IMMUNE MEDIATED THROMBOCYTOPENIA, DIAGNOSIS AND RECENT ADVANCEMENT IN ITS TREATMENT IN DOGS - A REVIEW

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ABSTRACT

Mononuclear phagocytic system causes the elimination of platelets with the help of antibodies and as result immune mediated thrombocytopenia (IMT), which there is activation of auto- or allo-antiglobulins against thrombocytes leads to its premature destruction by macrophagic engulfment in dogs. Immune system due to presence of other disorder may cause the destruction of platelets so by confirming the absence of secondary disorder is an evidence that immune system is responsible for causing destruction of platelets and this type of thrombocytopenia is primary immune mediated thrombocytopenia and it can be treated by suppressing immune system with the help of immune suppressive therapy while the thrombocytopenia caused by presence of any other disorder is secondary immune mediated thrombocytopenia which can be treated by removing that disorder which is responsible or due to which this type of immune mediated thrombocytopenia has been occurred or we can also give immune suppressive drug. If dogs suffering from immune mediated thrombocytopenia do not show any response to the therapy then additional immunotherapy can be given and in some cases the examination is repeated again in order to find out any other cause involved which may be left to be identified.

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INTRODUCTION

Immune-mediated thrombocytopenia (IMT) is a common immunological disorder of dogs in which there is activation of auto- or allo-antiglobulins against thrombocytes leads to its premature destruction by macrophagic engulfment [1, 2]. The auto-antiglobulins are produced against self altered platelet surface antigen while allo-antiglobulins are produced against foreign platelet antigens or blood products as a result of pregnancy, transfusion, or drugs [4]. The defined mechanisms for this decrease includes; increased platelet destruction, decreased platelet production by the bone marrow, splenic sequestration of platelets, and increased platelet consumption or loss during infection or inflammation [3]. Regarding its molecular mechanism the humoral immune system activate against platelet surface glycoproteins (PSG-IIb/IIIa and PSG-Ib/IX), while less commonly against glycoproteins (GP-IV and GP 1a-1ib) complexes. The clinical picture of IMT patients is obvious with petechiations, agonal hemorrhage (PMH), gingival bleeding, epistaxis, hemochromasia, hematuria, and mucosal bleeding occurs but life threatening situation is rare in acute IMT. A wide variety of therapeutic regimens are currently used for the treatment of IMT because it’s a common clinical entity in both humans and animals such as dogs, pigs, and horses [5-10]. IMT may be primary or secondary depend on the presence/ absence of underlying disease. If underlying disease is absent it is primary and if present then secondary. Primary IMT is also known as idiopathic thrombocytopenic purpura (ITP) [1, 8]. Spleenectomy resolves thrombocytopenia in 70% to 75% of chronic cases, but in a small group of cases (5% of all cases), the thrombocytopenia becomes refractory to therapeutic intervention including steroid therapy, intravenous immune globulin and immunosuppressive chemotherapeutic agents [15]. The consequences demonstrated from clinical studies showed that immune mediated inflammatory causes destructive articular changes of joints leading to rheumatoid arthritis [56].

Predilacted Breeds of Dogs:

IMT is a common acquired condition in miniature, toy poodles and other toy breeds. One hypothesized reason of high incidence may be the relative popularity of these breeds as domestic pets, hence a big trouble now-a-days for pet lovers.

Diseases and conditions in which it happens in dogs:

IMT may occur as a primary condition or secondary condition or happened in association with other maladies, Like it act as a primary entity in idiopathic thrombocytopenic purpura (ITP), but may also be secondary in case of certain drugs, toxic, microbial, and rickettsial agents. The associated diseases and conditions include: septicemia, lymphoproliferative disorders, incompatible blood transfusions, malignancy, and autoimmune diseases, including autoimmune hemolytic anemia, systemic lupus erythematos (SLE), and rheumatoid arthritis. These maladies and conditions closely relate the animal thrombocytopenias with the analogous human disorders and can be used as models for comparative studies.

Age and gender predilaction in dogs for IMT:

The onset of malady could occur in any age of dog but the most susceptible one is about 5 years. While talking about gender susceptibility, the number of females which are platelet IgG positive, irrespective to their fertility i.e. whether intact or spayed, is twice that of males. But interestingly the sex ratio is equal in antibody negative cases.

Time dependent severity:

Most cases of positive IMT are chronic and recurrent, although some acute onset IMTs have also been observed. Of the acute cases, however, most were negative for IgG against platelets.

Immune mediated thrombocytopenia (IMT) and immune mediated hemolytic anemia (IHA):

IMT shares many features with immune mediated hemolytic anemia IHA [7]. As both are the disorders of Type II hypersensitivity in which the humoral response i.e. IgG or IgM binds with the epitopes on the surface of the platelet or erythrocyte. This opsonization leads to the activation of complement (C) proteins [16-17]. So, the affected haemocytes are either lysed intravascularly or phagocytosed by the monocyte-macrophage system [8, 17-21].

PF3:

The positive test samples for IMT may be due to specific antiplatelet antibody or by nonspecific agents, such as trace amounts of thrombin or fibrinogen degradation products, which can also damage the platelet membrane and cause the release of PF3 in the assay. That is why the plasma of control and patient samples are used as the source of globulin fractions for the test, rather than serum as described by Karpatkin and Siskind [7]. The immunoglobulins against platelets in PF3-positive samples can only be identified they are removed or decreased by adsorption with mono-specific, antiserum. In most of cases, the positive samples have IgG act as antiplatelet antibodies; the remainder are either IgA or IgM antibodies [7, 10]. Recent researches show other types of immunoinjury assays designed for the detection of human platelet isoantibodies [13] but it is disappointed. But these assays are not much reliable because of false Positive and false negative results than the PF3-release test originally used [10]. The new tests like platelet migration inhibition (PMI) assay are now used for canine samples [22]. One new research in human has showed that ITP is due to delayed-type hypersensitivity reactions and serum blocking factors [11, 12]. Further studies on animal models may provide additional information on the basic mechanisms of this autoimmune phenomenon.
Pathophysiology of IMT

Megakaryocytes are the precursors of platelets, to whom fragmentation leads to the formation of thrombocytes which are then poured into the blood flow. Their average life span in circulation is 8 to 12 days. Aged platelets are detected and removed from the circulation by the tissue and splenic macrophages [1, 23]. IMT, as described earlier, a disorder of accelerated platelet destruction, where Abs are produced and binds to the platelet surface, enhancing platelet clearance by the mononuclear phagocytic system. Else than spleen, liver also plays a role [1, 2]. The term thrombocytopenia is used when platelets destruction exceeds compensatory production of platelets by the fragmentation of megakaryocytes in the bone marrow [1]. Antiplatelet antibodies are directed against normal platelet surface antigens [2]. Immune mediated is the humoral response of the body defense mechanism against Platelet integrin alpha IIB beta 3 (formerly glycoprotein IIb/IIIa) present on the platelet' surface which is immunogenic and thought to be the most frequent target antigen [24]. Antiplatelet antibodies (IgGs) not only damage the platelets but also alter their function [1].

Types of Thrombocytopenia:

On the basis of knowing of etiology two types of IMT is described.

Primary immune-mediated thrombocytopenia

Primary immune-mediated thrombocytopenia has also been called idiopathic thrombocytopenic purpura. In this spontaneous autoimmune disorder, autoantibodies are directed against specific portions of the platelet membrane. It occurs in the absence of an underlying disease and can only be diagnosed once causes of secondary immune-mediated thrombocytopenia have been ruled out [1, 2].

Secondary immune-mediated thrombocytopenia

In secondary immune-mediated thrombocytopenia, antibodies bind to antigens adsorbed to the platelet surface or immune complexes nonspecifically bind to the platelet [24]. Secondary immune-mediated thrombocytopenia occurs as a result of an underlying disorder [1, 2]. Many underlying causes of secondary immune-mediated thrombocytopenia have been reported. Often there is an association between a disease and thrombocytopenia, but the causative role has not been confirmed experimentally. Conditions that have been associated with immune-mediated thrombocytopenia include autoimmune disorders, drug therapy, blood product transfusion, vaccine administration, various neoplasms, and infectious agents [1].

Thrombocytopenogens:

1) Drug and blood product therapy

Although any drug could provoke this disease, several drugs have been associated with immune-mediated thrombocytopenia in dogs, including auranofin (gold salts), cefazedone, and trimethoprim-sulfonamide combinations [1, 2]. Drugs generally act as haptens, which combine with platelets to form a drug-platelet complex that is antigenic [26]. Drug-related immune-mediated thrombocytopenia usually develops weeks to months after initial therapy, resolves within two weeks of discontinuing therapy, and does not recur unless the drug is readministered [1, 2]. Severe thrombocytopenia caused by the production of antiplatelet antibodies has been reported in dogs within weeks after blood product transfusion [3].

2) Vaccine administration

Canine postvaccinal immune-mediated thrombocytopenia has been suspected, but evidence of a direct causative role of vaccines in immune-mediated thrombocytopenia is lacking [1].

3) Neoplasia

Thrombocytopenia is commonly associated with various hematopoietic and solid neoplasms, including lymphoma, mammary adenocarcinoma, mast cell tumor, hemangiosarcoma, nasal adenocarcinoma, and fibrosarcoma [1, 27]. Antibody against tumor antigens that are closely related to platelet membranes may initiate platelet destruction. In some dogs with solid neoplasms, thrombocytopenia resolves after tumor remission [1].

4) Infectious agents

Viral, bacterial, rickettsial, protozoal, and parasitic infections may also play a role in inducing immune-mediated thrombocytopenia [2]. Infections may result in immune-mediated platelet destruction by exposing antigenic sites on platelet surfaces or through immune-complex injury to platelet membranes [26]. Antibodies that can bind to platelets have been identified in dogs with ehrlichiosis, babesiosis, leishmaniasis, and dirofilariasis [2, 26, 28]. The pathogenesis of thrombocytopenia with several infectious diseases is multifactorial, involving decreased production by the bone marrow and splenic sequestration, in addition to immune-mediated destruction [1]. In acute ITP, the autoantibodies are associated with bacterial or, more commonly, viral infections. It has been postulated that these antibodies are emerging from antibodies against viral or bacterial antigens and are cross-reacting with platelets. Recently, it has been suggested that the autoantibodies in childhood ITP are a result of the persistence of proinflammatory cytokines and T-cell response following a viral or environmental trigger that leads to the emergence of previously suppressed autoantibodies [29]. The key pathology then is a failure to suppress these autoantibodies. This failure may be because CD25+ T-regulatory cells are not fully mature in young children and, therefore, cause autoantibodies to produce [30].
Differential diagnosis of immune mediated causes with non-immune mediated causes

When evaluating a patient with suspected immune-mediated thrombocytopenia, be sure to exclude non-immune-mediated causes. Other mechanisms of thrombocytopenia include decreased platelet production due to bone marrow disorders, platelet sequestration, non-immune-mediated platelet destruction, platelet consumption, and platelet loss. The cause of thrombocytopenia may be multifactorial.

Neoplasia may cause thrombocytopenia through immune-mediated and non-immune-mediated mechanisms. Non-immune-mediated mechanisms include platelet consumption, splenic sequestration, hemorrhage, myelophthisis, and bone marrow suppression by chemotherapy or radiation. Hemolytic uremic syndrome, a rare cause of thrombocytopenia, is readily differentiated based on renal failure, microangiopathic hemolytic anemia, and fever. Decreases in platelet number resulting from splenic sequestration are generally modest. Similarly, thrombocytopenia resulting from blood loss is generally mild and transient, although moderate to severe thrombocytopenia has been reported with anticoagulant rodenticide intoxication [1, 2]. Immune-mediated thrombocytopenia usually causes severe thrombocytopenia often < 50,000 platelets/µl [1]. A severe, inherited thrombocytopenia has been reported to occur in up to 50% of Cavalier King Charles spaniels [31]. Platelet counts can be as low as 20,000/µl, but many affected dogs have macrothrombocytes, and this disorder is not associated with a bleeding tendency. Perform manual platelet counts in dogs of this breed with thrombocytopenia, as large platelets may result in falsely low platelet counts as measured by impedance analyzers. Bone marrow disorders can cause thrombocytopenia, but if thrombocytopenia is the sole abnormality on a complete blood count (CBC), bone marrow disorders are unlikely. With the exception of early estrogen toxicity, bone marrow disorders that cause thrombocytopenia typically cause concurrent leukopenia, with or without anemia[1, 2]. The presence of a marked, nonregenerative anemia or neutropenia could suggest bone marrow disease; alternatively these findings may be seen with immune-mediated disease.

The autoantibody in ITP is either IgG or IgM and is directed against a variety of platelet glycoproteins, most commonly GP IIb/IIIa and GP Ib/IX complex. Recently, it has been suggested that the generation of CD4+ and CD25+ regulatory T -cells in the thymus during a critical proinflammatory response may prevent autoantibody formation. It is now clear that both decreased platelet production and increased platelet destruction are important in the pathogenesis of ITP. Helicobacter pylori infections have been suggested to be involved in the pathogenesis of chronic ITP in studies done in Italy and Japan.5 The mechanism for the production of autoantibodies due to H. pylori infection is unknown; however, 43% to 75% of patients with chronic ITP have H. pylori infections. The eradication of H. pylori infections with antibiotic treatment has improved platelet counts in 84% to 100% of ITP patients with platelet counts >30,000 [5,10].

Megakaryocytes and ITP

The number of megakaryocytes is normal to increased in the bone marrow of patients with ITP; however, decreased platelet production is reported in more than one-third of adults with ITP. Many studies have confirmed that the autoantibodies in ITP interfere with platelet production or platelet release from the bone marrow. Decreased megakaryopoiesis has been reported in certain pediatric and adult patients who demonstrate anti-GP Ib/IX alone or in combination with anti-GP IIb/IIIa autoantibodies. Thrombopoietin levels are normal or slightly increased in some ITP patients resulting in normal to increased megakaryocytes. It has been postulated that the suppression of megakaryocyte production by autoantibodies may be associated with increased apoptosis in adult ITP. In childhood ITP, the autoantibodies may not recognize the same epitopes on platelets due to a less-mature immune system [29].

Alloimmune Thrombocytopenia

Post-Transfusion Purpura

Rare form of alloimmune thrombocytopenia is Post-transfusion purpura (PTP in which there thrombocytopenia occurred is severe along bleeding following transfusion of blood or blood products [32]. Post-transfusion purpura is caused by previously immunized patients antibody-related platelet destruction results in post transfusion purpura Patients who have sensitized previously i.e. multiparous women [33].In most of cases antibodies are directed against platelet antigen A-1a (HPA-1a),it has been detected that in the serum of patient of patient of acute thrombocytopenia there is reaction of antibodies with other platelet glycoproteins.e. GP Ia/Ila, HPA-5a, and HPA-5b. After 3 to 12 days of transfusion there is occurrence of severe thrombocytopenia [32]. The pathophysiology of thrombocytopenia is not cleared however, it is postulated the immune thrombocytopenia in PTP is caused by alloantibodies which have HPA-1a-like specificity (pan-reactive antibodies) [19]. In the serum of patient detection of antibodies against platelet-specific antigens confirmed the presence of PTP in a patient.IVIG is generally used as treatment of choice.If IVIG is ineffective then we use corticosteroids and plasmapheresis to stop the bleeding [33, 34].

Neonatal Allo-immune Thrombocytopenia

Fetomaternal incompatibility of platelet antigens is responsible for causing neonatal alloimmune thrombocytopenia(NAITP). Destruction of the fetal platelets antibodies is due to cros sage of maternal antibodies. Intracranial bleeding (20%) occurs in severe thrombocytopenia, cause hydrocephalus and fetal death [35]. In 50% of cases first pregnancy is affected [29].The offending alloantibodies are commonly maternal anti-HPA-1a IgG alloantibodies (75% of cases), which are directed against platelet glycoproteins IIb/IIIa, Ib/IX, Ia/Ila, and CD109. 97.5% of the antibodies are against glycoprotein IIb/IIIa complex in whites [22]. NAITP is diagnosed by determining the genotyp of the maternal and paternal platelet antigens and antibody identification in maternal plasma that reacts with the paternal platelets. Treatment of the mother with corticosteroids and IVIG causes increase in platelets number in fetal in subsequent pregnancies and intracranial hemorrhage is prevented as well [35].According to the current guidelines, the affected neonate is treated by transfusion of antigen-compatible platelets or washed maternal platelets. If platelets which are antigen-negative unavailable then we do transfusion of platelets which are antigen positive from random donor because
NAITP is severe. This treatment is effective, and it causes increase in platelet count and it also cause stoppage of severe bleeding. Platelet antigen compatibility cause is not known. It is speculated some of the antigen-positive platelets cause absorbance of offending alloantibodies and for the normal functioning leave the rest of platelets [36].

### Heparin-Induced Thrombocytopenia

Due to heparin therapy in 1% to 4% patients there may be heparin induced thrombocytopenia. When we conduct therapy with either unfractionated heparin (UFH) or low molecular weight heparin (LMWH) then thrombocytopenia occurs within 5-14 days of stoppage of therapy. The offending antibodies against Heparin-PF4 (platelet factor 4 complexes) are directed. There is the occurrence of thrombocytopenia and platelet activation and the release of microparticles from the platelets (causing generation of thrombin) due to attachment of complex with platelet FC receptors. Arterial or venous thromboses are complications of HIT. HIT treatment is done by discontinuing heparin and by antithrombin drugs as an alternative.

### Drug–Induced Thrombocytopenia

Antibodies are introduced by vast no of drugs against platelets causing thrombocytopenia and it include quinidine sulfate, quinine sulfate, vancomycin, amoxicillin, and other drugs. The thrombocytopenia caused by drug is for brief time and get reversible when we stop using drug [30]. In 1973 according to Rhodes, Dixon and Silver thrombocytopenia and thrombosis occur after the 7 days of start of heparin therapy and it reveals that the heparin therapy by the involvement of immune system is responsible for this complication [37]. There was severe thrombocytopenia in 1% to 4% patients and especially those who received first time glycoprotein IIb/IIIa antagonists such as abciximab, eptifibatide and tirofiban. There was immune-mediated pathogenesis in thrombocytopenia caused by drug[38]. There are three different types of immune-mediated thrombocytopenia caused by drugs Immune thrombocytopenia caused by quinine, heparin, and thrombocytopenia due to heparin and thrombosis, and thrombocytopenia within hours after a first exposure to antagonist of glycoprotein IIb/IIIa. These syndromes differ in their development, severity, sign and symptoms, tests used for their diagnosis and their treatment as well. There is uncommon binding of antibodies to drug and complex of drug which is bound to platelet glycoprotein occur in immune-mediated thrombocytopenia caused by i.e. quinine Macrophages with the help of mononuclear system causes the elimination of platelets coated with antibodies (by identifying Fc tail of antibodies) from the blood circulation. Thousands of copies of glycoproteins (IIb/IIIa and Ib/IX) are present on platelets. and there is severe thrombocytopenia in 85% to 90% of patients caused by antibodies and the platelet count at nadir is less than 20,000/mm3. In immune thrombocytopenia caused by carbamazole: in that instance, the thrombocytopenia is of moderate type while the platelet count at nadir is 60,000/mm3 and there is complex formation between drug and glycoprotein, platelet endothelial cell adhesion molecule occur[1]. Immune mediated thrombocytopenia which is due to quinine also causes hemolytic uremia, neutropenia, disseminated coagulation intravascularly in some patients. There are about three dozen drugs which may be involved in causing immune-mediated thrombocytopenia[2]. When we give vancomycin to a patient occasionally then after 7 days of therapy there is the occurrence of is thrombocytopenia. When the drugs such as quinine if given continuously then the thrombocytopenia occurs abruptly and there is decrease in platelet count rapidly and as well as hypersensitivity reactions. This type of rapidly developing thrombocytopenia can occur in a patient who had previously received the drug many weeks or even years earlier. We can treat patient by stopping the use of drug and supportive treatment on the basis of severity of bleeding can be done by immunoglobulin (IV) which helps to increase platelets. The intracranial bleeding may cause fetal hemorrhage but it is rare. The similarity of immune-mediated thrombocytopenia due to glycoprotein IIb/IIIa antagonists and classic drug is of bleeding risk, intensity, and hypersensitivity reactions. In some cases the thrombocytopenia occurs after the administration of drug within hours and sometimes there was no evidence of exposure in past was present. Drugs such as eptifibatide and tirofiban cause conformation change, neoeptipe in the glycoprotein complex by binding of natural antibodies against glycoprotein (glycoprotein IIb/IIIa). Abciximab (chimeric Fab fragment) can cause thrombocytopenia. Abciximab bind reversibly to platelets and there is thrombocytopenia even after 7 days of first exposure of drug. In the thrombocytopenia caused by abciximab the platelet number can be increased by transfusions as compared to thrombocytopenia caused by tirofiban or eptifibatide. There is clumping of platelet occur in the patients of thrombocytopenia after the administration of drug which is not EDTA-dependent antibodies(natural antibodies) and in this type of conditions we do not give any treatment. Thrombocytopenia caused by heparin is an example of antibody-mediated disease. The thrombocytopenia is caused by heparin-dependent IgG antibodies that bind to multimolecular complexes (consisting of PF4). Platelets are activated by antibodies through their FcγIIa receptors, and platelet derived procoagulant microparticles are released as result. Coagulation reactions and thrombin generation is carried out by these microparticles. Arterial thrombosis in limb and cerebral arteries, and venous thromboembolism, adrenal hemorrhagic necrosis, skin lesions injection site of heparin, hypersensitivity reactions after an IV(bolus) heparin, and consumption coagulopathy.

Thrombosis is not further stopped by stopping heparin therapy and it necessitates its inhibition by thrombin or by acting non-heparin anticoagulants to prevent its generation rapidly. Coumarin (warfarin) which is an anticoagulant increases the chance of microvascular thrombosis (causing venous limb gangrene and skin necrosis) considerably and in acute thrombocytopenic phase it is contraindicated.

There is the appearance of infected antibodies in blood for brief period of time, it shows that on beginning heparin therapy the thrombocytopenia starts rapidly in the patients due to exposure of heparin within the previous several week. Sometimes when the heparin therapy is stopped then within a week or two week after therapy has been stopped the thrombocytopenia occur and it is also called delayed onset heparin induced thrombocytopenia. Unlike the reactions od drugs which are induced by the purpura have very rare long term effects as thrombocytopenia induced by heparin is due to thrombosis caused by long term sequelae. Drug dependent antibodies detected in laboratory are of no value. The binding of antibodies to the platelets which is either drug dependent or depends

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upon drug metabolites is detected in the classic syndrome and it results in sensitivity which is moderate it may be due to absence of relative drug metabolites in test system. Thrombocytopenia caused by glycoprotein IIb/IIIa antagonists and by heparin is difficult to differ in non infective antibodies from infective antibodies. There is the formation of potentially pathogenic heparin-dependent antibodies by the heparin and cause the activation of platelets which is a biologic property. In many hospitals commercial enzyme immunoassays is used to find out antibodies against the platelet factor-4 heparin complexes

**Diagnosis:**

**Signalment**

Dog of any age can suffer with immune-mediated thrombocytopenia but it occurs mostly in the dogs of middle age. Any breed or cross breed of the dog can be affected by immune mediated thrombocytopenia but according to a report in german shepherds, toy, standard poodles, miniature, and standard cocker spaniels, old English sheepdogs; and German shepherds there are high rates of incidence [1,2,25]. In some breeds of dogs there are high rates of incidence may be due to genetic reasons. Occurrence of immune-mediated thrombocytopenia takes place in both sexes but female dogs are affected 2 times more than male dogs [1].

**Clinical presentation**

Usually the bleeding is not checked in dogs who have immune-mediated thrombocytopenia but if there are signs of i.e. Anorexia, lethargy, and weakness we do evaluation of bleeding[1,25]. Sometimes there is the appearance of no symptoms who are affected. The immune mediated thrombocytopenia in such dogs can identified by CBC, when done there are decreased number of platelets which shows the presence of disease. Immune-mediated thrombocytopenia due to any other disease (secondary immune mediated thrombocytopenia) by checking or finding out the signs of other disease i.e. in lymphoma or dyspnea there is peripheral adenopathy and disseminated histoplasmosis [25]. There are signs of hemorrhage in dogs having immune-mediated thrombocytopenia [1]. Sometimes in immune-mediated thrombocytopenia there is unexpected hemorrhage also occurs [1,26]. When there is bleeding in the central nervous system then it causes the development of hemorrhage which may be fatal [2, 41-43]. Dogs having immune-mediated thrombocytopenia are given serious attention due to risk of bleeding. In dogs there may be severe GI hemorrhage due to acute thrombocytopenia which is the major cause responsible for the death of dogs with acute immune-mediated thrombocytopenia [1].

**History and physical examination:**

We check the complete history of patients of immune mediates thrombocytopenia in order to find out the cause which may be i.e. medication or vaccination. If there is any medication or treatment find to be the cause of decrease in the function of platelet i.e. aspirin then stop using such medications. It is advised to go to that area which is endemic for diseases i.e. ehrlichiosis and dirofilariasis and it should be known that how to use preventives of tick, heartworm and flea in such areas [1]. During physical examination or after taking history of patient the signs of other coexisting disease are also noted. The site, time period, and intensity of bleeding and episodes are found out. Information about blood transfusions in past is also collected. The patient is completely examined physically and eye examination is also involved. Abnormal hemostasis if present then it is noted. In order to find out melena we check the feces. Dogs having immune-mediated thrombocytopenia may have mild or very low fever. There may be enlargement of spleen [41]. And according to some authors the splenomegaly may be due to presence of any coexisting disease [1,2].

**Lab Tests:**

**CBC and blood smear**

CBC is used to find out thrombocytopenia by counting platelet automatically and blood smear can also be used to find out thrombocytopenia [25]. The range reference range of platelet is between 200,000 and 500,000/μl [1]. As compared to other breeds the number of platelet is low in healthy greyhounds and usually in Shiba Inus [44]. Clumping occur due to presence of macroplatelets overlapping to the size of erythrocyte may be responsible for low count of platelets. When there is overlapping then it is very difficult to differ the type of cell and as result platelets are counted as erythrocyte. Blood smear can be used to verify the low platelet count [2, 25]. In order to evaluate the platelets number on a blood smear quickly is done by counting platelets number/high power field (HPF) (100X). If there are 5 to 10 fields having average platelets number then to calculate the platelet count we carry out the multiplication of average platelets number/HPF by 15,000 per μl [25, 26, 1]. In normal count of platelets there are more than 12 platelets/HPF [26, 1]. Clumping of platelets is find out by examining feathered edge of smear. In case of presence of clumps the platelets number is not accurate on examination [1, 25]. Clumping if cannot be inhibited then slide is presented to pathologist for examination in order to check that the platelet numbers are appropriate or not. Size of platelet is also checked because the platelets which are large and immature, macrothrombocytes occur as result of response of bone marrow and there is also increase in function of hemostasis [1, 25]. While small platelets microthrombocytes are formed as result of platelet destruction caused by immune system [2, 50]. In the presence of EDTA platelets get swell but the reason behind this finding is uncertain [24]. Average size of platelets in blood can be estimated by the mean volume of platelets. When the number of platelets is less than 50,000 per μl then there is the great chance of hemorrhage. When the platelet number is less than 30,000 per μl then there is the occurrence of spontaneous hemorrhage. Disorders i.e. vasculitis or defects in platelet function may cause decrease in platelets [1, 25]. In thrombopoiesis flow cytometry is used for the quitation of reticulated platelets and generally for routine examination this test is not done [23]. Anemia may be present because of hemorrhage or concurrent immune-mediated hemolytic anemia (Evans’ syndrome). There is the existence of immune-mediated hemolytic anemia in 20% dogs having immune mediated thrombocytopenia [2]. Anemia in thrombocytopenic patients i.e having immune-mediated hemolytic anemia can be diagnosed by determining the presence of agglutination or spherocytes. In Evan’s
syndrome schistocytes may be present and there is existence of disseminated IV coagulation, but their presence is increased in splenic hemangiosarcoma and in other conditions i.e. vasculitis [45]. Normal count of leukocyte may be normal or stress leukogram may be present. In some patients there may be the presence of hemolytic anemia which is immune mediated and increased neutrophils with a left shift [2]. There are less inflammatory leukogram in patients of immune mediated thrombocytopenia and there is increased neutrophils, left shift, or toxic change occur in inflammatory disease.

Additional diagnostic testing:
Single test for the diagnosis of immune-mediated thrombocytopenia is not enough so we do other tests as well for definitive diagnosis [25]. For the diagnosis of thrombocytopenia the other cause due to which thrombocytopenia occurred should be eliminated [1, 2].

Blood testing and urinalysis:
Chemistry profile of serum and urinalysis are also done except CBC [1, 41] i.e. heartworm testing [1].Severe thrombocytopenia is caused by Anaplasma platis in dogs, but no symptoms are shown by dogs [24]. Prothrombin time, an activated partial thromboplastin time, and degradation products of fibrin or concentration of D dimer are measured to determine that the thrombocytopenia is caused by hemostatic abnormalities or not [25]. The abnormalities which are present clinically in disseminated intravascular coagulation are increased coagulation times, schistocytes, enhanced degradation of fibrin, or D-dimer concentrations, and decreased fibrinogen concentrations [1, 2]. If the platelet count is < 10,000 per ul then there can be false increase in clotting time [1, 25]. There is increased bleeding time from buccal mucosa in primary hemostasis and it get more increased in patients in which the platelet count will be very low i.e. platelet counts <30,000 per ul. In patients suffering with marked thrombocytopenia this test is not done.

Imaging:
We do radiography of thorax and abdomen and ultrasonography of abdomen in order to find out the neoplasia cause [1]. Cytologic examination of enlarged organs, such as spleen, lymph nodes liver is done by doing aspiration with fine needle. Such sampling is safe if the platelet count is enough i.e. platelet count> 50,000 per μl and if the platelet count is no according to test requirements then this technique is not safe [25].

Bone marrow aspiration:
Bone marrow aspiration is not done if leukopenia either with anemia or without anemia is absent [2]. We do bone marrow aspiration when the cause involved is not known or if the platelet production is not appropriate [1, 23]. Biopsy and as well as bone marrow aspiration can be done in thrombocytopenia but it may cause bruising and there may be less chance of hemorrhage (severe hemorrhage) [1, 2, 25]. The site for the aspiration is proximal humerus due to ease of less muscle mass and application of pressure in order to obtain hemostasis. In bone marrow there is increase in total number of megakaryocytes in case of thrombocytopenia and there is increased production of platelets by bone marrow in order to sow response [1]. After 3 to 5 days of episode of acute thrombocytopenia the response is shown by bone marrow [25]. There is the rare chance of aplasia in dogs due to immune mediated megakaryocyte [2].

Definitive diagnosis:
On the basis of intensity (moderate to severe) the thrombocytopenia is diagnosed clinically and find out that there is not any presence of abnormalities of hemostasis or non immunologic platelet collectin (sequestration), consumption or degradation and either there is minor or major increased in the volume of platelets [1, 2, 25].

Treatment:
Thrombocytopenia mediated by immune system is a highly fatal disorder [1]. Mortality rates of There are 25% to 30% death rates due to thrombocytopenia or relapse [1, 2]. Euthanasia or acute hemorrhage is responsible for causing death [1]. Initially the rates of survival can be improved by assertive therapy. Sometimes prolonged treatment is required for immune mediated thrombocytopenia i.e. we do prolonged treatment in 25% of dogs suffering with immune-mediated thrombocytopenia and there is occurrence of euthanasia takes place in these patients due to side effects [1]. There is recurrence after some period of lessening of disease and it is common i.e. in 40% of dogs it occur [1, 2].

Initial supportive therapy:
Immune-mediated thrombocytopenia managed by preventing platelet destruction, Stop bleeding, and by treating coexisting disease if present [24]. There are various strategies used as a supportive treatment of thrombocytopenia which is severe. The patient is kept in hospital and rest is recommended for the patient, kept in cage which is padded and it is handledly in order to reduce the hemorrhage due to trauma [2, 46]. The gingival trauma is reduced by feeding soft diets. IM injections and ocystocentesis are avoided [41, 46]. In patients who have severe thrombocytopenia jugular vein puncture is also avoided and in order to confirm the homeostasis in patients of severe thrombocytopenia to any vein puncture or site of injection apply pressure directly for 5 minutes. We can use IV catheters and as well as jugular catheter and great care is required in case of jugular catheter for the placement [46]. Colloids, crystalloids and blood products are given to the hypovolaemic patients [2]. Synthetic colloids are used carefully for hemodilutions they may cause the reduction in the function of platelets and plasma is used plasma should be considered as an alternate [46]. The benefit GI protectants are not proven but still in patients who have symptoms of GI hemorrhage we give GI protectants [41].
Stop using drugs which may cause decrease in functions of platelets i.e. NSAIDS [46]. To treat the tick born disease doxycycline is given orally after every 12 hour in the dose of 5-10 mg/Kg through IV [47]. Either 1% prednisolone acetate or 0.1% dexamethasone sodium phosphate topically 3 to 4 times a day with 1% atropine topically 2 to 4 times in a day is given for the treatment of hyphema [46].

Transfusions:

Sometimes anemia caused by hemorrhage can be treated by transfusion. Transfusion is used to cause an increase in number of platelets. The platelet from plasma of blood rich in platelets or concentrates of platelets are Whole blood, platelet-rich plasma, or platelet concentrates are used in transfusion. There is short term increase in the number of platelets when we carry out transfusions by these products and there is re-occurrence of thrombocytopenia because the life of circulating donor platelets is very low, only few hours in thrombocytopenic patients [2, 1]. Immediate bleeding in patients who are in critical condition can be stopped by transfusion. We also do transfusion before carrying out surgery, i.e. splenectomy or any other procedure of this type. There may be increase in the number of platelets up to 10,000 per μl by giving the fresh plasma rich in platelets in the dose of 10 ml per kg [1, 46]. Additional transfusion is not done if there is no increase in platelet numbers or if there is no uniformity for at least 2 hour in the enhanced level of platelet numbers [1]. There is special centrifugation done in order to prepare plasma rich in platelets and concentrates of platelet and we can also buy products from blood banks [46]. There is the chance of increased blood volume and as well as transfusion reaction due transfusion [1]. In patients suffering from thrombocytopenia due to hemorrhage we prefer transfusion of whole blood [48]. The collected blood should be used with in 4 hour. There are harmful effects of refrigerator on quality of platelets of being viable that’s why the storage of blood is done on room temperature [48].

Immunosuppressive therapy:

The primary aim of treatment of immune mediated thrombocytopenia is to restore the normal homeostasis which can be obtained by immunosuppressive therapy. First of all bleeding is stopped by obtaining the appropriate level of platelets i.e. if platelet count > 50,000 per μl then there may be no bleeding. The long time is required in order to maintain the normal level of platelet [1].

Glucocorticoids:

Immediate effects are obtained by glucocorticoids which are mainly used in immunosuppressive therapy. Mononuclear phagocytic system is suppressed by glucocorticoids and as result the affinity of antibody for binding to the platelet is decreased and it is used as treatment of choice [25, 41]. Prednisone or prednisolone is given orally in a dose ranging from 2 to 4 mg per kg per day. Dexamethasone dose 0.2 mg per kg intravenously can be in every 24 hours as an alternative. Usually within the first week of start of therapy there is increase in platelet numbers [1]. Prednisone is administered continuously until the platelet count becomes normal and then reduce the dose of drug slowly. Platelet count is checked before reducing the dose of prednisolone and if there is decline in platelet count or if there is no increase in platelet count then do not reduce the dose of drug. If the platelet count is normal to an extent of 10,000 per μl then we can reduce the dose of drug. If we reduce the dose of drug slowly then there is the appearance of side effects and there is the occurrence of relapse of immune mediated thrombocytopenia if the dose of drug is reduced rapidly [2]. Prednisolone is continued for two to four weeks generally as initial immunosuppressive dose. In case of adequate platelet count the dose is continued for another two to four weeks after reducing up to 50%. The dose is adjusted in order to obtain the lowest dose which effectively maintains the adequate number of platelets. The maintenance dose can be given after every 48 hour in the dose of 0.5 to 1 mg per kg if needed. At high doses prednisolone cause side effects are increased thirst, hyperventilation, increase urination and other signs of hyperadrenocorticism, i.e. baldness, muscle atrophy, susceptibility of infection and weakness. If the reduced dose of prednisolone causes the decrease in number of platelet then the dose is increased to an extent so that the platelet count becomes normal by the drug effectively. If the effective dose of prednisolone is not suitable for prolonged usage then we give immunosuppressive medication additionally.

Vincristine:

Vincristine belongs to class of alkaloids (vinca alkaloids) which is responsible for transient increase in platelet numbers by releasing platelet through megakaryocyte. It helps patient in early discharge from hospital by increasing the initial platelet count. Except enhanced release of platelets from bone marrow there are some mild immunosuppressive effects are shown by vincristine as well. Vincristine is given as a single IV dose of 0.02 mg/Kg. Within 2-3 days there is increased in platelet numbers. Vincristine can also be given as infusion or by incubation with a platelet transfusion but these routes of administrations are not completely investigated. Vincristine is administered through placement in an appropriate catheter as it is a powerful blistering agent. Its single dose may be responsible for causing some side effects as well. Platelet functions are also decreased by vincristine in vitro while it is investigated that there is no change in function of platelets by vincristine in vitro.

Other immunosuppressants:

Vincristine belongs to class of alkaloids (vinca alkaloids) which is responsible for transient increase in platelet numbers by releasing platelet through megakaryocyte [1]. It helps patient in early discharge from hospital by increasing the initial platelet count [42]. Except enhanced release of platelets from bone marrow there are some mild immunosuppressive effects are shown by vincristine as well. Vincristine is given as a single IV dose of 0.02 mg per Kg [1, 42]. Within 2-3 days there is increased in platelet numbers [1]. Vincristine can also be given as infusion or by incubation with a platelet transfusion but these routes of administrations are not completely investigated [1, 2]. Vincristine is administers through placement in an appropriate catheter as it is a powerful blistering agent. Its single dose may be responsible for causing some side effects as well. Platelet functions are also decreased by vincristine in vitro while it is investigated that there is no change in function of platelets by vincristine in vitro.
agent. Its single dose may be responsible for causing some other side effects as well [1, 42]. Platelet functions are also decreased by vincristine in vitro while it is investigated that there is no change in fuction of platelets by vincristine in vitro [1].

**Other therapeutic options:**

Except immunosuppressive treatment other approaches for treatment are gamma globulin(IV), plasmapheresis spleenectomy and danazol. Spleenectomy is not thoroughly investigated in a large number of dogs. For the management of immune-mediated thrombocytopenia, spleenectomy is not known to be safe in dogs and success rates are greatly variable [1, 2]. PCR and blood smear preparation is done for several weeks before and after spleenectomy in order to find out Babesia canis, Mycoplasma haemocanis [1].

Hepatic mononuclear phagocytic system may be responsible for causing immune-mediated thrombocytopenia after the spleenectomy [1]. After spleenectomy there may be thrombocytopenia caused by immune system due to mononuclear phagocytic system. We inject gammaglobulins through IV route as an infusion and gammaimmunoglobulin bind with macrophage receptors competitively and this binding by mononuclear phagocytic system declines platelet elimination. This treatment is cost effective while the effects produced are for a brief time [1]. The dose of immunoglobulin injected intravenously is 0.5 to 1 g per Kg [52].

Plasmapheresis is used less due to its cost and difficulties in techniques involved [1].

Danazol (an androgen) is given orally after every 12 hour in the dose of 5 to 10 mg per kg and it causes down regulation of macrophage receptors, reduced production of antibodies against platelets and interfere with the elimination of platelet by affecting on mononuclear phagocytic system [1, 2]. Prednisolone and danzol can be given together for the treatment of immune-mediated thrombocytopenia [1].

The time of 3 to 6 months for the appearance of peak effect is required [1]. Responded to danazol is shown by a small number of dogs having refractory immune-mediated thrombocytopenia [54].

Functions of liver enzymes are monitored on monthly basis. Danazol's usage involve large-breed dogs due to its expense [1].

**Monitoring:**

In every 1 to 2 weeks of first two months platelet number is checked. Before and after any drug dose reduction platelet count is rechecked. After complete remission in patients platelet count is rechecked at the interval of 3 months. It is critical to monitor platelet count because the clinical signs of thrombocytopenia is not an evident unless there is severe or greater reduction in platelet count [1].

**Vaccination in affected dogs:**

Due to vaccine administration in patients there is the chance of immune-mediated thrombocytopenia that’s why we should avoid vaccine or vaccines responsible for it. Platelet counts reduction occur after administering distemper-hepatitis vaccines while peak affects appear after one week of vaccination and in patients of thrombocytopenia vaccination is preferably avoided due to this reason.

It is recommended that in patients of thrombocytopenia after recovery administer core vaccines only and should be administered at separately at different times. The platelet count should be monitored before and after vaccination for few weeks.

**REFERENCES**