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Clinicopathological Description of a Chronic Lymphocytic Leukemia in a Dog

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Abstract

Hematopoietic tumors are associated with diagnostic and therapeutic challenges and a poor prognosis. Furthermore, their pathophysiology in dogs is not well understood. Chronic lymphocytic leukemia has been rarely reported in golden retrievers, perhaps due to difficulties in diagnosis and care. An 8-year-old spayed female golden retriever was referred to our hospital with a 2-month history of fatigue, weakness, and selective appetite. The dog was diagnosed with chronic lymphocytic leukemia based on blood smear evaluation. Despite treatment, the dog deteriorated and the owners opted for euthanasia. Histopathological examination revealed atypical lymphocytes in the spleen, extramedullary hematopoiesis in the spleen and liver, bone marrow depletion, moderate neutrophilic myocarditis, and acute renal tubular necrosis. In an immunohistochemical panel, cells in the spleen and bone marrow were positive for anti-CD20, anti-CD45, anti-CD3, anti-IBA-1, anti-Ki67, anti-MUM-1, and anti-PAX-5. In conclusion, the leukemia diagnosis was confirmed through reactive histiocytosis, marked extramedullary hematopoiesis, and other clinical and laboratory findings. Herein, we report a case of chronic lymphocytic leukemia in a female dog with clinical, laboratory, and necroscopy findings.

Keywords

Canine, Cancer, Neoplasia, Therapeutics

1. Introduction

Canine hematopoietic neoplasms are a common diagnosis in clinical veterinary practice [1]. Lymphoid leukemias can be divided into acute lymphoblastic leukemia and chronic lymphocytic leukemia, based on the cytologic phenotype of lymphocytic cells, genetic aberrations, microribonucleic acid expression, and immunophenotype [2].

Lymphocytic leukemia is more common than acute myeloid leukemia or myeloproliferative disorders. The disease may occur in dogs of any age but predominates in middle-aged to older dogs, a mean of 7 to 10 years [3]. A significant sex predilection is not reported. The etiology of lymphocytic leukemia is unknown, but genetic factors are believed to be involved [3].

Hematopoietic tumors are associated with diagnostic and therapeutic challenges. Furthermore, their pathophysiology in dogs is not well understood [3]. Chronic lymphocytic leukemia has been rarely reported in golden retrievers [2]. Herein, we report a case of chronic lymphocytic leukemia in a female dog with clinical, laboratory, and necroscopy findings.

2. Case Description

An 8-year-old spayed female golden retriever weighing 37.6 kg was referred to the veterinary hospital with a 2-month history of fatigue, weakness, and selective appetite. Following the initial diagnosis, the dog was treated with prednisolone (2 mg/kg), dipyrone (25 mg/kg), and omeprazole (1 mg/kg) for 10 days without clinical improvement.

The dog was alert and dyspneic at presentation. Physical examination revealed pale mucous membranes (oral, ocular, and vulvar) and a high respiratory rate (84 breaths per minute).

Complete blood cell count detected normocytic and hyperchromic anemia (red blood cell count: 1.03×10^6 µL, reference range (RR): $(5.5 - 8.5) \times 10^6$ µL; hemoglobin concentration: 2.3 g/dL, RR: 12.0 - 18.0 g/dL; hematocrit: 8%, RR: 37% - 55%; mean corpuscular volume: 77.7 fL, RR: 60 - 77 fL; mean cellular hemoglobin concentration: 28.8%, RR: 32% - 36%, nonregenerative anemia, anisocytosis, and polychromasia, severe lymphopenia (1.7 µL; RR: 1000 - 4800 µL), and thrombocytopenia (80,800 × 10^3/µL, RR: $(160 - 430) \times 10^3$/µL). Blood count abnormalities include hemoparasitosis, autoimmune hemolytic anemia, and lymphoid neoplasia. The bone marrow had decreased erythroid and myeloid series with a prevalence of mature and non-dysplastic forms, reduced mature megakaryocyte numbers, discrete anisocytosis, polychromasia, and hyperplastic lymphoid tissue, with small and medium lymphocytes characterized by scarce cytoplasm, lymphoglandular

corpuscles, Grümprecht shadows, and macrophages filled with hemosiderin and erythrophagocytosis (Figure 1).

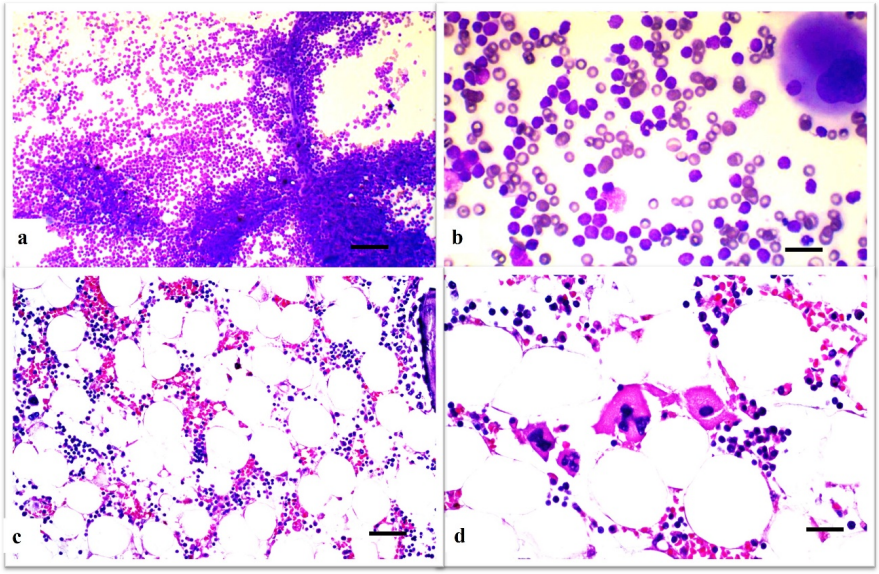

Figure 1. Golden retriever with leukemia. (a) Bone marrow myelogram with increased cellularity for dogs, predominantly small and medium-sized lymphocytes with few atypia, normal and mature megakaryocytes discreetly reduced in quantity. Giemsa, bar = 100 μm. (b) Young megakaryocytes are also present. The erythroid and myeloid series are intensely reduced in quantity, with a predominance of mature forms and without dysplastic alterations. There is discrete anisocytosis and polychromasia. The lymphoid series is intensely hyperplastic, with small and medium-sized lymphocytes showing cytoplasm in the cytoplasm, sometimes basophilic, coarse chromatin, and rare evident nucleoli. Discrete anisocytosis and anisokaryosis. There is a discreet amount of mitosis figures, lymphoglandular corpuscles, and Grümprecht shadows. Giemsa, bar = 40 μm. (c) Gross bone marrow histopathology showing cellular depletion. H & E, bar = 50 μm. (d) Mature and young megakaryocytes are shown in detail. H & E, bar = 20 μm.

Serum biochemical evaluation revealed: elevated urea concentration (86.00 mg/dL, RR: 21.40 - 59.92 mg/dL); increased activities of gamma-glutamyl transferase (9.8 IU/L, RR: 1.2 - 6.4 IU/L), alanine aminotransferase (131 IU/L, RR: 21 - 73 IU/L), and alkaline phosphatase (307 IU/L, RR: 20 - 156 IU/L); and reduced albumin concentration (2.4 g/dL, RR: 2.6 - 3.3 g/dL). Abdominal ultrasonography detected hepatomegaly with homogeneous hypoechogenic areas in the parenchyma, enlarged hepatic lymph nodes, splenomegaly, and accumulation of abdominal fluid; a sample of the latter was obtained by ultrasound-guided abdominal paracentesis and was a yellow, turbid ascitic fluid with a density of 1.028 and pH of 7.5 and hyperproteinemia, slight hyperglycemia, presence of occult blood (350 red blood cells/μL and 155 nucleated cells/μL) and high numbers of neutrophils, small reactive lymphocytes, and rare erythrophagocytosis.

Oxygen supplementation was provided and intravenous sodium bicarbonate (7.5 mL) was administered for 30 min in the veterinary hospital. Moreover, chlorambucil (20 mg/m^2) (Leukeram, Aspen, Brazil) was also included in the leukemia

treatment. Chronic lymphocytic leukemia in dogs is usually treated first with a protocol combining chlorambucil and prednisolone. This treatment is recommended for dogs with anemia, lethargy, anorexia, and weight loss [4] [5], as present in this dog.

The following day, the dog was alert and tachypneic, with pale mucous membranes. Abdominal paracentesis was done to drain accumulated fluid. Laboratory tests revealed persistent nonregenerative anemia, lymphopenia, thrombocytopenia, altered hepatic function, and hypoalbuminemia. The dog was given IV fluid therapy (5 mL/kg/hour) and packed red blood cells were transfused for 8 hours. Despite treatment, the dog deteriorated during hospitalization without clinical improvement or any indications of a better prognosis. In the absence of improvement, 24 hours later, focusing on the animal's welfare, the owner opted for euthanasia and post mortem examination

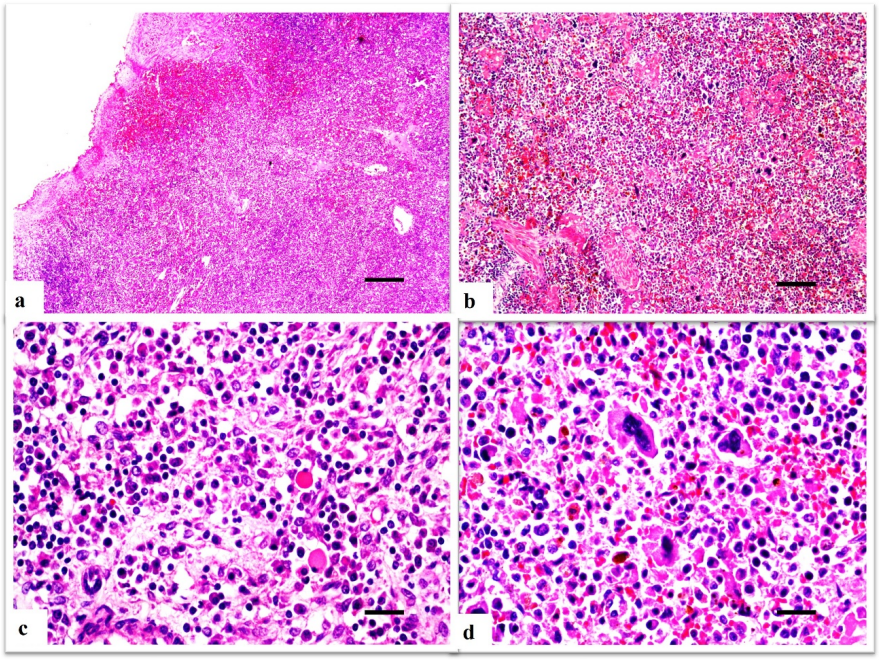

Figure 2. Golden retriever with leukemia. (a) Splenic histopathology. H & E, bar = 200 µm. (b) Focally extensive poorly demarcated round cells infiltration and extramedullar hematopoiesis showing moderate megakaryocytes in the spleen. H & E, bar = 100 µm. (c) Round, sparse-to-discrete eosinophilic, well delimited paracentral and peripheral nucleus, dense chromatin and conspicuous nucleolus, moderate anisocytosis, discrete anisokaryosis, pleomorphism and binucleate cells. H & E, bar = 50 µm. (d) Mature and young megakaryocytes are shown in detail. H & E, bar = 50 µm.

At necropsy, a 1.2-cm diameter white nodule in the spleen and diffuse hemorrhagic lymphadenomegaly were observed (**Figure 2**). Other findings were pale mucous membranes (oral, ocular, vulvar, and anal), a cyanotic tongue, icteric skin, hydroperitoneum, petechiae, hemorrhagic purpura in the thoracic ribs, pulmonary congestion, edema, focal epicardial fibrosis, and encephalic congestion. Portions of spleen, liver, kidney, and heart were fixed in 10% neutral-buffered

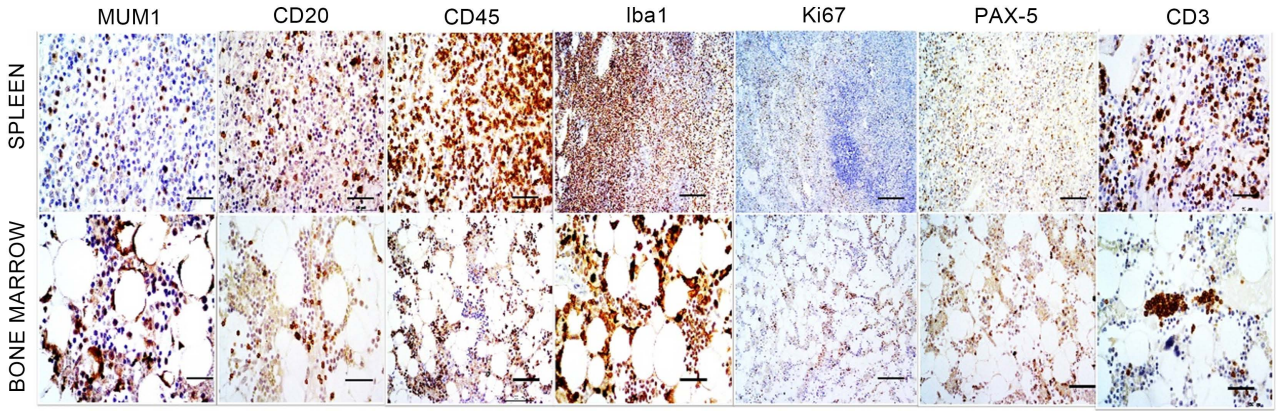

Figure 3. Golden retriever with leukemia. Immunohistochemical panel of spleen and bone marrow. MUM1: Positive in late B cells and activated T cells in spleen and bone marrow, bar spleen = 50 μm, bar bone marrow = 20 μm. CD20: Positive in reactive B lymphoid cells in the spleen and bone marrow, bar spleen = 50 μm, bone marrow = 50 μm. CD45: Positive in reactive lymphoid cells in the spleen and bone marrow, bar spleen = 50 μm, bone marrow = 50 μm. Iba1: Positive in reactive histiocytes bar spleen = 100 μm, bone marrow = 50 μm. Ki67: Sparse positivity, bar spleen = 100 μm, bone marrow = 100 μm (10×). PAX-5: Positive in reactive B lymphoid cells, bar spleen = 100 μm, bone marrow = 100 μm. CD3: Positive in activated T cells in the spleen and bone marrow 20 μm, bone marrow = 20 μm.

formalin for 24 hours. Tissue sections were routinely processed, embedded in paraffin, 3 - 5 μm sections were cut and stained with hematoxylin and eosin (H & E), and evaluated with light microscopy.

Histopathological examination revealed atypical lymphocytes in the spleen, extramedullary hematopoiesis of the spleen and liver, and bone marrow depletion (**Figure 1**). Moderate neutrophilic myocarditis and acute renal tubular necrosis were also visualized microscopically.

An immunohistochemical panel was done to evaluate T and B lymphoid proliferation. In the spleen and bone marrow, there were cells positive for anti-CD20 (lymphoid B marker), anti-CD45 (lymphoid marker in hematolymphoid process), anti-CD3 (T-lymphocyte receptor), anti-IBA-1 (histiocyte marker), anti-Ki67 (cell proliferation antigen), anti-MUM-1, and anti-PAX-5 (**Figure 3**). These immunohistochemical results suggested reactive histiocytosis with marked extramedullary hematopoiesis, which, in conjunction with other clinical and laboratory features, was consistent with a diagnosis of lymphocytic leukemia [6] [7].

3. Discussion

Canine hematopoietic neoplasms are a common diagnosis in clinical veterinary practice [7]. Leukemia can be divided into acute lymphoblastic leukemia and chronic lymphocytic leukemia, based on the cytologic phenotype of lymphocytic cells, genetic aberrations, microribonucleic acid expression, and immunophenotype [2]. Lymphocytic leukemia is more common than acute myeloid leukemia and myeloproliferative disorders. The disease may occur in dogs of any age but predominates in middle-aged to older dogs (mean of 7 to 10 years). A significant sex predilection is not reported. The etiology of lymphocytic leukemia is unknown, but genetic factors are believed to have a role [8]. In this case, clinical

evaluation, ultrasonography, laboratory examinations, myelography, necropsy, and histopathological analyses contributed to the diagnosis of lymphocytic leukemia, with neoplastic cell proliferation in the bone marrow [9].

Several biochemical abnormalities were detected in the present case. Initial stages of leukemia are often marked by elevated activities of transaminases and alkaline phosphatase due to injury caused by leukemic cell infiltration, leading to abnormal liver function [10] [11]. In addition, leukemia affects serum protein concentrations, including albumin and C-reactive protein [12]. Changes in liver function end points can indicate hepatomegaly, abnormal liver function, and potentially fatal complications, including liver failure [13].

Lymphoglandular corpuscles result from fragmentation of neoplastic lymphocytes and are a common finding in neoplasms of lymphoid origin. Grümprecht shadows correspond to degenerated nuclei, mainly of lymphocytes, and when present in large numbers, contribute to a diagnosis of leukemia. Myeloid and erythroid hypoplasia and slight megakaryocytic hypoplasia reflect involvement of other cell lineages that occur frequently in leukemia. This may be related to the intense proliferation of neoplastic cells in the bone marrow (myelophthisis). Also, small nuclear cells can be identified in leukemia [14].

The specific categorization of leukemias into various subtypes can be achieved with clinical signs, morphologic features, and immunophenotype [15]. Immunohistochemistry is very useful for leukemia diagnosis, as it enables identification of cell lineages and maturation phases, detection of specific genetic alterations, visualization of the extent of cell proliferation, and identification of therapeutic targets for clinical management [16] [17]. Although no single marker is definitive for a specific malignancy, a well-chosen antibody panel can provide data to diagnose and classify leukemias [18] [19]. In this case, our immunohistochemical panel revealed severe reactive histiocytosis and extramedullary hematopoiesis, highlighting the aggressivity and immaturity of the cells involved in the tumor and consistent with the severity of clinical signs.

Once a diagnosis is established, treatment of lymphoid neoplasm can be attempted, with several therapeutic protocols available. Treatment for chronic lymphocytic leukemia in humans and dogs usually involves administration of chlorambucil and prednisolone. Chlorambucil has a nitrogen mustard alkylating action inducing cytoreduction, is given orally, is readily available and has fewer side effects [20]-[22].

It is difficult to establish a prognosis, because studies and reports surrounding these cases are scarce, and effective treatments are poorly defined [18]. Thus, reporting these cases and further research on these topics are expected to improve the living conditions and increase the life expectancy of the patients.

In conclusion, we report herein a case of lymphocytic leukemia in a golden retriever and highlight the clinical and diagnostic complexities encountered with this neoplasm. This case provides clinicians and pathologists with an example of clinicopathological and immunohistochemical features of the tumor. Finally, this report highlights the need for further canine lymphocytic leukemia studies, given

the persistent challenges in disease management attributed to recurrent anemia, lethargy, anorexia, and weight loss, leading to a discouraging long-term prognosis.

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Author Contributions

MCBR, GVMB, ISM and BPB performed data curation and formal analysis; MCBR, GVMB, ACCR, and FBCM investigation, methodology, and writing original draft; ACCR, SCR, JPK, LGDB, FCSB, PTCGO, RKT, AM and NSR writing review and editing the manuscript. NSR supervised, validated, and visualized the entire study. All the authors have read and approved the final version of the manuscript.

Data Availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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