pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 35(4) : 150-154 (2018)
http://dx.doi.org/10.17555/jvc.2018.08.35.4.150

Journal of Veterinary Clinics
www.ksvc.or.kr

Chemotherapeutic Management in a Labrador Retriever with Cutaneous Nonepitheliotropic B-cell Lymphoma

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(Received: March 20, 2018 / Accepted: July 26, 2018)

Abstract : A 9-month-old, castrated, male Labrador Retriever was referred for generalized progressing cutaneous reddish mass lesions with bleeding, scale, crust, and pruritus. On the basis of histopathological findings and the results of immunochemical staining, cutaneous nonepitheliotropic B-cell lymphoma was identified. A cyclophosphamide–doxorubicin–vincristine–prednisolone (CHOP)-based chemotherapy regimen was initiated, and the patient initially showed partial response to vincristine and L-asparaginase, but the cutaneous lesions progressed gradually. After the first cycle of the CHOP-based protocol, lomustine was administered instead. The cutaneous lesions showed partial response to lomustine, but the treatment did not stop the progression of cutaneous lymphoma. The patient was euthanized due to neurologic signs, including reduced consciousness and seizures, 53 days after initial presentation. The postmortem histopathological examination showed systemic metastasis involving the lymph nodes, skin, kidney, ureter, liver, brain, temporal muscle, diaphragmatic muscle, conjunctiva, and oral cavity.

Key words **:** dog, cutaneous lymphoma, CHOP, lomustine (CCNU), B-cell.

Introduction

Lymphoma is the most common hematopoietic tumor in dogs and has 4 anatomic forms: multicentric, mediastinal, alimentary, and extranodal (renal, neural, ocular, and cutaneous) (5,14). Cutaneous lymphoma accounts for only 3% to 8% of all cases of canine lymphoma (10), and cutaneous nonepitheliotropic lymphoma is extremely rare in veterinary medicine (2,3,10,11). Although the etiology of canine cutaneous nonepitheliotropic lymphoma has not been established, it may be associated with genetic, molecular, infectious, environmental, and immunologic factors (3). The species with the highest risk of developing cutaneous nonepitheliotropic lymphoma are St. Bernards, Boxers, Irish Setters, German Shepherd Dogs, Cocker Spaniels, Basset Hounds, Scottish Terriers, and Golden Retrievers (8). In the majority of cases of cutaneous nonepitheliotropic lymphoma, treatment is unsuccessful, with the disease exhibiting short-term remission (1, 8). The median survival period is 4-8 months (1).

This report describes the clinical and histopathological features and response to chemotherapy in a Labrador Retriever with cutaneous nonepitheliotropic B-cell lymphoma that has been rarely reported in veterinary medicine.

Case Description

A 9-month-old, castrated male, Labrador Retriever was referred for generalized cutaneous reddish mass lesions with bleeding, scale, crust, and pruritus. Except for these cutaneous mass lesions, there were no remarkable findings in screening tests performed at a local animal hospital one month ago. Although the patient had been receiving prednisolone (Prednisolone; Korea Pharma., Seoul, South Korea; 0.5 mg/kg, PO, q 12 h) for 10 days, the cutaneous lesions gradually progressed. In our hospital, the initial physical examination showed generalized cutaneous mass lesions with erythema, bleeding, scale, and crust, especially on the trunk, ventrum, groin, thighs, and penis (Fig 1A). The patient did not show any superficial lymph node enlargement.

The results of a complete blood count were within the reference range, but serum biochemical assessments revealed the following abnormalities: elevated alanine aminotransferase level (271 U/L, reference range: 10-100 U/L), elevated gamma glutamyl transferase level (8 U/L, reference range: 0-7 U/L), hyperphosphatemia (phosphate: 7.2 mg/dL, reference range: 2.5-6.8 mg/dL), and hypoglobulinemia (globulin 2.3 g/dL, reference range: 2.5-4.5 g/dL). Thoracic and abdominal radiographs showed generalized subcutaneous mass lesions and enlarged sublumbar lymph nodes. Abdominal ultrasonography revealed irregular enlargement, heterogenous parenchyma, and vascular flow in the left medial iliac lymph node and left inguinal lymph node. Fine-needle aspiration was performed with the nodule of the dorsal skin, and

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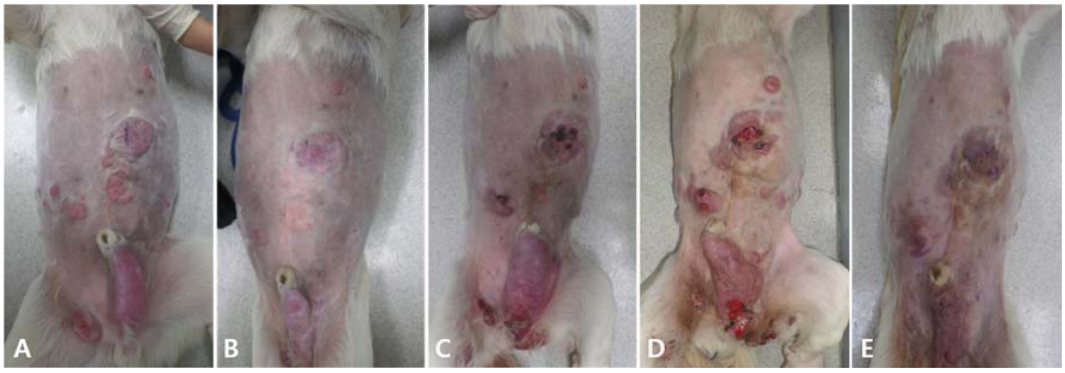

Fig 1. Changes in cutaneous lesions observed on ventrodorsal views following treatment with a CHOP-based protocol and lomustine. Before chemotherapy, generalized cutaneous lesions with erythema, bleeding, scale, and crust, especially on the trunk, ventrum, groin, thighs, and penis were observed (A). Nine days after initiation of the CHOP-based protocol, the measurable tumors showed about 50% reduction in size in response to vincristine and L-asparaginase (B), but 24 days after initiation of the protocol, the cutaneous lesions increased gradually in size and new lesions involving the oral mucosal membrane and conjunctiva were found (C). The CHOP-based protocol was then replaced by lomustine chemotherapy. Seven days after the first cycle of lomustine chemotherapy, the cutaneous lesions showed partial response, but rapidly deteriorated a week later (D). After the second lomustine cycle, the cutaneous lesions showed a 40% reduction in size whereas the conjunctival and oral cavity lesions showed progression (E).

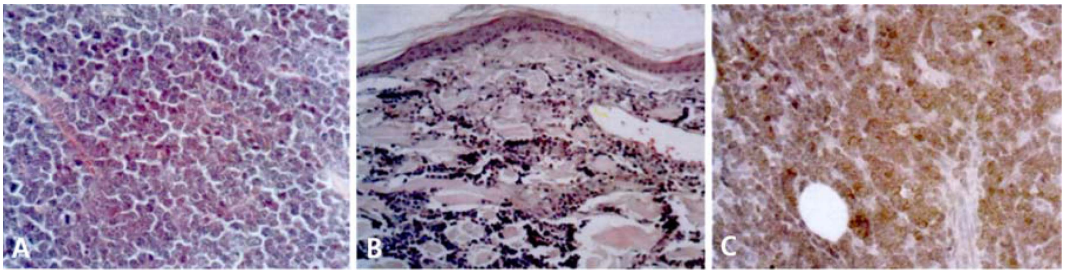

Fig 2. Histopathological images of skin biopsy specimens obtained after hematoxylin and eosin (A and B), and CD79a immunohistochemical staining (C), indicating a diagnosis of cutaneous nonepitheliotropic B-cell lymphoma. The mass consists of a proliferation of large round cells with scant cytoplasm (A). The tumor cells show no epitheliotropism (B) and are positive for CD79a but not CD3.

the samples were stained with the Wright stain (Diff-Quick). Microscopically, the samples showed marked proliferation of lymphoblasts. Based on the histopathological findings and the results of immunochemical staining, the tumor was diagnosed as a cutaneous nonepitheliotropic B-cell lymphoma composed of a proliferation of large round cells with scant cytoplasm in the dermis and subcutis. The tumor cells showed no epitheliotropism and were positive for the B lymphocyte marker (CD79a) but not the T lymphocyte marker (CD3) (Fig 2).

Treatment was initiated with a modified University of Wisconsin 25-week cyclophosphamide-doxorubicin-vincristine-prednisolone (CHOP)-based protocol consisting of vincristine, L-asparaginase, cyclophosphamide, doxorubicin, and prednisolone. Nine days after initiation of the protocol, the measurable tumors showed about 50% reduction in size in response to vincristine (Vincran®; Reyon Pharm., Anseong, South Korea; 0.5 mg/m^2 IV) and L-asparaginase (Leunase®; Kyowa Hakko Kirin Co., Shizuoka, Japan; 400 U/kg SC) (Fig 1B). However, 24 days after initiation of the protocol, the cutaneous lesions increased gradually and new lesions involving the oral mucosal membrane and conjunctiva were noted (Fig 1C). Thirty days after initiation of the protocol, the patient showed anorexia and moderate anemia (packed cell volume: 28.6%, reference range: 37%-55%; RBC count: 5.4 (10^{12}/L), reference range: 5.65-8.87 (10^{12}/L); hemoglobin: 12.6 g/dL, reference range: 13.1-20.5 g/dL). Serum biochemical assessments showed an elevated alanine aminotransferase level (415 U/L, reference range: 10-100 U/L), elevated alkaline phosphatase level (499 U/L, reference range: 23-212 U/L), and elevated gamma glutamyl transferase level (13 U/L, reference range: 0-7 U/L). Subsequent ultrasonography revealed an increase in the size of the left medial iliac lymph node, enlargement of the hepatic lymph node, and malignant changes in the mesenteric fat. The chemotherapy protocol was then replaced by lomustine. A total of 2 cycles of lomustine therapy (CeeNU®; BMS, Montreal, Canada; 68 mg/m^2 and 78 mg/m^2, PO respectively) were administered. Seven days after the first cycle, measurable cutaneous lesions showed more than 50% reduction in size, but the cutaneous lesions rapidly deteriorated a week later (Fig 1D). After the second cycle, cutaneous lesions showed a 40% reduction in size but the conjunctival and oral cavity lesions continued to show progression (Fig 1E). At 53 days after the initial presentation, a buffy coat smear examination revealed many lymphoblasts, indicating bone marrow metastasis. The dog was euthanized due to neurological signs, including reduced consciousness and seizures.

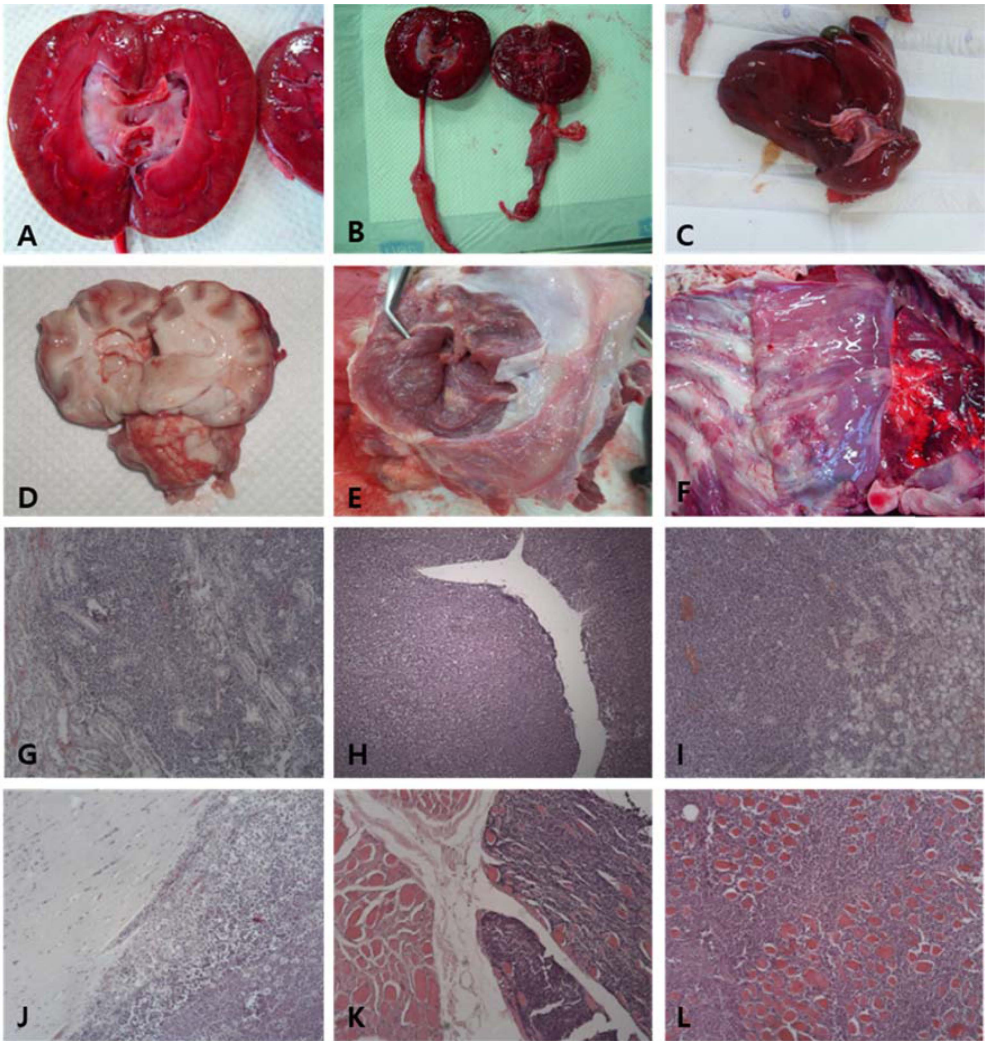

Fig 3. Necropsy (A-F) and histopathological findings (hematoxylin and eosin staining, G-L). Necropsy findings revealed metastatic lesions to the kidney (A), ureter (B), liver (C), brain (D), temporal muscle (E), and diaphragmatic muscle (F). Microscopically, metastasis of numerous tumor cells was identified in the kidney (G, ×100), ureter (H, ×40), liver (I, ×100), brain (J, ×200), temporal muscle (K, ×100), and diaphragmatic muscle (L, ×100).

Postmortem computed tomography and magnetic resonance imaging revealed metastasis to the diaphragmatic muscle, lymph nodes, skin, and brain. Cerebrospinal fluid (CSF) analysis showed an elevated protein level (100 mg/L; reference range, < 25 mg/L) and total nucleated cell count (15,500 cells/μL; reference range, < 5 cells/μL). Cytological examination of the CSF showed lymphoblast predominance. Necropsy findings revealed metastatic lesions involving the lymph nodes, skin, kidney, ureter, liver, brain, temporal muscle, diaphragmatic muscle, conjunctiva, and oral cavity (Fig 3A-3F). Microscopically, metastasis of numerous tumor cells to these organs was identified (Fig 3G-3L), and nonepitheliotropic B-cell lymphoma was again confirmed in the cutaneous masses.

Discussion

Nonepitheliotropic cutaneous lymphoma occurs in older dogs and usually presents as multiple, firm nodules that extend from the dermis to the subcutis (7,8). It is characterized by ulceration and alopecia that can be found anywhere on the body (11). Pruritus, oral mucosal involvement, erythema, and scaling, which are common features of epitheliotropic cutaneous lymphoma, are rarely observed in nonepitheliotropic cutaneous lymphoma (1,11). In this case, the patient was 9 months old, and cutaneous lesions with scale, crust, bleeding, and pruritus involving the oral mucosa were observed. These findings were different from those reported in previous canine cases of nonepitheliotropic cutaneous lymphoma (7).

Assessment of clinical signs, physical examination, laboratory examination, and cytological and dermatohistopathological examinations are helpful for diagnosing cutaneous nonepitheliotropic lymphoma. Immunohistochemical assessments can confirm whether the lymphoma arises from the B or T lymphocytes and predict the prognosis of cutaneous nonepitheliotropic lymphoma (14). While epitheliotropic cutaneous lymphoma always shows a T cell origin, nonepitheliotropic cutaneous lymphoma may originate from B or T lymphocytes and usually shows a T cell origin in dogs

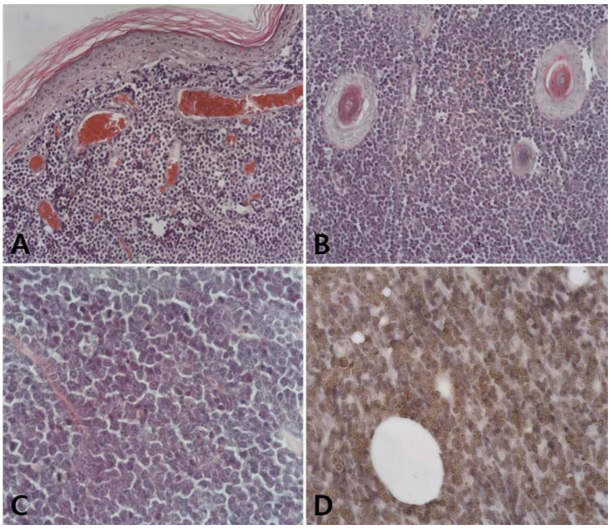

Fig 4. Microscopic examination of the cutaneous mass. Hematoxylin and eosin staining (A-C), CD79a immunohistochemical staining (D). The tumor cells do not infiltrate into the epithelium (A, ×200). Round cells with scant cytoplasm proliferate in the dermis, but hair follicles are not affected (B, ×200). Large and lymphoblastic tumor cells show high mitotic activity (C, ×400). Strong positive reaction to CD79a is seen in the nuclei of the tumor cells (D, ×400).

(1,3,8). We performed immunohistopathological examinations, and cutaneous nonepitheliotropic B-cell lymphoma, which is extremely rare in veterinary medicine, was diagnosed.

Treatment of cutaneous nonepitheliotropic B-cell lymphoma depends on the extent of the disease (8). For solitary lesions, surgical excision or radiation therapy may result in long-term local control or cures, and disseminated lesions can be treated with similar multiagent chemotherapy protocols as those used for cutaneous epitheliotropic lymphoma (1,8). In previous studies, systemic combination chemotherapy was administered in cases of cutaneous epitheliotropic lymphoma, and resulted in remission rates of 65%-84% with a median remission period of 8-13 months (13). Although one case report described treatment with prednisolone in a Golden Retriever with nonepitheliotropic B-cell lymphoma (3), there is no report describing chemotherapeutic trials for canine cutaneous nonepitheliotropic B-cell lymphoma. In the present case, treatment was initiated with the modified University of Wisconsin 25-week protocol. The patient showed partial response during early treatment using vincristine and L-asparaginase, but the response lasted for only 14 days.

Lomustine is an alkylating nitrosourea compound that shows effectiveness in dogs with relapsed lymphoma (9). Currently, lomustine is thought to be the first-line therapy for canine cutaneous epitheliotropic lymphoma (4). Previous studies have reported a response rate of 78%-83% and a median response duration of 88-94 days after a mean of 4 treatment cycles (range, 1-12 cycles) with lomustine at 60-70 mg/m^2 every 3 weeks in canine cutaneous epitheliotropic lymphoma (6,12,13). In the present case, we administered 2 cycles of lomustine therapy (68 mg/m^2 and 78 mg/m^2) to the patient. Although the cutaneous lesions showed partial response whenever we administered lomustine, the response duration was short and systemic metastasis was observed in the postmortem histopathological examination. Thus, we hypothesize that both the CHOP-based protocol and lomustine therapy could not stop the progression of cutaneous nonepitheliotropic B-cell lymphoma.

In conclusion, this report describes the clinical and histopathological features and the response to chemotherapy in a Labrador Retriever with cutaneous nonepitheliotropic B-cell lymphoma. Additional studies are needed to establish an effective treatment regimen for cutaneous nonepitheliotropic B-cell lymphoma in dogs.

Acknowledgement

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2017R1D1A1B03034904).

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