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Clinicopathological profile of canine thrombocytopenia

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Abstract
Platelets are the cellular elements in blood along with the erythrocytes and leukocytes. Both increase as well as the decrease in the number and functioning of circulating platelets is detrimental to life of the individual. Increase in the number and functioning of platelets lead to thrombosis in vital arteries and obstruct the blood flow. Decrease in the number and functioning of platelets lead to bleeding and death. Compared to human beings, canines are more often presented with more cases of platelet reduction or thrombocytopenia than thrombotic episodes. Hence this article is aimed at discussing the current concepts and understanding of the causes, mechanism, diagnosis and management of thrombocytopenia in dogs.

Keywords: Canine, ehrlichiosis, epistaxis, platelets, thrombocytopenia, thrombopathia

Introduction
In clinical practice and in critical care unit, dogs are often presented with signs of thrombocytopenia like petechiae to epistaxis. Severe reduction of platelet counts such as less than 10000 platelets/ µL are seen in few of such cases. Grindem *et al.* (1991) [1] have reported thrombocytopenia as the most common acquired haemostatic and platelet disorder in dogs. Thrombocytopenia can occur as a primary pathology as in decreased production of platelets as in conditions like aplastic bone marrow or it could arise secondary due to several other etiologies in dogs.

Platelets
Platelets are formed from bone marrow from the megakaryocytes and appear smaller in size than RBCs and WBCs in a stained blood smear. They appear as pleomorphic structures-round, ovoid or discoid with granular contents inside. Due to the absence of nucleus and pleomorphic nature, the cell may even appear as a cytoplasmic fragment or artefact to the beginners. In a healthy dog, platelets in general measure 2.2 - 3.7 microns in diameter and 0.5 microns thick and circulate at a concentration of approximately 200,000/µl to 600,000/µl (Rebar *et al*., 2001) [2]. Platelets have a variable lifespan of 7 to 9 days after which they are phagocytised and cleared by macrophages of the mononuclear phagocyte system.

Functions of platelets
The most well known function directed to platelets is their role in blood clotting mechanism or haemostasis. Platelets maintain haemostasis by sealing the damaged endothelium and formation of blood clot. Platelets also promote wound healing by the release of factors like Platelet derived growth factors (PDGF).

Major causes of Thrombocytopenia
The various causes leading to thrombocytopenia is grouped under four major categories and enlisted by Benjamin, (1978) [3] as follows.

Decreased platelet production
Platelet count is reduced due to impaired thrombopoietic activity of the bone marrow due to aplastic and hypoplastic bone marrow, effect of drugs, toxins, poisoning, exposure to radiation and in tumours like leukemia when the bone marrow is replaced by tumour tissue. Platelet production is also drastically reduced in various infectious diseases as Ehrlichiosis in dogs.

Increased platelet destruction
Platelets may get destructed in large proportion in conditions like: Due to immune mediated

destruction of platelets like Immune mediated Thrombocytopenia (ITP) and non immune mediated conditions like effect of administered drugs and toxins formed in the body, Disseminated intravascular coagulation (DIC) and idiopathic thrombotic thrombocytopenia.

Sequestration of platelets

Despite normal production of platelets, sometimes platelets may get accumulated in the internal organs without being available in the circulation for performing their functions. It happens during various infections, hypothermia and in hypersplenism where the spleen is hyperactive.

Loss of platelets

Platelets are lost during haemorrhage viz. during trauma when platelets are also lost along with other components like the erythrocytes.

Of all the causes, thrombocytopenia is observed most commonly with hemoprotozoans and immune dysregulation in our findings. Souza *et al.* (2016) [4] reported that severe thrombocytopenia in dogs is mostly caused by immune-mediated thrombocytopenia.

Parasitic thrombocytopenia

Thrombocytopenia due to hemoprotozoans and rickettsial organisms is frequently encountered in canine practice. *Ehrichia canis* located in monocyte is most frequently observed with a blood picture of relative monocytosis and thrombocytopenia. In addition, the hemoprotozoans *Babesia vogeli* and *Babesia gibsoni* located in RBCs and the extracellular parasite *Trypanosoma evansi* also cause thrombocytopenia. Another tickborne pathogen, *Anaplasma platys* is devoted to infect platelets and cause thrombocytopenia. It is seen as single to multiple, basophilic round to oval dots or cluster in the platelets. Again there is every possibility for these microorganisms to infect individually or to occur as a mixed, concurrent infection to result in thrombocytopenia.

Immune mediated thrombocytopenia

Immune mediated thrombocytopenia is the condition in which the body's immune system is dysregulated, because of which the body attacks its own platelets resulting in thrombocytopenia by massive destruction of the circulating platelets and its precursor cells in the bone marrow. It may be due to infectious and non infectious causes as well as sometimes can occur as idiopathic in nature. Immune mediated thrombocytopenia can be either primary or secondary type. Primary immune-mediated thrombocytopenia (ITP) is mostly of idiopathic type wherein no underlying cause can be ascertained and hence it is also called as idiopathic thrombocytopenia. Secondary immune-mediated thrombocytopenia occurs mostly due to infections like ehrlichiosis, babesiosis, leptospirosis and due to adverse effect of drugs administered etc. Day (1998) [5] reported that secondary IMTP occur due to neoplasia, infection or reactions from drugs or vaccination.

Drug-induced Thrombocytopenia

Drug-induced thrombocytopenias end up by the use of various extraneous agents used for therapeutic intervention in pets like the antibiotics, harmones etc. Antibodies are produced against such compounds which then bind with the platelets and cause destruction of platelets. Certain compounds also cause suppression of thrombopoiesis in bone marrow. However the platelet counts may revert to normal if the administration of drug is withdrawn.

Congenital thrombocytopenia

In addition to the acquired infectious and non infectious causes, certain dogs may also be born with congenital thrombocytopenia when the animal may have more predisposition to irresistible bleeding throughout their life even due to small bruises which are attributable to inadequate platelet count by birth.

Disorders in platelet function

Platelet Function Disorders i.e. Thrombocytopathia or Thrombopathia is suspected in dogs with signs of petechiae and haemorrhage but with normal platelet count or otherwise the magnitude of platelet reduction is not proportionately low to cause severe bleeding.

Platelet functional disorders can be of congenital like Von Willebrand Disease and Glanzmann Thrombasthenia or due to acquired causes like administration of certain drugs which interact with available platelets and the interaction complex is inefficient to facilitate hemostasis. Von Willebrand Disease in dogs occurs by virtue of a defective or flawed von Willebrand factor (vWF). Glanzmann thrombasthenia is yet another variant of thrombocytopathia seen in humans as well as in dogs. Haysom *et al*. (2016) [6] have reported that mutations in either of the genes encoding GPIIb or GPIIIa can result in Glanzmann thrombasthenia. It results in irresistible bleeding in dogs. Lollar (2012) [7] reported that GT is associated with a platelet aggregation defect causing mucocutaneous bleeding in dogs which can be even life-threatening. Solh *et al*. (2015) [8] stated that failure of haemostasis in this condition is due to impaired platelet aggregation and clot retraction.

Acquired platelet dysfunction may manifest as increased functioning of platelets or decreased functioning of platelets. In pathological conditions like ischemia, platelets become hyperactive and develop higher propensity to end in undesired thrombosis inside the blood vessels which may even become life threatening depending on the size of the thrombus, the blood vessel and absence of collateral blood supply to combat the ischemia. Non functional to less functioning platelets may be seen in uremia, canine ehrlichiosis, snake poisoning, cirrhosis, during administration of certain drugs etc wherein petechiae to haemorrhage may be seen in dogs as the circulating platelets fail to maintain hemostasis.

Diagnosis of thrombocytopenia

Thrombocytopenia in dogs is suspected on physical examination by observing the presence of petechiae, purpura, and echymoses in the skin especially in the hairless ventral surface of the body. Raw bleeding from any of the natural orifices esp. the nostrils (epistaxis) and other orifices like urogenital opening, scleral and retinal haemorrhage is suggestive of severe thrombocytopenia where the platelet count has been drastically reduced to less than 10,000/µl of blood. Other indicators suggestive of thrombocytopenia include Prolonged bleeding time and abnormal clot retraction wherein Buccal Mucosal Bleeding Time (BMBT), Evaluation of Bone marrow aspiration smears, Estimation of PT, APTT, Platelet factor III, intrinsic and extrinsic clotting factors and Coomb's test are done to confirm thrombocytopenia (Benjamin, 1978) [3].

For all studies focussed on platelets and coagulation profiles it

is preferable to collect blood with sodium citrate as the anticoagulant.

Blood smear evaluation of thrombocytopenia

Critical screening of blood smear is important in evaluating the number of platelets as well as their size, shape and distribution.

A blood smear with more than ten platelets on the average of ten 100×oil objective fields in a monolayer is considered to have adequate platelets in the circulation. If the smear has less than seven platelets on the average of ten 100× oil objective fields in a monolayer of blood cells, it is considered to be a case of thrombocytopenia. A value of 15000 is ascribed to finding one platelet in average of ten oil immersion fields (Valenciano *et al*., 2013) [9]. Hence if the average is three, then the platelet count in the animal is extrapolated to 45,000/cmm. In all cases and more necessarily in cases suspected for thrombocytopenia, if platelets are not discernible in the body of the smear, tail end is searched well for the presence of platelet clusters. The clusters are more often visualised in the tail or feathered end of the smear due to density distribution of cells.

Morphologic variation of Platelets

If the platelets appear bigger in size than the normal size, they are known as megaplatelets, macroplatelets, giant platelets or stress platelets which are suggestive of thrombopoiesis in the animal to combat thrombocytopenia or abnormal thrombopoiesis. Martin *et al*. (1983) [10] reported platelets with larger volume are produced in response to thrombocytopenia. Bommer *et al.* (2008) [11] also reported that large platelets are observed in peripheral blood when bone marrow is intact and when it is overstimulated to produce platelets. If the platelets appear smaller they are known as microplatelets. Latimer (2011) [12] stated that microplatelets occur in iron deficiency anemia, bone marrow aplasia, immune mediated thrombocytopenia or as an artefact when the samples are stored in EDTA anticoagulant for more than 24 hours.

Sometimes platelets may appear to have cytoplasmic projections in the form of tail or flagellum or undulating membrane with condensed granules or loss of granules. They are called activated platelets and they are formed as an artefact during collection and processing. They look like fibrin material or even like trypanosomes in the extracellular space in blood smear.

Reticulated platelets (RP) are seen in blood smears stained with new methylene blue. They are the young platelets similar to the reticulocyte counterpart of RBCs and serve as indicators of erythropoiesis. Rebar *et al*. (2001) [2] opined that finding notable numbers of elongated, cigar-shaped platelets is suggestive of focal or generalized systemic haemorrhage and poorly granular platelets or platelets with few large granules suggestive of developmental abnormalities which warrant examination of bone marrow aspiration smears.

Halmay *et al.* (2005) [13] reported the following platelet abnormalities in Giemsa and PAS-stained blood smears. The most common platelet abnormalities were polychromasia and the presence of giant platelets in cases presented with bleeding or haemolysis; Anisocytosis in hepatic, splenic or intestinal neoplasms and in certain endocrinopathies; Microcytosis in immune-mediated thrombocytopenia, hepatic neoplasms and endocrine disorders; Extreme platelet activation in haemolysis, hepatopathies, neoplastic diseases and sepsis; Vacuolisation in thymic haemorrhage, pancreatitis, diabetes mellitus and Cushing's syndrome and two forms of pathologic granulation the pseudonuclear and the spot-like formation of granules.

Platelet indices

Like the erythrocytic indices- Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC), platelet indices are obtained by running the blood samples in Auto Haematology Analyzer for the estimation of plateletcrit (PCT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet Large cell ratio (P-LCR).

Like Packed cell colume which gives information on the concentration of packed RBCs in blood sample, PCT gives information on the concentration of packed platelets in known volume of blood sample. Moraes (2001) [14] reported that PCT is the volume percentage that platelets match on a total volume of blood and it is directly related to the total number of platelets.

MPV is a measurement index of the size of the platelets. Feldman *et al*. (2000) [15] stated that MPV gives information on the estimated platelet size and it is inversely proportional to the number of platelets. The range of MPV in healthy dogs is 6.1 - 10 femtoliters and an increase in MPV is suggestive of responsive thrombopoiesis which may give a clue to secondary platelet destruction, some myeloproliferative diseases, and hyperthyroidism (Rebar *et al*., 2001) [2]. Schwartz *et al.* (2014) [16] suggested that presumptive primary immune-mediated thrombocytopenia in dogs is characterized by increased MPV levels due to large immature platelets` production during a regenerative process. Bharanidharan *et al*. (2017) [17] reported an increased MPV in Babesiosis induced thrombocytopenia in dogs and further suggested that MPV can be employed while screening samples suspected for babesiosis.

Decrease in MPV is an indicator of reduction in size of the platelets which can be visualised by screening the blood smear. Absence of bigger platelets in blood smear of cases with pre-existing thrombocytopenia is of unfavourable prognosis as it gives the information that the bone marrow is not responding to meet the platelet demand. Topper and Welles, (2003) [18] stated that thrombocytopenia associated with a decreased MPV is suggestive of insufficient megakaryocytes or bone marrow failure.

Platelet Distribution Width (PDW) is an indicator of variation in platelet size and is interpreted in the light of platelet morphology seen in blood smears. PDW throws information on the disparity in the size of platelets whether they are very small or large. Matos *et al*. (2008) [19] claimed PDW as a more sensitive indicator than MPV as PDW values get elevated due to small variations in platelet morphology even before MPV values are altered, similar to what occurs with the anisocytosis index, RDW. Bommer *et al*. (2008) [11] concluded that PDW is more sensitive to increased amount of large platelets than MPV, partly because MPV values can be influenced by the simultaneous presence of smaller platelets and cell debris.

Platelet Large cell ratio (P-LCR) indicates the percentage of large sized platelets in the blood stream (Silveira *et al.* 2003) [20]. Souza *et al*. (2016) [4] reported that both PDW and P-LCR increase in dogs with thrombocytopenia and they are more sensitive than MPV. The authors further stated that estimation of P-LCR is important in thrombocytopenic dogs especially when associated to the presence of large platelets in blood smears. The presence of large size platelets has to be

interpreted with caution as they may be a positive indicator as adaptive phenomenon to combat thrombocytopenia as well as deleterious, when high proportion of large and reactive platelets facilitate an undesirable thrombotic cascade to end in fatality.

Follow up of thrombocytopenia

Diagnosis of thrombocytopenia is followed by identification of one or more causes of thrombocytopenia enlisted above and management to treat the primary and secondary causes of thrombocytopenia viz. antihemoprotozoan drugs, whole blood transfusion, platelet transfusion etc. Post treatment and management, thrombopoietic rate in the animal is assessed to gauge the improvement in platelet profile. Aspirates from the bone marrow are screened to quantify thrombocytopoiesis. Active thrombopoiesis is recognised by visualising the bigger sized platelets in the blood smear or the platelet progenitor cells i.e. the megakaryocytes in the bone marrow impression smear.

Active thrombopoiesis is more evident when the cause of thrombocytopenia is due to increased destruction of platelets. However, if the cause of thrombocytopenia is of bone marrow origin as in aplastic anaemia then minimal thrombopoietic activity is witnessed which is an indicator of poor prognosis. Hence clinicopathological investigation like blood smear screening for identifying the morphologic variations of platelets and estimation of platelet indices is an evitable component in diagnosis and post treatment follow up of thrombocytopenic dogs to tailor the therapeutic strategy and to assess the prognosis.

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