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For the Degree of Master of Veterinary Medicine

Immune-mediated Etiology of Acquired von Willebrand Syndrome in a Jindo-Dog

Department of Veterinary Medicine
GRADUATE SCHOOL
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Abstracts

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An 8-year-old male Jindo dog, weighing 20 kg, with a 3-day history of severe pain in a thoracic mass-like lesion, was presented to the Veterinary Medical Teaching Hospital of the Jeju National University. Laboratory workup revealed non-regenerative anemia, markedly prolonged activated partial thromboplastin time (aPTT) and elevated C-reactive protein (CRP). The dog was hospitalized and medically managed with a phosphatate-binding agent, darbepoetin alfa, maropitant, and received supportive care with fluid therapy (plasma solution containing metoclopramide) for the management of chronic kidney disease (CKD). In addition, the administration of fresh whole blood transfusion or vitamin K1 did not correct the patient's prolonged aPTT or clinical signs. However, hematocrit (HCT), aPTT, CRP, and clinical signs were dramatically improved by the administration of an immunosuppressive dosage of glucocorticoid. Twelve days after treatment, corticosteroids were discontinued and replaced with leflunomide owing to severe

gastrointestinal adverse effects. Subsequently, the prolongued aPTT and elevated CRP level returned. The patient died two days later because of concurrent CKD. Coagulation factors VIII, IX, and von Willebrand factor were evaluated using ELISA, and only the von Willebrand factor showed a significant change. These results support the diagnosis of immune-mediated acquired von Willebrand syndrome (AvWS).

Keywords: AvWS, immune-mediated disease(IMD), coagulopathy, dog

Contents

Ⅰ. Introduction

Bleeding disorders are common in emergency veterinary medicine, and are classified into inherited and acquired types. With the exception of immune-mediated thrombocytopenia (IMT), disseminated intravascular coagulopathy (DIC), and rodenticide poisoning, bleeding disorders in dogs are predominantly inherited [1]. The most common hereditary bleeding disorder in humans and dogs is von Willebrand disease (vWD), followed by hemophilia A and B [2]. These inheritecd bleeding disorders are associated with defect of breakdown in certain component of hemostasis [2]. There are some reports that show that vWD can be both inherited and acquired in humans [3], but acquired vWD is still rare [4]. In human beings, acquired von Willebrand syndrome(AvWS) is related to various conditions including lymphoproliferative diseases, severe hypothyroidism, certain drug administration(e.g. valproic acid), thrombocytosis, myeloproliferative diseases and immune mediated diseases. Also, congenital and acquired cardiovascular defects including aortic stenosis or artificial heart valves are associated with AvWS [14].

Studies on acquired coagulopathy in dogs are rare, especially on AvWS, with only a few individual reports [5, 6]. All of these previous reports are related to angiostrongylosis infection, which responded to fenbendazole administration.

This case report describes the case of a patient with acquired coagulopathy who responded to immunosuppressive therapy. This case is unique in that there was no evidence of infection, unlike in previous reports in veterinary medicine [5, 6].

Ⅱ. Materials and Methods

Patient

An 8-year-old male, 18 kg Jindo dog was presented to the Veterinary Medical Teaching Hospital of the Jeju National University with history of severe pain on a thoracic mass-like lesion for 3 days, decreased appetite, and hesitation to move. In addition, the patient had been previously diagnosed with chronic kidney disease (CKD; IRIS stage 4).

Laboratory examinagtion

Blood and serum chemistry

Whole blood samples in potassium-EDTA tubes for CBC (IDEXX ProCyte Dx Hematology Analyzer; IDEXX Laboratories, ME, USA) and whole blood samples in Heparin tubes for serum biochemistry (IDEXX Catalyst one chemistry Analyzer; IDEXX Laboratories, ME, USA) were collected.

CBC test was done at the day of presentation(day 0) and day 1, 3, 6, 7, 8, 9, 20, 21, 22, 23 Serum biochemistry test was also done at day 1, 3, 6, 9, 10, 14, 20, 21, 22, 23.

Coagulation profile

Whole blood samples in Citrate tubes for Coagulation testing(VETSCAN VS pro Coagulation Analyzer; ABAXIS, CA, USA) were collected. Coagulation test was done at day 0, 7, 8, 9, 10, 11, 14, 20, 21, 22, 23.

Urinalysis

Urinalysis was done at the day 0. Urine samples were obtained by cystocentesis for urine analysis(UA) including dipstick chemistry (Cybow-50s, DFI, Gimhae, Republic of Korea). Urine specific gravity (USG) was measured using a clinical refractometer (Atago, Tokyo, Japan).

Canine anemia pathogen PCR test

Whole blood sample in potassium-EDTA was referred for canine anemia PCR (GreenVet, Yong-in, Republic of Korea).

ELISA

Blood concentrations of vWF and factors VIII and IX were measured by enzyme-linked immunosorbent assay (ELISA; All from MyBiosource, CA, USA) with canine control plasma (prepared from six healthy dogs) using citrated plasma samples stored at -70℃ until test.

Ⅲ. Results

1. Physical examination

The dog showed intense pain during palpation of a thoracic mass-like lesion (3×12 ㎝). No evidence of trauma was found. patient showed severe panting, pale mucosa, and a delayed capillary refill time (>3 sec; RI >2 sec); vital signs, including systemic blood pressure, temperature, and pulse rate, were normal.

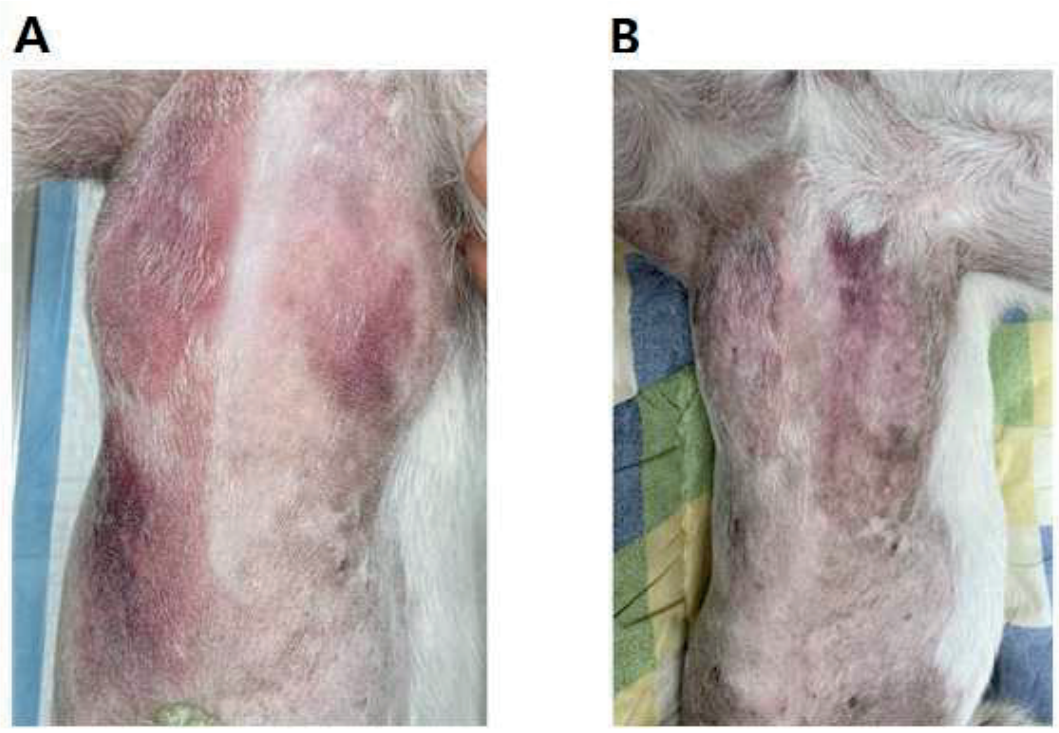

Figure 1. (A) Diffuse musculo-subcutaneous hematomas on thoracic region at day 3 (B) improvement of thoracic lesion after immunosuppressive treatment (day 11)

2. Laboratory test

Blood and serum chemistry

The test revealed non-regenerative anemia (hematocrit 19.5%; RI 37.3-61.7%; reticulocyte $9.7\times10^3/\mu\ell$; RI $10-110\times10^3/\mu\ell$) and thrombocytopenia (platelet count: 90×10^9/L;RI148-484×10^9/L). Results from serum biochemical analysis revealed severe azotemia (creatinine 9.8 ㎎/㎗; RI 0.5-1.8 ㎎/㎗; blood urea nitrogen >130 ㎎/㎗; RI 7-27 ㎎/㎗) and hyperphosphatemia (phosphorus; >16.1 ㎎/㎗; RI 2.5-6.8 ㎎/㎗). C-reactive protein (CRP) levels were elevated (8.9 ㎎/㎗; RI, 0-1 ㎎/㎗).

Table 1. Blood and serum chemistry results at presentation

CBC	Results	Normal ranges	Unit
RBC	3.61	5.65-8.87	10^{12}/L
HCT	19.5	37.3-61.7	%
#Reti	9.7	10-110	10^3/uL
WBC	16.03	6-17	10^9/L
#Neut	9.32	2.95-11.64	10^3/uL
PLT	90	200-500	10^9/L
Serum chemistry	Results	Normal ranges	
BUN	>130	7-27	mg/dL
Crea	9.8	0.5-1.8	mg/dL
PHOS	>16.1	2.5-6.8	mg/dL
T P	5.9	5.2-8.2	g/dL
ALB	2.7	2.2-3.9	g/dL
ALT	46	10-125	U/L
CRP	8.9	0.1-1	mg/dL

With the administration of prednisolone, the patient showed improvement with hematocrit elevation, shortened aPTT (164 sec; RI 75-105 sec) and decline in CRP (1.2 ㎎/㎗; RI 0-1 ㎎/㎗) (Figure 2 A-C).

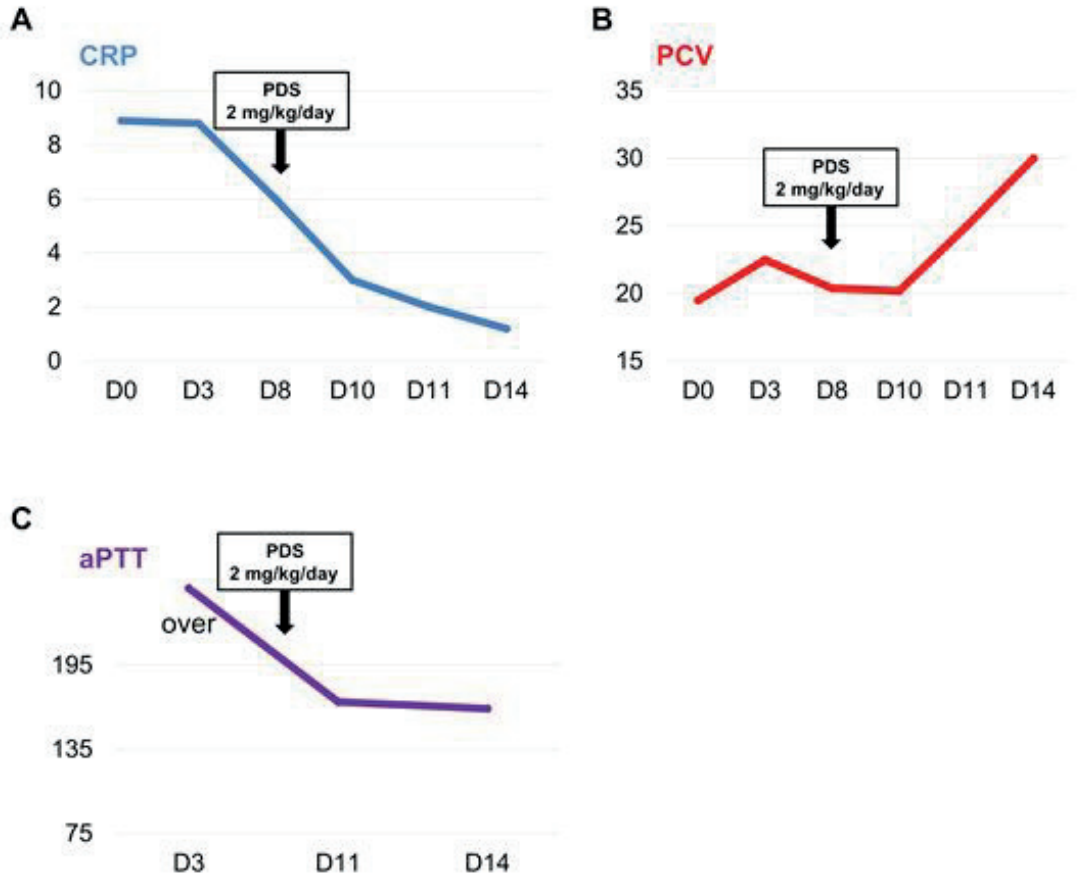

Figure 2. Changes of (A) serum CRP concentration, (B) PCV, and (C) aPTT of the patient.

Coagulation profile

Prothrombin time (PT) was within the reference range, but activated partial thromboplastin time (aPTT) was markedly prolonged (>200 sec ; RI 75-105 sec)at presentation(Table 1). The patient showed no improvement in coagulation profile after vitamine K injection and fresh whole blood transfusion.

Table 2. Coagulation profile at presentation

Coagulation Test	Results	Normal ranges	Unit
PT	16.2	14-19	sec
aPTT	>200	75-105	sec

Urine analysis (UA)

Urine analysis showed isostheuria (urine specific gravity; 1.012).

Table 3. Urinalysis at presentation

Urine alalysis	Results	Normal ranges	Unit
USG	1.012	1.015-1.025	

Canine anemia pathogen PCR test

To rule out infectious reason for anemia, Polymerase chain reaction (PCR) panel for canine anemia pathogen was done. The result was all negative.

Table 4. Result of canine anemia pathogen PCR test

No	Set	Pathogens	result	Class	Ct
1	KFT 1	*Anaplasma*	negative	-	-
2	KFT 2	*Ehrilchia*	negative	-	-
3	KFT 3	*Babesia* spp.	negative	-	-
4	KFT 4	*Lyme*	negative	-	-
5	KFT 5	*Bartonella* spp.	negative	-	-
6	KFT 6	*Hemotropic mycoplasma*	negative	-	-
7	KFT 7	*Rickettsia*	negative	-	-
8	KFT 8	*Leptospira*	negative	-	-
9	KFT 9	*Hepatozoon* spp.	negative	-	-
10	KFT 10	*Theileria* spp.	negative	-	-

ELISA

These are the result of serum concentration of Facotor VIII, Factor IX. and vWF by using ELISA Kit. For a differential diagnosis of coagulopathy, including von Willebrand Disease (vWD) and hemophilia A or B, the blood concentrations of vWF and factors VIII and IX were measured by enzyme-linked immunosorbent assay (ELISA; All from MyBiosource, CA, USA) with canine control plasma (prepared from six healthy dogs) using citrated plasma samples stored at -70℃ until test. The results of these analyses revealed a severe vWF decrease in the patients' sample after discontinuation of prednisolone (vWF:Ag = 61.9% to 19% ; RI 70-180%) with normal concentrations of factor VIII and IX coagulants (Figure 3 A-C).

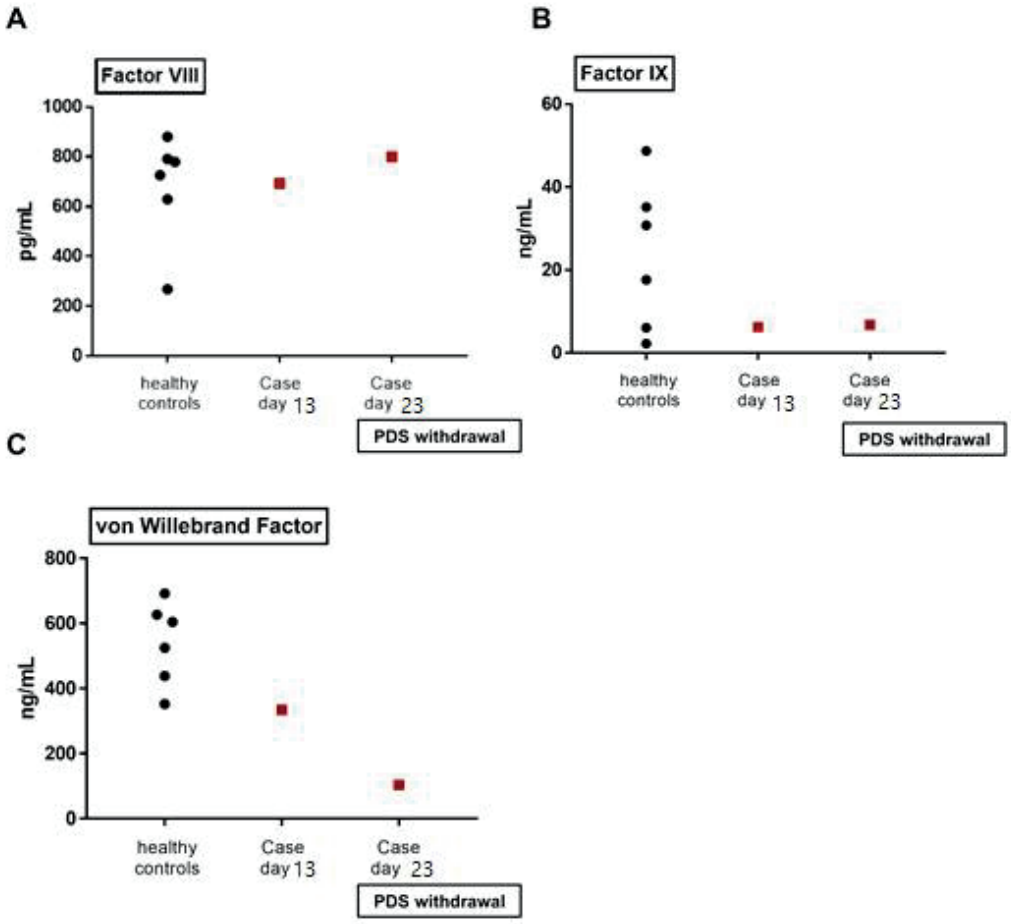

Figure 3. Serum (A) factor VIII concentration, (B) factor IX concentreation, and (C) von Willebrand factor of the patient before and after discontinuation, compared to normal healthy dogs.

Ⅵ. Discussion

Based on findings including physical examination, laboratory tests and imaging, thoracic lesion was suspected as musclo-subcutaneous hematoma.

At presentation, the patient was diagnosed with non-regenerative anemia and thrombocytopenia. In addition, patient has severe azotemia and hyperphosphatemia with elevated C-reactive protein (CRP) levels. Prothrombin time (PT) was within the reference range, but activated partial thromboplastin time (aPTT) was markedly prolonged (>200 sec ; RI 75-105 sec) (Table 1). Polymerase chain reaction (PCR) panel for canine anemia was all negative. Radiography and abdominal ultrasonography revealed no remarkable findings.

The dog was hospitalized at day 0 and treated with lanthanume (30 ㎎/㎏ two times a day, PO) as phosphatate binding agent, darbepoetinf (0.5 ㎍/㎏, SQ), maropitantg (1 ㎎/㎏ one time a day, IV), continuous rate infusion of metoclopramideh (0.02 ㎎/㎏/hr), and fluid therapy for management of CKD. Medication for coagulation disorder, vitamin K1 (1.1 ㎎/㎏, SQ) was administered with fresh whole blood transfusion (300 mL, DEA 1 positive). Remifentanilj (2-5 ㎍/㎏/hour, continuous rate infusion), which was substituted with fentanyl transdermal patch (50 ㎍/h), and gabapentin (10 ㎎/㎏ twice daily, PO) were administered for pain management.

Since the possibility of infection could not be ruled out, the dog was given doxycycline (5 ㎎/㎏ twice a day, PO). Despite these therapeutic attempts, the patient did not show any improvement.

A tentative diagnosis of immune-mediated coagulopathy (IMC) was made based on human IMC consensus [6, 7]. There was no evidence of infection or history of drug administration. In addition, fresh whole blood transfusion or vitamin K administration did not correct the patient's coagulation disorders or clinical signs. Therefore, the dog was treated with an immunosuppressive

dosage of prednisolone (1 ㎎/㎏ twice a day, PO). With the administration of prednisolone, the patient showed improvement with hematocrit elevation, shortened aPTT (164 sec; RI 75-105 sec) and decline in CRP (1.2 ㎎/dℓ; RI 0-1 ㎎/dℓ) (Figure 2 A-C).

Twelve days after treatment, prednisolone was discontinued and replaced with leflunomide owing to severe gastrointestinal adverse effects, including vomiting, diarrhea, and buccal ulcers. Then, aPTT was returned to >200 sec (RI, 75-105 sec), and CRP levels increased. The patient died two days later because of concurrent CKD.

Because of the clinical improvement of the patient and laboratory results after administration of prednisolone, immune-mediated coagulopathy (IMC) was suspected. For a differential diagnosis of coagulopathy, including vWD and hemophilia A or B, the blood concentrations of vWF and factors VIII and IX were measured by enzyme-linked immunosorbent assay (ELISA; All from MyBiosource, CA, USA). The results of these analyses revealed a severe vWF decrease in the patients' sample after discontinuation of prednisolone (vWF:Ag = 61.9% to 19% ; RI 70-180%) with normal concentrations of factor VIII and IX coagulants (Figure 3 A-C). These results are compatible with the immune-mediated etiology of AvWS.

This report describes the case of a patient with coagulation disorder who responded to immunosuppressive therapy. Autoimmune coagulation disorder refers to circulating anticoagulants of alloantibodies that inhibit the blood coagulation system [8]. The most common factor that can produce autoantibodies in humans is factor VIII, known for acquired hemophilia A. Other factors, including vWF, IX, V, XI, XII, and XIII, have also been reported for each autoantibody related to coagulation factor deficiency [9]. Among these, factors VIII, IX, and vWF are the most common factors associated with bleeding disorders in dogs [10, 11]. Therefore, these factors were selected for the measurement of blood concentration using ELISA.

In this case, with and without PDS, only the vWF was changed without changes in other factors. Therefore, it was suggested that the patient AvWS. This is a rare, non-inherited coagulation disorder similar to congenital vWD [12]. In humans, AvWS can occur in association with several underlying diseases, including lymphoproliferative, cardiovascular, solid tumors, hypothyroidism, and autoimmune disorders [13]; however, the patient did not show any evidence of these diseases. In addition, there was no response to several therapeutic attempts, including antibiotic therapy and fresh whole-blood transfusion. Therefore, immune-mediated coagulopathy was suspected. Although the patient was azotemic, prednisolone (1 ㎎/㎏ twice a day, PO) was administered to relieve the pain caused by musculo-subcutaneous hematomas from persistent bleeding.

Prolongation of aPTT, as was observed in this case, is not a common feature of vWD. Because vWF increases the half-life of Factor VIII in the blood, aPTT may be extended due to decreased Factor VIII in severe vWD [14]. Due to the absence of samples prior to taking PDS, which is a limitation of this report, it was not possible to confirm whether Facotr VIII was actually low before PDS treatment and affected the elongation of aPTT.

Several mechanisms have been proposed to explain the pathogenesis of AvWS. Most of these effects are explained by the increased clearance of vWF from the plasma. This includes cell absorption, proteolytic degradation, and autoantibody binding to vWF [15, 16]. Various underlying causes associated with these mechanisms include myeloproliferative neoplasias, cardiovascular disorders, lymphoproliferative disorders and autoimmune disorders [15]. Another mechanism described in AvWS arises from the decreased synthesis of vWF, as observed in hyperthyroidism [11]. In the past, AvWS was considered very rare, but in recent years, it has been thought that the actual incidence of AvWS is potentially underestimated [17].

There have been some case reports on AvWS in veterinary medicine.

Whitley *et al* reported a case of cerebral and conjunctival hemorrhage with vWF factor deficiency associated with angiostrongylosis in a dog. After treatment with fenbendazole and amoxycyillin clavulanate, the patient showed clinical improvement, with normal vWF levels. Even after 600 days, the vWF level and other coagulation results were normal [4]. Hausmann *et al* reported a similar case of vWF deficiency in an angiostrongylosis patient who was also deficient in Factor VIII. As in the previous report, the factor levels normalized after fenbendazole treatment [5].

To my knowledge, this is the first report of suspected immune-mediated coagulopathy of AvWS in a dog that is unrelated to infection.

Ⅴ. Conclusion

Although it should be noted that this is an individual case and that this patient was diagnosed as CKD IRIS stage 4, this case had the following characteristics related to AvWS.

In physical examination, there was an evidence of hemorrhage and musculo-subcutaneous hematoma. In blood and serum chemistry, the patient showed anemia and thrombocytopenia with about 100,000 platelet count on blood smear. There was no evidence of concurrent infection associated with anemia. In coagulation profile, Prolongation of PT and normal aPTT was observed. Also, there was no response with coagulation profile result after fresh whole blood transfusion, vitamine K1 administration. In ELISA, Factor VIII, Factor IX concentration was in normal range, but vWF concentration was significantly decreased after discontinuation of PDS. In response of treatment, No clinical response to fresh whole blood transfusion of vitamine K injection. But the patient Showed improvement in clinical sign, PCV elevation, coagulation test after immunosuppressive treatment. However, after the discontinuation of glucocorticoides, aPTT prolongation reccured.

In this case, IMC was suspected through consensus, and it was confirmed by improvement of symptoms through immunosuppressive treatment and deterioration after drug discontinuation. Finally, AvWS was diagnosed by ELISA.

In conclusion, when diagnosing and treating patients with coagulation disorders, immune-mediated causes should not be ruled out, and it is recommended to perform further tests including coagulation factor concentration tests for definitive diagnosis.

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Ⅶ. 국문초록

면역매개성으로 발생한 후천적 폰빌레브란트 증후군 진돗개의 증례

조희수

(지도교수 : 윤영민)

제주대학교 일반대학원 수의학과

체중 20kg의 8세 수컷 진도견이 3일 전부터 흉부에 종괴와 유사한 병변에 심한 통증을 호소하여 제주대학교 수의과대학병원에 내원하였다. 실험실 검사에서 비재생성 빈혈, 현저하게 연장된 활성화 트롬보플라스틴 시간(aPTT) 및 상승된 C-반응성 단백질(CRP)이 확인되었다. 환자는 입원하여 darbepoetin alfa, maropitant로 내과적으로 관리하고 CKD 관리를 위해 수액 요법(메토클로프라미드가 함유된 수액)으로 대증 치료를 받았다. 여기에 추가적으로 신선 전혈 수혈과 비타민 K1의 투여도 진행했지만, 환자의 연장된 aPTT 또는 임상 징후를 교정하지 못했다. 그러나, 글루코코르티코이드의 면역억제 용량 투여에 의해 헤마토크릿(HCT), aPTT, CRP 및 임상 증상의 즉각적인 개선이 이루어졌다. 치료 7일 후, 심각한 위장 부작용으로 인해 코르티코스테로이드를 중단하고 레플루노마이드로 대체하였다. 그 후, aPTT와 CRP 수치가 다시 상승하였다. 환자는 병발한 CKD로 인해 이틀 후 사망하였다. 응고인자 VIII IX, von Willebrand factor를 ELISA로 평가한 결과 von Willebrand factor에서만 유의한 변화를 보였다. 이러한 결과는 면역 매개 후천성 폰 빌레브란트 증후군(AvWS)의 진단을 뒷받침한

다. 내가 아는 한, 이것은 감염과 관련이 없는 개의 면역 매개 원인의 AvWS 에 대한 첫 번째 증례 보고이다.

키워드: 후천성 본빌레브란트 증후군, 면역매개성질병, 지혈장애, 개