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Case report

IDIOPATHIC THROMBOCYTOPENIA IN DOGS - CASE REPORT**

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Abstract: Thrombocytopenia represents a significant reduction in number of blood platelets in the circulation of mammals. The causes of thrombocytopenia in dogs and cats are: various infectious factors, viruses, bacterias, parasites, various pathological conditions of the liver, spleen, bone marrow or autoimmune diseases. Sometimes, thrombocytopenia causes many different factors or the real cause can not be detected, and its origin is called idiopathic. In our practice, in the course of haematological analysis of blood, we encounter a reduced number of platelets in the blood of dogs and cats. Then we are facing the great challenge of diagnosing and treating possible idiopathic thrombocytopenia in animals.
In our case, we have a Miniature poodle whose problems began at the age of 2.5. The dog had the following symptoms: inapetency, somnolence, temperature of 38.8^{0}C, *pale oral mucosa* with petechiae and behavioral changes. After the first hematological blood tests were performed, the results of the parameters indicated thrombocytopenia in this dog. Diagnosis of the disease is supported by symptoms and differential diagnosis, so we started with frequent monitoring of haematological parameters.
We included adequate therapy with the first symptoms of the disease in our case of idiopathic thrombocytopenia in a young dog. The therapy was successful, hematological parameters and the quality of life improved, and the dog is now eight years old. The treatment of idiopathic thrombocytopenia is a challenge for every small animal veterinarian and for this reason in this paper we share our experiences with colleagues.
Keywords: dog; thrombocytopenia; hematology; therapy;

INTRODUCTION

This disease is also called idiopathic thrombocytopenic purpura because it causes purple stains on the skin of a diseased dog and is therefore frustrating for the owners. It is more common in female dogs, in middle-aged dogs, and in the following dog breeds: Poodles, English shepherds, and Cocker spaniels, while cats can also get suffer of this disease. Platelets stop bleeding from damaged blood vessels. The most common symptoms of idiopathic thrombocytopenia in pets are bruising of the skin that appear for no reason, small bleeding (petechiae) in the mucous membranes of the eyes, nose and mouth, blood in urine and feces,

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and bleeding in the gut or brain in severe cases. These bleedings caused by thrombocytopenia can lead to other diseases and problems (Lichtenberg D. 2016).

The term idiopathic is often used to describe a disease with no identifiable cause of its occurrence. Causes of thrombocytopenia may include: various infectious agents, viruses, bacteria, parasites, various pathological conditions of the liver, spleen, bone marrow, neoplastic processes, the influence of toxins or autoimmune diseases. Lately, the cause of idiopathic thrombocytopenia is associated with vaccinations of dogs, although it is extremely rare. Idiopathic thrombocytopenia is most commonly autoimmune disease, bleeding followed by immunosuppressive platelet destruction or a disruption of platelet production in the bone marrow (Clabough L. D. et al., 1991, Herrtage M. et al., 2017). In dogs, idiopathic thrombocytopenia develops spontaneously or secondarily after infectious or neoplastic diseases. Differential diagnosis of idiopathic thrombocytopenia in both cases is based on the exclusion of known causes or other diseases. Corticosteroids with prednisolone are usually the first-line treatment option and this method is generally accepted. However, the effect of steroids is not predictable in each dog. Prednisolone doses vary considerably between cases of idiopathic thrombocytopenia in dogs where high doses are generally used at the beginning of therapy. Like any other medicine, prednisolone may be associated with unwanted effects such as increased thirst, increased water intake and urination, increased appetite, weakness in the body, and vomiting and diarrhea in dogs. If these unwanted effects are rare and mild, treatment with prednisolone is allowed for a longer period of time. Approximately two-thirds of the dogs achieve a complete or partial response to corticosteroids, while one third of them have no response. In these situations, alternative therapies such as platelet transfusion or intravenous immunoglobulin application must be applied or splenectomy as the most radical treatment. In dogs, transfusion reactions are rare after the first transfusion. However, if the dog has already received a blood transfusion, the risk of undesired reactions is significantly increased with each subsequent tranfusion (Clabough L. D. et al., 1991).

The spleen is the main center of autoimmune response and its surgical removal from the organism of a dog suffering from idiopathic thrombocytopenia is one of the good but radical solutions. Splenectomy is suggested in extremely severe cases of idiopathic thrombocytopenia with no response to prior treatment. All these options for the treatment are selected individually, from one case to another, and there are no generally accepted methods and protocols because the disease rarely occurs, 5.2% of all other blood diseases in dogs. Because of the insufficient knowledge and information, the rate of mortality in dogs suffering from this disease is very wide. Over the past years, great attention has been paid to research in order to find a solution to this disease because the pathogenesis and expected therapeutic application are very similar in humans (Spahr E. J. and Rodgers G. 2007).

After diagnosis and therapy, the dog stops getting treatment, but the disease can return after remission. After the stabilization phase of treatment the doses are usually slightly reduced, with careful monitoring of the platelet count. During this progressive dose reduction there is a risk of recurrence of clinical signs that may be observed in approximately 25% of patients. In these cases, the dog is placed on additional, long-term, oral combination therapy with prednisolone and other immunosuppressive medications along with obligatory occasional monitoring of the dog's complete blood count (Papadantonakis N. and McCrae R.K. 2016).

Unfortunately, despite the treatment, a large number of dogs may die or be euthanized at the onset of the disease or after the first repetition of its symptoms because the clinical picture is very poor. This is mainly seen in severe illnesses caused by idiopathic thrombocytopenia

associated with complications such as coagulation disorders or severe gastrointestinal bleeding. Fast and adequate therapy and frequent monitoring during the hospitalization of dogs are associated with a reduced risk of mortality in dogs with idiopathic thrombocytopenia. One of the future treatment goals is the initiation of peptide domains on the surface of megakaryocytes which would activate receptors for synthesis and increase platelet count.

The combination of the drug used today to induce proliferation of megakaryocytes in dogs is Eltrombopag administered orally from 25 to 75 mg / day and Romiplostim 1-10 μg/kg subcutaneously once a week. This therapy carries a risk because a dog can bleed and it can be life-threatening, and there is no alternative in this case, beside transfusion. The response to this therapy is very good and the platelet count is increased from 10 to 150h103 / mm3. The relapse is reducing the platelet count to low values a few days after the applied therapy. It is believed that relapse occurred if the platelet count is less than 150h103 / mm3. Also, the dog is classified as if he did not respond to the therapy if his platelet count did not increase above 150h103 / mm3 after the application of the therapy. Romiplostim is a cure licensed in human medicine and a comparable product is not available in veterinary medicine. Therefore, this medicine can not be used in veterinary medicine without a prescription (Kohn B. et al., 2016).

MATERIAL AND METHODS

In our case, we have a Miniature Poodle called Tia whose problems began at the age of 2.5. The dog had the following clinical symptoms: inapetency, somnolence, lethargy, temperature of 38.80S, pale oral mucosa with petechiae and behavioral changes. After the first blood hematological analysis was performed on 12.04.2013. results of the parameters indicated idiopathic thrombocytopenia.

It should not be forgotten that multiple causitive agents of the disease may be present at the same time, so it is necessary to determine complete therapy. From this analysis we can see that all haematological parameters have been changed, and that platelets have the greatest deviation from physiological values. We decided to use SNAP 4D test for four diseases: ehrlichiosis, cerebrospinal fluid, Anaplasmosis and heartworm disease (dirofilaria), which gave negative results. Ehrlichiosis, anaplasmosis, babesiosis are most commonly suspected in the differential diagnosis. Negative quick test does not necessarily mean that the dog is free of the disease it has been tested for. The most reliable methods for diagnosing possible thrombocytopenia causes are PCR, ELISA and IFAT test. After the analysis of the dog's blood, we determined the therapy.

Blood tests were performed in two days. The results of the haematological analysis were poor, so we realized that the disease progresses. We decided to transfuse blood from a healthy non-sedated dog in an amount of 10 to 15 ml of full blood / kg body weight after which the dog was better. With the help of the therapy (described below), the dog is still alive, with reduced platelet counts and a discipline of life that underlies reduced exposure to possible injuries, proper nutrition and therapy. Differential diagnosis in our case is very important because the dog is not burdened with unnecessary medication.

The differential diagnosis of idiopathic thrombocytopenia was performed as follows:1. Suspicion of babesiosis: it is excluded because we did not find any agent on blood spread, there was no characteristic leukopenia and associated clinical symptoms (hemoglobinuria, icterus).

2. Suspecion of leptospirosis: Due to the increased concentration of leukocytes there is definitely a suspicion of leptospirosis, especially if the disease is coincidental with other diseases and should not be neglected. In our case, fortunately, the kidneys were in function.

3. Suspicion of ehrlichiosis: this disease becomes more and more present in our country, the vectors are ticks and the disease has a zoonotic character. Ehrlichia spp. are gram-negative bacteria, round or pleomorphic obligate intracellular cocci. They are very similar to rickettsia, but there are phylogenetic differences due to which they are closer to bacteria. Because of some of the characteristics some some species of Ehrlichia were reclassified into the genera Anaplasma and are now called *Anaplasma phagocytophilum* and *Anaplasma platys*.
These two anaplasmas are important for infection in dogs. The former infects granulocytes whereas the latter infects platelets They cause severe thrombocytopenia and pose a threat to humans. In Europe in 1995, the first case of human granulocytic ehrlichiosis (HGE) was recorded. Ehrlichia chaffeensis causes human monocytic ehrlichiosis (HME). Tick are not the only vectors for Ehrlichia, E. sennetsu, was isolated in Japan. Human infection results from the consumption of fresh-water fish containining metacercaria. Morulae in anaplasma can be found in monocytes and granulocytes only 1 to 5 days from the onset of infection although they produce antibodies that are created from 7 to 28 days of infection and can be detected for a long time in the blood. Ehrlichiosis is a disease that we must not forget when treating thrombocytopenia. Our stance is that whenever we suspect idiopathic thrombocytopenia we give therapy for ehrlichiosis because it is very dangerous and unpredictable. The clinical picture indicates a fall in platelets because of the bleeding of oral mucosa, gums and gingiva, nosebleeds, eye fundus bleeding and eventually it can have a fatal outcome. It can be diagnosed with a quick test (SNAP 4D test), but its negative result does not mean the absence of disease. A large number of drugs: antibiotics, antiinflammatory drugs, cardiovascular drugs and hormones can cause disorders in platelet formation or accelerate their destruction.

RESULTS AND DISCUSSION

In our case, we came to the final diagnosis of the diiphase thrombocytopenia after frequent haematological and biochemical blood tests that included a large number of parameters as well as several rapid tests. Changes in all hematological parameters from 12 April 2013 to 23 May 2015 are given in Table 1. After a clinical examination and suspicion of illnesses described in the differential diagnosis we decided to treat the diseases that in acute phase caused the symptoms of thrombocytopenia. We applied imidocarb at a dosage of 2 to 4 mg / kg of body weight first day after examination examination, 10 mg / kg of doxicycline, 1 mg / kg of prednisolone, AD3E vitamins, B complex, vitamins K and C at prescribed doses. We did a blood transfusion. The next day the dog was feeling better. We continued to give doxycycline tablets for 20 days. After hematologic control and control clinical examinations, the therapy was redesigned. Prednisolone doses changed over time depending on the health status of the dog. After 14 days we reduced the dose of prednisolone by 25%, and after 30 days it was halved. Each dose reduction was gradual before complete exclusion from the treatment and recommendation to be gradually included in therapy only if needed with regular controls. We also recommended easily digestible diet.
Some clinicians consider it useful to do splenectomy as the spleen plays a role in the formation of platelet antibodies. We did not do this because we thought that it was not necessary on the basis of the response to the therapy, nor did we use other types of immunosuppressants.
In hematological analysis (Table 1), we can clearly see that the number of white blood cells at the beginning of the disease is high and its values diminished throughout the course of therapy. This is best seen on granulocytes then

monocytes and lymphocytes. The explanation for this phenomenon is the hyperreactivity of the immune system which attacks and destroys platelets. Immunosuppressants that are given therapeutically reduce the activity of cellular and humoral immunity. The values of erythrocytes in the acute phase are slightly lower, while they are later stabilized. The same happens with the values of hemoglobin. Platelets are extremely low with transient improvement after therapy, but they are always significantly below physiological values. Changes in the values of the haematological parameters of our analysis coincide with the literature, where there is also a decrease in erythrocyte, hemoglobin and thrombocyte values in idiopathic thrombocytopenia (Papadantonakis N. and McCrae RK 2016).

The analysis showed that the values of MCH and MPV were increased. AP was within the physiological limits, while AST and ALT were slightly elevated. It should not be forgotten that cortisol therapy has an influence on the value of these enzymes. Creatinine values were within physiological limits while urea was elevated. Bilirubin was elevated in some analyzes, not in the acute phase when the number of erythrocytes was lower, but later after 3 years of therapy. Because the liver was loaded with cortisol. liver transaminase levels showed the same. Glucose, total protein and globulin were stable, while cholesterol was slightly elevated. Pancreatic amylase and GGT were stable and they show conditions of the liver and pancreas. Sodium, potassium, calcium and phosphorus were within the limits of physiological values with a small increase in the value of calcium. Therapy of idiopathic thrombocytopenia in dogs with corticosteroids leads to liver load and disruption of its parameter values (Spahr E. J. and Rodgers G. 2007).

Table 1. Tabular display of hematologic parameters of the dog with idiopathic thrombocytopenia during the period 12. 4. 2013 - 23. 5. 2015.

(The red fields show the values above the reference range, the blue ones show the values below the reference range, while the green fields are the reference values)

параметар	12.4. 2013.	13.4. 2013.	14.4. 2013.	15.4. 2013.	16.4. 2013.	3.5. 2013.	13.9. 2013.	11.10. 2013.	16.10. 2013	21.10. 2013.	8.1. 2014.	22.4. 2014.	19.7. 2014.	30.8. 2014.	9.1. 2015.	23.5. 2015.	рефер. вредн.
WBC $10^3/mm^3$	17.3	21.3	27.0	23.3	30.1	13.1	17.4	11.5	18.6	15.88	9.1	12.45	16.76	14.61	11.6	16.5	6-12
RBC $10^6/mm^3$	5.22	4.58	4.73	4.27	4.43	6,2	5.89	5.98	5.56	6.39	8.30	7.48	6.27	6.87	7.86	5.68	5.4-8.5
HGB g/dl	12.1	11.3	11.4	9.9	15.5	18.9	16.0	14.7	14.1	15.7	20.1	18.3	14.2	16.8	17.4	13.1	13-20
HCT %	37.2	34.9	36.1	32.8	32.4	45.1	43.7	44.1	38.4	44.8	55.4	52.9	45.2	47.4	53.7	40.4	37-57
PLT $10^3/mm^3$	0	0	10	0	10	30	39	48	10	12	56	152	30	15	4	15	200-460
MCV μm^3	71	76	76	77	73.3	72.2	74	74	69.2	70.0	66.7	70.7	72.1	68.7	68.3	71.2	64-77
MCH pg	23.2	24.6	24.0	23.3	24.4	30.4	27.2	24.6	25.4	24.6	24.2	24.4	22.7	24.4	22.1	23.0	17-23
MCHC g/dl	32.5	32.2	31.5	30.3	33.3	41.9	36.7	33.3	36.8	35.1	36.3	36.0	31.5	35.6	32.4	32.4	31-36
RDW %	14.2	13.8	14.1	14			13.9	13.7	14.0	13.8	14.7	15.0	14.7	15.1	13.6	13.8	14-17

MPV µm^3	8.7	7	6.1	7.4			8.4	8.0	17.9	20.9	14.1	12.1	21.2	8.6	36.0	30.5	6.7-11.1
LYM %	21.7	11.7	12.0	20.3	17.0	15.4	14.1	16.1	19.2	18.7	26.8	23.5	24.3	19.3	15.6	13.20	12-30
MON %	8.9	4.5	3.0	8.0	6.0	2.0	2.9	5.7	5.5	5.3	7.5	6.1	4.3	6.2	6.9	5.3	3-14
GRA %	69.4	83.8	85.0	71.7	77.0	82.6	83.0	78.9	70.1	70.40	59.70	66.0	65.0	63.8	72.6	72.60	60-80
BAZ %					0	0			3.6	0.4	1.0	0.2	0.3	0.4	0.8	0.13	0-2.5
EOS %			5.5		0	0.1			0.4	4.8	4.1	3.6	5.2	5.6	3.4	7.4	2-10
LIM 10^3/mm^3	3.7	2.4	3.2	4.7			2.4	1.8	3.6	2.97	2.44	2.92	4.07	2.82	1.81	2.12	1-3.6
MON 10^3/mm^3	1.5	0.9	0.8	1.8			0.5	0.6	1.2	0.84	0.68	0.76	0.72	0.90	0.81	0.84	0-0.5
GRA 10^3/mm^3	12.1	18.0	23.0	16.8			14.5	9.1	13.2	11.18	5.43	8.22	10.89	9.99	8.46	11.65	3-10
ALP U/l	20				33	349			26.1			10.6	7.8	116.6	21.0	19.4	10-100
AST U/l	109				46.6	73			23.8			32.0	28.2	57.7	37.5	37.0	<19
ALT U/l	45.2				42.1	199			82.2			57.2	58.4	65.8	72.5	86.7	<39
UREA mmol/l	4.21				14.2	9.4		13.6	15.8			11.6	13.8	11.1	24.5	18.2	<9
KREAT µmol/l					73	86		91	77.5			97.5	93.9	93.7	105.9	141.5	0-100
ALBUM g/l					38.6				32.0			35.0	34.0	31.0	30.0	33.0	25-44
BILIRU µmol/l					3.2				23.3			6.8	3.7	7.6	10.7	44.9	1.7-10
GLOB g/l									37			34	33	41	40	42	21-37
PROT g/l					62	66			69			69	67	72	70	75	60-82
GLUK mmol/l					3.9	5.8			5.3			4.8	4.9	5.4	5.7	3.7	3-6
Na mmol/l					147								142	139	141		135-148
K mmol/l					3.97								3.91	4.41	4.22		3.5-5.3
Ca mmol/l					2.67				1.9			2.5	3.1	3.0	3.0	2.7	1.9-2.6
P mmol/l									1.6			1.2	1.4	1.7	1.3	2.1	0.9-2.0
HOLES mg/dl									269.9			337.0	259.9	255.4	268.5	248.0	120-253
AMILA U/l					950				979			993	1152	1449	1497	1036	280-1420
GGT U/l							5	5									<20

CONCLUSION

Using clinical examination, we are often unable to reach a final diagnosis. Therefore, we need laboratory, imaging, or diagnostics with fast tests and even more complex isolation systems of agents that sometimes have no justification in terms of the cost and feasibility. With the experience, careful observation of symptoms, elimination of other pathogenic agents, and proper choice of therapy, we had success in treating idiopathic thrombocytopenia in dogs. This work contributes to fellow veterinarians to access diagnostics and treatment of idiopathic thrombocytopenia in dogs with as much safety and information as possible.

LITERATURE

1. Kohn B., Bal G., Chirek A., Rehbein S., Salama A. (2016): Treatment of 5 dogs with immune-mediated thrombocytopenia using Romiplostim. BMCVetRes; 12:96.
2. Lichtenberg D. (2016): Immune-Mediated Thrombocytopenia: A Frustrating Disease. Pet Health Network.
3. Herrtage M., Warland J., Kent A., Bazelle J. (2017): Immune-Mediated Thrombocytopenia (ITP) in Dogs. Pet Health Network.
4. Curtis B. R., Kaliszewski J., Marques B. M., Saif W. M., Nebelle L., Blank J., McFarland J., Aster H. R. (2006): Immune-mediated thrombocytopenia resulting from sensitivity to oxaliplatin. American Journal oh Hematology. Vol. 81(3):199-201.
5. Clabough L. D., Gebhard D., Flaherty M. T., Whetter L. E. (1991): Immune-mediated thrombocytopenia in horses infected with equine infectious anemia virus. Journal of Virology. Vol. 65(11): 6242-6251.
6. Spahr E. J. and Rodgers G. (2007): treatmnt of immune-mediated thrombocytopenia purpura with concurrent intravenous immunoglobulin and platelet transfusion. American Journal oh Hematology. Vol. 83(2):122-125.
7. Papadantonakis N. and McCrae R. K. (2016): Immune-mediated Thrombocytopenia. Nonmalignant Hematology. Springer. Vol 14(2):209-220.

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