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Essential thrombocythaemia in two dogs

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Summary

In this report two dogs with essential thrombocythaemia (ET) are described. Both dogs were presented more or less at the same time with a combination of reduced exercise tolerance and pale mucous membranes without any report of blood loss. Moderate-to-severe, Coomb's-negative anaemia and thrombocytosis (> 1249 x 10^9/l) were present. In addition, the peripheral blood smear revealed the presence of basophilia and large numbers of abnormally shaped megakaryocytes in the bone marrow of both dogs.

Treatment with vincristine (0.7 mg/m² once intravenously) and hydroxyurea (500 mg/m² p.o. per day) was started. Because of insufficient response to treatment after 3 weeks, the dosage of hydroxyurea was increased in both dogs to 2000 mg/m² p.o. per day. The dogs deteriorated further, however, and were euthanized at 6 weeks after the start of treatment. Blood examination revealed pancytopenia in both dogs, most likely due to the myelosuppressive effects of high-dose hydroxyurea.

A survey of veterinary literature on ET is presented , including a comparison of ET in humans.

Samenvatting

Essentiële thrombocythemie bij twee honden

In dit case report worden twee honden met essentiële thrombocythemie (ET) beschreven. Beide honden werden in dezelfde periode aangeboden met de problemen een verminderd uithoudingsvermogen en bleke slijmvliezen. Er werd geen bloedverlies gemeld. Een matige tot ernstige, Coomb's negatieve anemie en een thrombocytose (>1249 x 10^9/l) waren aanwezig. Bijkomend werd er in een bloeduitstrijkje basofilie gezien en werden er in een beenmergaspiratiebiopt grote aantallen abnormaal gevormde megakaryocyten gevonden.

Er werd een behandeling met vincristine (0.7 mg/m² eenmalig intraveneus) en hydroxyurea (500 mg/m² p.o per dag) gestart. Vanwege een onvoldoende respons op de behandeling na drie weken werd de dosis hydroxyurea in beide honden verhoogd naar 2000 mg/m² p.o. per dag. De honden gingen klinisch verder achteruit en werden zes weken na de start van de behandeling geëuthanaseerd. Bloedonderzoek liet in beide honden een pancytopenie zien die waarschijnlijk het gevolg was van de myelosuppressieve effecten van de hoge dosering van hydroxyurea.

Er wordt een overzicht van de veterinaire literatuur over ET gegeven en er wordt een vergelijking met ET bij de mens gemaakt.

Introduction

Essential thrombocythaemia (ET) is one of the more uncommon myeloproliferative syndromes in dogs. In humans, it is characterized by a disorder of uncontrolled megakaryocyte and platelet production, specific biochemical and bone marrow abnormalities, a sustained high platelet count with a peripheral platelet population of platelets with a bizarre morphology, and a clinical course commonly associated with haemorrhage and / or thrombosis (1). ET can be considered as a preleukaemic stage, although the risk of transformation into either myelofibrosis with myeloid metaplasia or acute leukaemia is lower than 5% in humans (2). The term ET is synonymous with ideopathic thrombocythaemia, primary thrombocythaemia, primary haemorrhagic thrombocythaemia, primary thrombohaemorrhagic thrombocythaemia, and thromboblasthaemia. Other myeloproliferative disorders are polycythaemia vera, agnogenic myeloid metaplasia, and chronic granulocytic leukaemia (3).

The diagnosis of ET in humans is a challenge and is predominantly established by the exclusion of other (thrombocythaemic) disorders, such as polycythaemia vera, chronic myeloid leukaemia, idiopathic myelofibrosis, and reactive thrombocytosis (4). Excitement and exercise cause thrombocytosis due to either splenic contraction or increased blood flow. Thrombocytosis also can be seen with trauma, haemorrhage, neoplasia, gastrointestinal diseases, fractures, splenectomy, iron deficiency, various inflammatory conditions, and rebound thrombocytosis following idiopathic or drug-induced thrombocytopenia, Cushing's disease, glucocorticoid therapy, and antineoplastic agents (5). Strict criteria for the diagnosis of ET in humans were established in 1986 and updated in 1997 by the Polycythemia Vera Study Group of the National Cancer Institute (Table 1) (6, 7). The diagnosis of ET in dogs is probably even more difficult than it is in humans. Although most of the criteria are the same as those used in human medicine, the question remains whether all criteria are as relevant.

Hydroxyurea is the treatment of choice for ET, but recently newer therapeutic agents, including interferon alpha, anagrelide (an oral agent that has a platelet-lowering effect in humans) and pipobroman (an alkylating agent) are being used (8). Sometimes also antithrombotic agents such as aspirin are used (1, 8). Simpson and others (9) reported the successful treatment of a suspected case of ET in a dog with the combination of vincristine, cytosine arabinoside, cyclophosphamide, and prednisolone. After 6 months the dog had a normal red cell and platelet count. Hopper and others (10)

Table 1. Criteria for the diagnosis of essential thrombocythaemia in humans developed by the Polycythemia Vera Study Group of the National Cancer Institute (6, 7).

1. Platelet count > 600,000/µl (>600 x 10^9/l)
2. Normal erythrocyte mass/hemaetocrit
3. Stainable iron in bone marrow or failure of iron trial
4. No Philadelphia chromosome
5. Absence of collagen fibrosis on bone-marrow examination and no evidence of splenomegaly and leukoerythroblastic reaction
6. No cytogenic or morphological evidence for a myelodysplastic syndrome
7. No known cause of reactive thrombocytosis

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reported a dog with ET that also had basophilia in the peripheral blood. Treatment with radiophosphorus resulted in a rapid decline in the numbers of megakaryoblasts and megakaryocytes in the bone marrow and platelets and basophils in the peripheral blood. Degen and others (11) treated a dog with ET with radiophosphorus and melphalan, which was switched to hydroxyurea (40 mg/kg q 24 h) 11 months after the start of therapy, due to the unresponsiveness of the megakaryocytes. The dog died 32 months after the start of therapy due to an uncontrollable atrial fibrillation. Bass and others (12) reported the treatment of a 10.5-year-old Shih Tzu with hydroxyurea (16.25 mg/kg q 24 h), which was stopped after 2.5 months because of severe life-threatening anaemia. Although the dog did not receive any further medications it remained clinically normal.

In this case report the diagnosis and the treatment of two dogs with ET are described and discussed. A survey of veterinary literature on ET is presented, including a comparison with ET in humans.

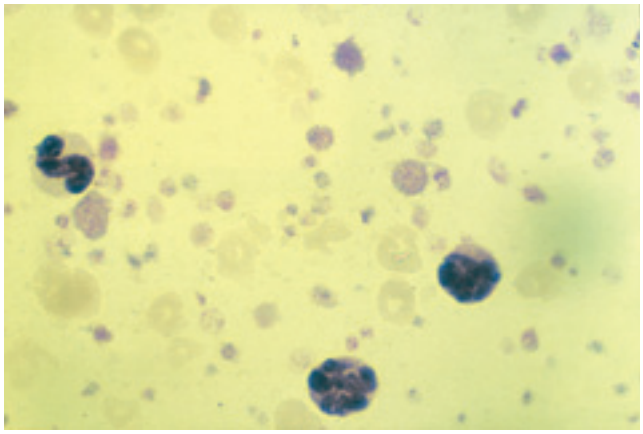

Figure 1. Peripheral blood smear of Dog1 reveals a severe thrombocytosis and basophilia. May-Grünwald Giemsa (MGG). Magnification 1000x.

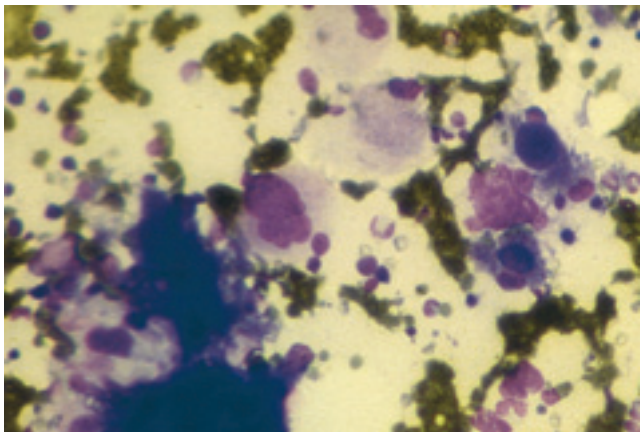

Figure 2. Bone marrow smear of Dog1 reveals increased numbers of megakaryocytes. MGG. Magnification 400x.

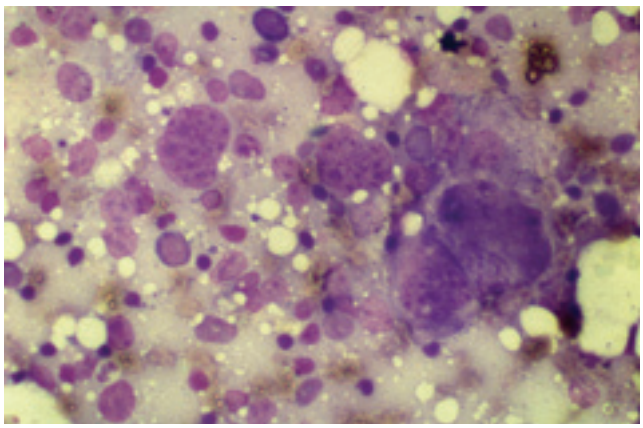

Figure 3. Bone marrow smear of Dog1 reveals abnormal shaped, unusually large megakaryocytes. MGG. Magnification 1000x.

Materials & Methods: Case Management

Signalment, History & Clinical examination

DOG 1.

A 5-year-old, intact female English Sheepdog, 16.5 kg, was referred to the Utrecht University Clinic of Companion Animals (UUCCA) for reduced exercise tolerance, episodic fever, weight loss, and pale mucous membranes. The dog still had a good appetite. No blood loss was reported and the animal had not been in an area endemic with blood parasites such as Babesia and Ehrlichia. The dog had been put on prednisolone (1.25 mg/kg per day) 2 weeks earlier by its referring veterinarian.

Physical examination revealed a pulse rate of 144 per minute, a rectal temperature of 39.6°C, and pale mucous membranes. Capillary refill time could not be measured.

The dog had severe, non-regenerative Coombs`-negative anaemia with severe thrombocytosis and basophilia (Table 2) (Fig. 1). To determine the reason for the thrombocytosis and the low reticulocyte count, a bone marrow aspiration biopsy was taken from the ileac crest. On cytological examination of the bone marrow biopsy a large number of abnormal shaped megakaryocytes was seen (Fig. 2 & 3).

The osmotic fragility test was performed as described by Dacie (13). The plasma thrombopoietin (TPO) level was measured with a quantitative sandwich enzyme immunoassay technique (R&D Systems, Inc. Minneapolis, United States of America) with intra- and interassay coefficients of variation (CV) of 5.2% and 9.3%, respectively, for human TPO. The minimal detectable dose of TPO is less than 15 pg/ml. However, this assay has not been validated for dogs.

To establish reference values for TPO in dogs, blood samples were taken from 6 clinically normal dogs, aged 4 to 7 years. The mean ± SEM was 70 ± 12 µg/l. Dog1 had a TPO of 518 µg/l.

DOG 2.

A 12-year-old, intact male mixed breed, 13.4 kg, was referred to the UUCCA in the same week as Dog 1 for a seizure 2 weeks before referral and pale mucous membranes. The dog had a good appetite. No blood loss was reported. The dog was imported out of Hungary at the age of 1 year. The dog has not been out of the Netherlands for 9 years. During the last 2 months, the dog had been given carprofen (1 mg/kg orally per day) for lameness of the right hind leg because of an orthopaedic problem in the knee.

Physical examination revealed a pulse rate of 100 per minute, pale mucous membranes, and a capillary refill time less than 1 second. No abnormalities were found on abdominal palpation. On heart auscultation a systolic murmur was heard at all valve areas. The right knee was painful at passive movement and tibia compression test was positive, suggesting rupture of the cranial cruciate ligament. At the ventral base of the tail a mass was discovered, which had been

there for probably 4 months. Fine-needle-aspiration biopsy was taken of this mass and tumour of a perianal gland was diagnosed on cytological examination. Results of blood examination are listed in Table 2.
The dog had moderate, Coombs`-negative anaemia with severe thrombocytosis and basophilia. A bone marrow aspiration biopsy was taken from the right femur bone And revealed a large number of abnormally shaped megakaryocytes.
The osmotic fragility test and the platelet aggregation test were performed as described by Dacie (17). The procedure for the platelet aggregation test was adapted by using of 10-fold increased concentrations of ADP (ADP; Chrono-log) and collagen (collagen; Chrono-log), resulting in 25 and 50 µmol/ml for ADP and 30 and 50 µM for collagen, respecti-

Table 2. Haemograms from 2 dogs with probable essential thrombocythaemia.

Haematological and Biochemical parameters	**Initial presentation**		**Week 3**		**Week 6**		**Reference values**
	Dog 1	Dog 2	Dog 1	Dog 2	Dog 1	Dog 2	
Haematocrit (l/l)	0.11	0.22	0.14	0.16	0.05	0.07	0.42-0.57
RPI *	0.48	2.2	0.04	1.0	<0.1	0.14	0.1-2
Leucocytes (x109/l)	11.1	10.3	11.2	8.0	1.4	1.6	5.9-13.8
Segm neutrophils (x109/l)	2.4	6.5	6.7	6.0	1.2	1.0	3.00-11.50
Band neutrophil (x109/l)	0.2	0.0	0.1	0.0	0.0	0.0	0.0-0.30
Juveniles (x109/l)	0.0	0.0	0.0	0.0	0.0	0.0	0-0
Lymphocytes (x109/l)	4.9	2.3	2.7	1.5	0.2	0.6	1.00-4.80
Monocytes (x109/l)	0.9	0.1	0.1	0.2	0.0	0.0	0.15-1.35
Eosinophils (x109/l)	0.0	0.0	0.2	0.2	0.0	0.0	0.10-1.25
Basophils (x109/l)	2.7	1.4	1.3	0.0	0.0	0.0	0-0
Platelets (x109/l)	1249	2037	1723	1573	19	2	150-400
Platelet aggregation		**normal**	**normal**				
Thrombopoietin (µg/l)	518						70±12 (Mean±SEM)
PT (sec) **	7	7					7
APTT (sec) ***	14	15					14
Fibrinogen (g/l)	2.7	4.4					1.2-3
Osmotic fragility 10%	193			165			125-163
Osmotic fragility 90%	127			114			90-128
Coombs test							
• a-IgG	Negative			Negative	Negative	Negative	
• a-IgM	Negative			Negative	Negative	Negative	
• Complement	Negative			Negative	Negative	Negative	
Fe (µmol/l)			58				6.3-37
TIBC (µmol/l) &			54				58-96
Urea (mmol/l)	7.3	5.1					3.0-12.5
Creatinine (µmol/l)	73	69					<50+1.2xBW#
AP (U/l) ^	218	68					25-117
Fasting bile acid (µmol/l)	26	16					0-8
Total protein (g/l)	69			70			54-70
Albumin (g/l)	27			33			25-37
Potassium (mmol/l)	4.6						3.6-5.0
Sodium (mmol/l)	147						141-149
Comments bloodfilm	Giant platelets, hypochromatic microcytic	Giant platelets, hypochromatic microcytic	Giant platelets, atypical basophils	Giant platelets, atypical basophils		Hypochromic, polychromic, anisocytosis	

* RPI = Reticulocyte Production Index
** PT= Prothrombin Time
*** APTT = Activated Partial Thromboplastin Time
& TIBC = Total Iron Binding Capacity
^ AP = Alkaline Phosphatase
# BW = Body Weight

vely. Because the stored blood sample was lost, TPO was unfortunately not measured.

Treatment

In both dogs treatment was started with vincristine (vincristine-sulphate, PCH) 0.7 mg/m^2 intravenously and hydroxyurea (Hydrea; Bristol-Myers Squibb) 500 mg/m^2 per os (p.o.) per day. Dog 1 received a blood transfusion with 450 ml A 1.1 and 1.2 negative packed cells, and in addition, the dose of prednisolone (prednisolon; Alfasan) was lowered to 0.9 mg/kg per day.

Follow-up

At 3 weeks after the start of treatment Dog 1 had very pale mucous membranes. The dog was still very anaemic, the platelets had further increased, although the basophilia was somewhat reduced. A platelet aggregation test showed no abnormalities (Table 2). It was decided to give Dog 1 a second blood transfusion with 450 ml A 1.1 and 1.2 negative packed cells. Prednisolone was further lowered to 0.6 mg/kg per day.
Dog 2 looked stable at 3 weeks after the start of treatment according to the owner, although blood examination (Table 2) demonstrated increasing anaemia. The platelet count was somewhat decreased but was still very high, the basophilia had disappeared. Treatment apparently did not have any effect on the haematocrit and platelets. For both dogs it was decided to increase the dose of hydroxyurea to 2000 mg/m^2 p.o. per day and to start with ferrofumarate (ferrofumaraat; Genfarma BV) 9 mg/kg p.o. per day because of hypochromic microcytic anaemia.
Six weeks after the start of treatment, both dogs were euthanized by the referring practitioners because of severe exercise intolerance and very pale mucous membranes. An EDTA-blood sample was sent to the clinic for control of the haematocrit, reticulocytes, platelets, leucocytes, and Coombs test (Table 2). In both dogs severe pancytopenia, platelets included, was found.

Discussion

As mentioned earlier, the diagnosis of ET is a challenge. In the two dogs described here, the diagnosis was made on the basis of the elevated platelet count and bone marrow megakaryocytic hyperplasia in the absence of any demonstrable evidence of lymphoid or myeloid neoplasia. Both dogs were treated with vincristine and hydroxyurea, the treatment of choice, but without an apparent effect on the physical condition of the patients. This was supported by the results of blood examination.
The platelet count in Dog 1 and Dog 2 was 1249 x 10^9/l and 2037 x 10^9/l, respectively, which is much higher than the 600 x 10^9/l (0,6 x 10^6/µl) included in the Polycythemia Vera Study Group (PVSG) criteria for ET. Iron deficiency anaemia can cause thrombocytosis, but the underlying mechanism is not clear up till now. Hypochromic microcytic anaemia was reported in both dogs. The serum iron concentration was slightly increased, while the total iron-binding capacity was decreased in Dog 1. In human patients with iron-deficiency anaemia, serum iron levels are decreased and iron binding capacity is increased. In dogs serum iron concentrations are decreased and the iron-binding capacity is normal or even slightly increased (14). The low iron-binding capacity and increased plasma iron concentration seen in Dog 1 were inconsistent with iron-deficiency anaemia. The Philadelphia chromosome abnormality does not occur in dogs and is thus not a criterion for ET in dogs. The bone marrow aspiration biopsies did not reveal any signs of fibrosis. Since megakaryocytic hyperplasia with dysmegakaryocytopoiesis, and abnormal platelet function and morphology are rarely associated with reactive thrombocytosis, it seems unlikely that the persistent thrombocytosis in both dogs was related to the cause of the reactive thrombocytosis.
The TPO concentration in Dog 1 was 7.5-fold higher than the mean value for healthy control dogs. In contrast, in humans TPO concentrations are not or only slightly increased. Circulating levels of TPO are inversely related to platelet mass (15), and normal or elevated levels of platelets inhibit the action of TPO by binding to circulating TPO. Disease-related abnormalities in the ability of platelets to clear TPO, may alter TPO levels, e.g., diminished clearance of TPO by abnormal platelets may account for the elevated platelet counts seen in myeloproliferative syndromes such as ET.
Basophilia has been reported in a dog with ET (10). It has also been reported in a dog with acute megakaryocytic leukaemia (16). Basophilia and eosinophilia are often present in humans with chronic myeloproliferative syndromes (17). Degen and others (11) described a dog with ET who had eosinophilia, but not basophilia. Both our dogs had moderate basophilia. As the basophils in the peripheral blood were morphologically normal and the basophilic precursor cells found in the bone marrow biopsies were also morphologically normal, there was no proof for basophilic leukaemia. In one dog (Dog 2) the basophils disappeared from the peripheral blood after 3 weeks of treatment with vincristine and hydroxyurea (500 mg/m^2).
No signs of bone marrow depression were seen after 3 weeks of treatment with low-dose hydroxyurea (500 mg/m^2), whereas severe pancytopenia was found in both dogs after 6 weeks of treatment with high-dose hydroxyurea. Hydroxyurea starts to act after 3 to 5 days (12). An explanation for the severe pancytopenia may be that the dose of hydroxyurea had been increased 4-fold 3 weeks after the start of therapy. The regimen used was comparable to that used in human medicine. In humans with ET, 2-4 g/day of hydroxyurea can be used without any side effects. The average body surface area of an adult human is about 1.9 m^2. This results in a dose of 2100 mg/m^2 per day. The dose hydroxyurea used in both dogs was about 2000mg/m^2, less than the dose used in humans. As this dose gave rise to signs of overdose in both dogs, it would appear that dogs are more susceptible for hydroxyurea than humans. Interestingly, Degen and others used a dose of 40 mg/kg, equivalent to approximately 1200 mg/m^2, for many months without signs of pancytopenia (11). An alternative drug could be anagrelide. This drug may interfere with megakaryocyte maturation, resulting in a reduced production of platelets. In an uncontrolled series of human patients treated with anagrelide, a substantial reduction in life-threatening events was associated with control of thrombocytosis (18). As far as we know, this drug has never been used in dogs. It may be reasonable to start testing this drug in dogs with ET, according to the results for human patients.
Myeloproliferative disorders are uncommon in dogs, but have been reported. An increased awareness of these syndromes should help clinicians in the diagnosis of these disorders.

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