotyping of lymph node and bone marrow using polymerase chain reaction for antigen receptor rearrangement identified a stage V lymphoma originating from T-cell. Despite of systemic chemotherapy using L-asparagenase, vincristine, cyclophoaphamide and prednisolone, neurologic deficits came out and progressed. Cerebrospinal fluid analysis revealed neoplastic lymphocytic pleocytosis indicating central nervous system involvement of lymphoma. The postmortem diagnosis was confirmed based on the histology and imunohistochemistry.

Keywords : central nervous system, dog, multicentric lymphoma, T-cell

Introduction

Lymphoma is one of the most common malignant tumors in dogs [5, 6, 12]. Multicentric form is the most common anatomic location and T-cell tumors have shorter remission and survival times than B-cell origin [3, 10]. Additionally central nervous system (CNS) involvement indicates a poor prognosis [6]. This case report describes the clinical signs, cytology, immunophenotyping using polymerase chain reaction for antigen receptor rearrangement (PARR), pathologic findings and identification of tumor cell surface antigen on immunohistochemical staining of a T-cell originated multicentric lymphoma secondarily affecting brain.

Case report

4-year-old, intact female Cocker spaniel dog was presented with sudden onset of abdominal distension and respiratory distress. Abnormal respiration initiated one month ago and in spite of empirical treatment including glucocorticoid, dyspnea relapsed. Physical examination revealed labored respiration, tachypnea, abdominal distension, and pale mucous membrane. Additionally swollen submandibular and axillary lymph nodes were palpated. A complete blood count showed moderate leukocytosis ($42.62 \times 10^3/\mu l$; reference range, 6.0 to 17.0×10^3 nucleated cells/μl) with a mild left shift (852 bands; reference range, 0 to 300 bands/μl) and lymphocytosis ($13.21 \times 10^3/\mu l$; reference range, 1.0 to $5.0 \times 10^3/\mu l$). A few lymphoblasts were observed on blood film evaluation. A moderate normocytic-normochromic, non-regenerative anemia (packed cell volume 27%; reference range 37 to 55%) and thrombocytopenia ($21 \times 10^3/\mu l$; reference range, 200 to $900 \times 10^3/\mu l$) were also noted. The abnormalities in serum biochemistry were a mild elevation of blood urea nitrogen (35.0 mg/dl; reference range, 9.2 to 29.2 mg/dl), a moderately elevated alkaline phosphate (1454 U/l; reference range 47 to 254 U/l), severe elevation of

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lactic dehydrogenase (3128 U/l; reference range, 20 to 109 U/l) and mild hypoproteinemia (4.5 g/dl; reference range, 5.0 to 7.2 g/dl). On thoracic radiography, increased radio density in cranial mediastinal region, tracheal elevation, and interlobar fissure due to pleural effusion were observed (Fig. 1). Hepatosplenomegaly was evident and radio density in abdominal cavity indicated the possible presence of abdominal fluid. Ultrasonographic findings were consistent with radiographic findings. Thoracocentesis was performed and the serosanguinous pleural effusion was reddish, slightly cloudy and showed pleocytosis (1×10^4 total nucleated cells/μl with low protein content, 1.8 g/dl). The prominent cells were small to medium size lymphocytes and some lymphoblasts with low mitotic figure were noted (Fig. 2A). The cytologic features of fine-needle aspirates of axillary and sternal lymph nodes and liver were same as pleural fluid, indicating lymphoma (Fig. 2B). Bone marrow aspirates showed similar neoplastic lymphocytic infiltration. PARR of lymph node aspirate and bone marrow were conducted (Clinical Immunology Laboratory, Department of Pathology, Colorado State University, USA). A clonally expanded T-cell population was detected in both samples. Based on the diagnosis of World Health Organizatio, stagemulticentric lymphoma was made [18]. The clinical staging as substage b was supported by the presence of systemic abnormalities. After whole blood transfusion for correction of anemia, treatment for lymphoma was initiated using a modified L-CHOP (L-asparaginase, cyclophosphamide, vincristine, doxorubicine, and prednisolone) protocol for dogs described previously [21]. On day 3, respiratory abnormalities disappeared and appetite and activity were returned to normal. No more pleural fluid was detected and swollen lymph node and hepatosplenomegaly were restored to normal size on radiography and ultrasonography. But on day 18, the client observed single episode of incoordination and neurologic signs were progressed. On day 26, brain

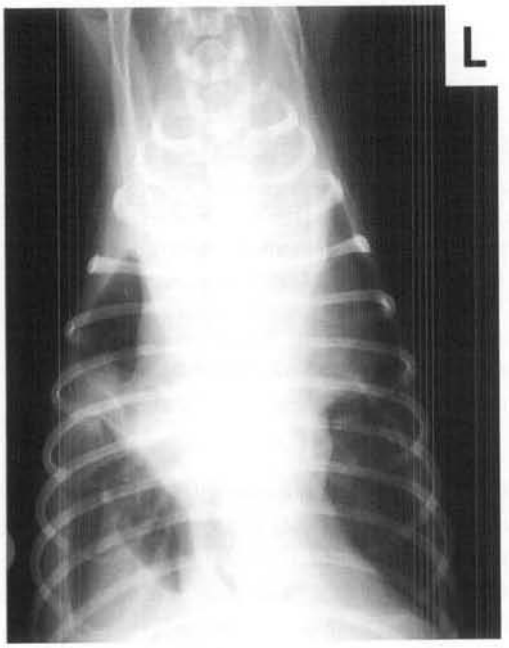

Fig. 1. Thoracic radiography. Large volume of fluid in the pleural space. Note interlobar fissures and widening of the cranial mediastinum. The cardiac silhouette is not visible.

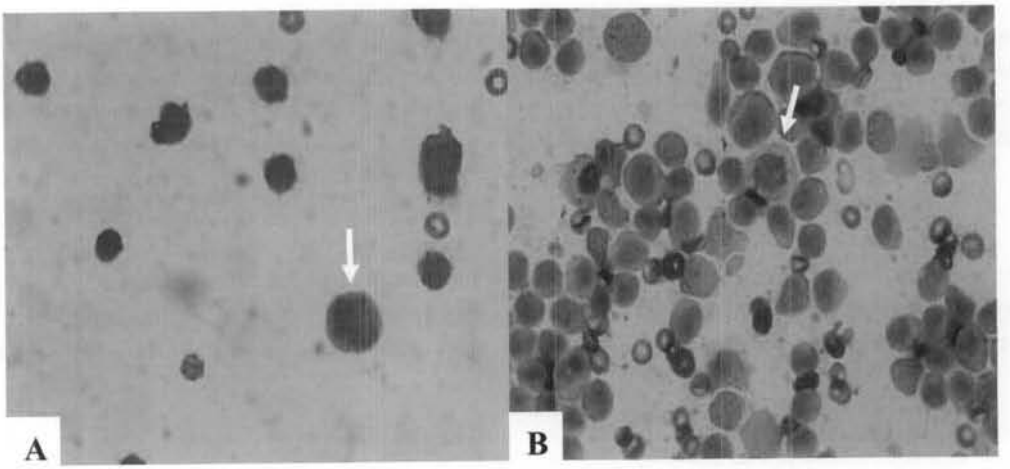

Fig. 2. Microscopic examination of pleural effusion (A) and liver (B). Monomorphic population of large lymphoblasts with mitotic figure is shown (arrows). The nuclear chromatin is fine and nucleoli are visible (Diff-quick stain).

magnetic resonance imaging (MRI) was performed but no abnormality was noted. An episode of tonic seizure developed 2 days later and pre-ictal sign was sustained. On re-examination, behavioral changes as hiding, wandering and depression, generalized weakness, and intermittent tetraparesis were observed and swelling of submandibular lymph nodes were palpated. Cerebrospinal fluid (CSF) analysis demonstrated that nucleated cell count (195 nucleated cells/μl; reference range, 0 to 5 cells/μl) and protein content (35 mg/dl; reference range, less than 20 mg/dl). On microscopic evaluation, a lymphocytic pleocytosis with high mitotic figure, a character of lymphoma was found (Fig. 3) and it highly indicated a CNS involvement of tumor. Cytosine arabinoside (Cytarabine; Choong Wae Pham, Korea) at a dosage of 300 mg/m^2 was given subcutaneously but despite of the supportive therapy, the neurologic dysfunction was progressed acutely. The dog was euthanized by owner's request. At necropsy, hepatosplenomegaly and enlargement of sternal and mesenteric lymph nodes were found; no other significant gross abnormalities were noted. Microscopically, the cerebrum had intense cellular infiltrates of a uniform population of neoplastic round cells in the meninges, perivascular space, and vascular wall (Figs. 4A and B). Apoptosis of the tumor cells was frequent. The sternal lymph node and mesenteric lymph nodes were replaced by the similar neoplastic cells. The spleen had extramedullary hematopoiesis. The bone marrow exhibited hyperplasia of both myeloid and erythroid; however, no neoplastic cell was noted. On immunohistochemistry, the tumor cells strongly expressed CD3 (Fig. 5) yet negative for CD79a, which was suggestive of a lymphoma of T-cell origin.

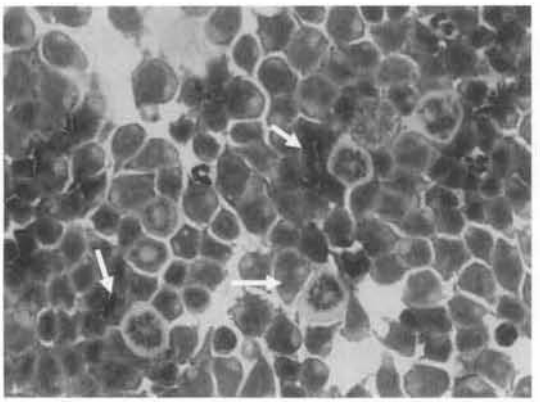

Fig. 3. Cytological microscopic examination of cerebrospinal fluid. A mixed population of medium lymphocytes and large lymphblasts accounts a great part of the cell population. Blast cells often contain prominent nucleolus and mitotic figures are visible (arrows) (Diff-quick stain).

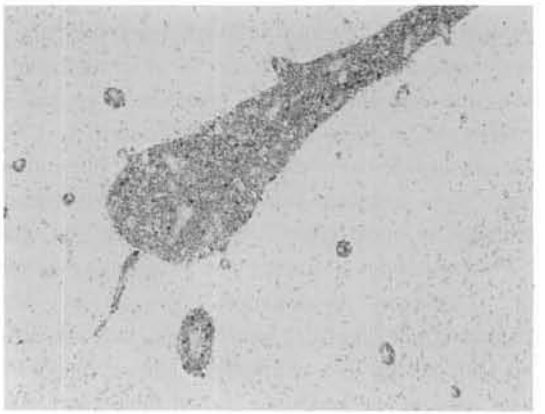

Fig. 5. Immunohistochemical staining with anti-CD3 antisera. CD3-positive cells infiltrated in the meninges and perivascular spaces (NovaRed stain. ×200).

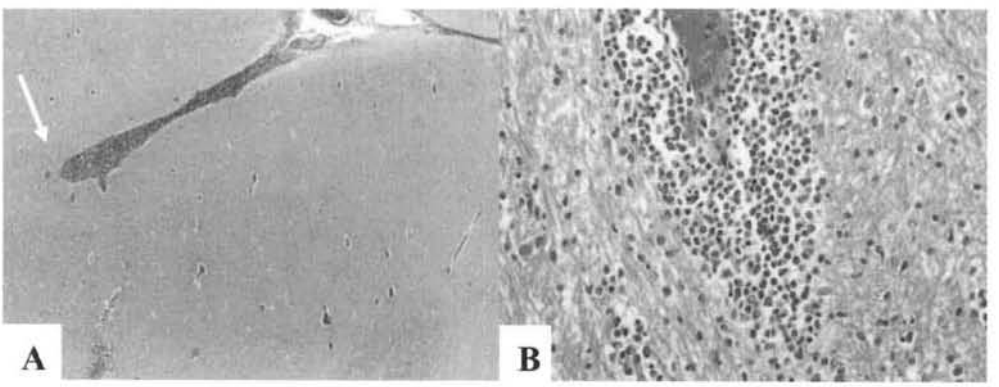

Fig. 4. Histopathology of cerebrum. Note the perivascular neoplastic cuffing (A). Typically, the tumor cells surrounded blood vessels (B) (H&E stain. A: ×100, B: ×400).

Discussion

Lymphoma traditionally occurs as a multicentric disease. In this case, initial pleural effusion and hepatosplenomegaly were conspicuous but superficial lymphadenopathy was not consistent. Not only liver and splenic involvement but bone marrow involvement was identified by PARR at the time of diagnosis. Clinical stage of V b implies the worst prognosis [4, 13]. All canine patients with multicentric lymphoma showed at least partial response to chemotherapy irrespective of cell type [21]. This patient responded to chemotherapy initially but lymphadenopathy was relapsed very soon after initial chemotherapy and moreover, in spite of systemic chemotherapy progressive neurologic deficits were worsened rapidly. She died on day 31 after diagnosis. The chemotherapeutic drugs chosen cannot across the blood-brain barrier, CNS infiltration of neoplastic cells progressed. Histopathology revealed CNS involvement although neoplastic cells in bone marrow were destroyed after initial chemotherapy. Histopathology identified the presence of neoplastic lymphocytes in brain tissue, thus metastasis to CNS was confirmed. Dural metastasis may be difficult to detect with computed tomography or MRI without formation of mass lesions [1, 15, 16]. The dog with CSF abnormalities consistent with lymphoma may not have significant abnormalities detected by MR imaging, but be diagnosed with lymphoma at necropsy and CSF cytology. A definitive diagnosis cannot be based on either method alone [1]. Imaging may not be able to distinguish CNS lymphoma from inflammation but cytology will of the neoplastic cells are present. Consequently, CSF analysis is essential in cases of suspected CNS metastasis of lymphoma. In brain lymphoma, typical lesions involve the meninges or spinal cord and are locally invasive [19]. The lesions are typically proliferative masses of neoplastic lymphocytes but there may be no space occupying masses on gross postmortem [9]. Marked infiltration of neoplastic cells on the cerebral meninges and around small blood vessels in the gray and white matter are detected on histopathology [2, 9], which was identical with this case. The neoplastic cells were positive for CD3 and negative for CD79a, which indicated T-cell origin and is correspondence with the result of PARR. T-cell originating lymphomas are relatively rare than B-cell [7, 11]. The PARR detects neoplastic lymphocytes more often than do microscopic methods and can be useful for detecting or phenotyping of lymphoma [14]. The definitive diagnosis of T-cell lymphoma could be made by histopathology, immunohistochemistry, and PARR. Dogs with T-cell lymphoma are more likely to show systemic signs [8]. Additionally lymphomas with bone marrow involvement have risk of neoplastic CNS infiltration in human [2, 17]. Stage V, substage b, and T-cell origin are all negative factor to prognosis. L-CHOP protocol was chosen, but it showed only brief remission of clinical signs. The long-term survival for dogs with multicentric lymphoma with CNS involvement is guarded and most patient show only a partial or brief remission of clinical signs [19]. After the progress of neurologic sign, cytosine arabinoside was given additionally as it could pass the blood-brain barrier (BBB), but it had no effect. Alkylating agent, Carmustin can cross the BBB therefore it is used in the treatment of CNS tumor [20]. In human lymphoma, carmustine has capacity to prevent the occurrence of cerebral metastasis and is suggested an effective alternative option in combination with other antitumor drugs in canine multicentric lymphoma [20]. If CNS metastasis could be predicted based on bone marrow involvement, another chemotherapy protocol including carmustine should be considered, since CNS metastasis might be delayed or prevented and the survival time was extended. PARR with bone marrow aspiration is a simple and useful option to staging of canine lymphoma and it may be a valuable key in choice of chemotherapy protocol. Like this case, stage V T-cell lymphoma especially involved bone marrow can be diagnosed using PARR and may have the possibility of CNS involvement, thus chemotherapeutic agents that can pass BBB can be considered to be the antitumoral agent of choice.

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