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Case Study: Supportive Diagnostic Procedures in A Canine Lymphoma Case at Hikari Veterinary Clinic

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Abstract
Lymphoma is one of the most common neoplastic diseases in dogs, typically characterized by the enlargement of lymph nodes due to malignant lymphocyte proliferation. This study aims to describe the sequence and use of supportive examinations for confirming lymphoma diagnosis in dogs at Hikari Vet Clinic. This study methods used a case study approach involving a 12-year-old male Mini Pinscher weighing 5.1 kg, diagnosed with lymphoma based on clinical symptoms and diagnostic tests. Data were collected through physical examination, hematology and blood chemistry analyses, ultrasonography, and radiography. The examinations were conducted from August to November 2024. The results showed that the patient had multiple enlarged lymph nodes, including the popliteal, submandibular, axillary, and inguinal lymph nodes. Hematology findings indicated leukocytosis and granulocytosis, accompanied by reduced red blood cell count, hemoglobin, hematocrit, and platelet levels, consistent with anemia. Blood chemistry analysis showed elevated levels of alkaline phosphatase (ALP) and glutamate pyruvate transaminase (GPT), while glucose and total cholesterol were decreased. Ultrasonography revealed enlarged, round lymph nodes with irregular margins and a hypoechoic appearance, and dorsoventral radiographs confirmed lymph node enlargement in the cervical region. This study concludes that hematology, biochemistry, ultrasound, and radiography examination procedures are very important to be carried out sequentially and correctly in order to establish a diagnosis.

INTRODUCTION

Tumors, defined as the abnormal growth of tissue mass in an animal's body, represent a serious pathological condition with a significant incidence in the field of veterinary medicine.Although generally identified with the formation of a mass, Skor *et al.* (2021) explain that tumor tissue does not always manifest as a solid mass but can also occur in hematopoietic cells or as carcinoma in situ. The occurrence of tumors is not limited to specific

animal species; Madsen *et al.* (2017) reported that tumors can be found in both vertebrate and non-vertebrate animals. Specifically in dogs, Martini *et al.* (2018) note that tumor cases account for 9.5% to 51% of all tumors occurring in animals. Among the various types of tumors affecting dogs, lymphoma is one of the most frequently encountered cases. Lymphoma itself is a specific medical term referring to a type of malignancy (tumor) that originates from the cells of the lymphatic system.

The systemic nature of lymphoma makes it a highly concerning disease. The lymphatic system, which has interconnected circulation networks, facilitates the spread of tumor cells throughout the entire body (Amanda 2019). Although the etiology of lymphoma is not yet fully understood, genetic risk factors and certain breeds have been identified. Comazzi *et al.* (2018) mention that dog breeds such as Doberman Pinscher, Boxer, and Golden Retriever have a higher risk of developing lymphoma. Age also plays a crucial role; lymphoma generally affects older dogs, often around 10 years of age. Statistical data reinforces this observation: Sagome *et al.* (2018) show that lymphoma cases in dogs under 1 year old only account for 1.5% of cases, but drastically increase to 84% in 10-year-old dogs. Mahhiddine *et al.* (2022) support this finding by stating that 80% of lymphoma cases in dogs are characterized by spread to the lymph nodes and other organs, emphasizing the systemic and aggressive nature of the disease. Therefore, for the accurate diagnosis of lymphoma and effective treatment planning, a physical examination must be supplemented with a series of supporting examinations (diagnostic tests).

Supporting examinations are vital medical procedures, performed based on specific clinical conditions, with the primary goal of providing additional information to help the veterinarian reach a more accurate and definitive diagnosis (Amaliyah *et al.* 2016). These procedures can include diagnostic examinations such as imaging, and laboratory tests (Basariyadi 2016). In the context of disease management, supporting examinations play a key role in determining the most optimal handling and treatment measures for the patient (Simarmata *et al.* 2021). In lymphoma cases, essential supporting examinations include laboratory tests (such as hematology and blood chemistry), ultrasonography to assess internal organs and lymph nodes, and radiography (X-ray) to evaluate the spread and status of organs within the body cavities. All these supporting examinations must be performed by competent and authorized personnel, namely veterinary paramedics, under the direct supervision of a veterinarian.

Veterinary paramedic play a crucial role in companion animal health services, functioning as both veterinary assitant and laboratory technicians (Green 2024). Their responsibilities include accurate documentation of medical records, continous monitoring of hospitalized patients, preparation and compounding of prescribed medications, and direct assistance during clinical examinations (Yuniswan *et al.* 2022). In diagnostic procedures, veterinary paramedic are responsible for patient preparation, ensuring diagnostic equipment is functional and properly calibrated and performing specimen collection, thereby supporting veterinarians in obtaining accurate, and reliable diagnostic data. As emphasized by Carnicelli *et el.* (2024), the competence of veterinary paramedics is essential to ensure that supporting diagnostic examinations are conducted efficiently and produce valid results.

In lymphoma cases, such as those handled at Hikari Vet Clinic, the involvement of veterinary paramedics is highly necessary across the entire spectrum of using diagnostic supporting examination tools. This includes execution and assistance with laboratory examination equipment (such as hematology and blood chemistry analyzers), operation of ultrasonography equipment for soft tissue imaging, and assistance with the use of radiography equipment (X-ray) for imaging bones and body cavities. The skills of veterinary

paramedics in preparing patients who may require special handling (such as proper restraint without anesthesia) and ensuring equipment parameters (such as USG frequency or X-ray kVp/mAs factors) are set according to applicable protocols, are determinants of diagnostic data quality. Therefore, an in-depth study of the roles and procedures performed by veterinary paramedics in the series of supporting examinations for lymphoma cases is important to understand the standard operating procedures and their contribution to successful diagnosis.

RESEARCH METHODOLOGY

Time and Place

The data collection activities were carried out from August 5 to November 5, 2024, The case study was conducted at Hikari Vet Clinic. A case study is a method performed to explore a case to gather detailed and in-depth information using various data collection procedures during a specific period and activity (Yohanda 2020). The case study was performed on one 12-year-old male Mini Pinscher dog, observed to be suffering from lymphoma at Hikari Vet Clinic. The case study data results were obtained based on anamnesis, medical records, physical examination, and results from hematology tests, blood chemistry, ultrasonography, and radiography. The quantitative data results were obtained and discussed in the form of descriptive analysis.

Tools and Materials

The equipment used in the case study were EDTA tubes, plain tubes, 1ml syringes, Rayto RT-7600 hematology analyzer, Fuji Film biochemistry analyzer, Edan DUS 60 ultrasonography unit, and Radiography unit. The necessary materials were one Mini Pinscher dog with lymphoma, 1ml blood sample, and gel.

Work Procedures

The hematology test was performed by preparing the patient for blood collection. Blood sample was drawn from the left hind leg's saphenous vein. The sample was put into a purple-top tube (EDTA) and labeled. The blood sample was examined using a hematology analyzer. The blood chemistry test was performed by drawing a 1 ml blood sample from the saphenous vein, which was put into a plain tube and labeled. The blood sample tube was centrifuged for 15 minutes at a speed of 3000 rpm to produce serum, which was then tested using a biochemistry analyzer. The blood chemistry test using the biochemistry analyzer took 10 minutes.

The ultrasonography examination used a portable B-mode USG unit with a curved transducer. The ultrasonography examination was performed by preparing the curved transducer. The ultrasonography unit was set to an appropriate frequency of about 7.5 MHz for a small dog (Noviana *et al.* 2018). Animal preparation involved clipping the hair on the ventral abdomen area across the entire cranial abdomen between the xiphoid process of the sternum and the umbilicus, and the lateral abdomen area at the 11th and 12th intercostal spaces (Iman *et al.* 2022). The examination was performed with the animal in a dorsoventral recumbency position. Gel was applied to the abdominal skin surface when scanning with the transducer.

The X-ray examination was performed by turning on the machine and setting the kVp and mAs. Radiographic imaging of the patient's thorax area used 60 kVp and 2 mAs, while the abdomen area used 52 kVp and 2.4 mAs. This is consistent with Sikumbang *et al.* (2018), where the kVp and mAs for thorax imaging are 60 kVp and 2 mAs. Abdomen imaging was performed with 52 kVp and 2.4 mAs (Meomartino *et al.* 2021). The Focus Film Distance (FFD) used in the X-ray examination was set to 100 cm (Ashary *et al.* 2023). The paramedics

assisting the X-ray examination wore an apron which serves as personal protective equipment to shield the body from radiation (Fitriana *et al.* 2023). The X-ray examination was performed with two views: right lateral recumbency and dorsoventral positions. The patient was handled without anesthesia during the X-ray examination.

RESULT AND DISCUSSION

Signalment and Anamnesis

Signalment and anamnesis (history) are data obtained based on interviews with the animal's owner. Signalment consists of the patient's identity, including medical record number, patient name, animal species, breed, age, and hair color. Signalment serves as the patient's identifier to facilitate the storage of data or medical record documents in the clinic. Anamnesis functions to record the patient's health history and detail the complaints experienced by the patient. A male Mini Pinscher dog named Bambi, with medical record number 192/10. The patient is twelve years old, categorized as geriatric, and has a tan-colored coat. The patient presented to Hikari Vet Clinic on August 13, 2024.

The patient presented with a physical condition featuring lumps on the neck and legs, which were identified as enlarged superficial lymph nodes. The owner stated that the lumps were first noticed about two months ago and have gradually increased in size, indicating a progressive growth rate of the mass. In addition to the lumps, the owner also reported a change in behavior, where the patient rested or remained still more frequently (lethargy) than before, although the patient's appetite and drinking habits were reported to be good. Lethargy in a geriatric dog with multiple lumps can be a significant clinical sign, indicating a systemic disease affecting the patient's quality of life. The patient's past health history did not show any other significantly relevant chronic diseases, and the patient received routine vaccinations and deworming according to schedule. The rapidly enlarging lumps and lethargy strongly suggest a neoplastic process, especially lymphoma, which is common in geriatric dogs. Based on this anamnesis, patient Bambi was directed to undergo a thorough physical examination to obtain objective clinical data.

Physical Examination

Physical examination is a clinical assessment performed directly upon the patient's arrival. Physical examination involves 4 methods: inspection, palpation, percussion, and auscultation. The physical examination aims to directly detect clinical signs in the patient and evaluate the patient's general condition (status present). The results of the physical examination showed the patient's body temperature was 39.1℃, which is within the upper limit of normal or slightly elevated, and a body weight of 5.1 kg. A mild increase in body temperature can be caused by a systemic inflammatory process accompanying tumor development. Evaluation of hydration status via skin turgor and Capillary Refill Time (CRT) showed good hydration. Mucous membranes (conjunctiva and gingiva) appeared pink, indicating adequate perfusion and oxygenation. Ectoparasites were not found on the patient's body. Auscultation of the thoracic cavity showed normal heart and lung sounds, but the patient was breathing abdominally (respiration involving excessive use of abdominal muscles), which could indicate respiratory distress or discomfort in the chest or abdomen, although subsequent radiography showed normal lungs.

The most significant finding was during palpation. When the lumps were palpated, the patient showed no signs of pain (no painful or non-painful response), which is a common characteristic of lymph node enlargement due to lymphoma (Vail *et al.* 2020). The general physical condition revealed lumps, which were enlarged multiple superficial lymph nodes. The enlarged lymph nodes included the left and right popliteal lymph nodes ±2 cm, the left

and right submandibular lymph nodes ±3.5 cm, the left and right axillary lymph nodes ±1.5 cm, and the left and right inguinal lymph nodes ±3.4 cm. Enlargement in more than two lymph node locations, especially superficial lymph nodes, clinically points towards suspected multicentric lymphoma, which is the most common form of canine lymphoma. Palpation of the lumps revealed a tumor mass that was round, smooth, with a rubbery to firm consistency. According to Paquin *et al.* (2023), lymph nodes affected by lymphoma will feel hard and rubbery. Irregular, hard, and rubbery lymph nodes occur due to the infiltration of tumor cells (Kaseb and Babiker 2023). According to Maini and Nagalli (2023), normal lymph nodes are regular oval- shaped, soft, smooth, and movable upon palpation. The multiple lymph node enlargements, rubbery- firm consistency, and lack of pain in a geriatric dog highly support the clinical suspicion of lymphoma. Based on the physical examination performed, patient Bambi was directed to supporting laboratory tests and imaging examinations.

Supporting Laboratory Examinations (Hematology and Blood Chemistry)

Supporting hematology examination was performed based on the physical examination. Hematology tests are fundamental supporting examinations performed using blood samples. The purpose of the hematology examination is to detect and measure the progression of lymphoma. Lymphoma in dogs causes an increase in lymphocyte count or leukocytosis (Colombe *et al*. 2022). This leads to decreased red blood cell production due to tumor cell invasion into the bone marrow (Sorenmo 2020). Blood tests can serve as indicators for diagnosing and monitoring the progression of lymphoma in dogs. Hematology examination was performed using a Haematology analyzer. The Haematology analyzer is an automated digital instrument that yields rapid results and can perform a complete blood count including hemoglobin, erythrocytes, erythrocyte indices, leukocytes, platelets, and hematocrit (Arini *et al.* 2023).

The results of hematology indicated leukocytosis (increase in white blood cell count) and granulocytosis (increase in granulocyte count), accompanied by reduced red blood cell count (RBC), hemoglobin, hematocrit, and platelet levels, consistent with anemia and/or thrombocytopenia. The increase in leukocytes suggests an ongoing response such as infection or inflammation (Yang *et al.* 2024). However, in the context of lymphoma, leukocytosis can occur due to the release of inflammatory mediators or, less commonly, neoplastic lymphocytosis (lymphoma cells circulating in the blood). According to Sauerbrei *et al.* (2018) and Kumar *et al.* (2018), an increase in granulocyte parameters (granulocytosis) indicates the presence of a chronic disease, a secondary infection that may affect the immune system, or tumor indication. In this case, elevated granulocytes may be associated with immune system disorders, given that the patient is over twelve years old. Reduced red blood cell count, hemoglobin, hematocrit, and platelet levels decreased, consistent with anemia. Henriques *et al.* (2021) stated that anemia is mostly associated with chronic diseases linked to decreased iron availability (Anemia of Chronic Disease/ACD) or tumor cell invasion into the bone marrow (myelophthisis). The decrease in platelets (thrombocytopenia) can also be caused by increased platelet consumption due to subclinical disseminated intravascular coagulation (DIC) or bone marrow invasion.

Tables 1. Hematology Result

Parameter	Results	Range Normal	Unit	Information
WBC	25.25*	5.5 - 19.5	10^3/u	H
Lym #	4.57	0.80 - 5.10	10^3/u	
MID #	2*	0.00 - 1.80	10^3/u	H
GRA #	18.68*	2.10 - 12.6	10^3/u	H
Lym %	18.1	12.0 - 30.0	%	
MID %	7.9	2.0 - 9.0	%	
Gra %	74	60.0-83.0	%	
RBC	3.47*	5.10 - 8.50	10^6/L	L
HGB	7.9*	11 - 19	g/dL	L
MCHC	33.9	29.0 - 38.0	g/dL	
MCH	22.8	20.0 - 26.0	pg	
MCV	67.4	62.0 - 72.0	fL	
RDW- CV	12.9	11.0 - 15.5	fL	
RDW-SD	45.4	33.2 - 56.1	%	
HCT	23.4*	33.4 - 56.1	%	L
PLT	96*	100 - 518	1	L
MPV	8.4	7.0 - 12.9	fL	
PDW	17.4	10 - 18	fL	
PCT	0.081	0.001 - 0.5	%	

Remarks : H (*High*) dan L (*Low*)

Blood chemistry is a supporting examination that serves to detect abnormalities in organ function and identify complications. Blood chemistry analysis showed elevated levels of alkaline phosphatase (ALP) and glutamate pyruvate transaminase (GPT), while glucose and total cholesterol were decreased. GPT (glutamate pyruvate transaminase) is an enzyme primarily found in the liver, playing a role in amino acid metabolism, particularly in the conversion of alanine to pyruvate. The increase in ALP can be related to stress conditions in the patient or disorders of the liver or bile ducts. The chronic inflammation suffered by the patient can cause stress, leading to the release of adrenaline and cortisol hormones by the body, which can stimulate liver cells, bones, kidneys, and bile ducts to produce ALP (Rosida 2016). Increased ALP caused by stress (steroid-induced ALP) is often seen in chronically ill dogs. Oikonomidis and Milne (2023) stated that elevated ALP and GPT (liver enzymes) may be caused by tumor cell infiltration into the liver, systemic inflammation, or secondary effects of metabolic disturbances accompanying the neoplastic disease. Lymphoma infiltration into the liver (lymphomatous hepatomegaly) is a common finding in advanced stages. In dogs with lymphoma, glucose (hypoglycemia) and total cholesterol (hypocholesterolemia) levels below the normal range may indicate metabolic disturbances due to the systemic impact of lymphoma. Hypoglycemia can occur due to excessive glucose utilization by rapidly dividing tumor cells (Warburg effect) or due to the production of insulin-like growth factors (ILGF) by the tumor. Hypocholesterolemia may be a sign of malnutrition or secondary liver dysfunction.

Tables 2. Result of Biochemistry

Parameter	Result	Range Normal	Unit	Information
ALB	3.4	2.6 - 4.0	g/L	
TP	6.6	5.0 - 7.2	g/L	
ALP	1705*	13 - 83	U/L	H
GLU	69*	75 - 128	Mg/dl	L
TBIL	0.2	0.1 - 0.5	Mg/dl	
IP	4.3	1.9 - 5.0	Mg/dl	
TCHO	109*	111 - 312	Mg/dl	L
GGT	10	5 - 14	U/L	
GPT	95*	17 - 78	U/L	H
Ca	10.0	9.3 - 12.1	Mg/dl	
CRE	0.47	0.040 - 1.40	Mg/dl	
BUN	24.8	9.8 - 29.2	Mg/dl	

Remarks : H (*High*) and L (*Low*)

Supporting Imaging Examinations (Ultrasonography and Radiography)
Ultrasonography

Ultrasonography (USG) is an examination tool that uses sound waves emitted by a transducer (Sarotama *et al.* 2018). The ultrasonography used was a portable B-mode USG with a curved transducer. B-mode type, using ultrasound pulses captured by the transducer, provides an image in the form of dots on the monitor screen (Maslebu *et al.* 2016). The purpose of ultrasonography examination in lymphoma cases is to identify the position, size, and spread of the lymphoma, especially to internal (visceral) lymph nodes and parenchymal organs (liver, spleen). This is crucial for clinical staging of the lymphoma.

The ultrasonography results showed enlarged lymph nodes that were round with irregular edges and a hypoechoic organ appearance. Hypoechoic is a dark gray image on the ultrasonography display, indicating low sound wave reflection (Purmono 2020). Dogs with lymphoma often show lymph nodes that tend to be round, hypoechoic, and lack a distinct echogenic hilus, which is a sign of the loss of the normal internal structure of the lymph node due to tumor cell invasion (Duplan *et al.* 2023 and Belotta *et al.* 2019). The hypoechoic appearance occurs due to inflammation in the lymph node or infiltration of dense lymphoma cells, which reflect less ultrasonic sound waves than the surrounding fatty tissue (Borska *et al.* 2018).

The USG results (figure 1), though not included, for patient Bambi showed that parenchymal organs such as the liver, lungs (not well visualized by abdominal USG), kidneys, spleen, urinary bladder (*vesica urinaria*), and pancreas were generally normal in size and showed no significant changes in echotexture, except possibly in the internal lymph nodes. More detailed USG findings showed enlarged lymph nodes during the abdominal area examination, specifically the mesenteric lymph nodes, which are often affected in lymphoma. Enlarged lymph nodes were reported in the left abdominal area as 4 masses and 1 mass (likely referring to several groups of enlarged lymph nodes or different lymph node locations). In the right abdominal area, 4 masses were found, and enlarged lymph nodes were detected around the urinary bladder (medial iliac lymph nodes) with a size of 40 mm (4 cm). In the study by Teodori *et al.* (2021), the normal size of canine lymph nodes is around 19.5 mm to 24.6 mm (depending on the location), so the size of 40 mm clearly indicates significant, neoplasm-suspicious lymphadenopathy. The involvement of internal lymph nodes

(mesenteric, medial iliac) indicates that the disease has spread systemically, supporting the diagnosis of advanced multicentric lymphoma (Stage IV).

Figure 1. Findings of enlarged lymph nodes on ultrasaound

Radiography

Supporting radiographic examination is necessary to detect the swelling of lymph nodes in the mediastinal (chest) and abdominal regions, as well as to determine the location of tumor growth and the possibility of lung metastasis (Reski and Sugianto 2022). Radiography is an examination using X- rays that pass through the patient's body organs, producing an image based on density differences (Farizka *et al*. 2020). The examination uses digital radiography so that the results are immediately visible on the monitor screen. The X-ray examination was performed by handling the patient without anesthesia. The X-ray for the patient was conducted with two views: the right lateral recumbency and the dorsoventral positions.

The *right lateral recumbency* position of the abdomen aims to visualize the contour, shape, and position of the internal organs, and to help evaluate the spread of tumor mass (Kayanuma *et al*. 2020). The *dorsoventral* position aims to view the organ structures symmetrically, especially the lungs, heart, and mediastinal and tracheobronchial lymph nodes. In the *right lateral recumbency* position, the patient's head and front legs were handled by one paramedic, while the hind legs were handled by another paramedic. In the lateral recumbency position, the patient's spine was ensured to be straight. In the *dorsoventral* position, the patient was handled, with the *os mandibula* and *Os sternum* positioned to adhere to the X-ray table surface (Meomartino *et al.* 2021). During image acquisition, the patient's spine was straightened to reduce the possibility of overlapping internal organs (Teodori *et al.* 2021). Image acquisition for both positions was performed during the patient's inspiration so that the lung images would appear clear (Bergamaschi *et al.* 2023). When the lungs are filled with air, the image will be black (lucent); if the image is taken during expiration, it will increase the radio-opaque (white) density.

Figure 2. shows the results of the lung fields being black (*lucent*), while the bones and internal organs were white (*opaque*). This color difference is because X-rays cannot penetrate solid objects, causing the bones to appear white (*opaque*). Organs such as muscles, fat, skin, and others appear gray. The black color indicates the presence of air or gas, such as in the lungs and abdominal cavity. Wirahadikesuma *et al.* (2020) stated that radiodensity is determined by the thickness of an object; the thicker the object, the greater the radiodensity

it possesses, thus causing the X-ray beam to weaken after passing through the object and increasing the density of the radiographic image.

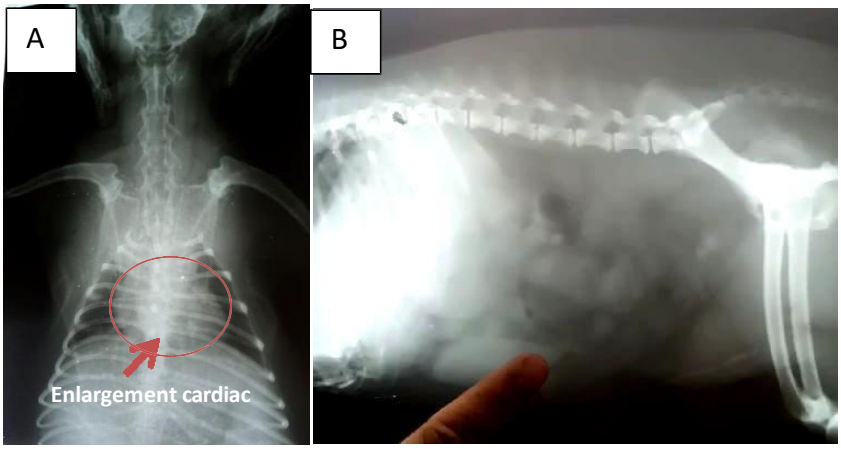

Figure 2. X-ray examination results for Bambi the dog. A) *dorsoventral;* B) *right lateral recumbency*

Based on the veterinarian's interpretation for patient Bambi, the X-ray results in the *right lateral recumbency* position showed that the size of the spleen, kidneys, liver, and intestines were still in normal condition with no obvious abnormalities (no significant hepatomegaly or splenomegaly). In the X-ray results in the *dorsoventral* position, enlargement was found in the lymph nodes in the patient's neck area, confirming the submandibular or prescapular lymph node enlargement seen during the physical examination. The lungs appeared normal with no nodules, ruling out lung metastasis. However, the heart examination revealed an enlargement of the heart size, or *cardiomegaly*. *Cardiomegaly* in a geriatric dog can be caused by chronic valve disease common in small breeds, but in lymphoma cases, *cardiomegaly* can be an important comorbidity finding in determining prognosis and anesthesia/treatment protocol. The respiratory tract (*trachea*) appeared slightly winding (*tortuous*) due to pressure or enlargement of the tracheobronchial lymph nodes around the main bronchus, which is sometimes the cause of abdominal breathing or a cough that was not specifically reported in the initial anamnesis.

CONCLUSIONS AND SUGGESTION

The use of supporting examination equipment for the lymphoma patient included laboratory testing for hematology and blood chemistry, ultrasonography imaging of the abdominal area using a curved transducer, and radiographic imaging using two views, the right lateral recumbency and dorsoventral positions. The stages of the supporting examination began with patient preparation, equipment preparation, and operator preparation. Supporting examination procedures must be carried out sequentially and correctly to aid in establishing a diagnosis. This research used a case study method with secondary data (medical records). A limitation of the case study was incomplete supporting examination data and the lack of biopsy examination, which affected the diagnostic analysis results.

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