Int J Clin Exp Pathol 2016;9(2):2389-2395
www.ijcep.com /ISSN:1936-2625/IJCEP0019316

Case Report

Diagnosis and pathological characteristics of canine lymphoma: report of 4 cases

Shan-Shan Jia[1,2], Zhen-Zhong Hou[1], Bo Meng[2], De-Bin Ma[2]

[1]College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China; [2]School of Science, Harbin University, Harbin 150086, China

Received November 6, 2015; Accepted January 3, 2016; Epub February 1, 2016; Published February 15, 2016

Abstract: This study aimed to analyze and sum up the clinical diagnostic method of canine lymphoma. Diagnostic methods applied on 4 cases of lymphoma were summarized and studied. Pathological tissue sections and immunohistochemistry were used as the primary diagnostic tools, while blood biochemical analysis, imaging, toluidine blue staining and IgH gene clonal rearrangement detection were used as adjunct diagnostic tools. Appropriate diagnostic methods were flexibly taken depending on the circumstances of the dogs, which provided a reference for clinical diagnosis.

Keywords: Canine lymphoma, diagnosis, histopathologic sections, immunohistochemistry

Introduction

Lymphoma, as the malignant tumor that originates in the lymph nodes or lymphatic tissue, accounts for 7%-24% of neoplastic conditions in dogs. Lymphoma can be present at all ages and breeds of dogs, but are more apt to occur in dogs aged 6-12 years. The typical manifestation of the superficial tissue is the painless, progressive enlargement of lymph nodes, which are smooth and in tough texture. Such lymphadenopathy is most common in the neck and supraclavicular lymph nodes, and also present in the axillary and inguinal lymph nodes. Concealed lymphadenopathy is the major initial manifestation in some dogs, usually in more evident forms when detected. The incidence of the disease may be due to genetic factors and the environment the animal lives in [1-4]. According to the site of its occurrence, lymphoma can be divided into multi-center lymphoma, gastrointestinal lymphoma, cutaneous lymphoma, central nervous system lymphoma and extra-lymph node lymphoma etc. [1]. Lymphoma diagnosis requires detailed history, physical examination, comprehensive laboratory tests and imaging examinations [5]. In this study, four cases of canine lymphoma encountered in practice were analyzed to summarize the manifestations and pathological characteristics, so as to provide reference for clinical diagnosis.

Case source and condition

Canine lymphoma cases treated in College of Veterinary medicine, Northeast Agricultural University between Jan 2013 and Dec 2014 were investigated, and data concerning age, sex, breed and chief complaint were recorded in **Table 1**.

Diagnostic methods and results

Different diagnostic methods were assumed according to the conditions and inflicted areas. Biopsy sample of the swelling site from Case 1 was extracted for the initial diagnosis by WG staining, which was then verified by HE, toluidine blue and immunohistochemical stainings. As per previous endoscopic results of Case 2, gastrointestinal tissue biopsy sample was extracted for the diagnosis by HE, toluidine blue and immunohistochemical stainings. Biopsy samples from mandibular lymph nodes and spleen tissue of Case 3 were taken for diagnosis via HE and immunohistochemical stainings. Case 4 was examined through blood tests, peripheral blood and ascitic smears, as well as

Table 1. The characteristics of 4 case canine lymphoma

Case	Breed	Sex	Age (years)	Chief complaint
1	Shih Tzu	Male	12	Right forelimb terminal swelling, abdominal hair loss, pigmentation and erythema, severe itching
2	Poodle	Male	8	Bloating, loss of appetite, vomiting after meals, previous gastric endoscopy revealed antral polyp
3	Hybrid	Female	8	Diarrhea, anemia, splenomegaly, thrombocytopenia
4	Hybrid	Female (already neutered)	13	Anemia, ascites, hepatosplenomegaly

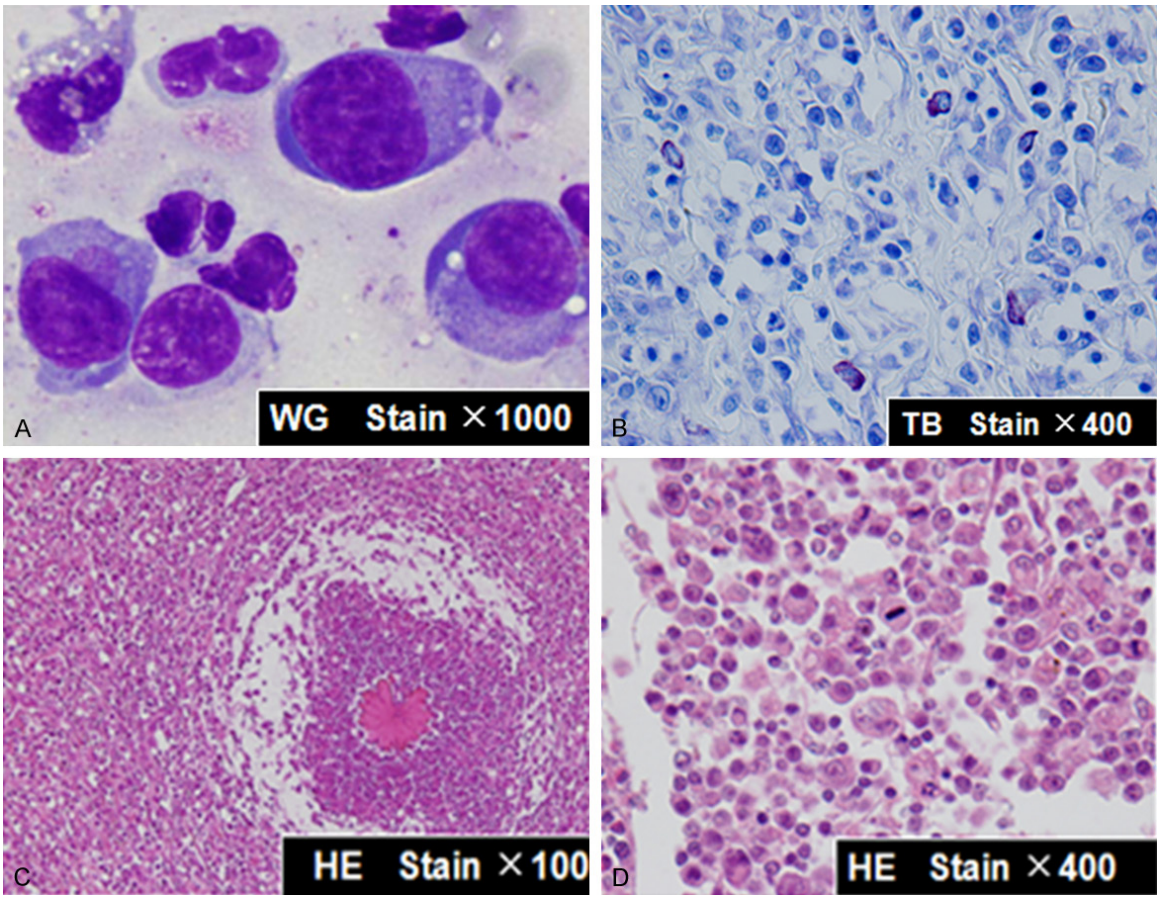

Figure 1. Pathological characteristics of case 1. A: Peroxidase staining of lesions; B: Toluidine blue staining of skin lesions; C: HE staining of skin lesions 100×; D: HE staining of skin lesions 400×.

immunohistochemical staining of spleen biopsy to establish diagnosis.

Diagnosis of case No. 1

Smear of forelimb lesions obtained by fine needle aspiration were examined by WG staining, and irregular round mast cells were visible, with large nuclei and parts of the nuclear membrane close to the cell membrane. Vacuole-like structures and blue-black peroxisomes were present in the cytoplasm (**Figure 1A**). However, only a very small amount of positive particles were observed in the toluidine blue staining (**Figure 1B**). Pathological tissue sections were observed under the microscope. Proliferating cells and eosinophilic granuloma could be found in the dermis. Eosinophilic infiltration-associated globular mass was visible in the 100× microscopic field (**Figure 1C**). Massive and diffuse high-density monocyte/lymphocyte proliferation scattered to the lymphatic sinus was visible in the 400× field (**Figure 1D**). Allowing for the eosinophil infiltration, mast cell tumor was

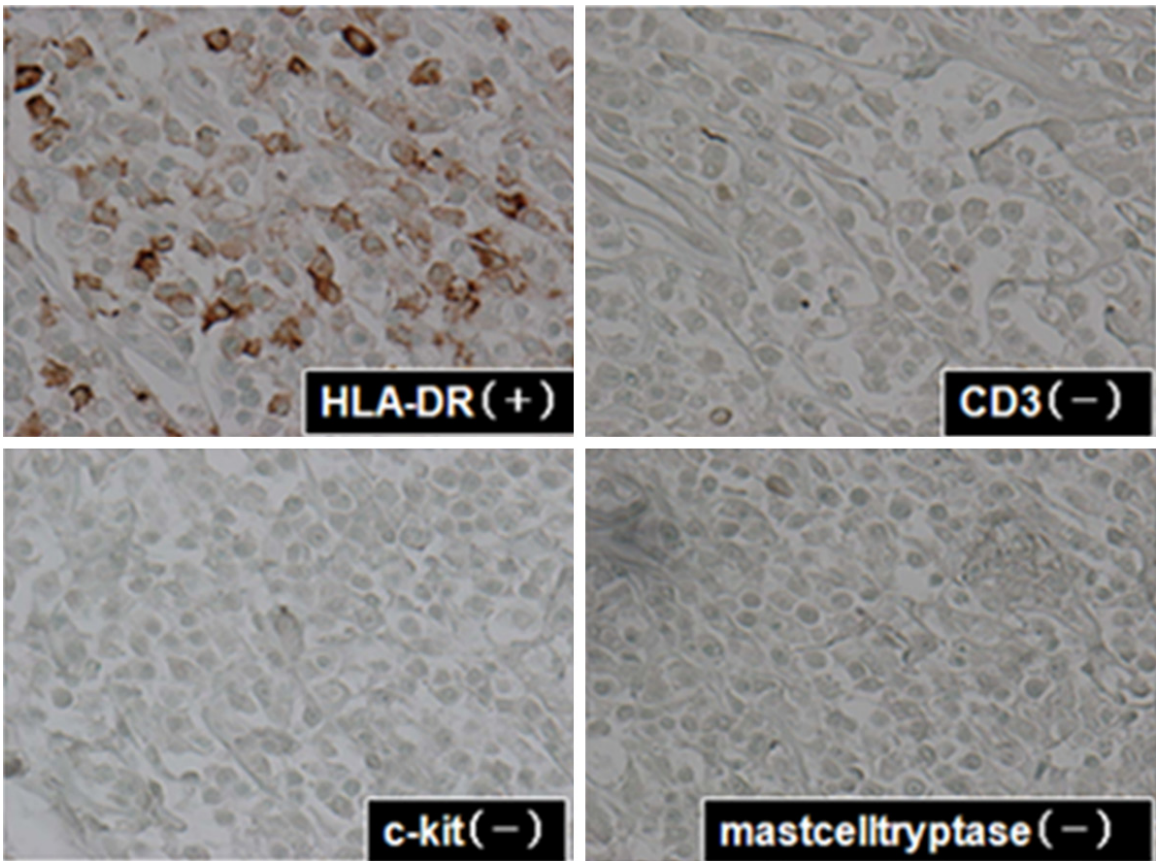

Figure 2. Immunohistochemical staining of skin tissuelesions.

considered as the initial diagnosis. So further immunohistochemical assays were conducted using c-kit, mast cell tryptase, CD3 and HLA-DR antibody. The results were positive in HLA-DR rendering, and negative in c-kit, mast cell tryptase, CD3 rendering (**Figure 2**). Such results denied the possibility of mast cell tumor and T-cell lymphoma, but established the diagnosis of non-epithelial tropism (derived from B cells) lymphoma.

Diagnosis of case No. 2

Samples of duodenal mucosa and gastric polyp from the dog were examined by HE staining, which showed duodenal edema, lymphatic duct dilatation and lymphocyte infiltration (**Figure 3A**). And atypical independent round proliferation of cells with prominent nucleoli, and eosinophil infiltration were present in polyps from the antral part of the stomach (**Figure 3B**). A few positive cells, which were observed in the results of toluidine blue staining (**Figure 3C**), were accordingly suspected as from mast cell tumors. CD3 expression proved positive in spleen tissue via immunohistochemical staining (**Figure 3D**). Based on the above findings, the diagnosis was T-cell lymphoma.

Diagnosis of case No. 3

By immunohistochemical staining, TCR-positive cells (**Figure 4A**) and CD3-positive cells (**Figure 4B**) were present in the polyp tissue. And the CD3-positive cells were found to invade the epithelium (**Figure 4B**). Atypical lymphocyte proliferation and myriads of binucleate cells were found in the HE staining of tissue sections from spleen lymph nodes of the dog (**Figure 4C**). Atypical lymphocyte proliferation was also observed in the HE-stained tissue sections of mandibular lymph nodes (**Figure 4D**). The dog was diagnosed with T cell lymphoma.

Diagnosis of case No. 4

According to the symptoms of Case 4, the blood biochemical tests (**Table 2**) showed that the values of white blood cell (WBC) and C-reactive protein (CRP) were higher, while the values of

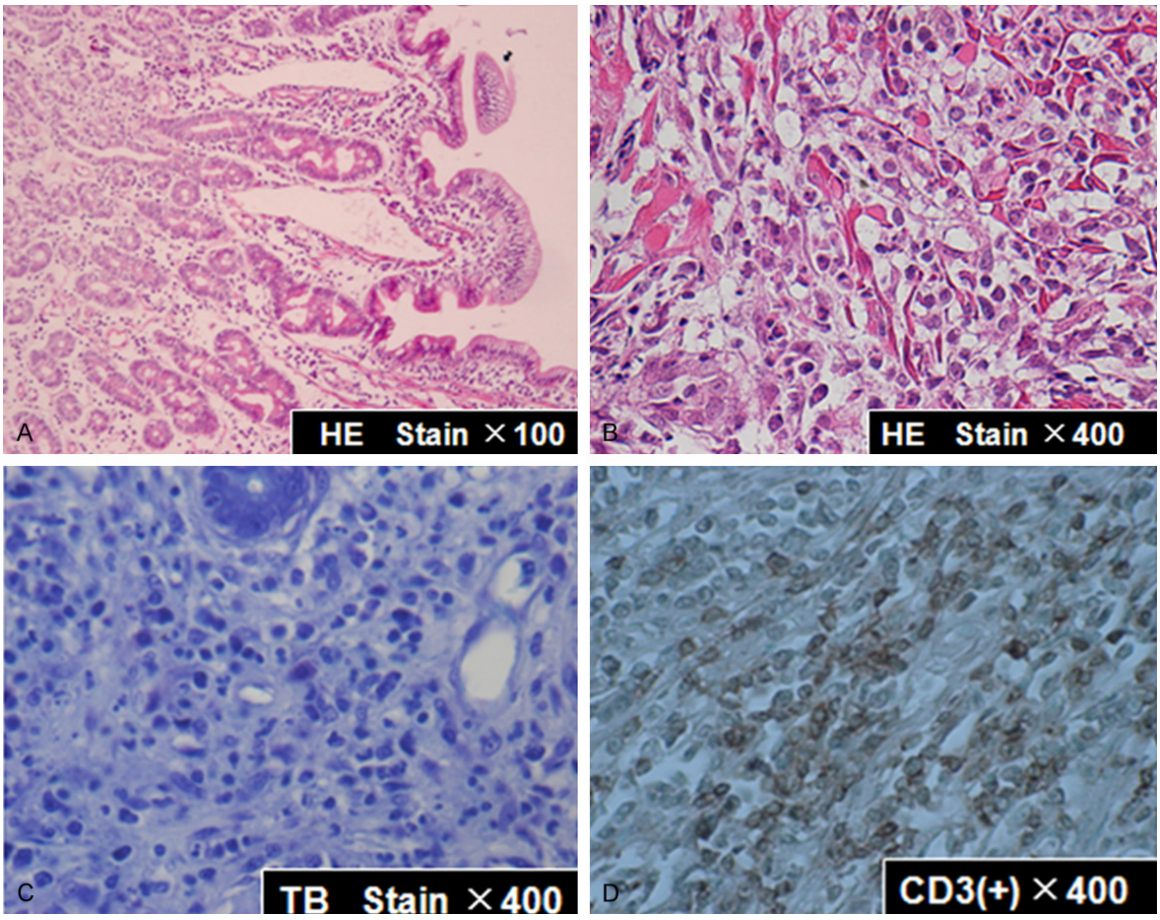

Figure 3. Pathological characteristics of case 2. A: HE staining of duodenal tissue; B: HE staining of antral tissue; C: Toluidine blue staining of antral tissue; D: Immunohistochemical staining of spleen tissue.

red blood cell (RBC), hemoglobin (Hgb) and hematocrit (Hct) were lower than normal. And the contents of platelet (PLT), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (Cre), albumin (Alb), total cholesterol (T-cho), P, K, Cl and Ca were all within the normal range. Peripheral blood smear examination (**Figure 5A**) showed that the reticulocyte production index (RPI) was 0.3. Combined with results of blood biochemical tests, the initial diagnosis was made as non-regenerative anemia. X-ray examination showed hepatosplenomegaly, with pleural effusion and ascites (**Figure 5B**). Red blood cells, neutrophils and eosinophils (**Figure 5C**) were visible in ascites by smear. A large number of independent circular proliferations of atypical cells, and eosinophilic granulocyte infiltration were visible in spleen biopsy (**Figure 5D**). Pax5, CD10, CD20, CD3 and Iba-1 were positive in the immunohistochemical staining of spleen tissue (**Figure 5E**). IgH and TCR were both positive by IgH gene clonal rearrangement detection (**Figure 5F**). The diagnosis was confirmed as T cell lymphoma.

Discussion

The locations of lymphoma are diverse, and its most common types consist of T cell lymphoma, multi-center lymphoma [5], B cell non-Hodgkin lymphoma [6], splenic lymphoma [7] et al. This study identified three cases of T cell lymphoma, and one case of non-epithelial tropism lymphoma (derived from B cell lymphoma). The clinical manifestations and pathological forms of lymphoma are various. And it's difficult to tell if the tumors are benign or malignant. Pathological diagnosis is the gold standard in determining all malignant tumors, regardless of the depth, location, size, shape and hardness of the lesion. Therefore, patho-

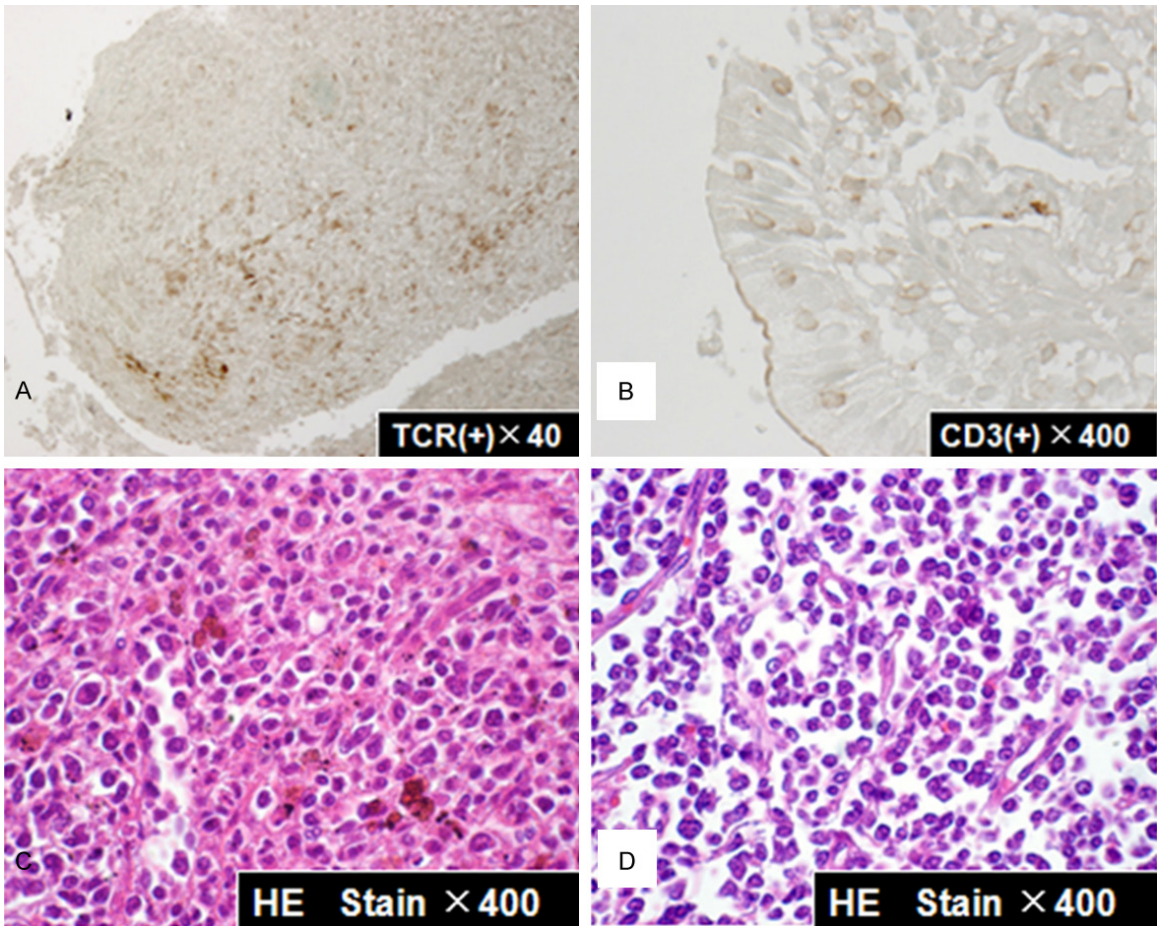

Figure 4. Pathological characteristics of case 3. A: Immunohistochemical staining of antraltissue (TCR); B: Immunohistochemical staining of antral tissue (CD3). C: HE staining of spleen tissue; D: HE staining of the tissue from mandibular lymph nodes.

Table 2. Results of blood biochemical tests

WBC 70.8×10^3/μl	Cre 0.7 mg/dl
RBC 3.41×10^6/μl	Alb 2.7 g/dl
Hgb 8.1 g/dl	T-cho 207 mg/dl
Hct 23%	Ca 9.8 mg/dl
PLT 54.4×10^4/μl	P 3.9 mg/dl
ALP 333 U/l	K 3.7 mEq/l
ALT 32 U/l	Cl 109 mEq/l
BUN 36.3 mg/dl	CRP 2.75 mg/dl

logical biopsy must be performed to diagnose or exclude lymphoma. The four cases in this study were all examined in tissue sections, which provided a good reference for diagnosis. Secondary lymphoma, as the late-stage presentation of a prior primary one, is most common in malignant cases [8, 9]. And splenic involvement is one of the systemic manifestations of malignant lymphoma [9]. Accordingly, spleen tissue biopsy samples from the three cases except Case 1 were all examined in this study. Toluidine blue staining can be used to display and identify mast cell hyperplasia-related disease. And it was adopted in this study for Cases 1 and 2, to determine whether or not they were mast cell tumors. The immunohistochemical method plays a key role in the diagnosis of lymphoma, for example, CD3, CD20, CD30, CD15, LCA and PAX-5 are often used as detection antibodies to diagnose the classic Hodgkin lymphoma [10]. In this study, three cases of lymphoma were diagnosed using the immunohistochemical method, which provided the basis for the final diagnosis. Imaging examinations and biochemical indicators of the blood are often used as supplementary tools for the diagnosis of lymphoma. In this study, the ascites in Case 4 were detected via imaging, anemia via blood tests, and positive eosinophils and basophils via ascitic laboratory tests.

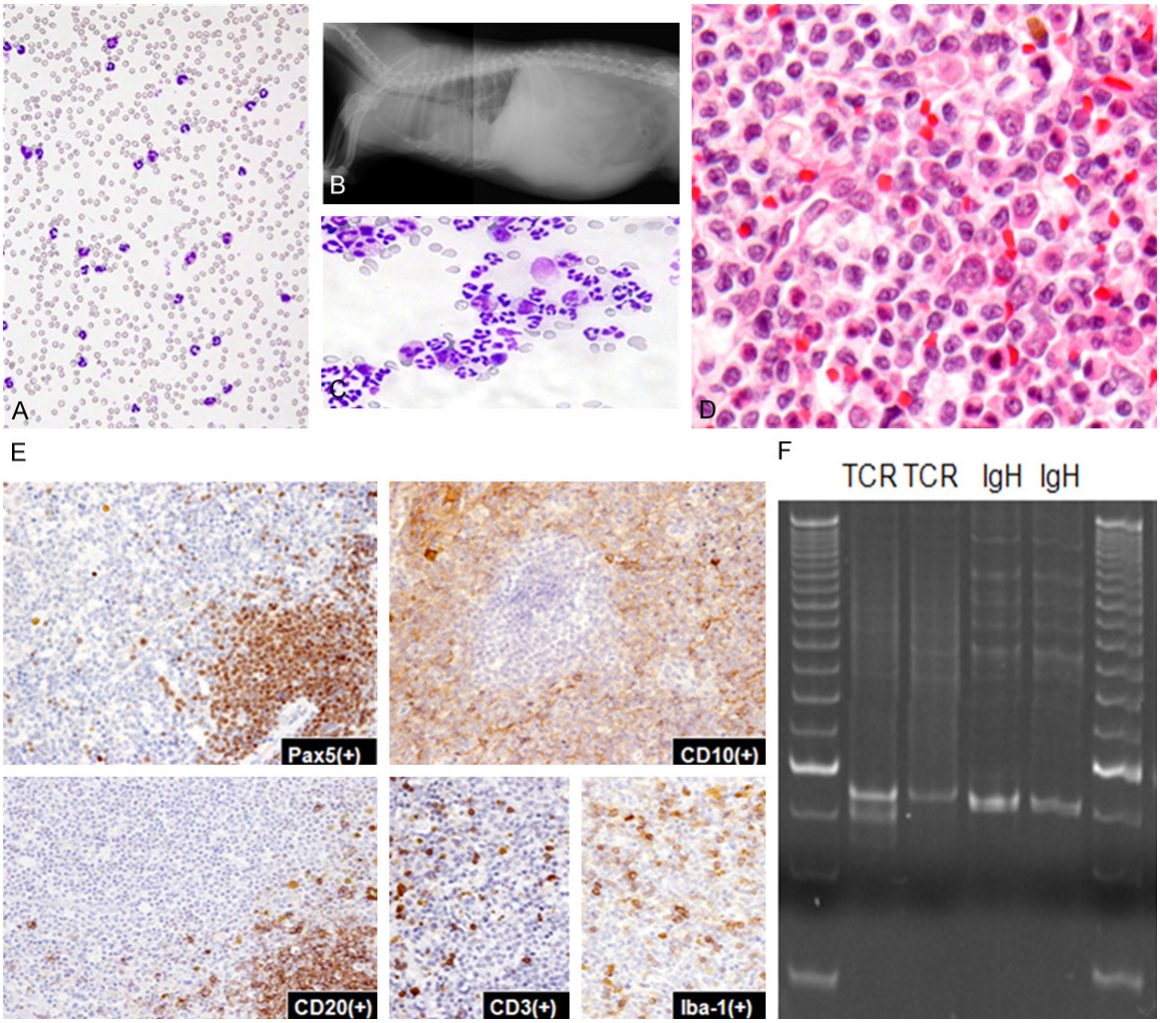

Figure 5. Characteristics of case 4. A: Blood smear; B: X ray examination of the chest and abdomen; C: Ascites smear; D: HE staining of spleen tissue; E: Immunohistochemical staining of spleen tissue; F: IgH gene clonal rearrangement detection.

The auxiliary diagnostic method of IgH gene clonal rearrangement detection used in the study has been widely applied both at home and abroad [11, 12]. The principle of this method is that most normal lymphocytes will undergo immunoglobulin heavy chain gene or T-cell receptor gene rearrangement. Since each normal B or T lymphocyte carries a unique gene rearrangement, the difference between such rearrangements leads to the test result of polyclonal rearrangements. While the vast majority of B or T cells in non-Hodgkin's lymphomas are from monoclonal origins, in which case IgH and TCR gene rearrangements are determined as monoclonal ones. In this study, IgH gene clonal rearrangement detection was used in the diagnosis of Case 4, with the advantages of less sample volume required, simplified test method, objective and accurate results, and easy deployment in more generalized application [13].

Disclosure of conflict of interest

None.

Address correspondence to: Zhen-Zhong Hou, College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China. Tel: +86-045155190676; Fax: +86-045155190363; E-mail: houzz_1963@163.com

References

[1] Stephen J, Withrow David M. Vail. Withrow and MacEwen's Small Animal Clinical Onocology [M]. Saunders Ltd, 2007.

[2] Jane M, Dobson B, Duncan X. Lascelles. Bsava Manual of Canine and Feline Oncology [M]. BSAVA, 2011.
[3] Carolyn J. Henry, Mary Lynn Higginbotham. Cancer Management in Small Animal Practice [M]. Saunders Ltd, 2010.
[4] Susan North, Tania Banks. Small Animal Oncology: an introduction [M]. Saunders Ltd, 2009.
[5] Chen YY, Dong J, Zheng L. Diagnoses of center lymphoma in 22 cases of canines. Zhong Guo Shou Yi Za Zhi 2013; 49: 59-60.
[6] Sun N. Diagnosis of non-Hodgkin's B-cell lymphoma in a canine. Animal Husbandry & Veterinary Medicine 2013; 45: 112.
[7] Wu WY, Xu BR, He L. Case reports of canine spleen lymphoma. Zhong Guo Xu Mu Shou Yi Wen Zhai 2013; 29: 191-192.
[8] Ji XM. Clinicopathological analysis in 2 cases of primary spleen malignant lymphoma. Bing Li Yu Lin Chuang 2009; 47: 128-133.
[9] Chen P, Jiang LS. Primary malignant lymphoma of the spleen (report of 9 cases). West China Medical Journal 2004; 19: 252.
[10] Zhou XG. Antibody selection in pathological diagnosis of lymphoma. Zhen Duan Bing Li Xue Za Zhi 2010; 17: 4-6.
[11] Daniel A. Molecular diagnostic approach to non-Hodgkin's lymphoma. J Mole Diagno 2000; 2: 178-190.
[12] Hodges E, Krishna M T, Pickand C. Diagnostic role of tests for T cell receptor (TCR) genes. J Clin Pathol 2003; 56: 1-11.
[13] Luo LK, Zhang XF, Lu QF. Application of IgH gene rearrangement detected in B cell lymphoma diagnosis. Quan Ke Yi Xue Lin Chuang Yu Jiao Yu 2005; 3: 204-209.