Clinical Pathology

Development of High-Grade B-Cell Lymphoma Concurrent with T-Cell Chronic Lymphocytic Leukemia in a Dog

Takumi OKAWA1), Hiroko HIRAOKA2)*, Yuko WADA1), Kenji BABÂ3), Kazuhito ITAMOTO4), Takuya MIZUNO1) and Masaru OKUDA1)

1)Laboratories of Veterinary Internal Medicine, Department of Veterinary Medicine, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan
2)Laboratories of Veterinary Clinical Pathology, Department of Veterinary Medicine, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan
3)Laboratories of Veterinary Parasitology, Department of Veterinary Medicine, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan
4)Laboratories of Veterinary Clinical Diagnostics, Department of Veterinary Medicine, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, Yamaguchi 753–8515, Japan

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ABSTRACT. Second malignancies are frequent complications in human patients with chronic lymphocytic leukemia (CLL). However, the clinical details and outcome of this phenomenon were unclear in their canine counterparts. Here, we report a dog with high-grade lymphoma concurrent with T-cell CLL. A 10-year-old male golden retriever presented with lymphadenopathies. The lymph nodes contained large-sized lymphocytes, raising suspicion of high-grade lymphoma. Meanwhile, small lymphocytic lymphocytosis in the peripheral blood was consistent with CLL. Interestingly, molecular biological analyses revealed that CLL cells were of the T-cell type, whereas lymphoma cells were of the B-cell type. Chemotherapy using the L-VCA short protocol was effective for 155 days, but the dog died on day 194 after diagnosis, despite rescue therapies.

KEY WORDS: canine, chronic lymphocytic leukemia, high-grade lymphoma, PARR, second malignancy.


Chronic lymphocytic leukemia (CLL) is a neoplastic clonal proliferation of small lymphocytes that manifests as a persistent peripheral lymphocytosis [17]. In human medicine, the development risk of second malignancies is three- to fivefold in patients with CLL [7, 11]. The most frequent event is CLL transformation to diffuse large B-cell lymphoma, also known as Richter’s syndrome (RS) [10, 16]. However, a wide range of lymphoid malignancies (Hodgkin’s lymphoma, prolymphocytic leukemia, acute lymphoblastic leukemia and multiple myeloma) and several solid tumors have been reported as second malignancies in CLL patients [7, 11]. In the veterinary field, publications regarding this phenomenon are minimal. Leifer et al. reported that among 22 CLL cases, three dogs had second malignancies (two lymphosarcoma and one undifferentiated round cell sarcoma) [6]. Another publication reported that three dogs developed high-grade lymphoma (two B-CLL dogs progressed to high-grade B-cell lymphoma, and one T-CLL dog progressed to high-grade T-cell lymphoma) among 43 CLL cases [3]. However, the clinical details and outcome of the disease were not included in these reports, causing the lack of information of this phenomenon in our canine counterparts. Here, we report the case of a dog with T-cell CLL that presumably developed high-grade B-cell lymphoma as a second malignancy.

A 10-year-old male golden retriever was referred to the Yamaguchi University Animal Medical Center (YUAMC) for evaluation of lymphadenopathies that had rapidly progressed over a month’s time (day 1 of presenta-
The dog did not show any clinical signs; however, physical examination revealed bilateral enlargement of superficial lymph nodes (LNs), except for the axillary LNs. Mild splenomegaly and enlarged iliac LNs were confirmed by radiography and ultrasonography. Complete blood count and serum biochemistry revealed small lymphocytic lymphocytosis (73,884/µl; Table 1 and Fig. 1) and increased levels of lactic dehydrogenase (169 IU/l) and C-reactive protein (1.60 mg/dl). The differential diagnosis included CLL/small lymphocytic lymphoma. Cytological examination of the enlarged right prescapular LN showed that almost all lymphocytes were large-sized immature lymphocytes. Morphologically, approximately 80% of all lymphocytes were centroblastic cells, the remainder being immunoblastic cells and a few small lymphocytes (Fig. 2A, B). Based on the updated Kiel classification [4], these findings coincide with the monomorphic subtype of the centroblastic category under high-grade B-cell lymphoma. Thus, the dog was diagnosed with multicentric high-grade lymphoma based on the cytological examination of the superficial LNs. However, this contradicted the clinical finding in the peripheral blood (PB), which showed CLL.

Therefore, a polymerase chain reaction for antigen receptor rearrangement (PARR) analysis (Kahotechno, Fukuoka, Japan) of the LN and PB samples was performed to detect the clonal expansion of lymphocytes [2]. Both samples exhibited rearrangements of the immunoglobulin heavy chain (IgH) gene (B cells) and the T cell receptor (TCR) γ gene (T cells) (Fig. 3A, B) [2]. We also examined the immunophenotype of the LN sample using flow cytometry. The results revealed that most of the lymphocytes in the LN were of B-cell origin (negative for CD3, CD4 and CD8 and positive for CD21, IgM and IgG, with less than 10% of them being Thy1 positive). Hence, we arrived at the following two hypotheses: either 1) both malignancies were of the same origin and might have had concurrent IgH and TCRγ gene rearrangements or 2) two separate types of lymphoproliferative malignancies had developed (incidentally, each sample contained both types of malignant lymphocyte). Based on the diagnosis of high-grade lymphoma, we started chemotherapy using the L-VCA short protocol [17]. On day 8, the patient had gone into partial remission. However, small lymphocytic lymphocytosis (51,615/µl) was still detected. Consequently, PARR analysis of PB was repeated. In contrast to the previous result, only monoclonal TCRγ gene rearrangement was detected (Fig. 3C), highly supporting hypothesis 2 and suggesting that two different types (T cell and B cell) of lymphoproliferative malignancies had developed in the dog.

To reach a definitive diagnosis, we attempted bone marrow examination on day 22. The bone marrow was normocellular, and the ratio of myeloid to erythroid cells was 0.84. The blast ratio in all nucleated blast cells (ANCs)

Table 1. Complete blood count on day 1

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>Day 1</th>
<th>Reference range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>×10⁶/µl</td>
<td>5.2</td>
<td>5.5–8.5</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>g/dl</td>
<td>12.3</td>
<td>12.0–18.0</td>
</tr>
<tr>
<td>PCV a)</td>
<td>%</td>
<td>40</td>
<td>37–55</td>
</tr>
<tr>
<td>MCV b)</td>
<td>fl</td>
<td>70.2</td>
<td>60–77</td>
</tr>
<tr>
<td>MCHC c)</td>
<td>g/dl</td>
<td>33.6</td>
<td>32–36</td>
</tr>
<tr>
<td>White blood cells</td>
<td>/µl</td>
<td>78,600</td>
<td>6,000–17,000</td>
</tr>
<tr>
<td>Band neutrophils</td>
<td>/µl</td>
<td>0</td>
<td>0–300</td>
</tr>
<tr>
<td>Segmented neutrophils</td>
<td>/µl</td>
<td>4,716</td>
<td>3,000–11,500</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>/µl</td>
<td>0</td>
<td>100–1,250</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>/µl</td>
<td>73,884</td>
<td>1,000–4,800</td>
</tr>
<tr>
<td>Monocytes</td>
<td>/µl</td>
<td>0</td>
<td>150–1,350</td>
</tr>
<tr>
<td>Platelets</td>
<td>×10³/µl</td>
<td>251</td>
<td>200–500</td>
</tr>
</tbody>
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a) PCV: packed cell volume. b) MCV: mean corpuscular volume. c) MCHC: mean corpuscular hemoglobin concentration.

* Schalm’s Veterinary Hematology, 6th ed.
was 4.6%. However, the percentage of mature, small lymphocytes similar to those in PB was 36.6% among ANCs. There was no increase in the number of immature lymphocytes. Lymphocytes from the bone marrow showed clonal expansion of only the TCRγ gene (data not shown). This led to a conclusive diagnosis of T-cell CLL concurrent with B-cell lymphoma.

On day 155, while the dog was still under treatment with the L-VCA short protocol, the superficial LNs suddenly enlarged. An increased number of large-sized lymphoma cells were detected in cytological samples of the enlarged LNs. Therefore, we resorted to rescue protocols, first using the L-asparaginase and lomustine combination therapy [14], followed by the D-MAC protocol [1] when the former protocol failed. Unfortunately, the dog did not improve and died on day 194.

In this case, we were not able to determine which malignant clone was first triggered. Commonly, canine CLL usually develops with no clinical sign. It was possible that our patient first developed T-cell CLL, followed by high-grade B-cell lymphoma as a second malignancy. Moreover, the survival duration of our patient was relatively short (194 days) compared with the survival of dogs with common high-grade B-cell multicentric canine lymphoma treated using the L-VCA short protocol (median survival duration, 396 days [17]) or T-cell CLL (median overall survival time, 930 days [3]).

The development of high-grade lymphoma, most commonly diffuse large B-cell lymphoma, in patients with CLL is regarded as a classical form of RS in human medicine [8, 11, 13]. Plasmablastic lymphoma and high-grade T-cell lymphoma have also been reported as unusual variants of RS [9, 12]. In the classification of the World Health Organization, “CLL” is referred to as monoclonal neoplastic B-cell proliferation, whereas the entity previously described that T-cell CLL is now known as T-cell prolymphocytic leukemia [5]. According to the classification in the veterinary field, canine T-cell CLL is still included in the CLL category [15], so this might lead to confusion when we use the term RS in our animal patients.

To the best of our knowledge, this is the first report describing a dog with T-cell CLL that presumably developed high-grade B-cell lymphoma as a second malignancy. Several etiological mechanisms have been reported in the development of second malignancy in human CLL, including genetic abnormalities, microsatellite instability and epigenetic abnormality [11]. In veterinary medicine, more case reports and analyses of etiological factors are required in order to clarify the similarity of this phenomenon between the canine and human.

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REFERENCES


