PRIMARY CARDIAC LYMPHOMA WITH CONCURRENT PERICARDIAL MICROFILARIASIS IN A GERMAN SHEPHERD DOG - A CASE STUDY

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Primary cardiac lymphoma as a cause for pericardial effusion and cardiac tamponade is uncommon in dogs. Diagnosis and treatment of cardiac lymphoma is challenging, with guarded prognosis. A German shepherd dog was presented with an enlarged abdomen, reduced appetite, dyspnoea and nocturnal coughing. Pulsus paradoxus, hypo-resonant thorax, ascites, hepato and splenomegaly were found in examination. Normal complete blood count, elevated blood urea nitrogen and albumin levels in biochemical panel and microfilaria in blood were identified. Pleural effusion, pulmonary edema and cardiomegaly in thoracic radiography, ascites in abdominal ultrasonography, pleural effusion, fibrous adhesions, severe pericardial effusion with cardiac tamponade in echocardiography were identified. Also, free-floating structures, hypoechoic masses were seen adhered to the myocardium, pericardium and base of the aorta in echocardiography. Pericardial fluid analysis identified Dirofilaria repens microfilaria and lymphoma cells. Levemisole was given to eliminate microfilaria and a modified COP chemotherapy protocol consisting cyclophosphamide, vincristine and prednisolone was initiated against the lymphoma. By day 3 of levamisole treatment, microfilaria in blood and in pericardial effusion was negative and by week 3 of chemotherapy, pericardial effusion and lymphoma were absent. Meanwhile, the patient developed Babesia gibsoni infection and immune mediated hemolytic anemia, treated with imidocarb dipropionate. A day after the week 4 of chemotherapy, sudden dyspnoea and pleural effusion were developed and the patient succumbed. The clients had a lapse in the oral chemotherapy during the last week. Chemotherapy is effective against cardiac lymphoma but the schedule must strictly be adhered. Concurrent hemoparasitism and immune mediated conditions may complicate the clinical outcome of primary cardiac lymphoma.

**Keywords:** Pericardial effusion, pericardial microfilariasis, primary cardiac lymphoma, cardiac lymphoma, echocardiography, pericardiocentesis, COP protocol, chemotherapy, levamisole.

Lymphoma originates typically from lymphoid tissues and commonly multicentric, affecting the peripheral lymph nodes (Zandvliet, 2016; Ponce et al., 2010; Vezzali et al., 2010). However, various tissues including the spleen, liver, gastrointestinal tract, bone marrow, mediastinum, skin or any other tissue in the body can get affected (Vail, 2003; Duncan, 1999; Raskin, 2001; Thrall 1987). Primary cardiac lymphoma (PCL) originates from the heart, the pericardium or from both sites (Rolla et al., 2002; Nascimento, 2007), and are atypical and rare in dogs (Zandvliet, 2016; MacGregor et al., 2005; Aupperle et al., 2007). Incidence of cardiac tumors in dogs is very low, approximating 0.19% of all tumors (Ware and Hopper, 1999). Incidence of PCL out of the total cardiac tumors is about 2.5% (Ware and Hopper in 1999).

The clinical signs associated with PCL are not based on the tumor type, rather they depend on the mass effect or the
development of pericardial effusion (PE) (Kisseberth, 2013). The most common outcome of cardiac tumors located on the surface of the myocardium, pericardium or large vessels is hemorrhagic effusion into the pericardial space (Treggiari, 2015; Kittleson and Kienle, 1998). The cause for PE in 60% the dogs is cardiac neoplasia (Berg and Wingfield, 1984). Severe PE causes tamponade of right atrium (RA) and right ventricle (RV) due to the increased pressure exerted on the right side of the heart by the accumulated effusion (Ware, 2011). Elevation of RV filling pressure leads to reduced pre-load, congestive heart failure (CHF) and reduced cardiac output (CO) (Kittleson and Kienle, 1998; Stafford et al., 2004; Cho et al., 1998; Benvenuti et al., 2001). CHF related clinical signs are observed in the affected dogs, including; exercise intolerance, lethargy, anorexia, weight loss, abdominal enlargement, dyspnoea and cough (Kittleson and Kienle, 1998). In general clinical examination (GCE), muffled heart sounds, femoral pulsus paradoxus, jugular venous distension and pulsation, positive hepatojugular reflux, ascites, hepatomegaly, tachycardia and tachypnoea are often observed (Berg, 1984; Tobias, 2010; Ware, 2011; Kittleson and Kienle, 1998). In thoracic radiography, generalized cardiomegaly, spherical cardiac silhouette, tracheal elevation, distended caudal vena cava are observed (Ware, 2011; Kittleson and Kienle, 1998). However, often these patients develop pleural effusion (PE), which might obscure the cardiac silhouette and caudal vena cava (Kittleson and Kienle, 1998). Echocardiography is the procedure of choice in identifying PE and the presence of cardiac neoplasia (Tobias, 2010; MacDonald et al., 2009; Hoit, 2007). An anechoic space separating the parietal and visceral pericardium, oscillation of the heart within the pericardial fluid and diastolic collapse of the RA, RV or both are seen in echocardiography of patients with PE (Hoit, 2007; Gidlewski, 2005). Cardiac tumors are identifiable when PE is present, as the free end of the tumor masses extending into the pericardial space appear echogenic against the anechoic pericardial fluid (Ware, 2011). Pericardiocentesis is required to perform in all patients with PE as a temporary remedy for cardiac tamponade resulting clinical signs (Kittleson and Kienle, 1998). Analysis of the PE is an important part of identifying the etiology of PE. Dogs with PE of neoplastic origin have sanguinous (port wine colour) effusions (MacGregor, 2005). Even though, most cardiac tumors are non exfoliative and cytological analysis of the effusion unprofitable, pericardial mesothelioma and cardiac lymphoma exfoliate, and therefore cytological analysis of the pericardial fluid is of diagnostic value (McDonough, 1992; MacGregor, 2005). Lymphoma is identifiable by the presence of large number of lymphoblasts (>50%) with granular dispersed chromatin, prominent multiple nucleoli, lower nucleus to cytoplasm ratio, basophilic cytoplasm, mitotic figures, eosinophilic free chromatin, and basophilic small lymphoglandular bodies (Duncan, 1999).

Studies on therapy of canine lymphoma mostly conducted on multicentric lymphoma, and data on atypical lymphoma are limited (Zandvliet, 2016). Chemotherapy is the effective treatment of choice for canine lymphoma and a large number of chemotherapy protocols are available at present (Zandvliet, 2016; Vail, 2003). Commonly used combination chemotherapy protocols for lymphoma are modifications of the CHOP protocol that is used in treating human lymphoma (Vail, 2003). The CHOP protocol contains cyclophosphamide, doxorubicin, vincristine and prednisolone (Vail, 2003). COP protocol is an alternative to the CHOP protocol, and frequently used on dogs in European countries, (Vail, 2003). Two modified versions of the original COP protocol are available as high dose COP and low dose COP (Cotter, 1983). COP protocol consists of cyclophosphamide, vincristine and prednisolone (Vail, 2003).

Isolation of *Dirofilaria repens* microfilaria (MF) from PE in is a very rare occurrence in dogs and only 1 publication is available to date reporting pericardial microfilariasis in 2 dogs of 8 and 14 years of age (Paździor-Czapula, 2018). In humans also, only a handful of publications available on pericardial microfilariasis, in which
**Wuchereria bancrofti** was identified as a causative factor for pericarditis, PE and cardiac tamponade (Sinha et al., 2015; Prasanthi, 2010).

The following case study discusses about a canine patient with PCL and concurrent pericardial microfilariasis, treated with the modified low dose COP chemotherapy protocol.

**CASE STUDY:**
A 41/2 year old 28 kg female German shepherd dog (GSD) was brought to the Veterinary Teaching Hospital (VTH), University of Peradeniya, Sri Lanka, with a 4 day history of enlarged abdomen, reduced appetite, dyspnoea, exercise intolerance, lethargy, nocturnal coughing and 1 month history of diarrhea. The patient was treated with furosemide and spironolactone, and abdominocentesis was performed by another veterinary institution, 4 days prior to the presentation. At the time of presentation to the VTH, the patient was severely dyspnoic and orthopnoic with a restrictive breathing pattern. The pulse quality was alternating from weak to normal with no pulse deficit. Heart rate was 100 beats/min, temperature was 100.7°F, respiratory rate was 60 breaths/minute, capillary refill time was less than 2 seconds, mucous membrane color was pale and the dehydration percentage was 6%. Wheezing sounds were heard during lung auscultation and a hypo-resonant dorsal thorax and dull to hyporesonant ventral thorax were identified during thoracic percussion. Abdomen was enlarged and fluid waves were felt during percussion. Splenomegaly, hepatomegaly and mild abdominal pain were detected during abdominal palpation.

Differential diagnosis (DD) list at this stage was: CHF, PE, hepatic impairment and protein losing enteropathy. Following diagnostic investigations were carried out to arrive at a definitive diagnosis:

- Complete blood count (CBC) obtained by cell counter analyzer, MS9-5V (Melet Schloesing Laboratories, Osnv, France) showed: normal white blood cell (WBC) count of 17.57 \(10^3/\mu l\) (5.21.5 \(10^3/\mu l\)), red blood cell (RBC) count of 4.68 \(10^9/\mu l\) (3.4-7.6 \(10^9/\mu l\)), hematocrit (Hct) of 26.7% (25-47%), mean corpuscular hemoglobin concentration (MCHC) of 36.8% (34-42%), normal hemoglobin concentration (Hb) of 9.8 g/dl (9-19 g/dl) and normal thrombocyte (THR) count of 317 \(10^3/\mu l\) (200-500 \(10^3/\mu l\)). Polychromic macrocytes (PCM) and ghost cells were seen during examination of Leishman stained blood smear viewed under 10 \times 100 oil emersion light microscopy (Olympus, CX21, Tokyo, Japan). Peripheral blood drop was positive for MF with 5 MF /10 \(\mu l\) of blood under 5 \times 10 power light microscopy.

Biochemical profile showed an elevated blood urea nitrogen (BUN) level of 45.36 mg/dl (10.00-28.00 mg/l), lower margin of the normal range total protein (TP) level of 5.09 g/dl (5.00-8.80 g/dl), reduced albumin (Alb) level of 1.47 g/dl (2.60-3.30 g/dl) and normal ALT level of 45.4 U/L (20 -120 U/L).

Left lateral and dorsoventral (DV) thoracic radiographs were taken by X ray high voltage generator (UD150L-40F UD 150L-40E, Shimadzu, Kyoto, Japan). Diffused soft tissue opacity of the thoracic cavity, unclear cardiac silhouette and floating lung lobes indicating pleural effusion (PIE), increased opacity of the hilar lung field and pneumo-bronchograms indicating lung consolidation by edema and slight tracheal elevation indicating left cardiomegaly were seen on the left lateral thoracic radiograph (Fig. 1a). DV thoracic radiograph further confirmed the presence of PIE by the disappearance of cardiac silhouette and the presence of pulmonary consolidation and edema by the presence of pneumo-bronchograms (Fig.1c).

Moderate ascites was observed during abdominal ultrasonography performed with a 5 - 10 MHz linear array transducer (MyLab30Vet, Esaote, Genova, Italy). Mild to moderate PIE, fibrinous pleural strands and severe PE with cardiac tamponade were observed in echocardiography examination carried out with a 4 - 7.5 MHz linear array transducer (MyLab30Vet, Esaote, Genova, Italy) (Fig. 2a and 2b). Cardiac size was significantly diminished during both diastole and systole. Unclassifiable multiple free-floating large irregular echogenic structures were seen within the pericardial fluid (Fig. 2a and 2b).
Irregular hypoechoic masses were seen adhered to the myocardium and pericardium at multiple sites resembling either fibrinous pericarditis, tumor masses or both (Fig. 2a and 2b). A complete echocardiography examination was not possible at this stage due to the presence of PE and PIE and the orthopnoea of the patient.

Pericardiocentesis was performed after sedation with intravenous (IV) 0.2 mg/kg diazepam, local anesthesia with subcutaneous (SC) 1 ml of lidocaine HCl 2% and adrenaline 1:80,000. Approximately 65 ml of pericardial fluid was drained with a makeshift PE draining apparatus made with a standard 17G cannula, an intravenous (IV) infusion set, a rubber connector from an IV infusion set, a 3 way stopcock and a 10 cc syringe (Fig. 3). Complete removal of the pericardial fluid was not possible due to the

Figure 1. 1a. and 1b: Left lateral thoracic radiographs. 1a: At presentation: diffused soft tissue opacity of the thoracic cavity (yellow asterisk), unclear cardiac silhouette and floating lung lobes (red arrow) indicate PE. Increased opacity of the hilar lung field (white arrow) and pneumo-bronchograms (blue arrow) indicate consolidation of lung fields. Slight tracheal elevation (yellow arrow) indicates left cardiomegaly. 1b: 3 weeks after chemotherapy: Normal radiodensity of the thorax (yellow asterisk), normal positioning of lungs (red arrow) and clear cardiac silhouette indicate clearance of PE. Absence of hilar lung opacity and pneumo-bronchogram indicate absence of lung edema (blue arrow). Ventral dipping of the trachea at carina indicates absence of left sided cardiomegaly (yellow arrow).

Figure 1c. and 1d: Dorsoventral thoracic radiographs. 1c: At presentation: disappearance of cardiac silhouette indicates PE, pneumo-bronchograms (black arrow) indicate consolidated lung fields. 1d: 3 weeks after chemotherapy: identifiable cardiac silhouette indicates clearance of PE (red arrow). Absence of consolidation of the lung field and pneumo-bronchograms indicate improvement of lung edema.
repeated clogging of the cannula by the accumulated debris and fibrin strands of the PE.

A 10 cc sample of the PE was centrifuged to yield 3 distinct layers of the sediment. A thin smear from the top 2 layers of the sediment was stained with Leishman stain and examined under 10 x 100 high power microscope revealed a large number of unsheathed MF recognized as *Dirofilaria repens* (Fig. 4c) and large round cells with prominent multiple nucleoli, mitotic figures, eosinophilic free chromatin, and basophilic small lymphoglandular bodies, tentatively diagnosed as lymphoma (Fig. 4a and 4b). A manual count of 196 MF /10 µl of PE was obtained under 5 x 10 power light microscopy.

Figure 2. RPSA echocardiographic view at presentation. 2a. During diastole and 2b. during systole, shows diminished overall size of the heart due to PE. Severe PE (yellow asterisk), free-floating large echogenic structures (black arrows), hypoechoic masses (pink arrows), PIE (red asterisk) and fibrin strands (yellow arrows), pericardium (blue arrow), LV (green arrow) and right ventricle (red arrow).

Figure 3. Makeshift pericardiocentesis device: 17 G IV cannula (yellow arrow), IV infusion set (black arrow), 3 way stopcock (pink arrow), 10 cc syringe (blue arrow) and rubber connector from an IV infusion set (red arrow).
The patient was treated as an in-house patient and the initial treatment plan was started as: prophylactic antibiotic treatment in the form of clavulanate amoxicillin 10 mg/kg intravenously (IV) twice daily (bid) was commenced to prevent possible bacterial pleuritis from pericardiocentesis. Levasimole at 10 mg/kg per orally (PO) once a day (sid) for 5 days with ondansetron at 0.5 mg/kg IV bid, against pericardial microfilariasis and as an antiemetic consecutively, furosemide at 2 mg/kg PO bid and spironolactone at 2 mg/kg PO sid to control pulmonary edema, theophylline at 10 mg/kg PO bid as a bronchodilator, amino acid supplementation (Amino plus (TIL), Healthcare PVT. Ltd., Andhra Pradesh, India) at 5 ml/10 kg PO bid for hypoalbuminemia created by the loss of Alb via third space fluid loss or due to secondary hepatic malfunction due to CHF, and lactulose 5 ml/10 kg PO three times daily (tid) to reduce the BUN level by removing ammonium ions at the gut level.

The cytology report issued 3 days after the submission confirmed the neoplastic cell type as lymphoma and the parasitology report confirmed the MF as *Dirofilaria repens*.

By the day 3 of levamisole treatment, the MF count was negative in a peripheral blood drop viewed under low power light microscopy and the sediment of the PE obtained on the same day contained only 2
dead MF /10 µl. Approximately 35 ml of pericardial fluid was removed on the day 3 of levamisole treatment. The echocardiography examination after pericardiocentesis showed a much larger cardiac contour than at the presentation (Fig. 5a) and reduced severity of cardiac tamponade. The pericardial fluid was comparatively debris free and the unclassifiable free-floating structures seen at the initial examination were absent (Fig. 5a and 5d).

The hypoechoic masses attached to the myocardium and the pericardium were distinguishable enough to measure (Fig. 5a and 6c). In addition, in left parasternal (LP) cranial LV outflow view, approximately 2.3 cm diameter irregular hypoechoic mass was visible at the level of the aortic root with the free end protruding into the pericardial fluid (Fig. 6a and 6b).

Once receiving the cytology report confirming the tumor masses in the heart as lymphoma, the modified low dose version of the COP chemotherapy protocol was initiated at the induction dose of cyclophosphamide at 50mg/m² PO every 48 hours (q48h), vincristine at 0.5 mg/m² IV every 7 days, and prednisolone at 40mg/m² PO once daily for 7 days, then at 20mg/m² PO q48h (Vail, D., 2003, Cotter, 1983). The induction dose was planned to be given for 8 weeks and the maintenance dose as an alternative week treatment for 4 months.

After the first day of chemotherapy, as the general condition of the patient had been

Figure 6. LP cranial LV outflow echocardiographic view of the heart. 6a, 6b and 6c: 3 days after levamisole treatment. 6a: 2.3 cm diameter large hypoechoic lymphoma mass (red double head arrow) was visible at the root of the aorta (orange arrow). 6b: zoomed view of the same mass in 6a. 6c: approximately 1 cm diameter mass (red double head arrow) attached to the pericardium (blue arrow). 6d: 3 weeks after chemotherapy: aortic root was cleared from the lymphoma mass. RV wall (red arrow), PE (yellow asterisk)
gradually improving up to then; including the improved appetite and reduction of ascites, the patient was managed as an outpatient with weekly follow up examinations. On each follow up, CBC, basic biochemical panel, urinalysis, thoracic radiography and echocardiography examinations were to be performed, and the weekly vincristine dose was to be given on the same day.

One week after chemotherapy, the volume of PE and the size of the large lymphoma mass at the base of the aorta were subjectively reduced. The patients overall condition including the appetite, alertness, responsiveness and activity level were further improved.

Two weeks after chemotherapy, the RBC, Hct, Hb levels and THR levels were reduced to 3.35 x 10^6/µl, 19.8%, 6.7 g/dl and 109 10^3/µl, respectively. Examination of a Leishman stained peripheral blood smear revealed *Babesia gibsoni*, anisocytosis, ghost cells, spherocytes and microagglutination. The findings indicated an ongoing babesiosis, concurrent immune mediated hemolytic anemia (IMHA) and possible immune mediated thrombocytopenia (IMTP). Imidocarb dipropionate at 6.6 mg/kg intramuscularly (IM) was given against *Babesia gibsoni* organism with chlorpheneramine maleate at 2 mg/kg IM as a prophylactic anaphylaxis treatment. The second dose of imidocarb dipropionate was scheduled to be given in 2 week time.

Three weeks after chemotherapy, in thoracic radiography, PIE was absent and the cardiac silhouette was clearly visible. The hilar pulmonary consolidation was cleared in both lateral and DV radiographs (Fig.1b and 1d). In echocardiography, PIE, PE and cardiac tamponade were completely absent, and the large lymphoma mass attached to the base of the aorta was not visible (Fig. 6d). The other multiple masses attached to the myocardium and pericardium also were not visible (Fig. 5b). As the PE was absent the pericardial cavity could not be separately identified (Fig. 5b). The RBC, Hct and Hb levels were further reduced to 2.88 x 10^6/µl, 17.4% and 6.5 g/dl, consecutively, but the THR level was normalized to 254 10^3/µl.

After the regression of tumor masses and the clearance of PE, follow up examinations consisted only of CBC, basic biochemical panel and giving the IV dose of vincristine. Monthly echocardiography examinations were scheduled instead of weekly.

Four weeks after chemotherapy, a week after imidocarb dipropionate treatment, the RBC, Hct and Hb levels were elevated to 3.31 x 10^6/µl, 20.5% and 7.3 g/dl, respectively. However, the THR level was again reduced to 146 10^3/µl. Vincristine dose was given at the time of presentation but no echocardiography was performed. The second imidocarb dose was given on the same day. A day after the fourth follow up, the patient was presented with the complaint of sudden localized thoracic subcutaneous edema. Furosemide was prescribed at 3 mg/kg PO and a complete echocardiography examination was scheduled to be performed 3 days later to reexamine the possibility of recurrence of the lymphoma and PE. On the day of echocardiography examination, the patient was severely dyspnoeic and an immediate thoracic ultrasound examination revealed significant PIE and recurrence of PE. Emergency thoracocentesis was performed and a large volume of purulent exudate was removed. However, the patient had a sudden respiratory arrest during the procedure and succumbed. Later, it was found out that the clients had a lapse in giving cyclophosphamide oral dose for a week.

**DISCUSSION**

Primary cardiac lymphoma (PCL) is a rare condition in dogs (Ware and Hopper, 1999). In a retrospective study conducted by MacGregor et al. in 2005, out of total 7,248 records, only 12 dogs met the inclusion criteria for PCL, and the prevalence rate of PCL was identified as 0.17% (MacGregor et al. 2005).

Large breed dogs are commonly affected with lymphoma (Teske et al., 1994b; Edwards et al., 2003; Villamil et al., 2009) and GSD is a breed that has a high risk in developing lymphoma (Ware and Hopper, 1999; Edwards et al., 2003; Teske et al., 1994b; Villamil et al., 2009, Zandvliet, 2016). GSD is also has higher incidence of
cardiac neoplasia (Ware and Hopper, 1999). The patient in this case study was a GSD, and therefore, in a high risk breed category to acquire lymphoma. Lymphoma and cardiac neoplasia commonly affect middle age to old dogs (Dorn et al., 1967; Vail, 2003; Ware and Hopper, 1990). However, cardiac lymphoma was identified in young dogs as well (Ware and Hopper, 1999). In the previous study by MacGregor et al., 2005, the age range of PCL dogs was 2 to 16 years with 8 years being the median.

In a retrospective study conducted by Ware et al., it was reported that majority of PCL dogs were less than 7 years old (Ware and Hopper, 1999). Most recently, a case study reported by Kimura et al. in 2018, PCL was diagnosed in a 10 month old dog. In the present case study, the dog was 4 1/2 years old and represents the younger age group of the susceptible age range.

Even though, there is no gender susceptibility recorded, intact females are found to be less susceptible to PCL than neutered females. Young female Golden retrievers and vizslas neutered less than 1 year of age found to have an increase risk for developing lymphoma (Torres de la Riva et al., 2013; Zink et al., 2014). Even though, no such relationship has been identified in neutered GSD and the susceptibility to acquire lymphoma, being a neutered female must have played part in acquiring PCL in the current patient.

The case study was presented with classical clinical signs associated with PE, cardiac tamponade and RHF. Pulse paradoxus, muffled heart sounds, ascites and hepatomegaly are indicative of the presence of PE and RHF. However, the etiological factor for PE cannot be identified by clinical signs only, as the signs associated with cardiac lymphoma were mainly caused by the presence of PE. Signs of PE created by other cardiac neoplasia or by idiopathic pericarditis are the same (Kisseberth, 2013). Orthopnoea, restrictive breathing pattern and the dull sounds heard during percussion of the ventral thorax of this patient were not related to PE. Those findings were related to PIE which is a common outcome of right CHF. The wheezing sounds heard during auscultation were possibly due the compression of lung and airways by PIE and hilar pulmonary edema. Hyporesonance of the lung fields was most probably due to the compression of the lung field by PIE.

In any canine cardiac lymphoma patient, CBC and serum biochemical profiles were routinely measured (Vail, 2003; Gavazza et al., 2008). Abnormal findings in the serum biochemical panel in these patients are often mild and non specific and not diagnostic for canine PCL (MacGregor, 2005). In a study carried out by MacGregor in 2005, on dogs with cardiac lymphoma, low serum Alb levels and low total protein levels were detected in 4 dogs, and 6 dogs had high ALT levels (MacGregor, 2005). No information available as to the RBC morphological abnormalities associated with cardiac lymphoma. But canine lymphoma patients have shown various abnormalities associated with RBC: presence of schistocytes (Madewell et al., 1980), eccentrocytes (Caldin et al., 2005), acanthocytes (Warry et al., 2013), signs of immune-mediated hemolytic anemia (IMHA) and immune mediated thrombocytopenia (IMTP) (Day, 1996, Keller, 1992). In this patient, PCM and ghost cells were seen at the time of presentation, indicating the presence of intravascular immune mediated hemolysis. None of the studies on either canine lymphoma or cardiac lymphoma, reported abnormal BUN levels. However, elevated BUN levels are associated with reduced renal perfusion caused by forward heart failure (Saklayen et al., 2002, Gluck et al., 2011, Madewell and Norrdin, 1975). The initially elevated BUN level in this patient was probably due to the reduced renal perfusion.

Right CHF is a major cause for PIE, in which, the emptying of pleural fluid collected by the parietal pleural lymphatic vessels into systemic veins and then into the RA is impaired (Wiener-Kronish, 1987). In PE, the RA filling pressure is increased, which causes inadequate drainage of the pleural cavity. Ascites observed during abdominal ultrasonography, was due to the same reason; reduced venous return and subsequent elevation of the hydrostatic pressure of the caudal vena cava and
dependant veins, due to elevated RA filling pressure.

Lung consolidation identified by the presence of pneumo-bronchograms in the hilar region could be due to the increased filling pressure on the LA and subsequent elevation of hydrostatic pressure in the pulmonary veins. In the left lateral radiograph, tracheal elevation was detected, which indicated LV enlargement. However, in echocardiographic examination, there was no LV enlargement. PE enlarges the overall cardiac silhouette, mimicking bilateral cardiomegaly, thereby elevating the trachea. Cardiac size was reduced in both systole and diastole in PE because of the high filling pressures exerted on the cardiac chambers by the accumulated PE. Cardiac tamponade is observed in severe PE, which collapses the right side, especially the RA during diastole.

The unclassifiable multiple free floating large irregular echogenic structures seen within the pericardial fluid were possibly the aggregation of MF, as 3 days after levamisole treatment, those structures were absent and the PE was debris free. *Dirofilaria repens* microfilariasis in PE is an extremely rare finding in dogs (Paździor-Czapula, 2018). In humans also, only one such incident was reported; a case study of a 66 old woman with pericardial dirofilariasis (Nambiar et al., 2018). Hemodynamic effects of pericardial microfilariasis is not discussed extensively. However, movement of MF can cause mechanical irritation and inflammation of the pericardium (Paździor-Czapula, 2018).

The absence of pericardial fluid masks the presence of cardiac tumors in echocardiography examination (Ware, 2011). The presence of a large volume of PE in this case, caused the cardiac size to diminish and the multiple small tumors attached to the LV wall were not clearly distinguishable before pericardiocentesis. The complete volume of the pericardial fluid could not be removed, therefore, the leftover fluid was sufficient to make the multiple lymphomas masses to be visualized.

Often cardiac lymphoma is diagnosed entirely by cytological examination of the pericardial fluid (McGreggor, 2005). In this case study, the diagnosis of PCL was made solely upon the cytological findings in a Leishman stained smear made of the sediment of the centrifuged pericardial fluid. No surface markers of the lymphoma cells were identified by immuno histochemical staining or no histopathology was performed, as a biopsy from the lymphoma masses was not obtained due to the risk of perforating the aorta or myocardium. Sub classification of the PCL as a B cell, T cell or null cell was not carried out in this case study. Sub classification would have helped in determining the prognosis of the patient (Vail, 2003).

The COP protocol over CHOP protocol was selected because of the adverse effect of cardiotoxicity associated with doxorubicin (Ramsey, 2008; Hallman et al., 2019). The low dose COP protocol was selected over high dose protocol because of the sterile hemorrhagic cystitis associated with cyclophosphamide (Charney et al., 2003; Setyo et al., 2017). The COP protocol was found to be effective in this patient as, the gradual reduction of PE and the size of the large lymphoma mass was observed 1 week following chemotherapy. Also, because by 3 weeks after chemotherapy, PE and the large lymphoma mass and other multiple lymphoma masses attached to the LV free wall and the pericardium were not visible in echocardiography examination.

No reports on *Babesia gibsoni* infection of canine PCL patients on chemotherapy were recorded up to date. Moreover, hemoparasitism occurring concurrently with PCL was not recorded except in the study of Macgregor, 2005, where 2 candidates were found with high serum *Ehrlichia canis* antibody titers, but were failed to confirmed by PCR. Concurrent hemoparasitism could bring about further complications in PCL patients, since immunosuppressive effects of prednisolone and cyclophosphamide could lead to increase of parasitemia.

According to World Health Organization (WHO) staging of lymphoma, PCL with PE falls under stage V and sub stage b; which is characterized by being extranodal and in an organ other than liver and spleen and with the presence of clinical signs consecutively. According to WHO, lymphoma above stage...
III, and with clinical signs have poor prognosis for remission and survival, compared to lower stage lymphoma (MacGregor, 2005; Teske, 1994; Ruslander, 1997). Accordingly, the PCL diagnosed in this patient was of stage V and sub stage b, therefore, with poor prognosis. Since, a postmortem examination was not carried out, the immediate cause of death of the patient was not identified. Recurrence of PIE and PE within 1 week could be attributable to the lapse of giving cyclophosphamide oral dose for 1 week. Even though, no relationship has been establish between babesiosis and PCL, concurrent IMHA and IMTP possibly could have contributed to the recurrence (Day, 1996, Keller, 1992). Following management practices are recommended to avoid similar lapses in the chemotherapy protocols in future: performing echocardiography examinations during each follow up visit is of extreme importance, since, the gradual build up of PIE, PE or the recurrence of the tumor mass, initially would go unnoticed during the GCE.

The clients should be thoroughly advised about the importance of adhering to the chemotherapy protocol for the required time period. Also, they should be advised to keep the chemotherapy medications in a separate container in order to keep them apart from any other medications the patient might be prescribed during the time period of chemotherapy. The clients should be advised to inform the veterinarian about any lapse in the chemotherapy protocol immediately and to make corrections accordingly.

CONCLUSIONS
Chemotherapy, if implemented properly according to recommended protocols like CHOP or COP, remission could be expected in PCL. However, concurrent hemoparasitism, IMHA, IMTP or abrupt discontinuation of the chemotherapy drugs even for a brief period could have fatal consequences.

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