Sequential development of B-cell lymphoma of alternative lineage after treatment for mantle cell lymphoma in a dog

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Abstract
An 11-year-old spayed female Chihuahua visited this hospital as a regular follow-up for the previous splenomegaly problem. Ultrasound examination revealed an enlarged spleen with a honeycomb appearance. Fine needle aspiration and polymerase chain reaction for antigen receptor rearrangement of lymphocytes suggested possible B-cell lymphoma, and the subsequent splenectomy and histopathology led to a diagnosis of mantle cell lymphoma. The patient was then monitored with no further treatments. On day 511, the peripheral lymph nodes were found enlarged, leading to a diagnosis of intermediate grade B-cell lymphoma. Genome sequencing analysis revealed that the splenic mantle cell lymphoma and lymph node lymphomas were dissimilar and that they arose independently of one another. Although our observations imply that splenic mantle cell lymphoma was treated successfully with splenectomy alone, a subsequent independent malignancy arose in the same patient.

Key words: canine, gene sequence analysis, mantle cell lymphoma, spleen, splenectomy

Conflict of Interest:
The authors declare that there is no conflict of interest regarding the publication of this paper

Mantle cell lymphoma (MCL), a B-cell neoplasm that develops within the mantle cuff, is an indolent lymphoma that progresses slowly [19]. This lymphoma is typically splenic and is of two types: one that forms a solitary mass or multiple masses, and the other in which nodules grow diffusely throughout the spleen [17]. The MCL is rare in domestic animals and is rarely recognized as a disease in its own right. This lack of recognition could be because tests specific for MCL are only run once the neoplasm has reached an advanced stage; at this point, mantle cells from the adjacent germinal center fuse, causing MCL to present more like a pervasive neoplasm than a neoplasm that develops close to the germinal center [19]. Lesions also occur in the liver, lymph nodes, and bone marrow (peripheral blood). Recently, MCL has been added to the World Health Organization (WHO) criteria for the veterinary field based on its listing for humans [19, 20]. Although the behavior and prognosis of MCL is not well known, splenectomy has yielded favorable outcomes in cases of splenic MCL [22].

Similar to MCL in humans, infiltration into the lymph nodes, spleen, blood, and marrow is common in dogs; remission after standard therapy is short, and the median survival is 4–5 years. Treatment and prognosis depend on risk classification.
For indolent MCL, the recommended initial therapy is follow-up. Conversely, for aggressive MCL, the recommendation is proactive treatment, such as a combination of molecular-targeted drugs and chemotherapy [2, 23].

The present study describes a case in which MCL in a dog was treated with splenectomy alone, and a novel lymphoma was found on the body surface lymph nodes of the animal during the follow-up.

An 11-year-old spayed female Chihuahua weighing 2.4 kg was brought to this hospital for a health check-up (day 0). She did not present with any clinical symptoms. We performed a general physical examination, complete blood count (CBC), blood chemistry examinations, urinalysis, thoracic/abdominal radiography (both three-view), cardiac ultrasound, and abdominal ultrasound. The ultrasound examination revealed a moderate enlargement of the spleen with diffuse hypoechoic pea-sized lesions resulting in a honeycomb appearance (Fig. 1). Cytological analysis of the spleen showed that the specimen primarily consisted of mature lymphocytes, interspersed with medium-to large-sized lymphocytes. Neither the body surface lymph nodes nor the intra-abdominal lymph nodes were enlarged at that time. The patient was asymptomatic, and the owner did not request further testing. Consequently, we elected to conduct monthly assessments.

On day 202, a splenic ultrasound revealed splenomegaly and fairly large hypoechoic lesions measuring approximately 1 cm in diameter, in addition to a well-defined honeycomb appearance. Cytological analysis of the splenic samples yielded the same findings as before. We confirmed the clonality of the B-cells following polymerase chain reaction (PCR) for the antigen receptor rearrangements (PARR) analysis of splenic lymphocytes using the cytological material. On day 237, the patient demonstrated progressive splenomegaly and irregular margins, but enlargement of other lymph nodes or abnormalities in other organs were not observed. We therefore performed a splenectomy. During the operation we resected a portion of the liver for biopsy, despite this organ appearing macroscopically normal. Histopathological examination of the spleen yielded a diagnosis of splenic MCL (small-cell type) (Fig. 2). Histopathology of the liver demonstrated aggregated lymphoid nodules around the portal and the central veins. Although inflammatory and neoplastic changes (early MCL lesions) were proposed as differential diagnoses, lymphocyte clonality in the liver tissue was negative. Splenectomy was performed with no adjuvant therapy. Instead, we conducted monthly examinations, consisting primarily of general physical examinations CBC, blood chemistry, and abdominal ultrasonography.

On day 511 (day 274 after splenectomy), we observed enlargement of the bilateral submandibular lymph nodes (<2 cm) and the right superficial cervical lymph node (0.7 cm). Cytology of the left submandibular lymph nodes showed that approximately 90% of the cells were medium-sized lymphocytes about the same size as neutrophils, with a small number of large and small lymphocytes. Neutrophils and macrophages were also observed. The medium- and large-sized lymphocytes had oval nuclei, sparse chromatin, a single nucleolus located in the center or slightly on the edge of the nucleus, and a small amount of pale basophilic cytoplasm around the nucleus. No mitotic figures were seen. Lymphoglandular bodies were observed in the background. A pathologic diagnosis of intermediate grade lymphoma was made. The PARR analysis of lymphocytes again revealed the B-cell clonality. We next performed several additional tests to determine whether the second neoplasm was related to the orig-
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Electrophoresis of the PCR products obtained using specific primers for the splenic and lymph node lymphocytes yielded distinct band patterns for the two lymphocyte samples (Fig. 3). A melting curve analysis showed that the peak temperatures (Tm) of the main bands differed (Fig. 4). Finally, we sequenced the products of PCR for the IgH* (IgH: immunoglobulin heavy chain) (Fig. 3); we used the primer pairs CB1 and CB2, which were described in the study by Burnett et al., respectively [4, 16]. In (B), bands are observed at approximately 118 bp in the IgH (1) and IgH (2) lanes, but not in (A). The tumor cells, from the spleen are therefore genetically distinct from those in the submandibular lymph node.

The subtypes of primary splenic lymphomas include, marginal zone lymphoma (MZL), MCL, large diffuse B-cell lymphoma, follicular lymphoma, peripheral T-cell lymphoma, and natural killer cell lymphoma [20]. The most common splenic B-cell lymphomas are the MZL and MCL [11, 15, 21]. In dogs, MCL accounts for 1.6% of all lymphomas [18]. Due to its indolence, MCL goes undetected until it progresses, with clinical symptoms sometimes not manifesting for up to 2 years [18, 21]. It was the case in this patient. Despite the abnormalities observed in spleen ultrasonography, our patient was asymptomatic when presented to this hospital for a health examination. Thus, our case indicates that regardless of the presence of symptoms at the time of examination at a primary care facility, regular screenings are important and should be recommended to pet owners to enable early detection and treatment of diseases.
Cytology for small cell type MCL shows presence of small lymphocyte nuclei, dense chromatin, round, shallow notches and depressions, ill-defined nucleoli, cytoplasm of extremely low volume, and a low mitotic index [17, 19, 21]. Thus, small cell type MCL is primarily composed of mature lymphocytes, which makes the diagnosis difficult using cytology alone. In the present case, combining cytologic material-based PARR of lymphocytes with cytology bolstered our suspicion of lymphoma and enabled us to proceed with further treatment despite the absence of clinical symptoms. Although needle biopsy is inexpensive and minimally invasive, it may not lead to proper diagnosis when used alone. In such cases, needle biopsy should be proactively combined with other testing methods.

In a previous report, a combination of splenectomy and chemotherapy was used to treat dogs with lymphoma that had triggered splenic rupture and splenomegaly [3]. However, multicentric lymphoma in dogs is typically treated systemically with chemotherapeutic agents [5, 7, 8, 13, 14]. According to the WHO classification [10], splenic lymphoma is classified as stage 4, which is a clinically advanced stage. Indolent B-cell lymphoma has a poor prognosis when it progresses and extends to a wide range of organs [11]; however, splenectomy alone without adjuvant chemotherapy can engender long-term survival in MCL localized to the spleen [22]. In the present case, we suspected indolent B-cell lymphoma based on the splenic ultrasonography findings, cytology findings, the PARR analysis of lymphocytes, and slow disease progression. Due to the lack of invasion into other organs, we elected to first perform splenectomy rather than initiating chemotherapy, which is the conventional treatment. The actual disease was diagnosed as splenic MCL, for which we secured a favorable postoperative course. Liver biopsy, which was performed simultaneously, revealed aggregated small lymphocytes but did not confirm lymphocyte clonality, no changes were observed in regular postoperative blood chemistry testing or in imaging examinations, indicating the possibility of inflammatory lesions. This result supports a previous finding that MCL localized in the spleen can be treated favorably with splenectomy alone [22].

Primary splenic indolent B-cell lymphoma that becomes progressive and extends outside the spleen is associated with poorer outcomes than B-cell lymphoma restricted to the spleen [1, 22]. Similar to other forms of lymphoma, chemotherapy has been indicated for B-cell lymphoma treatment [6, 9, 12]. Similar to humans, canine patients are also stratified according to specific risk factors. Therapies and prognoses differ according to these strata: follow-up is recommended for low-risk patients, while chemotherapy with rituximab ± a CHOP base is recommended for intermediate- and high-risk patients [23]. In the present case, on day 511 (day 274 after splenectomy), we confirmed mild enlargement of the peripheral lymph nodes and diagnosed the patient with B-cell lymphoma (intermediate grade) based on cytology and the PARR analysis of the lymphocytes. If this lesion was the result of progression of a primary splenic MCL, the subsequent course would be predicted to be poor, and proactive combination chemotherapy would be required. However, if it was a novel lesion, it would have been acceptable to consider the time of treatment initiation and the drug of choice based on the absence of symptoms, disease progression, and the intermediate grade of the lesion. Therefore, we performed gene sequence analysis to determine whether the splenic MCL and body surface lymph node lymphomas originated from the same lesion. Although we were able to analyze the gene sequence in the lymph nodes, the gene sequence in the spleen was unreadable. This unreadability may have been a result of an apparent mixing of two separate PCR products within the splenic sample. This mixture of PCR products was indicated by the presence of two bands with different migration properties, albeit this difference being subtle. There are two explanations for this apparent mixing of PCR products. First, V(D)J recombination may have occurred in the \( \text{IgH} \) gene of the B-cells at both the anatomical sites. Second, each B-cell type propagated clonally. However, the two PCR products demonstrated different electrophoresis patterns and Tm in the melting curve analysis, suggesting that the lymphomas in the spleen and the lymph node arose independently.

In the present case, we were able to detect an early stage MCL localized to the spleen in a dog by combining multiple testing methods for screening the asymptomatic patient, which enabled an early treatment. In addition, our results supported a previous study which showed that splenectomy alone without chemotherapy yielded favorable outcomes for the treatment of MCL localized in the spleen [22]. Although we could not fully compare the gene sequences of the splenic MCL and the subsequent lymph
Sequential development of B-cell lymphoma of alternative lineage after treatment for mantle cell lymphoma in a dog

node lesion, we used two other methods to demonstrate that they are most likely different from one another. This information was useful for designing the subsequent treatment regimen. In the future, we hope that research into developing effective therapies and prognoses by defining lymphoma subtypes will progress, thereby enabling optimal treatment to be tailored to individual patient.

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マントル細胞リンパ腫の治療後に別系統の
B細胞型リンパ腫が発生した犬の1例
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和文要約

11才、避妊手術済チワワが健康診断のために来院した。超音波検査を実施したところ、脾臓に蜂の巣状エコー所見が
認められ、FNAとリンパ球のPARRの結果からB-cellリンパ腫が疑われた。脾臓摘出術が実施され、マントル細胞リンパ
腫(MCL)と診断された。第511病日、体表リンパ節が腫大しB-cellリンパ腫と診断された。遺伝子シークエンス解析により、
脾臓のMCLとリンパ節のリンパ腫は異なり、それぞれ独立して発生したことが明らかになった。このことから、同じリン
パ節の悪性腫瘍にも関わらず、脾臓に局所したMCLは脾摘単独で良好に治療可能であると考えられる。

Key words: canine, gene sequence analysis, mantle cell lymphoma, spleen, splenectomy

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受付日: 2017年3月31日／採択日: 2021年11月10日