Case Report

Presumptive Ischemic Brain Infarction in a Dog with Evans' Syndrome

Angelo Pasquale Giannuzzi, Antonio De Simone, Mario Ricciardi, and Floriana Gernone

"Pingry" Veterinary Hospital, Via Medaglie d'Oro 5, 70126 Bari, Italy

Correspondence should be addressed to Mario Ricciardi; ricciardi.mario@alice.it

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A ten-year-old neutered female mixed breed dog was referred for pale mucous membrane and acute onset of right prosencephalic clinical signs. Brain magnetic resonance imaging was suggestive for right middle cerebral artery ischemic stroke. Based on cell blood count, serum biochemistry and serologic tests and flow cytometric detection of anti-platelets and anti-red blood cells antibodies, a diagnosis of immunomediated haemolytic anemia associated with thrombocytopenia of suspected immunomediated origin was done. Immunosuppressive therapy with prednisone was started and the dog clinically recovered. Two months later complete normalization of CBC and serum biochemistry was documented. The dog remained stable for 7 months without therapy; then she relapsed. CBC revealed mild regenerative anemia with spherocytosis and thrombocytopenia. A conclusive Evans' syndrome diagnosis was done and prednisone and cyclosporine treatment led to normalization of physical and CBC parameters. The dog is still alive at the time the paper submitted. Possible thrombotic etiopathogenetic mechanisms are illustrated in the paper and the authors suggest introducing Evans' syndrome in the differential diagnosis list for brain ischemic stroke in dogs.

1. Case History

A ten-year-old neutered female mixed breed dog, weighing 8,5 Kg (18,7 lbs), was referred to Pingry Veterinary Hospital (Bari, Italy) because of acute onset of disorientation and inability to stand the day before the clinical evaluation. On owner's opinion the clinical signs were improving and at the moment of the evaluation the dog was able to deambulate. General physical examination revealed pale mucous membrane with normal refill time. Systolic arterial blood pressure recorded by Doppler ultrasonography was 140 mm Hg. On neurological exam the dog showed mild depressed mental status, compulsive gait, right circling, decreased postural reactions on the left side, absent menace response on the left eye with normal cotton ball test in both eyes, and absence of conscious nociceptive perception of the left nasal mucosa. Neuroanatomic localization of the lesion was right prosencephalon.

Brain MRI was performed using a 0.25 Tesla permanent magnet (ESAOTE VET-MR GRANDE, Esaote, Genoa, Italy) with the dog in general anaesthesia. MRI sequences

protocol included a Fast SE T2-W acquired in sagittal and transverse plane, a fluid attenuate inversion recovery (FLAIR) image, and a SE T1-W acquired in transverse plane before and after intravenous administration of paramagnetic contrast medium (Magnebita, gadopentetate dimeglumine 500 mmol/mL, insight agents; 0.15 mmol/kg BW). T2W and FLAIR images showed a sharply hyperintense well demarcated lesion at the lateral surface of the right temporal lobe with right caudate nucleus involvement (Figures 1(a), 1(b), and 1(e)). These changes involved both gray and white matter with major involvement of cerebral cortex. The lesion appeared iso-hypointense on T1-W images with mild and irregular enhancement after contrast medium administration (Figures 1(c) and 1(d)). No mass effect was noted. The distribution of the lesion matched the territory of the right middle cerebral artery and its striate branches [1]. Based on MRI features and distribution of the lesions, the acute onset of the neurological abnormalities, and spontaneous improvement a presumptive diagnosis of ischemic territorial infarct was supposed. Inflammatory and neoplastic lesions were considered less likely.

FIGURE 1: Magnetic resonance images of the brain. Transverse T2-weighted (a), FLAIR (b), T1-weighted (c), and T1-weighted, after IV gadolinium administration, (d) images at level of the thalamus; (e) dorsal FLAIR image at level of the dorsal part of the lateral ventricles. There are extensive focal grey and white matter T2 and FLAIR hyperintensity in the right temporal lobe without mass effect (white arrows). The lesion appears isointense to the normal gray matter (c) with mild contrast enhancement (black arrow) (d). The signal changes are most marked within the cortical gray matter (a). On dorsal plane the lesion extends to the right caudate nucleus (arrowhead) (e). Based on MRI features and distribution, the lesion appeared compatible with ischemic infarct in the territory of the right middle cerebral artery and its striate branches.

FIGURE 2: Peripheral blood smear. The orange arrow shows an agglutination that appears as irregular clusters of red blood cells. The blue arrow shows a ghost cell that appears as a very pale small blood cell. The green arrow shows a spherocyte that is a smaller cell that lacks central pallor. The red arrow shows a nucleated red blood cell with the round nucleus and condensed chromatin. Modified Wright $\times 50$ objective.

No hemorrhages was observed by owners and thorax X-Ray and abdominal ultrasound were unremarkable.

CBC, serum chemistry, and urinary analysis were performed in a referenced laboratory. CBC was performed by automatic cell counter (ADVIA 120, Bayer) associated with the evaluation of blood smears stained with modified Wright technique (Aerospray slide stainer 7120, Delcon). Blood smears were evaluated by a board certified internist.

CBC revealed strongly regenerative anaemia, severe spherocytosis (Figure 2), a left shifted neutrophilia, and mild thrombocytopenia (Table 1). Serum chemistry showed slightly increased total bilirubin and increased C-reactive protein (CRP). The low level of haptoglobin despite inflammatory condition was consistent with hemorrhagic and/or hemolytic event. Serum protein electrophoresis revealed mild hypergammaglobulinemia (Table 1). Urinary analysis was unremarkable. Fibrinogen, D-Dimers, and fibrin/fibrinogen degradation products (FDPs) were increased (Table 1).

Based on haematological and physical findings IMHA associated with thrombocytopenia was suspected. Serology tests for *Leishmania infantum* (ELISA test), *Ehrlichia canis* (IFAT), and *Rickettsia conorii* (IFAT) were all negative.

Because of the suspected immune mediated condition a flow cytometry test for searching anti-platelets and anti-red blood cells antibodies was performed.

Although Coombs' test for the diagnosis of IMHS is available, its sensitivity is low in comparison with flow cytometry assays [2]. Actually flow cytometry is considered to provide more rapid, cost-effective, sensitive, objective method to determine erythrocytes-bound immunoglobulins [3–5] and PLT-bound immunoglobulins [1, 6–8] if compared with other assays.

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Evaluation of anti-RBC antibodies and anti-PLT antibodies was performed by using the Epics XL-MCL (Beckman Coulter) FCI instrument throughout validated tests of a referenced laboratory [9, 10].

Blood samples were treated following the laboratory's guidelines and they were evaluated within 24 hours after the blood collection.

IgM-anti-platelets and IgG-anti-red blood cells antibodies were detected on blood samples by flow cytometry (Figure 3). The lack of an evident or occult hemorrhage, together with the intense spherocytosis, intense reticulocytosis, and flow cytometric results, suggested IMHA as cause of the regenerative anemia. Although thrombocytopenia was mild and DIC and thrombosis may contribute to the lower thrombocyte count, the cytofluorometry result made IMT suspected too. Based on these findings of IMHA and IMT, Evans' syndrome was presumed and a treatment with prednisone (2 mg/kg/for day) was started. After 21 days no neurological abnormalities were noticed. Two months after the corticosteroid therapy, complete normalization of CBC, serum biochemistry parameters were present (Table 1). The dog remained stable for 7 months without therapy. Then he was one more time evaluated because of mild depression and lack of appetite. CBC revealed mild regenerative anemia, spherocytosis, and thrombocytopenia (Table 1) with normal D-Dimers. No other physical signs consistent with thrombotic events were noticed. Based on blood results and considering the outcome, thrombocytopenia in this dog was assumed as of immune mediated origin allowing a conclusive ES diagnosis. Suspecting a relapsed event, the dog was again treated with prednisone (2 mg/kg/for day) and cyclosporine (5 mg/kg/for day). After 4 months of treatment the dog was normal on owner's opinion and physical examination and CBC were unremarkable (Table 1).

2. Discussion

Ischemic stroke is a deprivation of blood flow leading to brain necrosis and most commonly occurs due to vascular occlusion by embolus or thrombus [11, 12]. These kinds of cerebrovascular accidents are commonly described in dogs [13–20] with few descriptions in cats [21, 22]. Although a large percentage of them have an unknown etiology, several underlying causes have been recognized including hypertension, endocrine, kidney, heart, and metastatic diseases [23].

In human being and dogs, IMHA and IMT have been associated with an increased incidence of thrombosis and seem to be a procoagulant condition [24–26].

ES is a pathological condition defined by the combination (either simultaneously or sequentially) of IMT and IMHA in the absence of known underlying aetiology [27].

The association of thrombosis and ES is extremely rare in human literature and the etiopathogenesis of thrombosis in these patients is not clear [28].

Cerebral venous thrombosis is reported in a man with ES probably not correlated with the therapeutic agent complications [28].

FIGURE 3: Cytofluorometric evaluation of anti-platelets and anti-red blood cells antibodies. (a) Forward versus side scatter plot showing the placement of the erythrocyte gate (E). (b) Fluorescence intensity histogram of the erythrocyte membrane-anti-RBC IgG bond. Negative and positive fluorescence peaks on the left and on the right, respectively. (c) Percentage of erythrocyte membrane binding IgG (F). (d) Forward versus side scatter plot showing the placement of the platelet gate (E). (e) Fluorescence intensity histogram of the platelet membrane-anti-PLT IgM bond. Negative and positive fluorescence peaks on the left and on the right, respectively. (f) Percentage of platelet membrane binding IgM (F).

TABLE 1: Cell blood count, serum chemistry, serum protein electrophoresis, and hemostatic profile: the first clinical evaluation and follow-up.

	The first evaluation	56 days after prednisone therapy	7 months after the first evaluation (relapse)	2 months after prednisone and cyclosporine suspension	Reference interval
RBC ($\times 10^6/\mu\text{L}$)	2.76	7.12	4.60	6.71	5.70–8.56
Hgb (gr/dL)	8.2	18.0	11.6	15.6	14.1–21.2
Hct (%)	27.2	60.6	36.8	50.3	39.0–59.2
WBC ($\times 10^3/\mu\text{L}$)	16.02	10.6	15.5	10.63	5.45–12.98
Band neutrophils ($\times 10^3/\mu\text{L}$)	139	0	0	0	0–286
Segmented neutrophils ($\times 10^3/\mu\text{L}$)	11954	7632	12710	8018	3555–9314
PLT ($\times 10^3/\mu\text{L}$)	146	339	121	396	176–479
Absolute reticulocytes count (/μL)	376464	15640	412620	76494	12320–100128
Bilirubin (mg/dL)	0.56	0.18	0.27	0.19	0.11–0.31
Haptoglobin (mg/dL)	1		98		1–96
RCP (mg/dL)	5.95	0.01	2.30	0.01	0.01–0.22
Gamma globulin (%)	21.3	14.4	14.9	13.9	6.4–14.5
Fibrinogen (mg/dL)	426		393		152–284
FDPs ($\mu\text{g/mL}$)	>20		<5		<5
D-Dimer ($\mu\text{g/mL}$)	0.61		0.23		0.01–0.34

In veterinary medicine few cases of ES have been documented in dogs [2, 29, 30]. In other reported cases of IMHA associated with thrombocytopenia in dogs the term “Evans’ syndrome” is not used because of the difficulty to evaluate the immune mediated destruction of platelets [30], and the underlying mechanism of thrombocytopenia is suspected ruling out other causes of thrombocytopenia [30, 31]. In our dog diagnosis of ES was based on the presence of anti-platelets and anti-erythrocyte antibodies detected by cytofluorometric method [4, 5, 9, 10].

In both people and dogs it is well understood that IMHA per se is associated with a hypercoagulable state and the thrombosis is a frequent complication of hemolytic condition [24, 32, 33].

Clinical evidences suggestive of TE have been detected up to 35% of dogs with IMHA [34] and thromboembolic complications have been recognized as a clinically important factor for mortality [35–37], estimated up to 70% [37, 38] of cases. Pulmonary TE seems to be a common consequence of IHMA, but thrombi also can be found in heart, liver, spleen, kidney, and pituitary gland [26, 35, 36, 39].

Multiorgan TE was found from 32.2% up to 80% of dogs with IMHA after postmortem examination [26, 35, 36, 40].

It is often difficult to distinguish in patients with IMHA the predisposing factors due to treatment (immunosuppressant medication, blood product transfusion) from the real rule of the pathological condition, with several observations being made during medical treatment.

Several mechanisms have been investigated in human and dogs related to vascular complications of haemolytic anemia [33, 34, 41, 42].

There is increasing evidence that the products of haemolysis are vasculotoxic and coagulation system is principally involved in initiating the thrombotic events [24, 41].

The presence of free haemoglobin during haemolysis predisposes to hypercoagulability state by binding NO. The extracellular haemoglobin reacts with NO transforming it to nitrate and consequently reducing the bioavailability of NO [43].

NO is vital for vasodilatation, is a potent inhibitor of platelet aggregation [44], and inhibits releasing of procoagulant protein, inflammatory mediators, and proliferative factors [45]. A state of decreased NO availability as induced by haemolysis, predisposed to thrombus formations by means of vasoconstriction, endothelial adhesiveness, platelet activation, and aggregation and vessel wall cellular proliferation [45].

Reduced NO activity is also due to releasing of arginase-1 in plasma from erythrocytes destruction. This ectopic arginase activity converts arginine to ornithine, reducing plasma arginine, the obligate substrate for nitric oxide synthase [45] in human patients with hemolysis due to sickle cell disease or thalassemia [46].

Activated platelets have been identified in dogs with IMHA by measuring surface expression of P-selectin, an intercellular adhesion molecule, using a flow cytometric analyzer [34, 47, 48]. PMPs formation also occurs following platelet activation in human [49] and dogs [41] during haemolytic disorders. PMPs are membrane surrounded fragments of platelets [49] that have the potential to exhibit procoagulant activity.

Membrane of PMPs expresses binding sites for fibrinogen, PTS, P-selectin, and other adhesive receptors such as GPIIb/IIIa [47, 49].

PTS is a phospholipid with a negative charge that can be found primarily on the inner platelet membrane during a resting state [50]. As microparticles circulate, they can interact with coagulation factors through the exposed PTS, bind to leukocytes with P-selectin, or form platelet aggregates through their GPIIb/IIIa receptors. These interactions may contribute to inflammation and coagulation, possibly predisposing a patient to a thromboembolic event [47, 49].

Microparticles displaying PTS derived from erythrocytes [51], platelets [52], and endothelial cells [53] have been observed in association with human haemolytic condition such as sickle cell disease and thalassemia [54].

IMT, without a concomitant IMHA, is also considered a risk factor for ischemic stroke in human medicine [55–57].

A Danish study on human patients with primary chronic IMT suggested more than twofold higher risk of venous TE compared to the general population [58].

Heightened platelet activation in dogs with IMHA has been reported. In these patients a concurrent thrombocytopenia was found probably as a consequence of immune-mediated platelet destruction, irrespective of IMHA [41].

Although in dogs the associations between IMHA, IMT, and increased risk of thromboembolic events have been described [25, 26] no reports exist about cerebrovascular complications of these immune-mediated disorders. Neurologic signs are rarely reported in dog with IMHA. There is only one report describing a dog that at the time of the initial evaluation had neurologic disease, characterized by head tilt and seizures [34].

In this paper we describe the onset of acute brain dysfunction with MRI lesions suggestive of ischemic strokes in a dog with ES.

To the authors' opinion IMHA and IMT should be considered in the list of possible underlying pathological conditions of the ischemic stroke in dogs.

Abbreviations

MRI:	Magnetic resonance imaging
IMT:	Immune-mediated thrombocytopenia
IMHA:	Immune-mediated haemolytic anaemia
NO:	Nitric oxide
PMPs:	Platelet microparticles
PTS:	Phosphatidylserine
ES:	Evans' syndrome
TE:	Thromboembolism.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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