Where did all the platelets go? Highlights of the Consensus statement on the diagnosis of immune thrombocytopenia in dogs and cats

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Immune thrombocytopenia (ITP) is the most common acquired disorder of primary hemostasis in dogs. It is a complex autoimmune disorder characterized by both antibody and T-cell mediated platelet and sometimes concurrent megakaryocyte destruction. The resultant thrombocytopenia leads to variable clinical signs ranging from none to a severe mucocutaneous bleeding diathesis. Treatment strategies should aim to restore adequate platelet count to prevent bleeding, but should not necessarily target a normal platelet count. Frontline therapy involves immunosuppressive glucocorticoids combined with adjunctive immunosuppressive therapy as needed. Here we review pathogenesis and diagnosis of ITP as guided by the recent American College of Veterinary Internal Medicine Consensus Statement on the Diagnosis of ITP in dogs and cats. A focus is placed on dogs simply because the disease is rare in cats and thus literature-based evidence for ITP diagnosis in cats is lacking.

1. ITP Pathogenesis

ITP is an autoimmune disease characterized by both platelet destruction and impaired megakaryocyte and platelet production.[1] The immune dysregulation resulting in ITP is incompletely understood, and likely complex. Little is known about ITP pathogenesis in dogs and cats, so we base most of our understanding of the disease on human patients and murine models. Traditionally, ITP has been thought of as a humoral disease.[2, 3] Autoantibodies targeting platelet surface glycoproteins lead to platelet clearance by the splenic and hepatic macrophages.[1] However, it is now recognized that T cells play a central role in platelet destruction in ITP.[1, 4-6] A proinflammatory T helper cell (Th)1, Th17, and Th22 cytokine milieu predominates in many ITP patients.[1, 3] T and B regulatory cells that normally serve to maintain self-tolerance are dysfunctional in ITP, enabling the onset of autoimmunity.[1, 7-11] In some patients, platelet destruction is not mediated by autoantibodies, but instead by autoreactive cytotoxic T lymphocytes.[1, 6, 12, 13]

Growing evidence indicates that ITP is not only a disorder of destruction, but also one of production. In some patients, antibodies and T-cells attack megakaryocytes, resulting in decreased platelet production.[12, 14] Megakaryocytes of dogs with ITP display signs of injury including foaminess, vacuolation, and reduction of cytoplasmic granularity.[15]

In addition to immune targeting of megakaryocytes and platelets, thrombopoietin (TPO) levels are often inappropriately normal in human patients with ITP. TPO is the major

regulator of platelet production and should be elevated in response to thrombocytopenia.[16-18] As platelets age, they are desialylated and subsequently recognized and cleared by the hepatic Ashwell-Morell receptor.[18] This removal in turn, drives hepatic TPO expression providing a feedback system: as more aged platelets are cleared, more TPO is produced. However, in ITP antibody-coated platelets are cleared by macrophages of the spleen and liver, so it is postulated that the Ashwell-Morell receptor is bypassed and does not trigger hepatic TPO production.[18] Circulating TPO levels in people with ITP are often inadequate and TPO levels in dogs with ITP have been recently determined to be inappropriately low as well.[16,19]

Cocker Spaniels and Old English Sheepdogs are predisposed to ITP, which suggests there may be genetic or hereditary variables that contribute to the development of ITP, at least in dogs.[20-23] ITP likely results from genetic factors predisposing to autoimmunity and some sort of environmental or infectious trigger, which often remains unidentified.

2. **Clinical Presentation**

ITP is the most common cause of severe thrombocytopenia in dogs. Although any dog can develop ITP, affected dogs tend to be young to middle age; Cocker spaniels, Poodles, and Old English Sheepdogs are predisposed.[20, 22-25] Primary ITP is rarely reported in cats, but has been described.[26-31] Interestingly, in people, dogs, and cats, signs of ITP vary widely. When bleeding does occur, it is typically surface bleeding of the skin and mucosal surfaces - cutaneous, oral, and gastrointestinal bleeding is most common in dogs.[21, 32] However, many patients with severe thrombocytopenia (<30,000 platelets/µl) have no clinical signs of bleeding and platelet count alone is not a reliable predictor of bleeding.[32] Why patients demonstrate variable bleeding is not understood and is likely multifactorial. Interference of platelet function by anti-platelet antibodies and the variable impact of thrombocytopenia on endothelial integrity likely play a role in bleeding presentation.[33-37]

3. **Diagnosis**

Because of the variability of ITP pathogenesis, ITP is a diagnosis of exclusion. Unfortunately, there is no one test for the disease, and as such, a systematic approach to excluding other causes of thrombocytopenia must be taken. In human medicine, ITP is defined as a platelet count of under 100,000/µl in the absence of other causes or disorders that may be associated with thrombocytopenia.[38] As part of the ITP Consensus, we developed five diagnostic questions in the Population Evaluation Comparison Outcome (PECO) question format to investigate whether in dogs and cats with thrombocytopenia (P), evaluation by a diagnostic test (E) compared with platelet count alone (C) improved differentiation of ITP from non-immune thrombocytopenia (O). The questions were then answered by an extensive process that involved systematic review of the available veterinary literature followed by a Delphi review.[39]

Answers to the PECO questions resulted in the development of algorithms for diagnosis of ITP in dogs (Figure 1) and cats (Figure 2). Diagnostic certainty levels for diagnosis of ITP

depending on the extensiveness of workup included *Possible*, *Possible with immunologic evidence*, *Probable*, *Probable with immunologic evidence*, *Diagnostic*, and *Diagnostic with immunologic evidence*.[39]

While extensive answers the PECO questions are found in the Consensus statement, key points will be highlighted below and in the seminar.

Fig 1. Scan to access ACVIM Consensus Diagnostic Algorithm for ITP in Dogs

Fig 2. Scan to access ACVIM Consensus Diagnostic Algorithm for ITP in Cats

3.1 Pre-analytic variation:

Pseudothrombocytopenia must be ruled out in any ITP suspect, especially in nonclinical patients and in cats. Cats often have pseudothrombocytopenia due to feline platelet reactivity and difficulty of some hematology analyzers to differentiate them from erythrocytes as they are similarly sized.[40] A manual platelet count estimate must be performed before any further workup is pursued. In brief, first assess the slide's feathered edge under low magnification for clumps, the presence of which suggests the platelet count is falsely low and warrants obtaining a new blood sample. If there are no clumps, a platelet count is estimated by averaging the number of platelets observed in 10 oil immersion fields (100×) and multiplying this by 15,000 to obtain the number of platelets per microliter.[41] For example, if there is an average of 4 platelets per 100x field, the estimated platelet count is 60,000/µl.

3.2 In dogs/cats with confirmed thrombocytopenia (P), compared with platelet count alone (C) do platelet indices (eg, mean platelet volume [MPV], immature platelet fraction [IPF], reticulated platelets, plateletcrit) (E) improve differentiation of ITP from non-immune thrombocytopenia (O)?

Overall, platelet indices do not help in the diagnosis of ITP, though some studies suggest that increased reticulated platelets may help differentiate ITP from non-immune thrombocytopenia.[42] Although studies regarding the utility of mean platelet volume (MPV) were conflicting, congenital macrothrombocytopenia should be suspected in dogs with chronic thrombocytopenia, macrothrombocytes, and absence of bleeding signs. Congenital macrothrombocytopenia due to a β1 tubulin gene mutation has been identified in the Cavalier King Charles Spaniel, Norfolk and Cairn Terriers, and several

other breeds.[43] Auburn University offers DNA testing that can help confirm congenital macrothrombocytopenia.

3.3 In dogs/cats with confirmed thrombocytopenia (P), does severe thrombocytopenia (E) compared with mild to moderate thrombocytopenia (C), improve differentiation of ITP from non-immune thrombocytopenia (O)?

While severe thrombocytopenia (<20,000 platelets/µl) should make a clinician suspicious of ITP, other causes of thrombocytopenia like consumption can cause equally severe thrombocytopenia. Dogs with ITP commonly have more severe thrombocytopenia than those with non-immune thrombocytopenia, but in all studies, there are overlaps in counts between diagnostic groups; one study found more severe thrombocytopenia in dogs with disseminated intravascular coagulation (DIC) than with ITP.[32, 44-46] Similarly, in case series, cats with presumptive ITP tend to have more severe thrombocytopenia than those with other causes of thrombocytopenia, however, platelet counts <50 000/µL are also reported in cats with neoplasia, infection, bone marrow disease, or traumatic hemorrhage.[47] Thus, severity of thrombocytopenia alone cannot confirm an ITP diagnosis.

3.4 In dogs/cats with confirmed thrombocytopenia (P), compared with platelet count alone (C) does the addition of bone marrow examination (E) help differentiate ITP from non-immune thrombocytopenia (O)?

Bone marrow analysis is not routinely recommended unless underlying marrow disease is suspected due to multiple cytopenias, there is a poor response to standard therapy, or if the clinician is suspicious of lymphoproliferative disease but cannot safely sample solid organs due to thrombocytopenia. Although no studies were identified that directly addressed the PECO question, one study of dogs with thrombocytopenia that underwent bone marrow examinations determined that diagnostic cytologic changes were less common in dogs with severe thrombocytopenia (<20,000 plts/µl) compared with those with platelet counts >20,000 plts/µl.[48] When needed, a sternal marrow aspirate can be considered as a less invasive alternative to sampling of the humerus or ilium.[49]

3.5 In dogs/cats with confirmed thrombocytopenia (P), compared with platelet count alone (C) do platelet/megakaryocyte-associated antibody assays (E) help differentiate ITP from non-immune thrombocytopenia (O)?

Unfortunately, platelet surface-associated immunoglobulin (PSAIG) testing is relatively insensitive and nonspecific, thus routine measurement of platelet antibodies is not currently recommended. This is consistent with the American Society of Hematology guidelines for diagnosis of ITP in people and likely reflects the heterogenous pathogenesis of ITP including ITP induced by T cells and not autoantibodies.[38] Several studies have found no difference in PSAIG between dogs with primary ITP and those with non-immune thrombocytopenia.[42, 50] Furthermore, PSAIG do not reliably

differentiate primary from secondary ITP.[45, 50] Positive platelet-associated antibody testing does confirm that there is an immune component to the thrombocytopenia, but it could be primary or secondary.

There is new evidence that, at least in human ITP, the platelet glycoprotein that is being targeted by antibodies will determine the pathway of platelet clearance. Antibodies to GPIbα lead to platelet desialylation (premature aging) and clearance of platelets independent of macrophages.[51] As a result, patients with anti-GPIbα do not respond well to intravenous immunoglobulin but may respond to sialidase inhibitors like oseltamivir (Tamiflu®).[3] There may be utility in determining the target of autoantibodies in treatment selection, but no such test is currently available for companion animals. We are currently assessing the role of platelet desialylation in canine ITP pathogenesis with a new flow cytometric assay.

3.6 In dogs/cats with confirmed thrombocytopenia (P), compared with platelet count alone (C) does the addition of hemostasis testing (eg, coagulation testing, platelet function testing, viscoelastic testing, fibrinolysis testing, D-dimer concentration) (E) help differentiate ITP from non-immune thrombocytopenia (O)?

Coagulation testing is essential to rule out consumptive causes of thrombocytopenia like DIC. Several studies support the utility of coagulation testing to differentiate patients with consumptive or toxic (rodenticide) coagulopathies from those with primary ITP.[46, 52, 53] Overlap in the degree of thrombocytopenia can occur between ITP and DIC.[46]

3.7 Investigation of secondary ITP triggers

Primary ITP must also be distinguished from secondary ITP due to infections, medications, or neoplastic causes. The consensus panel also systematically evaluated potential secondary triggers of ITP. Triggers for which the most evidence was found in dogs include: *Ehrlichia canis*, [54, 55] *Leishmania*, [56] Rangelia,[57, 58] *Babesia*, [59] and some medications (cefazedone and gold salts).[60, 61] An intermediate level of evidence was found supporting an association between ITP development and *Anaplasma*,[62, 63] solid tumors,[64] and potentiated sulfonamides.[65] Unfortunately, studies assessing ITP triggers in cats are scarce. Overall screening recommendations to rule out secondary ITP should include obtaining a thorough drug, travel, vector exposure and preventative history, a minimum database, abdominal and thoracic imaging, and infectious disease testing based on the geographic locale, making sure to include the above-listed agents where appropriate.[39] All sick cats should be tested for feline leukemia and feline immunodeficiency virus, even though evidence for their role in ITP development is unclear.[39] To improve the sensitivity of vector borne disease testing, PCR and serology combined are strongly recommended, as one study documented that combining these modalities increased sensitivity by up to 58%.[66]

4. **Prognostic Markers**

As ITP is a heterogenous disease with variable disease severity, treatment may should also be individualized to the patient. However, disease severity markers are needed for

individualized treatment. The PECO questions were also applied to help identify any prognostic markers in the following format: To investigate disease severity, *population* (P) referred to dogs and cats diagnosed with primary ITP and *outcome* (O) referred to disease severity encompassing bleeding risk, blood product usage, duration of hospitalization, time to platelet recovery, response to first-line treatment, or relapse.[39] In short, we determined:

a. Platelecrit may increase prior to platelet count as a sensitive indicator of platelet recovery.[67]
b. Admission platelet count in dogs with moderate to severe thrombocytopenia (<50 000/µL) should not be employed to predict disease outcome. However, there are no studies that compare disease severity in dogs with ITP with mild vs. moderate to severe thrombocytopenia. Thus no conclusions could be made regarding platelet count as an outcome predictor when comparing mild vs. moderate to severe thrombocytopenia.[39]
c. Evidence is contradictory regarding whether megakaryocyte hypoplasia on a bone marrow examination predicts disease severity; currently routine bone marrow evaluation is not recommended for prognostication.[20, 39, 68]
d. Serial monitoring of platelet/megakaryocyte associated antibodies in dogs with ITP might help to predict disease relapse, however, evaluation of platelet/megakaryocyte-associated antibodies for outcome prediction is not recommended.[39, 45]
e. Bleeding severity score may aid in the assessment of disease severity. Gastrointestinal bleeding, part of a published bleeding assessment tool, has been identified as a poor prognostic indicator in several studies.[20, 32, 39]

5. Summary

Unfortunately, diagnosis of ITP remains a diagnosis of exclusion. However, the consensus statement provides an organized, evidence-based algorithm to explore and rule out other causes of thrombocytopenia to make a diagnosis of ITP in a systematic manner.

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