Case Report

A Case Report of Canine Pure Red Cell Aplasia in Middle-Aged Male Pomeranian

Watanyathorn Uthasin[1*] Kripitch Sutummaporn[2]

[1]Prasu-arthorn small animal hospital, Faculty of Veterinary Sciences, Mahidol University, Phuttamonthon 4 Rd., Salaya, Nakhon Pathom 73170, Thailand
[2]Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Sciences, Mahidol University, Phuttamonthon 4 Rd., Salaya, Nakhon Pathom 73170, Thailand

[*]Corresponding author, E-mail address: watanyathorn.uth@mahidol.ac.th

Received: 2 March 2023; Revised: 13 April 2023; Accepted: 24 April 2023

Abstract

Canine pure red cell aplasia (PRCA) is a rare pathological condition in dogs related to a lack of erythroid precursor linages that cause a failure of red blood cell production, leading to severe anemia. Middle-aged dogs, especially females, have a great chance of developing acquired pure red cell aplasia. The clinical signs show pale mucous membrane, systolic murmur, tachypnea, tachycardia, weakness, syncope, and seizure. For diagnosis, the clinical pathological findings show the normocytic normochromic non-regenerative anemia, and the bone marrow pathological findings show a lack of erythroid precursor cells. According to the responsiveness of the immunosuppressive therapy, PRCA is proposed as one form of the immune-mediated anemia. Most PRCA are secondary diseases associated with recombinant human erythropoietin (rhEPO) treatment, infectious disease including parvoviral infection or modified-live parvovirus vaccination, or bone marrow cancer. In case of poor prognosis, the histopathology shows collagenous fibrosis in the bone marrow, and recurrent diseases after withdrawing the treatment commonly occur. In this case study, an 11-year-old non-castrated male Pomeranian dog was presented with a history of syncope. The non-regenerative anemia was found with a decrease of erythroid precursor in the bone marrow. The dog has been successfully treated with prednisolone 2 mg/kg once daily and tapered down every two weeks. The clinical signs of syncope and other blood parameters improved after 2 weeks of treatment.

Keywords: Pure red cell aplasia, non-regenerative anemia, syncope

ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดงในสุนัขโตเต็มวัย พันธุ์ปอมเมอเรเนียน เพศผู้

วธัญธร อุทาสิน[1*] ไกรพิชญ์ สุธรรมาภรณ์[2]

[1]โรงพยาบาลสัตว์ประศุอาทร คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
ถนนพุทธมณฑลสาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170
[2]ภาควิชาปรีคลินิกและสัตวศาสตร์ประยุกต์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
ถนนพุทธมณฑลสาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170

[*]ผู้รับผิดชอบบทความ E-mail address: watanyathorn.uth@mahidol.ac.th

Received: 2 March 2023; Revised: 13 April 2023; Accepted: 24 April 2023

บทคัดย่อ

ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดง (Pure red cell aplasia: PRCA) เป็นภาวะที่พบได้ไม่บ่อย ส่งผลให้สัตว์มีภาวะเลือดจางรุนแรง มักพบในสุนัขโตเต็มวัยโดยเฉพาะเพศเมีย สุนัขมักมาด้วยอาการเยื่อเมือกซีด หายใจเร็ว หัวใจเต้นเร็ว หรือมีภาวะหมดสติ เมื่อตรวจเลือดมักพบภาวะเลือดจางชนิดที่ไม่มีการตอบสนองจากไขกระดูก เม็ดเลือดแดงมักมีขนาดปกติ โดยคาดว่ามีความเกี่ยวข้องกับระบบภูมิคุ้มกันเนื่องจากในการรักษามีการตอบสนองต่อยากดภูมิคุ้มกันได้ดี มักเป็นโรคที่เกิดตามมาจากการใช้ฮอร์โมนอิริโทโพอิติน (rhEPO) กระตุ้นการสร้างเม็ดเลือด การติดเชื้อ เช่น พาร์โวไวรัส พยาธิเม็ดเลือด หรือ เป็นมะเร็งกระดูก เป็นต้น ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดงมีพยากรณ์โรคที่ไม่ดี โดยเฉพาะอย่างยิ่งในกรณีที่ไขกระดูกมีการสร้างคอลลาเจนมาแทนที่แล้ว และมักกลับมาเป็นซ้ำหลังจากหยุดยากดภูมิคุ้มกัน สุนัขพันธุ์ปอมเมอเรเนียน อายุ 11 ปี มาโรงพยาบาลสัตว์ด้วยอาการหมดสติเป็นครั้งคราว หลังตรวจพบว่ามีภาวะเลือดจางรุนแรงโดยไม่พบการตอบสนองของไขกระดูก จึงมีการเก็บตัวอย่างไขกระดูก พบภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดง และพบว่ามีการตอบสนองต่อการรักษาด้วยยากดภูมิคุ้มกันชนิดเพรดนิโซโลน ขนาด 2 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัมได้ดี ภายหลังการรักษา 2 สัปดาห์ ไม่พบอาการหมดสติและมีผลเลือดอยู่ในเกณฑ์ปกติ

คำสำคัญ : ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดง ภาวะเลือดจางที่ชนิดไม่มีการตอบสนองของไขกระดูก ภาวะหมดสติ

Introduction

Pure red cell aplasia (PRCA) in dogs is associated with the disease of erythroid precursor and progenitor leading to a secondary failure of the erythropoiesis (Lucidi 2022). The clinical signs are pale mucous membrane, systolic murmur, tachypnea, or tachycardia (Means 2016). Most PRCA has a greater incidence in middle-aged female dogs and has a common clinical sign of severe anemia (Lucidi 2022). The information related to breed predisposition is unknown (Brooks et al., 2022).

Anemia is the most common hematological abnormality defined as a reduced red blood cell (RBC) mass and is recognized when the hematological values of the red blood cell panels, including the packed cell volume (PCV), hematocrit (Hct), hemoglobin concentration (Hb), or RBC counts, are below reference intervals (Ettinger and Feldman 2000). Anemia contributes to the development of pale mucous membrane, tachypnea, tachycardia, anorexia, weight loss, fatigue, lethargy, depression, weakness, syncope, or seizures (Ettinger et al., 2017). The two main pathophysiological mechanisms, regenerative anemia and non-regenerative anemia, are the causes of pathological anemia and divided into hemorrhage, hemolysis, and decreased red blood cell production (Ettinger and Feldman 2000). To find the causes of anemia, distinguishing between regenerative and non-regenerative anemia is essential for clinical diagnosis (Means 2016). The cause of regenerative anemia is likely to be hemolysis or hemorrhage (Ettinger et al., 2017). Meanwhile, the non-regenerative anemia is due to the decreased red blood cell production, which can be related to an abnormal function of extramedullary or intramedullary diseases including immune-mediated diseases (Weiss 2008). In the persistent chronic non-regenerative anemia in dogs, the clinical information shows the ineffective erythropoiesis for at least five days for the clinical duration (Lucidi 2022). The clinical pathological findings show normocytic normochromic anemia correlated to the reduced erythrocytic production and the low number of erythrocytic precursors in the bone marrow (Brooks et al., 2022).

The immune-mediated non-regenerative anemia is associated with a persistent anemia with a lack of a reticulocyte (Lucidi 2022). The symptom can be classified by the stage of targeted erythroid cell in bone marrow into three diseases including pure red cell aplasia (PRCA), precursor-targeted immune-mediated anemia (PIMA) and immune-mediated hemolytic anemia (IMHA) (Lucidi 2022; Brooks et al., 2022).

For the diagnosis of PRCA, the hematological findings are usually severe non-regenerative anemia with normocytic normochromic RBCs; therefore, the canine Coomb's test and direct antiglobulin test (DAT) which detects antibodies or complement bound to RBC membranes, are expected to be negative, but positive results have been occasionally reported; consequently, characteristic of bone marrow is very useful to clarify the diseases (Lucidi 2022). Not only PIMA but also IMHA and erythroid hypoplasia are often misunderstood due to the similarity in clinical conditions (Lucidi et al., 2017). PRCA affects erythroid progenitors and the presence of only rare erythroid progenitors in bone marrow (Brooks et al., 2022). PIMA affects early erythroid precursors and rubiblasts through metarubicytes respectively, and IMHA directly affects the number of red blood cells; meanwhile, erythroid hypoplasia presence marks red blood cell disorders (Lucidi 2022).

PRCA usually found as an acquired disease that can be both a primary disorder or secondary to some other

disorder (Means 2016) and has been associated with recombinant human erythropoietin (rhEPO) treatment (Fiocchi et al., 2017), parvoviral infection, or modified-live parvovirus vaccination, *Ehrlichia canis* infection, myelodysplastic syndromes, and myelofibrosis (Lucidi 2022).

The therapeutic strategy for PRCA in dogs is directly involved to the using of the immunosuppressive therapy (To et al., 2021). Glucocorticoids are the drug of choice for treating the canine PRCA, including prednisone, prednisolone, or dexamethasone administration (Brooks et al., 2022). Prednisolone is an intermediate acting corticosteroid and well absorbed after oral administration; and peak plasma concentration is increased in 0.5-3 hours (Schijvens et al., 2019) with half-life of 12-36 hours (Plumb 2011). It is a lipophilic molecule that can easily diffuse across the cell membrane and bind to the glucocorticoid receptors in the cytoplasm then the glucocorticoid complex is formed and transferred into the nucleus (Plumb 2011). After entering the nucleus, the activated glucocorticoid complex binds to the DNA resulting in increased an expression of anti-inflammatory genes and decreased an expression of pro-inflammatory genes (Schijvens et al., 2019). Prednisolone is metabolized in the liver and excreted by the kidney (Plumb 2011). Adverse effects of long-term administration exogenous corticosteroids with immunosuppressive dose are usually common and severe including dry hair coat, weight gain, panting, vomiting, diarrhea, elevated liver enzymes associated with steroid hepatopathy, pancreatitis, gastrointestinal ulceration, lipemia, activation of diabetes mellitus, iatrogenic hyperadrenocorticism, and thromboembolism; also the dog may be seen with polydipsia, polyuria, and polyphagia (Plumb 2011). Moreover, the dogs with thromboembolism significantly have an approximately 25 times shorter survival time than the dogs without thromboembolism (Ettinger and Feldman 2000).

Case description

An 11-years-old non-castrated male Pomeranian dog, 4.1 kilograms in body weight, was presented at the Prasuarthorn animal hospital, Faculty of Veterinary Science, Mahidol University, with a history of syncope twice for 10 minutes on the day before presented. He was fully vaccinated and regularly checked by a parasitic prevention program. Any abnormal medical conditions were not previously noticed by owners, and the dog has no history of toxin ingestion.

On initial physical examination, the dog was bright, alert, and responsive. The heart murmur was noticed, but the lung sound was normal. The heart rate was 130 bpm with a systolic blood pressure of 110 mmHg, and the respiratory rate was 28 bpm. The mucous membrane was pale with CRT < 2 sec. The moderate dental tartar was observed from the oral examination. There were no ocular and nasal discharges. The uncomfortable clinical signs such as abdominal cramp, or discomfort were not found. The rectal temperature was 101°F.

For the clinical diagnosis, the disease investigations are shown in Figure 1. A hematological examination was completed by the Mindray BC-5300 Vet, an auto hematology analyzer, to rule out systemic diseases and showed severe normocytic normochromic anemia with non-regenerative. Blood smear shows few polychromasia, hypochromasia, poikilocytosis with macrocyte and microcyte which are associated to an iron deficiency. The serum biochemistry was tested by the Beckman Coulter AU-480 chemistry analyzer and resulted in unremarkable. The reticulocyte percentage was corrected

with a normal PCV (45%), and the corrected reticulocyte percentage is 0.033% indicating non-regenerative anemia. The full results of the blood examination are shown in Table 1. The repeated complete blood count was examined three days after the first visit are shown in Table 2. The hematological examination showed the lower of all red blood cells, hemoglobin, and hematocrit than the result from the first visit. The second corrected reticulocyte percentage is 0.06% interpreted as non-regenerative anemia. All blood smear test results stay consisted; however thrombocytopenia occurring from decrease production, increase consumption, increase destruction, and increase sequestration was found, so the test kit for *Ehrlichia canis, Anaplasma phagocytophilum, Borrelia burgdorferi* antibody, and *Dirofilaria immitis* antigen was performed to investigate the blood parasites (SNAP 4Dx test kit®, IDEXX, Thailand). The Polymerase Chain Reaction (PCR) was also done to confirm *Ehrlichia canis*, *Anaplasma* spp., *Babesia* spp., and *Hepatozoon canis* infection. The PCR results were all negative. The flow cytometric assay for detecting the platelet-IgG was also utilized to rule out IMT and the result was negative.

The immune-mediated hemolytic anemia (IMHA) was investigated by the direct canine Coomb's test and the flow cytometric assay for detecting the RBC-IgG. Both assay results were negative.

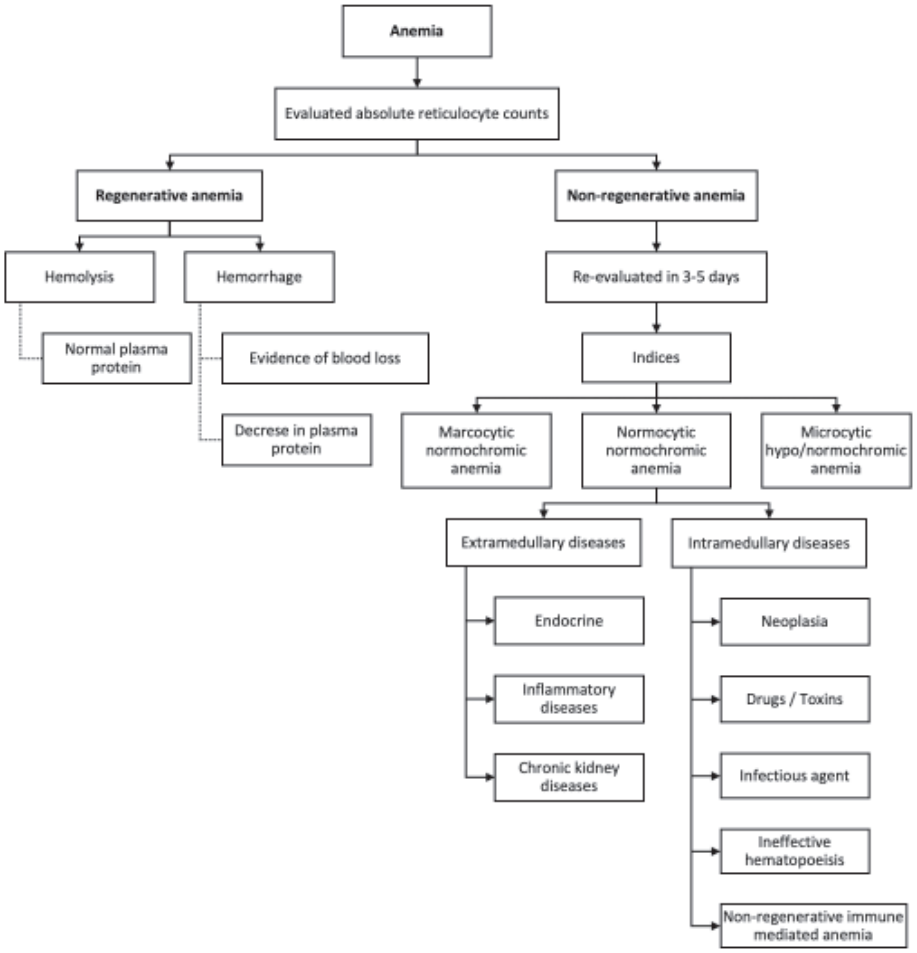

Figure 1. Flow chart of disease investigation. Anemia can be divided into regenerative and non-regenerative by absolute reticulocyte counts, which should be re-evaluated in 3 to 5 days. The non-regenerative anemia can be separated by indices to investigate causes of anemia.

Table 1. Results of blood examination on 14 July 2022, the first day of the visit.

Parameters	14/07/2022	Reference	Unit
Complete blood count			
WBC	8.28	6.0-17.0	10^3/uL
Monocytes	0.66	0.15-1.35	10^3/uL
Neutrophils	5.96	3.00-11.50	10^3/uL
Band neutrophils	0	0-3	%
Lymphocytes	1.57	1.00-4.80	10^3/uL
Eosinophils	0.08	0.10-1.25	10^3/uL
Basophils	0	0.00-0.10	10^3/uL
RBC	2.18	5.0-9.0	10^6/uL
Hb	4.8	10.0-18.0	g/dL
Hct	14.9	35-55	%
MCV	68.0	60-77	fL
MCH	22.0	20-25	pg
MCHC	32.3	32-36	g/dL
PLT	226	200-500	10^3/uL
RDW	20.6	12-15	%
Plasma protein	6.6	6.0-7.5	g/dL
Platelet smear	Adequate		
Reticulocytes	0.1	0-1.5	%
Blood morphology			
Hypochromia	2+		
Polychromasia	Few		
Anisocytosis	2+		
Macrocyte	1+		
Microcyte	1+		
Blood chemistry			
BUN	16	7-27	mg/dL
Creatinine	1.11	0.5-1.8	mg/dL
ALT	88	10-100	U/L
ALP	66	23-212	U/L
Total protein	6.4	5.2-8.2	g/dL
Albumin	2.9	2.7-3.8	g/dL

Table 2. Results of blood examination on 16 July 2022, the second day of the visit.

Parameters	16/07/2022	Reference	Unit
Complete blood count			
WBC	9.14	6.0-17.0	10^3/uL
Monocytes	0.09	0.15-1.35	10^3/uL
Neutrophils	6.76	3.00-11.50	10^3/uL
Band neutrophils	0	0-3	%
Lymphocytes	2.28	1.00-4.80	10^3/uL
Eosinophils	0	0.10-1.25	10^3/uL
Basophils	0	0.00-0.10	10^3/uL
RBC	2.08	5.0-9.0	10^6/uL
Hb	4.8	10.0-18.0	g/dL
Hct	13.9	35-55	%
MCV	67.1	60-77	fL
MCH	23.1	20-25	pg
MCHC	34.4	32-36	g/dL
PLT	170	200-500	10^3/uL
RDW	21.8	12-15	%
Plasma protein	7	6.0-7.5	g/dL
Platelet smear	Adequate		
Reticulocytes	0.2	0-1.5	%
Blood morphology			
Hypochromia	2+		
Polychromasia	Few		
Anisocytosis	1+		
Macrocyte	1+		
Microcyte	Few		

Thoracic radiography was conducted by the Philips Optimus 65 had shown in Figure 2, and abdominal radiography had shown in Figure 3 to rule out cardiopulmonary diseases and internal abdominal mass. Thoracic radiography illustrated unremarkable pulmonary diseases and mild cardiomegaly in the vertebral heart score (VHS) of 10.5 with a pulmonary knob, echocardiography should be further investigated. For abdominal radiography, calcification was found in both renal pelvises. There is no evidence of renomegaly, hepatomegaly, or splenomegaly.

Ultrasonography was executed by the GE Healthcare Logiq E10S to investigate renal blood flow and rule out abdominal mass. The ultrasonography result is homogeneous diffuse hypoechoic parenchyma with a prominent portal vein and rounding of the caudal tip of the liver as shown in Figure 4. The hepatic vein was dilated by 4 mm in Figure 5. Hyperechoic of the renal cortex and decrease of corticomedullary distinction with normal color blood flow at interlobar vessels of both kidneys within normal kidney size in length 3.7 cm in Figure 6. Gall bladder, spleen, adrenal glands, pancreas,

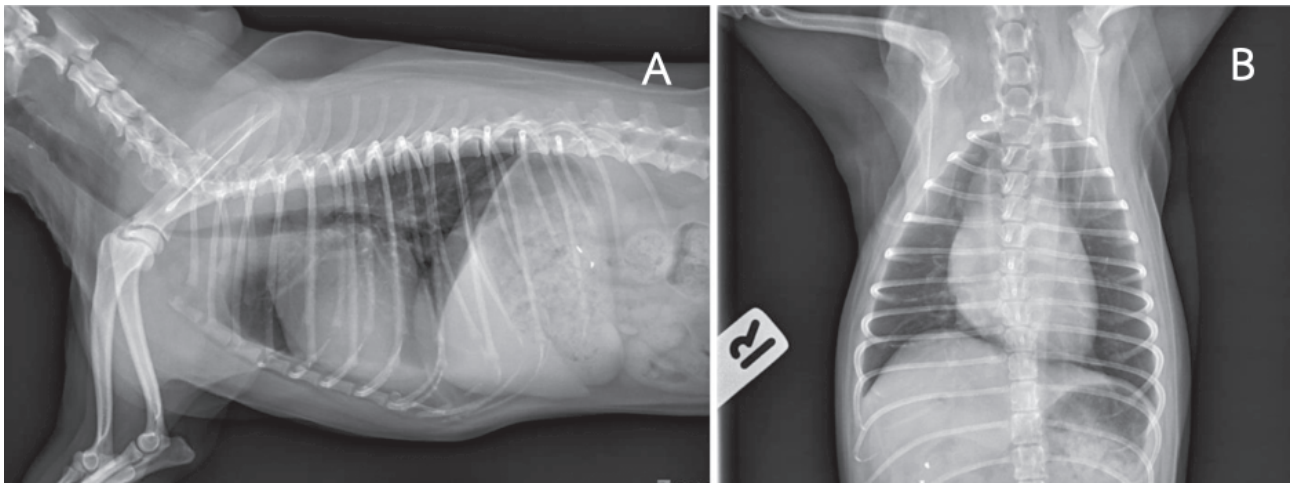

Figure 2. Lateral (A) and ventrodorsal (B) view of thoracic radiography suggested mild cardiomegaly in a vertebral heart score of 10.5.

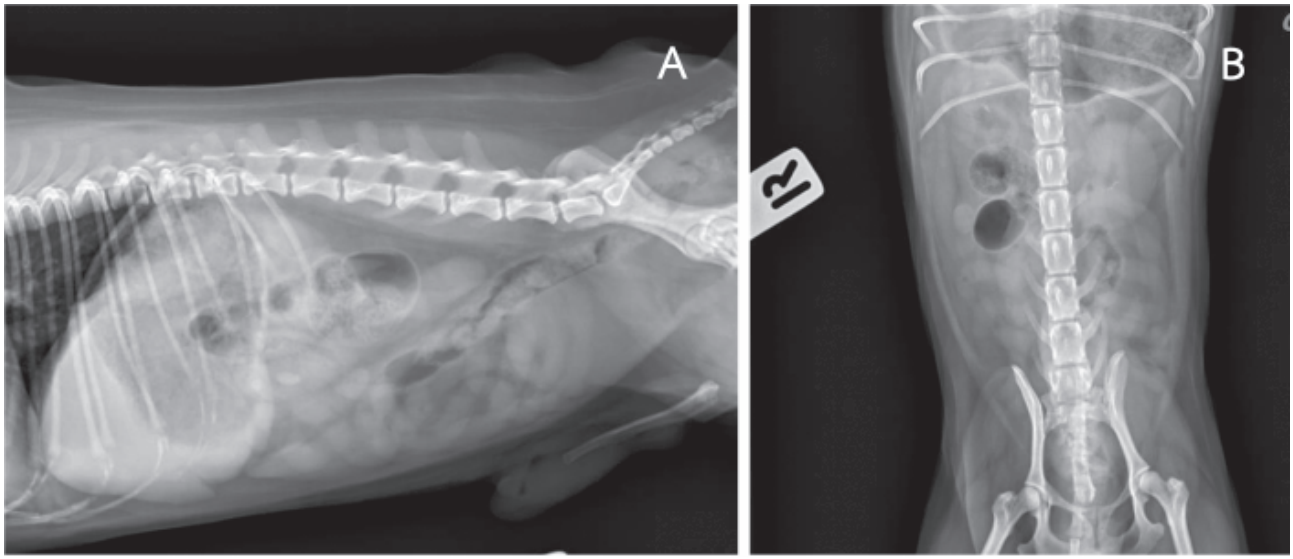

Figure 3. Lateral (A) and ventrodorsal (B) view of abdominal radiography which is unremarkable.

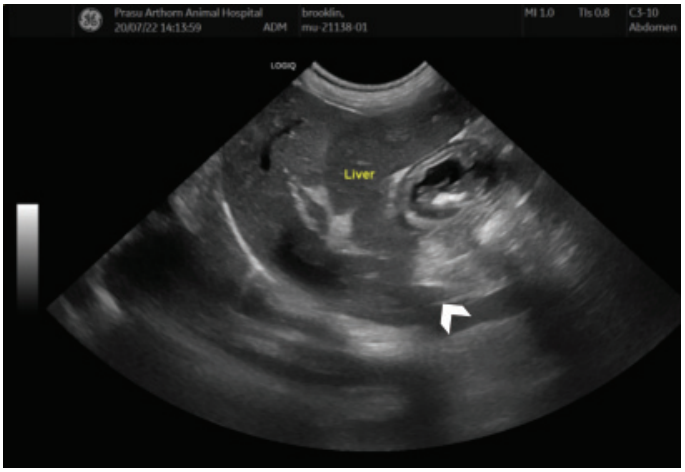

Figure 4. Rounding of the hepatic caudal tip showed a hepatic congestion (white arrowhead).

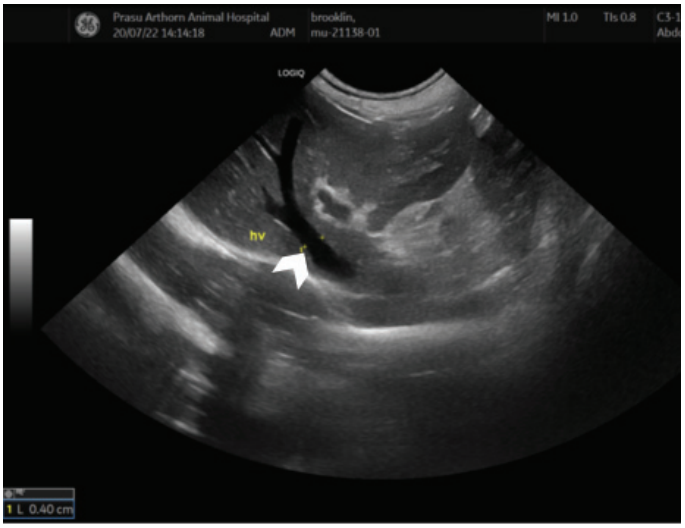

Figure 5. Hepatic vein was dilated in diameter of 4 mm specified a hepatic congestion (white arrowhead).

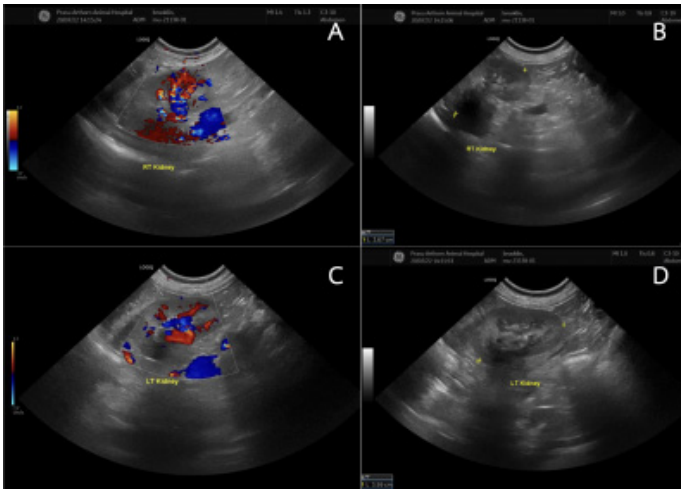

Figure 6. Right kidney with color flow (A), right kidney with no color flow (B), left kidney with color flow (C), and left kidney with no color flow (D) are decrease of corticomedullary distinction with normal color blood flow at interlobar vessels of both kidneys within normal kidney size both in length 3.7 cm that indicates an early stage of CKD.

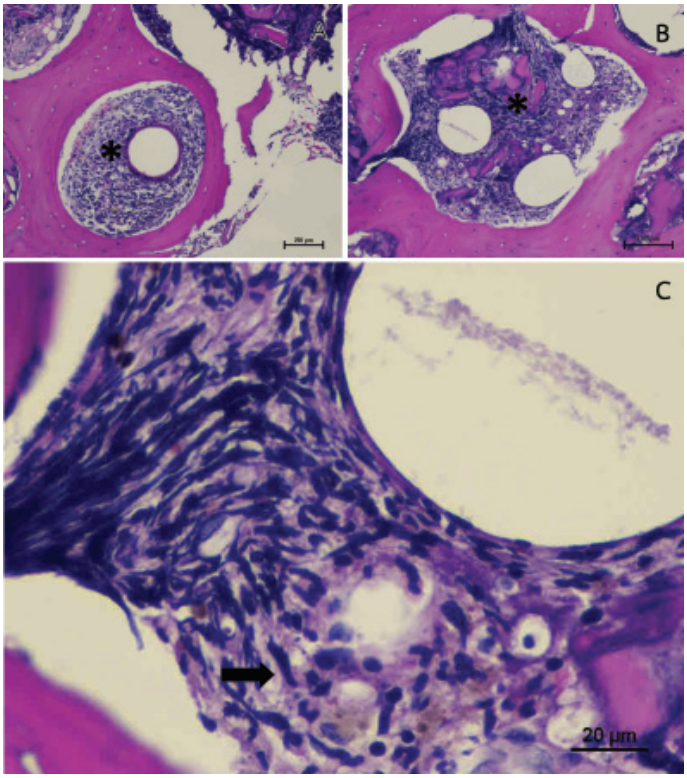

Figure 7. Bone marrow biopsy with H&E stain 4x (A), 10x (B), and 40x (C) respectively. Osteodystrophy The progenitor cells were replaced as fibrous tissues produced by fibroblasts, (asterisk). The fibroblastic spindle-shaped cells proliferated in the marrow cavity (arrow).

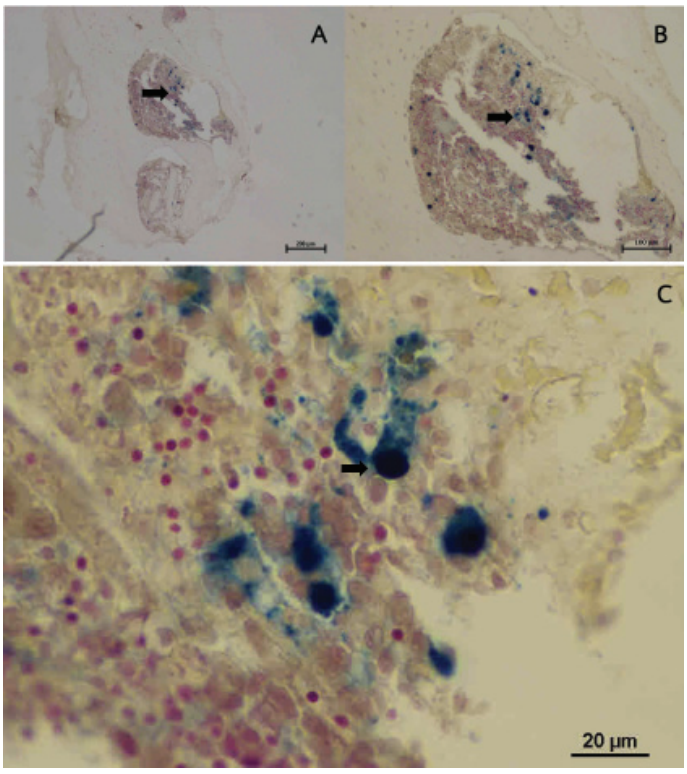

Figure 8. Bone marrow biopsy with Prussian blue 4x (A), 10x (B), and 40x (C) respectively. The iron-storage cells were stained to identify the iron-storage resources in the bone marrow. The blue-stained positive cells were depleted in the bone marrow cavity (arrows).

urinary bladder, and abdominal lymph node are no remarkable lesions. According to the ultrasonographic findings, passive congestion and renal nephropathy with early stage of chronic kidney disease (CKD) was diagnosed, there are no remarkable organomegaly, or neoplasia were found. To confirm an organomegaly or internal mass, a whole-body CT scan was done and resulted in unremarkable.

To investigate the cardiogenic diseases, echocardiography was performed by the Mindray Merge DC-70. The 2D measurement found that left atrial and aortic (LA: Ao) ratio is 1.34, and the normalized left ventricular internal dimension in diastole (LVIDDN) is 1.08. Therefore, both ratios are in a normal range. Doppler measurement found a mitral valve thickening and mild regurgitation without mitral valve prolapses. Marked tricuspid regurgitation (Max 3.8 m/s) and trivial pulmonic regurgitation was reported. Aorta, Pulmonary artery, and vein were not congested. The systolic and diastolic LV functions were non-remarkable lesions, with no aortic regurgitation. The diagnosis for echocardiography is myxomatous mitral valve disease stage B1 (MMVD B1) with intermediated pulmonary hypertension, cardiogenic syncope should be considered.

According to the diagnosis result above, there are no significant causes of severe non-regenerative anemia. A core bone marrow biopsy was collected from the left proximal humerus taken to investigate erythrocytic progenitors and precursors. The biopsy was processed as formalin-fixed embedded samples, sectioned, and stained with hematoxylin and eosin stain (H&E) and Perls Prussian blue stain. The bone marrow has a low number of erythroid lineages that were replaced by fibrous tissues and adipocytes, indicating osteodystrophy in Figure 7. The Perls Prussian blue was performed to identify non-heme iron deposition, and there was a non-heme iron deposition such as ferritin and hemosiderin in the bone marrow parenchyma in Figure 8. There are no remarkable abnormalities in cells such as leukemia or neoplasm found in this case, the bone marrow biopsy results in leading to the definitive diagnosis as pure red cell aplasia (PRCA).

For the treatment, a blood transfusion was completed while the diagnosis was in process and brought hematocrit from 13.9% to 32.9%. After the blood transfusion, the level of the hematocrit still decreased to 26.1% in two weeks. The prednisolone administration at 2 mg/kg per oral once a day was started with a repeated blood examination after the two weeks of the treatment, and the dog has a good response. Hematocrit was increased to 35.3%. After that, prednisolone was tapered down 25% every two weeks and recheck hematocrit. Hematocrit is still in reference interval. The comparison of hematocrit between before and after the treatment of prednisolone are shown in Table 3.

Table 3. Comparing of blood examination results between before and after the treatment.

Parameters	20/07/22*	4/8/22**	18/8/22***	7/9/22****	Reference	Unit
Complete blood count						
WBC	8.45	13.42	8.63	10.88	6.0-17.0	10^3/uL
Monocytes	0.52	0.8	0.43	0.32	0.15-1.35	10^3/uL
Neutrophils	6.16	10.1	6.12	8.26	3.00-11.50	10^3/uL
Band neutrophils	0	0	0	0	0-3	%
Lymphocytes	1.77	1.98	1.98	2.06	1.00-4.80	10^3/uL
Eosinophils	0	0	0.08	0.21	0.10-1.25	10^3/uL
Basophils	0	0	0	0	0.00-0.10	10^3/uL
RBC	4.62	3.64	4.46	5.81	5.0-9.0	10^6/uL
Hb	10.9	8.8	10.7	14.8	10.0-18.0	g/dL
Hct	32.9	26.1	35.3	45.3	35-55	%
MCV	71.1	71.8	79.1	78	60-77	fL
MCH	23.6	24.2	24	25.5	20-25	pg
MCHC	33.2	33.7	30.3	32.7	32-36	g/dL
PLT	153	190	308	443	200-500	10^3/uL
RDW	14.8	15.1	15.3	14.2	12-15	%
Plasma protein	8.6	7	8	7	6.0-7.5	g/dL
Platelet smear	Decrease	Adequate	Adequate	Adequate		
Hypochromia	-	Few	Few	-		
Polychromasia	-	Few	-	-		
Anisocytosis	Few	Few	1+	1+		
Macrocyte	Few	Few	1+	1+		
Microcyte	-	-				

*After blood transfusion, **Monitor in 2 weeks during the diagnosis, the hematocrit was decreasing, prednisolone was started after the blood examination, *** 2 weeks after start prednisolone 2 mg/kg, **** 2 weeks after tapering prednisolone down prednisolone 25%.

Discussion

Our case study is a middle-aged male dog that PRCA commonly affected (Lucidi 2022), presented at the animal hospital with a history of syncope associated with both cardiovascular system and severe anemia. For physical examination, the initial problem lists including a pale mucous membrane and systolic cardiac murmur in accordance with clinical signs of PRCA (Means 2016). From echocardiography results, the diagnosis is MMVD stage B1 with intermediate pulmonary hypertension and cardiogenic syncope was indicated, and it is probably associates to respiratory disease or hypoxia due to the tricuspid regurgitation that is related to systolic cardiac murmur (Reinero et al., 2020), consequently, cardiogenic syncope may not be the primary cause. The result of blood examination shows severe non-regenerative anemia which has two main causes dividing into extramedullary disease including endocrine disease, chronic kidney disease, inflammation, and neoplasia, and the other is intramedullary disease (Ettinger et al., 2017). According to the history taking and all diagnosis, extramedullary disease can be ruled out. The blood biochemistry shows unremarkable to renal and liver functions in the line with radiographic results have no significant of neoplasia lesion or abnormal shape of internal organ. Although, the ultrasonography result shows an early stage of CKD. 60% of CKD has particularly common in developed an anemia in dogs at IRIS stages 3 and 4 and frequently increase in end stage of renal disease (Lippi et al., 2021). For intramedullary disease, the bone marrow is characterized by complete lack of erythroid precursors typically as in PRCA (Means 2016) leading to our diagnosis.

According to a study of Brooks et al., the result demonstrated that the level of IgG fraction of serum of a dog with PRCA inhibited the generation of erythroid precursors in vitro correspond to the production of antibodies against membrane proteins expressed on erythroid progenitors and precursors, cytotoxic T-cell-mediated and molecules affecting the erythroid lineage and suppress erythropoiesis (Brooks et al., 2022). While the mechanism of primary PRCA remains unclear, most cases responsive to immunosuppressive therapy suggest that PRCA in dogs is immune-mediated anemia.

PRCA in dogs can be divided in to congenital and acquired. Most of them are acquired diseases, considering the limited data of young patients; it is difficult to identify congenital PRCA (To et al., 2021). As acquired PRCA can be secondary to a wide variety of conditions including blood parasite infections which can be ruled out from PCR and rule out rhEPO treatment by history taking, but parvovirus vaccination or infection remains one of the primary causes of PRCA. Some of puppies with parvovirus infection develop erythroid and myeloid hypoplasia, and the parvoviral infection or vaccination also causes PRCA in humans and affects erythropoietic precursor cells which can be tested by measuring IgG and IgM levels and by testing for the parvovirus DNA (Lucidi 2022). The limitation of this case is the lack of the test of parvovirus antigen detection due to the limitation of laboratory testing and the dog having no clinical signs associated with canine parvovirus. Parvoviral antigen from the bone marrow biopsy should be tested by fresh tissue sample while our sample is fixed in 10% formaldehyde which requires a specific primer. For blood examination, parvoviral antigen can be tested only if the dog is in viremia. If parvovirus infection can be ruled out, the cause of PRCA of this dog will be primary hematologic disorder, termed idiopathic PRCA. Furthermore, PRCA can be secondary to others disease such as mast cell

tumor (Akiyoshi 2021), or there is a study reported in human that PRCA are related to lymphoma (Choi et al., 2010).

For thrombocytopenia, the decrease production of thrombocytes can occur following an iron deficiency besides an iron supplement. There is a report in human studying about an iron supplement leading to thrombocytopenia (Eisa et al., 2021). Moreover, when the immune system affected to megakaryocyte-erythroid progenitor cells instead of erythroid progenitor cells, the production of thrombocytes will decrease and the destruction of it will increase by immune-mediated disease leading to severe thrombocytopenia termed immune-mediated thrombocytopenia (IMT). The thrombocytopenia of this dog has a better chance of being an iron deficiency according to mild thrombocytopenia and normocytic normochromic anemia were found.

The traditional immunosuppression approach in human began with an oral corticosteroid usually prednisone in doses 1 mg/kg. Overall, this approach appears to have a 40% response rate. (Means 2016). In most human cases, cyclosporine A is the first choice providing the best response rate but requires a concentration monitoring (150 to 250 ng/mL), and the second choice is cyclophosphamide (Lobbes 2023). In the meantime, the drug of choice for treating PRCA in dogs is corticosteroid comprising prednisolone (Brooks et al., 2022) with a combination of corticosteroids, adjuvant immuno-suppressive or immunomodulatory drugs that may be necessary in some cases. Nevertheless, it depends on the time it takes to respond to the treatment. Our case study uses prednisolone, a drug of choice for PRCA, 2 mg/kg per oral once daily then taper down 25% every two weeks; the dog has good response to the treatment. For long-term using corticosteroid, our case study was developed some of these predictable adverse effects which is steroid hepatopathy, polydipsia, polyuria, and weight gain from polyphagia; no thromboembolism were found during the treatment.

In addition, one or multiple blood transfusions may be essential therapy. The response time to the corticosteroid treatment of this dog is within 14 days and bringing the hematocrit back to the reference interval. The disease remission is within 128 days. The median initial response time was 38 days, and the time required for the PCV to return to the reference interval was 118 days; around 77% of dogs treated with immuno-suppressive therapy were in complete recovery (Brooks et al., 2022). The shorter response time than the median time may related to the blood transfusion before using corticosteroid treatment

An approximately 20% of remission dogs relapsed at a median of 302 days during weaning of immunosuppressive therapy (Brooks et al., 2022). The presence of collagenous fibrosis was significantly aggravating the disease. There is a study reported that the median survival time after PRCA was diagnosed without collagenous fibrosis is 1429 days, greater than two times of dogs with collagen myelofibrosis, whose median survival time is 578 days (Ettinger and Feldman 2000).

Conclusion

According to clinical signs of non-regenerative normocytic normochromic anemia, and lack of erythroid lineage from bone marrow biopsy, pure red cells aplasia has a high probability to be the final diagnosis. The dog responded well to the treatment of prednisolone 2 mg/kg per oral once daily and other clinical signs were clinically improved. Monitoring for relapse of this disease is still in progress. PRCA has a poor prognosis, especially with

collagenous fibrosis in the bone marrow, recurrent diseases after withdrawing the treatment commonly occur. The purpose of this case report is to make clinicians aware of possible causes of this disease and improve future definitive diagnosis.

Acknowledgments

The authors would like to express their sincerely gratitude to all staff at the Prasu-arthorn animal hospital, especially, Assistant Professor Rungrote Osathanon for being the consultant, Wachira Trakoolchaisri, DVM, the cardiologist for the diagnosis of cardiogenic disease, Pruksa Julapanthong, DVM, the ultrasonologist for the diagnostic ultrasound, and all the patient's support through collaboration.

References

Akiyoshi M. Precursor-targeted immune-mediated anemia in a dog with a stage IV mast cell tumor and bone marrow infiltration. Vet Clin Pathol. 2021;50:151-7.

Assenmacher TD, Jutkowitz LA, Koenigshof AM, Lucidi CdA, Scott MA. Clinical features of precursor-targeted immune-mediated anemia in dogs: 66 cases (2004-2013). J Am Vet Med Assoc. 2019;255(3): 366-76.

Brooks MB, Harr KE, Seelig DM, Wardrop KJ, Weiss DJ. Schalm's Veterinary Hematology. 7th ed. Pondicherry: Wiley; 2022.

Casadevall N, Nataf J, Viron B, Kolta A, Kiladjian J-J, Martin-Dupont P, et al. Pure red cell aplasia and antierythropoeitin antibodies in patients treated with recombinant erythropoietin. N Engl J Med. 2002;346(7):469-75.

Choi JH, Oh YH, Park IK. A case of pure red cell aplasia associated with angioimmunoblastic T-cell lymphoma. Cancer Res Treat. 2010;42(2):115-7.

Eisa MS, Al-Tikrity MA, Babikikir MM, Yassin MA. Thrombocytopenia secondary to iron deficiency anemia responding to iron therapy. Clin Case Rep. 2021;9(7):1-4.

Ettinger SJ, Feldman EC. Textbook of veterinary internal medicine. 7th ed. St. Louis: Elsevier; 2000.

Ettinger SJ, Feldman EC, Cote E. Textbook of Veterinary Internal Medicine: Diseases of the dog and the cat. 8th ed. St. Louis: Elsevier; 2017.

Fiocchi EH, Cowgill LD, Brown DC, Markovich JE, Tucker S, Labato MA, et al. The Use of Darbepoetin to Stimulate Erythropoiesis in the Treatment of Anemia of Chronic Kidney Disease in Dogs. J Vet Intern Med. 2017;31(2):476-85.

Javinsky E. Hematology and Immune-Related Disorders. In: Little SE, editor. The Cat: Clinical medicine and management. St. Louis: Elsevier; 2012. p. 643-703.

Lippi I, Perondi F, Lubas G, Gori E, Pierini A, D'Addetta A, et al. Erythrogram Patterns in Dogs with Chronic Kidney Disease. Vet Sci. 2021;8(7):123.

Lobbes H. Pure red cell aplasia: Diagnosis, classification and treatment. Rev Med Interne. 2023;44(1):19-26.

Lucidi CA, de Rezende CLE, Jutkowitz LA, Scott MA. Histologic and cytologic bone marrow findings in dogs with suspected precursor-targeted immune-mediated anemia and associated phagocytosis of erythroid precursors. Vet Clin Pathol. 2017;46(3): 401-15.

Lucidi CA. Precursor-Targeted Immune-Mediated Anemia and Pure Red Cell Aplasia in Dogs and Cats. In: Brooks MB, Harr KE, Seelig DM, Wardrop KJ, Weiss DJ, editors. Schalm's Veterinary Hematology. 7th ed. Pondicherry: Wiley; 2022. p. 307-12.

Means J, Robert T. Pure red cell aplasia. Blood. 2016; 128(21):2504-9.

Parrish CR. Pathogenesis of feline panleukopenia virus and canine parvovirus. Baillidres Clin Haematol. 1995;8(1):57-71.

Plumb DC. Plum's Veterinary Drug Handbook. 7th ed. Wisconsin: Wiley; 2011. p. 2973-86.

Reinero C, Visser LC, Kellihan HB, Masseau I, Rozanski E, Clercx C, et al. ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. J Vet Intern Med. 2020;34(2):549-73.

Sawada K, Fujishima N, Hirokawa M. Acquired pure red cell aplasia: updated review of treatment. Br J Haematol. 2008;142(4):505-14.

Schijvens AM, Ter Heine R, de Wildt SN, Schreuder MF. Pharmacology and pharmacogenetics of prednisone and prednisolone in patients with nephrotic syndrome. Pediatr Nephrol. 2019;34(3):389-403.

Stokol T. Idiopathic pure red cell aplasia and nonregenerative immune-mediated anemia in dogs: 43 cases (1998-1999). J Am Vet Med Assoc. 2000; 216(9):1429-36.

To I, Boelsterli PR, Walter JJ, Rutland BE. Suspected primary pure red cell aplasia in a 4-month-old intact male mixed Bernese moutain dog. Can Vet J. 2021;62:408-12.

Weiss DJ. Bone marrow pathology in dogs and cats with non-regenerative immune-mediated haemolytic anaemia and pure red cell aplasia. J Comp Pathol. 2008;138(1):46-53.

Weiss DJ, Wardrop KJ. Schalm's Veterinary Hematology. 6th ed. Iowa: Wiley; 2011.