Concurrent Epistaxis, Retinal Bleeding and Hypercoagulability in Dog with Visceral Leishmaniosis

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ABSTRACT

Canine visceral leishmaniosis (CVL) caused by *Leishmania infantum* is a common endemic zoonotic disease in the Middle East, including Israel. The common clinical signs, include, among others, ocular lesions and epistaxis. This report describes a case of CVL in a Boxer dog, which was presented to the hospital due to severe epistaxis, retinal hemorrhage, detachment and uveitis. The platelet count and platelet-crit were increased, and the clotting times were normal. Thromboelastometry demonstrated marked hypercoagulability. The bleeding tendency in this dog was therefore present concurrently with a hypercoagulable state and was likely a result of vasculitis secondary to circulating immune complex (CIC) deposition, which probably interfered with the platelet-vascular endothelium interactions. The dog improved with systemic prednisone and allopurinol therapy, as well as topical ophthalmic treatment. No bleeding episodes were noted from the time treatment was initiated. Nevertheless, the dog remained hypercoagulable during the follow-up period. Epistaxis and retinal hemorrhages in dogs with CVL may result from platelet dysfunction and thrombocytopenia, as previously reported. Nevertheless, based on the present findings, we suggest that in a subset of such cases, when thrombocytopenia is absent and hypercoagulability is demonstrated, bleeding may occur secondary to vasculitis. Thromboelastomety is therefore indicated in dogs with CVL, even in cases showing a bleeding tendency.

Keywords: Canine; *Leishmania infantum*; Thromboelastometry; Hemostasis; Coagulation.

INTRODUCTION

Canine visceral leishmaniosis (CVL) caused by *Leishmania infantum* is a common endemic zoonotic disease in the Middle East, including Israel, transmitted to dogs by sandflies (1-5). In endemic areas, many infected dogs harbor subclinical infections, with a minority of susceptible dogs showing clinical signs (4). The balance between the Th1 to Th2 cellular immune responses and the cytokine profile are important in the pathogenesis of natural CVL (4, 6-8). Boxers are particularly susceptible to CVL, due to single nucleotide polymorphism caused by mutations in the *Slc11a1* (Solute carrier family 11 member a1) gene (haplotypeTAG-8-141) promoter region (9). Impaired T-cell regulation, with deranged B-cell activity, leads to marked circulating immune complex (CICs) production (10). Their deposition on blood vessel walls with activation of the complement cascade, leads to vasculitis, polyarthritis, uveitis and glomerulonephritis (GN) (4, 5, 11, 12). Renal CICs deposition eventually leads to overt renal failure, the main cause of death in CVL (4).

CVL is a chronic systemic disease (4, 11), with highly variable clinical signs, commonly including skin lesions, lymphadenomegaly, weight loss, weakness or lethargy, inappetance, splenomegaly, polyuria, polydipsia (PU/PD), ocular lesions, diarrhea, vomiting, epistaxis and lameness (4, 5, 13-15). Common laboratory abnormalities include hyperproteinemia, hyperglobulinemia, hypoalbuminemia, decreased A/G

ratio, proteinuria, mild to moderate non-regenerative anemia, lymphopenia, thrombocytopenia, azotemia, increased alkaline phosphatase and alanine transaminase activities, leukocytosis, leukopenia and positive Coombs' and antinuclear antibody tests. CVL is confirmed by demonstrating the amastigotes in cytological or histological samples, by culture and molecular testing (4, 5). Positive serological tests have good sensitivity and specificity for diagnosing clinical CVL (4).

Epistaxis has been variably reported dogs with CVL, ranging from 3.8% to 16% of cases (4, 5). Nevertheless, CVL is a common cause of epistaxis in endemic areas reported in 29/61 dogs with epistaxis in Greece (16). The pathogenesis of epistaxis in CVL is complex. Although hemostasis has been extensively studied in clinical CVL, results are inconsistent.

Thrombocytopenia, including immune-mediated thrombocytopenia (IMT), occurs in up to approximately 50% of cases (4, 14, 17, 18).The hemostatic alterations in CVL are related to the severity of clinical signs and to thrombocytopenia (18). Nevertheless, thrombocytopenia was present in only 52% of CVL cases with epistaxis (16), and therefore, cannot be a regarded as a sole mechanism of epistaxis. Moreover, in another study of epistaxis in CVL, the proportion of thrombocytopenia was lower, albeit insignificantly, in the epistaxis group versus the dogs in which epistaxis was absent (19). Epistaxis in CVL is associated with hypergammaglobulinemia, decreased platelet aggregation in response to collagen, hyperviscosity, and nasal mucosal ulceration (19, 21). Platelet dysfunctions were described in CVL, including aggregation deficiency in response to ADP and collagen (18, 20). The buccal mucosal bleeding time (BMBT) of dogs with CVL and epistaxis was significantly prolonged compared to healthy controls (21), suggesting ongoing platelet dysfunction and vasculitis. An immunological component has also been suspected in in kala-azar patients, associated with the presence of platelet-bound antibodies (22). Thrombocytopenia and thrombocytopathia in CVL may result from vessel wall changes due to vasculitis, altered thrombopoiesis, or increased platelet destruction following renal and hepatic failure (15, 18, 23).

Prednisone, with allopurinol and meglumine-antimonate therapy in dogs with naturally-acquired clinical CVL has led to a faster improvement of platelet aggregation versus those treated only with anti-leishmanial therapy, possibly due to prednisone's action on specific anti-platelet antibodies (24).

Additionally, secondary hemostasis abnormalities were reported in dogs with CVL (4).The activated partial thromboplastin time (aPTT) was prolonged in dogs with CVL vs. controls, but was within reference interval (RI) (25). In experimentally infected dogs with CVL, prolonged thrombin time and increased fibrinogen/fibrin degradation products were observed (26). Other studies failed to demonstrate any differences in clotting times and D-dimer, von-Willebrand factor (vWF) and fibrinogen concentrations in dogs with CVL versus healthy controls (21, 26). Conversely, a recent study has noted hyperfibrinogenemia in 13/29 dogs with CVL, while thromboelastometry (TEM) supported hypercoagulability in symptomatic dogs (27).

Figure 1: The dog at presentation to the Hebrew University Veterinary Teaching Hospital. Note the severe epistaxis from the right nostril.

This report describes a dog with CVL, presented with concurrent epistaxis and a hypercoagulable state.

CASE REPORT

A 6.5-year old male intact Boxer dog from a rural area in Central Israel was referred to the Hebrew University Veterinary Teaching Hospital (HUVTH) due to intermittent epistaxis from the right nostril over a period of a month, inappetance, weight loss, PU/PD over the last six months, impaired vision and splenomegaly.

It was currently vaccinated and infrequently dewormed or treated against external parasites. Six months prior to presentation, it was examined at the referring clinic due to PU/PD,

weight loss and listlessness. CBC and serum chemistry were unremarkable, with exception of mild azotemia (creatinine 1.6 mg/dL; reference interval [RI] 0.5-1.3 mg/dL). It was tentatively diagnosed with chronic kidney disease (CKD), and treated with an antibiotic (brand, dose and treatment duration unknown). It improved clinically, but continued to lose weight. One month prior to presentation, episodic epistaxis from the right nostril was observed with sneezing while discharging blood clots. Over the last week prior to presentation, the dog seemed to have vision deficits, bumping into objects, even at daylight, and showed selective appetite and listlessness, weight loss and worsening PU/PD. Three days prior to presentation, it was re-examined by the referring veterinarian due to ongoing profound epistaxis from the right nostril, depression, weight loss and PU. Physical examination showed splenomegaly, pale mucous membranes, dehydration (6%) with normal vital signs.

Complete blood count (CBC) (Table 1) showed a mild normocytic normochromic anemia, with thrombocytosis (platelets 424x10^9/L; RI 143-400x10^9/L). Blood smear examination showed mature neutrophils with no cytoplasmic toxicity, very mild polychromasia, spherocytosis (20-30%) and microscopic agglutination. Serum chemistry (Table 2) showed azotemia (creatinine 3.4 g/dL; RI 0.3-1.3), hypophosphatemia, severe hypoalbuminemia (albumin 1.4 g/dL; RI 3.0-4.4), hyperglobulinemia and decreased A/G ratio. Serology for *E. canis* IgG (Immunocomb Ehrlichia, Biogal, Galed, Israel) was negative. Survey radiography of the nasal cavity was unremarkable. Abdominal ultrasonography showed marked, diffuse homogenous splenomegaly and unremarkable kidneys. Urinalysis showed proteinuria (4+) and urine specific gravity of 1.016 (Table 3).

At presentation to the HUVTH, the dog was quiet, alert and responsive, and showed poor body condition (2/9), gen-

Table 1: Hematology and hemostatic test results of a dog with leishmaniosis, epistaxis and hypercoagulability.

Analyte (units)	Day 0[1]	Day 3[2]	Day 17	Day 78	Reference interval
Leukocytes (x10^9/L)	8.29		12.06	11.43	5.2-13.9
Neutrophils (x10^9/L)	6.3		6.30	9.09	3.9-8.0
Lymphocytes (x10^9/L)	1.33		2.01	0.68	1.3-4.1
Monocytes (x10^9/L)	0.50		0.99	0.43	0.2-1.1
Eosinophils (x10^9/L)	0.17		0.15	0.43	0.00-0.60
Basophils (x10^9/L)	0.00		0.01	0.02	0.00-0.10
Large unstained cells (x10^9/L)	0.00		0.05	0.16	0.00-0.30
Red blood cells (x10^{12}/L)	2.48		3.21	2.86	5.70-8.80
Hemoglobin (g/L)	58		73	60	129-184
Hematocrit (L/L)	0.169	0.170	0.244	0.207	0.370-0.570
Mean corpuscular volume (fL)	68.1		76.2	72.5	58.8-71.2
Mean corpuscular hemoglobin (pg)	23.4		22.8	20.9	20.5-24.2
Mean corpuscular hemoglobin concentration (g/L)	343		299	288	310-362
Red blood cell distribution width (%)	14.6		13.3	13.8	11.9-14.5
HDW (g/L)	22.1		16.9	18.6	14.0-21.0
Platelets (x10^9/L)	424		392	155	143.3-400.0
Mean platelet volume (fL)	17.4		15.0	20.0	7.0-11.0
Platelet distribution width (%)	NA		60.7	59.7	40.6-65.2
Platelet crit (L/L)	NA		0.59	0.31	0.1-0.4
Prothrombin time (sec)	NA	8.23			6.0-8.5
Activated partial thromboplastin time (sec)	NA	13.8			11.5-19.5
Fibrinogen (g/L)	NA	0.433			0.15-0.4
Antithrombin activity (%)	NA	87.9			87.0-140.0
D-dimer (mg/mL)	NA	429			<250

[1] at the referring clinic, three days prior to presentation to the hospital; [2] at presentation to the hospital; HDW, cellular hemoglobin concentration distribution width; NA, not available.

Table 2: Serum chemistry results of a dog with leishmaniosis, epistaxis and hypercoagulability

Analyte (units)	Day 0[1]	Day 17	Day 78	Reference interval
Albumin (g/L)	1.4	2.07	1.78	3.0-4.4
Globulin (g/L)	8.9	5.73		2.2-4.4
Albumin/globulin ratio	0.20	0.36		0.6-1.2
Total protein (g/L)	10.3	7.8		5.5-7.6
Alanine transaminase (U/L)	26	246		19-67
Alkaline phosphatase (U/L)	30			21-170
Amylase (U/L)	2863			103-1510
Aspartate transaminase (U/)	28			19-42
Creatine kinase (U/L)	147			51-399
g-glutamyltranspeptidase (U/L)	1.1			0-6
Creatinine (mg/dL)	3.4	2.5	2.5	0.3-1.2
Urea (mg/dL)	185	199	90	10.7-53.5
Glucose (mg/dL)	99			64-123
Calcium (total) (mg/dL)	8.8			9.7-11.5
Phosphorus (mg/dL)	9.4		6.85	3.0-6.2
Potassium (mmol/L)	5.2		5.5	3.6-5.3
Sodium (mmol/L)	143			145-154
Chloride (mmol/L)	118			108-118
Cholesterol (mg/dL)	221			135-361
Triglycerides (mg/dL)	88			19-133
Total bilirubin (mg/dL)	0.2			0.0-0.2

[1] at the referring clinic, three days prior to presentation to the hospital.

Table 3: Urinalysis results of a dog with leishmaniosis, epistaxis and hypercoagulability

Analyte (units)	Day 0[1]	Day 3[2]	Day 17	Day 78
Specific gravity	1.016		1.023	
Protein	4+		4+	
pH			6.5	
Glucose			Negative	
Ketones			Negative	
Urobilingen			Normal	
Bilirubin			Negative	
UPC (mg/mg)[2]		22.6	12.0	9.16
		Sediment		
Leukocytes			1-2/X10 field	
Erythrocytes			2-4/X10 field	
Sperm			Rare	
Bacteria			Negative	

[1]: at the referring clinic, three days prior to presentation to the hospital;
[2], urine protein/creatinine ratio; reference intervalis <0.4.

eral muscle atrophy, pale mucous membranes, stertor, sneezing and splenomegaly, normal heart rate and body temperature and mild tachypnea (respiratory rate 32 breaths/min).

Ophthalmic examination showed anisocoria, with the right pupil larger than the left one. In the right eye, there were an inconsistent menace response, partial direct pupillary light reflex (PLR), and a questionable consensual PLR when the right eye was stimulated. Ophthalmoscopic examination revealed vitreal liquefaction and inflammatory haze, partial retinal detachment, and two retinal petechiae near the optic disc. Findings in the left eye included lack of a menace response, direct PLR, and consensual PLR when stimulating the left eye. Ophthalmic examination revealed keratic precipitates and inflammatory debris on the anterior lens capsule, vitreal hemorrhage and extensive retinal detachment with multiple retinal petechiae. Intraocular pressure (IOP) in the right and left eyes was 6 and 5 mm Hg, respectively (RI 15-25 mm Hg). The ophthalmic diagnoses included anterior uveitis in the left eye, and bilateral vitreitis, retinal hemorrhage and retinal detachment. The prognosis for regaining vision in the right and left eyes were guarded and poor, respectively.

The systolic/diastolic arterial blood pressures (ABPs) were 155/102 mmHg (systolic BP RI <180). Serum chemistry confirmed the hypoalbuminemia, hyperglobulinemia and azotemia (Table 2). The urine protein to creatinine ratio (UPC) was markedly high (Table 3). Serology for *L. infantum*

Table 4: Thromboelastometry (Ex-TEM) results in a dog with leishmaniosis, epistaxis and hypercoagulability

Analyte (units)	Day 3[1]	Day 17	Day 78	Reference interval
Clotting time (sec)	42	38	42	31-97
Clot formation time (sec)	17	17	22	58-282
Alpha (degrees)	87	87	85	48-78
Amplitude at 10 min (mm)	77	77	73	32-66
Amplitude at 20 min (mm)	81	81	74	34-70
Maximal clot force (mm)	82	83	80	39-72
Maximal lysis (%)	0	0	3	0-62
Lysis at 30 min (%)	100	100	100	92-100
Lysis at 45 min (%)	100	100	100	74-100
Lysis at 60 min (%)	NA	NA	100	54-99
Maximal velocity	75	72	57	6-22
Time to maximal velocity (sec)	55	47	55	33-162
Area under curve	8085	8114	7874	3912-7149
Maximal clot elasticity	452	476	396	64-274
Time to maximal clot force (sec)	1825	2353	3829	1269-2878
Clot formation rate (degrees)	87	87	86	55-79

[1] at presentation to the hospital; NA, not available.

Table 5: Thromboelastometry (In-TEM) results in a dog with leishmaniosis, epistaxis and hypercoagulability.

Analyte (units)	Day 3[1]	Reference interval
Clotting time (sec)	168	97-203
Clot formation time (sec)	29	50-287
Alpha (degrees)	84	46-78
Amplitude at 10 min (mm)	74	28-63
Amplitude at 20 min (mm)	79	36-68
Maximal clot force (mm)	79	42-70
Maximal lysis (%)	5	0-17
Lysis at 30 min (%)	99	99-100
Lysis at 45 min (%)	96	99-100
Maximal velocity	45	4-25
Time to maximal velocity (sec)	187	113-256
Area under curve	7774	4273-6972
Maximal clot elasticity	368	91-265
Time to maximal clot force (sec)	1192	1726-2822
Clot formation rate (degrees)	85	47-79

[1] at presentation to the hospital.

(ImmunoRun Leishmania, Biogal, Galed, Israel) was positive, and this was confirmed by a positive in-house ELISA (1).

Whole blood obtained in 3.2% trisodium-citrate tubes was used for plasma-based hemostatic tests (prothrombin time [PT], aPTT, antithrombin activity [ATA], fibrinogen concentration (ACL 9000 coagulometric analyzer; IL, Milan, Italy; and D-dimer concentration; Tina-quant D-dimer Gen 2, Roche; Cobas Integra 400 Plus, Roche, Mannheim, Germany). Thromboelastometry (ROTEM delta analyzer; Rotem, Munich, Germany) was performed using citrated whole blood within 15 minutes from collection, with recombinant tissue factor (Ex-TEM) and a contact activator (kaolin; In-TEM). Citrated plasma was harvested and analyzed within 30 minutes from collection. The clotting times were within RI, while ATA was borderline low and D-dimer and fibrinogen concentrations were above their RIs (Table 1). The In-TEM and Ex-TEM both showed evidence of marked hypercoagulability (Tables 4 and 5; Figure 2A and 2B).

At that point, the dog was also diagnosed with CKD secondary to CVL, with both a tubular component (manifested by azotemia) and a glomerular component (based on hypoalbuminemia and markedly increased UPC).

The dog was then discharged with the following medications: acepromazine (PromAce, Boeringer, Ingelheim, Germany; 12.5 mg PO PRN to decrease blood pressure in case of epistaxis), enalapril (Enaladex, Dexcel, Or Akiva, Israel; 20 mg PO q12h; antihypertensive and to decrease proteinuria), amlodipine (Teva, Petach-Tikva, Israel; 5 mg PO q24h; antihypertensive), allopurinol (Alloril, Dexcel, Or Akiva, Israel; 400 mg q12h; antileishmanial), famotidine (Teva, Petach-Tikva, Israel; 20 mg PO q24h; to prevent gastric ulceration), metoclopramide (Pramin, Rafa, Jerusalem, Israel; 10 mg PO q8h; antiemetic), mirtazapine (Teva,

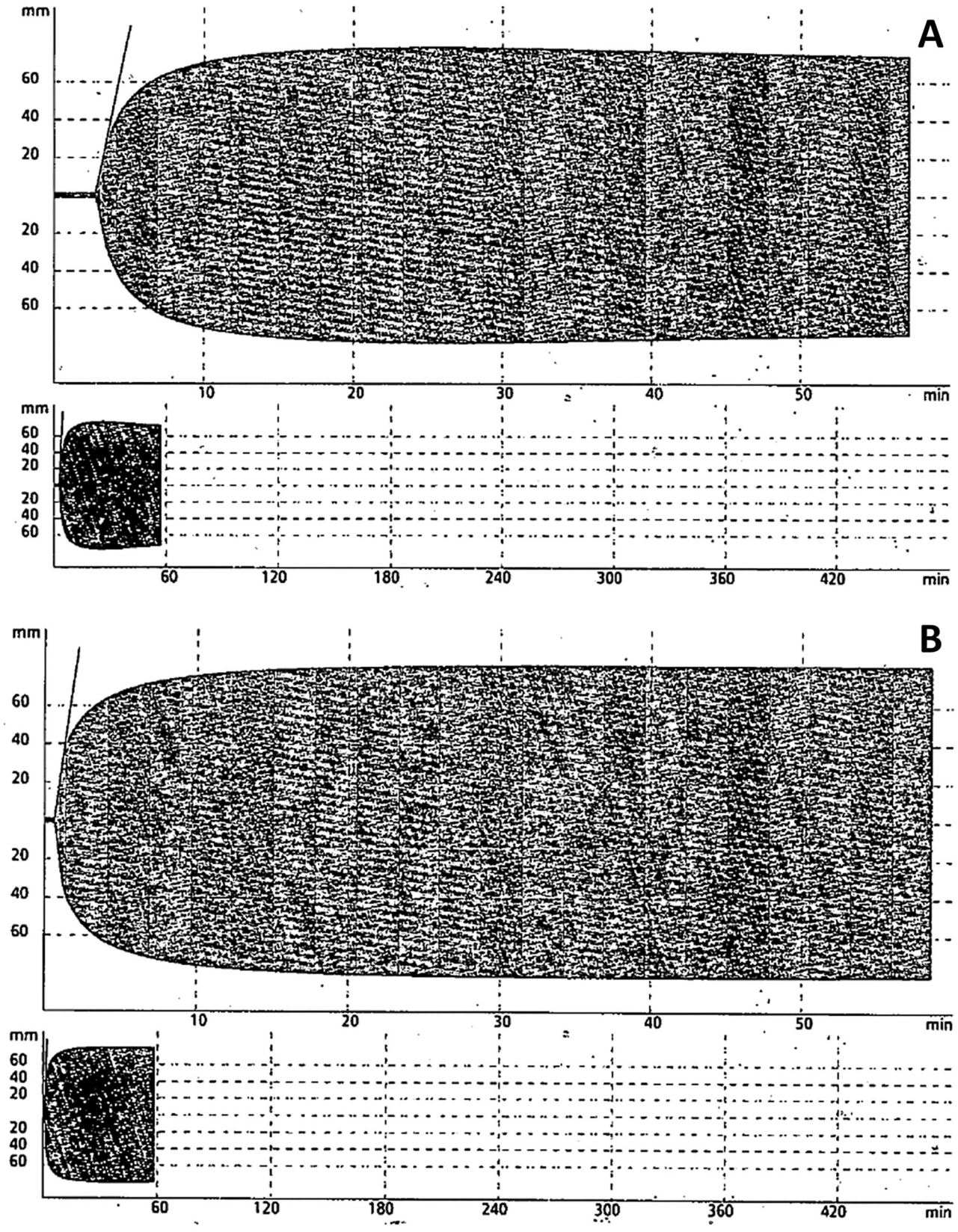

Figure 2: Thromboelastometry performed with kaolin (In-TEM) (A) and recombinant tissue factor (Ex-TEM) (B), at presentation to the hospital. Note the high alpha angle and maximal clot force.

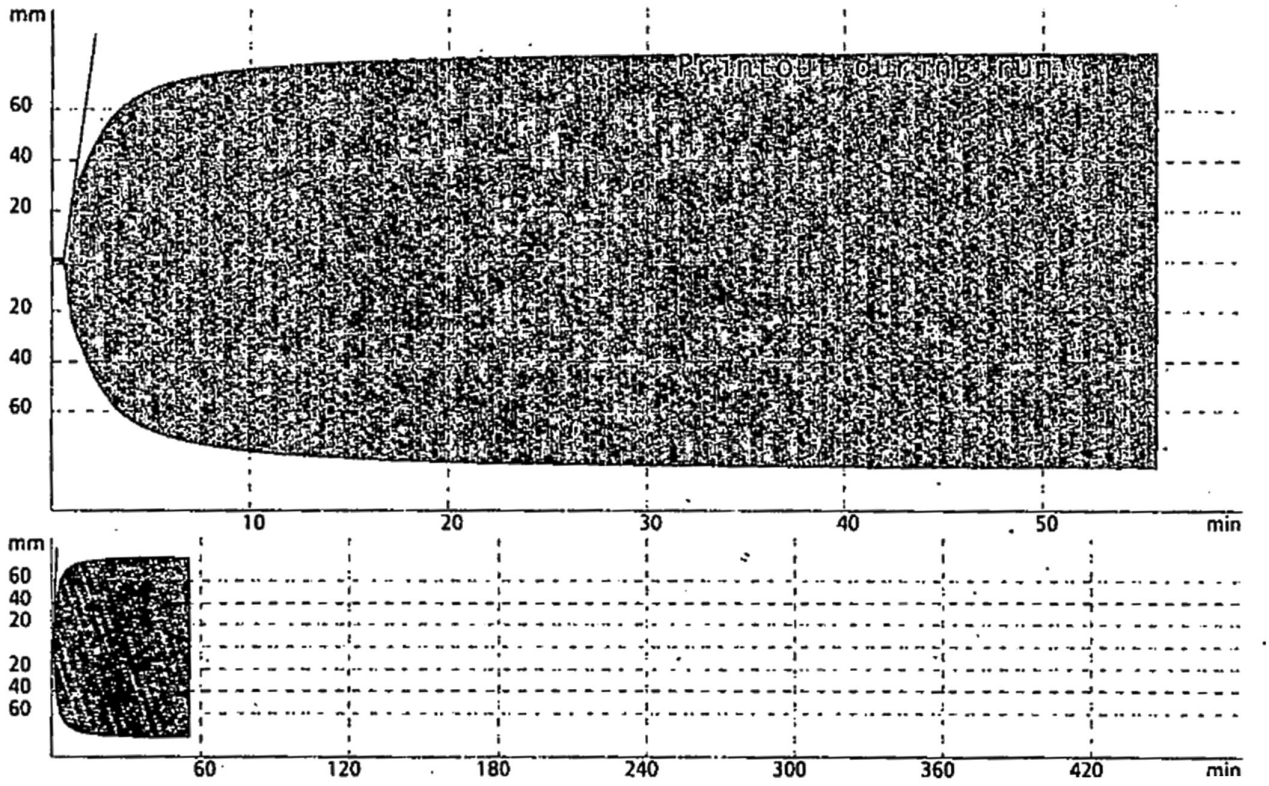

Figure 3: Thromboelastometry performed with recombinant tissue factor (Ex-TEM), at day 17 during treatment. Note the steep alpha angle and high maximal clot force.

Petach-Tikva, Israel; 15 mg PO q24h; appetite stimulant), prednisone (Rekah, Holon, Israel; 60 mg PO q24h × 3 days, tapered to 30 mg PO q24h x3 days, and later to 15 mg q24h x14 days; anti-inflammatory, to treat the uveitis and the putative vasculitis), ophthalmic prednisolone solution (Pred Forte, Allergan, Westport, Ireland;1 drop q8h bilaterally) and a renal prescription diet (k/d, Hill's, Topeka, KS).

The dog was reexamined 15 days later. No epistaxis episodes were noted by the owners since the last visit. The owners reported that the dog's general condition had improved; his vision had improved and the PU had decreased. It has gained one kg of body weight. The systolic/diastolic ABPs were 143/60 mmHg. Ophthalmologic examination of the right eye showed normal menace response, direct PLR and consensual PLR when the right eye was stimulated. Vitreous degeneration was seen, but the retina had fully reattached and there was no evidence of retinal hemorrhage in the left eye, the menace response and dazzle reflex, direct PLR and consensual PLR when stimulating the left eye were all absent. Results of the ophthalmic examination in the left eye were similar to the findings in the previous visit, except that blood clots had formed in the vitreous, and a superficial corneal ulcer was present. IOP in the right and left eyes was 8 and 7 mmHg, respectively.

Blood samples were obtained for CBC, chemistry and Ex-TEM (Tables 1, 2 and 4, respectively). The hematocrit had increased, but there was a macrocytic hypochromic mildly regenerative anemia. The platelet count had decreased slightly, although the platelet crit was still >RI. Albumin and globulin concentrations had improved, but were still abnormal. The Ex-TEM showed evidence of marked hypercoagulability (Table 4; Figure3). The UPC had decreased significantly, but was still markedly higher than the RI (Table 3).

The dog was prescribed all the above-mentioned oral medications except for the systemic prednisone which was discontinued. Due to the corneal ulcer, topical chloramphenicol-polymyxin B (Phenimyxin, Vitamed, Binyamina, Israel, 1 drop q6h) and ofloxacin (Oflox, Allergan, Westport, Ireland; 1 drop q6h) was prescribed to the left eye.

The dog was rechecked 60 days later, and was still receiving the systemic medications. According to the owners it was doing better, with improvement in its vision and drinking and

urinating less. No epistaxis episodes were reported. However, 2 weeks after the dog's last visit to the hospital the owners noted an increase in the size of the left eye. Their attending veterinarian discontinued the topical treatment, and instead prescribed Dorzolamide-Timolol (Cosopt, Rafa, Jerusalem, Israel; 1 drop q12h) and latanoprost (Glautan, Vitamed, Binyamina, Israel; 1 drop q24h) ophthalmic solutions to the left eye. On presentation, the systolic/diastolic ABPs were 183/116 mmHg .Ophthalmologic examination of the right eye showed similar findings as in the previous visit. The left eye was buphthalmic, and its pupil miotic and dyscoric; posterior synechia and a shallow anterior chamber, evidence of iris bombé, were evident. IOP in both eyes was 6 mm Hg.

There was no improvement in serum albumin and creatinine concentrations; however, serum urea concentration had decreased significantly (Table 2). The UPC had decreased, but was still considerably greater than the RI (Table 3). There was a moderate macrocytic hypochromic anemia, and the platelet count and the platelet crit were within their RIs (Table 1). The Ex-TEM still showed evidence of hypercoagulability (Table 4).

The dog was prescribed allopurinol (400 mg PO q12h), mirtazapine (15 mg PO q24h), clopidogrel (Teva, Petach-Tikva, Israel; 75 mg PO q24h; to decrease platelet activity), enalapril (20 mg PO q24h), amlodipine (5 mg PO q24h), telmisartan (Micardis, Rafa, Jerusalem, Israel; 20 mg PO q24h; to decrease blood pressure and proteinuria). Ophthalmic treatment of the left eye included dorzolamide-timolol solution q12h and discontinuation of the latanoprost. The dog was scheduled a recheck in two months, but was lost to follow-up.

DISCUSSION

This dog was diagnosed with CVL based on the typical clinical and laboratory abnormalities and positive serological testing (4). Its weight loss, PU/PD, azotemia, proteinuria, hypoalbuminemia and hyperphosphatemia at presentation were assumed to be consequences of protein losing nephropathy (PLN) which was secondary to GN, with resultant CKD (4, 11). The GN, epistaxis and uveitis are common findings in CVL, occurring due to marked hyperglobulinemia with increased CICs, leading to generalized vasculitis (4, 5, 10-15). Severe hyperglobulinemia, with hypoalbuminemia and decreased A/G ratio were also presently noted, in agreement with previous findings (4, 5). Interestingly, cutaneous lesions, observed in 81-89% of CVL cases (4) were absent in this case. Nevertheless, the CVL stage of this dog was probably LeishVet stage IV (very severe disease) (28), with presence of a high antibody titer and International Renal Interest Society (IRIS) markedly proteinuric stage-III CKD (4). This is consistent with the reported susceptibility of Boxer dogs to CVL (9).

The interesting feature in this case was the concurrent evidence of bleeding, manifested by the severe epistaxis, vitreal and retinal hemorrhage and consequent retinal detachment, with hypercoagulability. The latter was reflected by both the In-TEM and Ex-TEM, both demonstrating many measures supportive of a hypercoagulable state, including increased alpha angle, maximal clot force (MCF), amplitudes at 10 and 20 minutes, maximal velocity, maximal clot elasticity, clot formation rate and area under the curve. These results are in agreement with a recent study of dogs with CVL, demonstrating increased MCF and alpha angle in symptomatic cases (27). However, in that study, the clinical signs were not reported, so it is unclear if any of the dogs had concurrent bleeding tendency or epistaxis (27).

Additionally, this dog had normal clotting times, in the face of bleeding, with increased platelet count and plateletcrit and hyperfibrinogenemia. The latter three likely contributed to the hypercoagulability. Based on that, we believe that the retinal bleeding and epistaxis did not result from thrombocytopenia or secondary clotting abnormalities, and were instead secondary to other mechanisms reported in CVL, including thrombocypathy and vasculitis, or in case of the epistaxis due to nasal turbinate mucosal ulceration (4, 11, 17-19). Decreased platelet aggregation was reported in CVL (18), however, the TEM in this case did not support this phenomenon. Rather, hypercoagulability was demonstrated at presentation, with severe epistaxis and retinal bleeding as well as later, throughout the follow-up period. Although rhinoscopy and nasal biopsies were presently not performed to diagnose nasal mucosal ulceration, with the concurrent vitreal and retinal bleeding and the prompt and positive response to prednisone treatment, this mechanism seemed unlikely. Therefore, the most likely putative mechanism responsible for bleeding in this dog was vasculitis, which characterizes many CVL cases (4, 10-12). The blood vessel wall bound CICs interfered with platelet-endothelium interactions, resulting in bleeding. This interaction probably cannot be evaluated

by *in vitro* hemostatic tests, such as citrated plasma-based clotting times or even by TEM, which may show normal coagulability, or even hypercoagulability, as noted presently and previously (27). In contrast, the BMBT, not carried out in this case, assesses the *in vivo* platelet-vascular endothelium interactions, and has been found to be significantly prolonged in dogs with CVL with epistaxis, compared to healthy controls (21), supporting a defect in this interaction.

The present hypercoagulability, reflected by repeated TEM measurements was probably multifactorial, due to the combined effect of hyperfibrinogenemia, thrombocytosis, increased plateletcrit, mild hypoantithrombinemia and anemia. In dogs with CVL, most TEM measurements are positively correlated with the platelet count and fibrinogen concentration, and negatively correlated with the hematocrit (29). Fibrinogen is a positive acute phase protein (30). Hyperfibrinogenemia, likely due to systemic inflammation has been noted in 45% of symptomatic dogs with CVL (27). Systemic inflammation in symptomatic CVL was also a likely mechanism of reactive thrombocytosis, mediated primarily by interleukin-6 (31), which is increased in dogs with CVL (32). The present mild hypoantithrombinemia, likely resulted from GN, leading to marked proteinuria (33) as well as due to systemic inflammation (34, 35). The latter, along with CKD and the epistaxis, resulted in moderate to severe anemia, noted throughout the disease course. Anemia likely contributed to the hypercoagulability, as reflected by the TEM (28, 36). The mildly increased D-dimer concentration in this dog reflected increased thrombin and plasmin activities, likely due to bleeding and clot formation.

In light of the grave ophthalmologic prognosis for vision and the severe uveitis and retinal detachment, and with the suspected ongoing vasculitis responsible for the retinal bleeding and epistaxis, systemic and topical glucocorticoid therapy was initiated, along with allopurinol treatment to inhibit the parasite. Additional anti-lesihmanials, such as meglumine-antimonate and miltefosine might have been considered; however, both are unavailable in Israel and are not recommended in stage 4 CVL (28). In agreement with a previous study, where combined prednisone-allopurinol therapy led to faster improvement in platelet aggregation compared to anti-leishmanial therapy alone (24), this dog improved clinically under such therapy, with no additional epistaxis noted, while vision in the right eye improved due to retinal reattachment. Prednisone had a positive effect likely through its anti-inflammatory actions, decreasing immunoglobulin, CICs and anti-platelet antibody production, thereby reducing the vasculitis and improving platelet function and the endothelium-platelet interactions. This positive effect was also manifested by improvement over time of the UPC and serum albumin concentration, suggesting that the GN was also responding to therapy. Nevertheless, treatment failed to save vision in the left eye, probably because the uveitis and retinal bleeding induced irreversible changes.

In conclusion, this is a complex case of CVL with concurrent epistaxis, retinal bleeding and hypercoagulability, in which the typical cutaneous abnormalities that characterize CVL were absent.

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