Supporting information 7: Evidence Summaries and Consensus Recommendations PICOs with Minimal Evidence

5. In dogs/cats with pITP (P), is treatment with dexamethasone (I) compared with use of prednisone or prednisolone (C) associated with different primary or secondary outcomes (O)?

Dogs:

a. There is no evidence available suggesting improvement in any outcome when dexamethasone is compared to prednisone or prednisolone in dogs with primary ITP.
b. For management of primary ITP in dogs, use of either prednisone/prednisolone or dexamethasone can be considered.
    i. Injectable dexamethasone may be considered in those patients where oral glucocorticoid therapy is not tolerated or parenteral therapy is preferred.

Strength of Evidence: *No evidence exists.* ***Strength of Recommendation:*** *Weak.* **Degree of consensus:** 39/39 Delphi Round 2.

Evidence Summary:
No prospective controlled studies were identified that directly addressed the PICO question. In the studies where treatment allocation is described, dogs not able to receive oral medications were typically administered injectable corticosteroids (usually dexamethasone), whereas dogs able to receive oral medications were typically administered prednisone/prednisolone.[1-5] Often, dogs receiving any type of corticosteroid are analyzed as one group rather than comparing outcomes between specific glucocorticoid drugs. Variation in inclusion and exclusion criteria and concomitant use of other medications in addition to corticosteroids preclude comparison of outcomes in patients receiving either prednisone or dexamethasone.

One study described the outcome of dogs with immune-mediated hematological diseases initially administered dexamethasone versus prednisone,[6] but the study included dogs with IMHA and thrombocytopenia (<50,000/µL), not dogs with pITP specifically, and was not designed to address whether one treatment was superior to the other. In that study, 12 dogs received dexamethasone, 15 received prednisolone, and 8 dogs administered dexamethasone initially later received prednisolone. Dogs treated with prednisone had a significantly longer hospitalization [median 7d (range 2-17)] versus the non-prednisolone group [median 2.5 d (1-5)]. The investigators concluded that this was likely confounded by the high rate of non-survival in dogs initially receiving dexamethasone, because this group could not tolerate oral prednisolone or died before transitioning to oral therapy.

While some studies in human ITP report a benefit to commencing therapy with high dose oral dexamethasone (0.6 mg/kg/day for 4 days vs. 1-2 mg/kg/day prednisone for 2-4 weeks),[7] the 2019 American Society of Hematology (ASH) guidelines for treatment of ITP in people suggest that the use of either high pulse dose dexamethasone (4 days) or prednisone (0.5 – 2.0 mg/kg/day for ≤ 6 weeks) is acceptable.[8] If rapidity of platelet count response is valued, then dexamethasone is recommended by the ASH guidelines.[8] No studies describing high dose pulse dexamethasone therapy in dogs with ITP were identified.

Cats:

a. There is no evidence in cats with primary ITP that treatment with dexamethasone compared with the use of prednisone or prednisolone is associated with different primary or secondary outcomes.
b. In cats with primary ITP, the use of either dexamethasone or prednisolone can be considered.
    i. In cats, prednisolone is preferred to prednisone.
    ii. Dexamethasone may be considered in cats that fail to respond to oral prednisolone.
    iii. Injectable dexamethasone may be considered in those patients where oral glucocorticoid therapy is not tolerated or parenteral therapy is preferred.

Strength of Evidence: *No evidence exists.* ***Strength of Recommendation:*** *Weak.* **Degree of consensus:** 38/38 Delphi Round 2.

Evidence Summary:
We identified no prospective controlled studies comparing the outcome of cats with primary ITP treated with dexamethasone versus prednisone or prednisolone. Several studies have described treatment with dexamethasone, prednisolone, or other corticosteroids in treatment of cats with primary ITP, but outcomes cannot be compared across these studies.[9-11]

One case report described a single cat with primary ITP that consistently failed to respond to oral prednisolone and other immunosuppressive drugs over a period of more than half a year, but had a rapid and sustained improvement in platelet count when oral dexamethasone was commenced.[12] Similarly, in a case series of 4 cats, 1 cat was initially treated with oral prednisolone for 5 days without response, at which point prednisolone was switched to dexamethasone and the cat's platelet count normalized within two days.[10] In contrast, in the same study, the only cat initially administered intravenous dexamethasone instead of oral prednisolone did not survive to discharge, while the 3 cats treated initially with oral prednisolone survived to discharge.[10] This comparison is likely confounded by illness severity influencing the choice to give dexamethasone initially.

It should be noted that oral prednisolone is preferred in cats because it has more reliable bioavailability than oral prednisone.[13]

7. In dogs/cats with pITP (P), is initial treatment with high doses of prednisolone or prednisone (> 2 mg/kg/day) (I) compared to more conservative dosages (2 mg/kg/day or 50-60 mg/m^2/day (dogs)) (C) associated with different primary or secondary outcomes (O)?

Dogs:

a. There are no studies of dogs with pITP evaluating effect on any outcome that compare treatment with high dose corticosteroids with more conservative doses of corticosteroids.
b. In dogs for management of pITP, use of high doses of corticosteroid is not recommended compared to more conservative doses of corticosteroid.

Strength of Evidence: *No evidence exists.* ***Strength of Recommendation:*** *Weak.* **Degree of consensus:** 35/37 Delphi Round 2.

Evidence Summary:
There is no research comparing the outcome of dogs with primary ITP treated with high doses of prednisolone or prednisone (>2 mg/kg/day) compared to more conservative dosages (2 mg/kg or 50-60 mg/m^2/day). Three studies had retrievable information related to the PICO, although none directly answered the PICO question as they did not directly compare the outcomes of dogs receiving high versus conservative doses of steroids.[14-16] The studies varied greatly in reporting methods and in other treatments administered precluding comparisons of outcomes across populations.

Cats:

a. There are no studies of cats with pITP evaluating effect on any outcome that compare treatment with high dose corticosteroids with more conservative doses of corticosteroids.
    i. In cats, prednisolone is preferred to prednisone.
    ii. Studies in cats with other similar immune diseases, such as immune-mediated hemolytic anemia, suggest that higher doses of prednisolone (3-4 mg/kg/day) may be needed in some cats.
b. In cats for management of pITP, although the use of high doses of corticosteroid is not supported by evidence in cats with ITP, higher doses may be considered based on experience

with other immune diseases.

Strength of Evidence: *No evidence exists.* ***Strength of Recommendation:*** *Weak.* **Degree of consensus:** 34/39 Delphi Round 2. One evaluator suggested that if higher glucocorticoid doses are initially employed, they should be tapered after a few days to standard immunosuppressive dosages, while another evaluator was concerned that cats do not tolerate higher steroid doses.

Evidence Summary:
There is no research comparing the outcome of cats with primary ITP treated with high dose prednisone/prednisolone compared to more conservative dosages. Several studies had retrievable information related to the PICO question, but none directly addressed it.[9-11,17,18] The studies varied greatly in reporting methods and in other treatments administered precluding comparisons of outcomes across populations. In general, cats require higher doses of glucocorticoids than dogs to attain similar effects. Studies have shown a decreased density of glucocorticoid receptors in tissues of cats as compared to dogs, as well as decreased glucocorticoid receptor binding affinity.[19,20]

10. In dogs/cats with pITP (P), is treatment with melatonin (I) compared with no melatonin therapy (C) associated with different primary or secondary outcomes (O)?

Dogs and cats:

a. There is insufficient evidence to determine if melatonin affects patient-centered outcomes in dogs or cats with primary ITP.

Strength of Evidence: *No evidence available.* ***Strength of Recommendation:*** *Weak.* **Degree of consensus:** 17/17. Delphi Round 3.

Evidence Summary:
No studies evaluating the effects of melatonin on outcome in dogs with ITP were identified, and melatonin is not currently FDA-approved for oral usage in dogs or cats. No studies were identified describing administration of melatonin to cats with ITP and studies in healthy cats have focused primarily on reproductive health. Melatonin's impacts on the immune system are potentially far reaching and have been reviewed elsewhere,[21-25] but some pertinent effects in various experimental settings include enhancement of MHC II mediated antigen presentation, increased natural killer cell numbers, promotion of hematopoiesis, and increased or decreased production of pro-inflammatory cytokine and interleukin-10 depending on the context.[21,24-29] Evidence evaluators could not reach consensus on whether melatonin is safe for use in dogs. Some felt there was little risk of using melatonin as an adjunctive intervention, others noted its potential for immune stimulation as a potential adverse effect in the context of immune-mediated disease.[21,22,27,30]

There are currently no published controlled clinical trials of melatonin in human ITP patients, but a single case series documented modest increases in platelet counts in three patients with refractory ITP following addition of melatonin.[23] However, the study design precludes evaluating efficacy, and notably, platelet count did not normalize in any of these patients.

Studies in healthy dogs have evaluated the effects of oral melatonin supplementation on expression of inflammatory cytokines, and on platelet parameters. In these studies, oral melatonin for 14 days had no effect on interleukin-2 or interferon gamma,[31] while 28 days of supplementation did not affect mean platelet volume or plateletcrit relative to baseline.[32] A single case report described a dog that developed secondary ITP following cyclophosphamide administration that was successfully treated with vincristine, prednisolone, and melatonin, but it is unclear what impact, if any, melatonin had on the resolution of ITP in this dog.[33]

References:

1. Williams DA, Maggio-Price L. Canine idiopathic thrombocytopenia: clinical observations and long-term follow-up in 54 cases. J Am Vet Med Assoc. 1984;185:660-663.
2. Jackson ML, Kruth SA. Immune-mediated Hemolytic Anemia and Thrombocytopenia in the Dog: A retrospective study of 55 cases diagnosed from 1979 through 1983 at the Western College of Veterinary Medicine. Can Vet J. 1985;26:245-250.
3. O'Marra SK, Delaforcade AM, Shaw SP. Treatment and predictors of outcome in dogs with immune-mediated thrombocytopenia. J Am Vet Med Assoc. 2011;238:346-352.
4. Scuderi MA, Snead E, Mehain S, et al. Outcome based on treatment protocol in patients with primary canine immune-mediated thrombocytopenia: 46 cases (2000-2013). Can Vet J. 2016;57:514-518.
5. Balog K, Huang AA, Sum SO, et al. A prospective randomized clinical trial of vincristine versus human intravenous immunoglobulin for acute adjunctive management of presumptive primary immune-mediated thrombocytopenia in dogs. J Vet Intern Med. 2013;27:536-541.
6. Goggs R, Boag AK, Chan DL. Concurrent immune-mediated haemolytic anaemia and severe thrombocytopenia in 21 dogs. Vet Rec. 2008;163:323-327.
7. Xiao Q, Lin B, Wang H, et al. The Efficacy of High-Dose Dexamethasone vs. Other Treatments for Newly Diagnosed Immune Thrombocytopenia: A Meta-Analysis. Front Med (Lausanne). 2021;8:656792.
8. Neunert C, Terrell DR, Arnold DM, et al. American Society of Hematology 2019 guidelines for immune thrombocytopenia. Blood Adv. 2019;3:3829-3866.
9. Garon CL, Scott MA, Selting KA, et al. Idiopathic thrombocytopenic purpura in a cat. J Am Anim Hosp Assoc. 1999;35:464-470.
10. Bianco D, Armstrong PJ, Washabau RJ. Presumed primary immune-mediated thrombocytopenia in four cats. J Feline Med Surg. 2008;10:495-500.
11. Wondratschek C, Weingart C, Kohn B. Primary immune-mediated thrombocytopenia in cats. J Am Anim Hosp Assoc. 2010;46:12-19.
12. Tasker S, Mackin AJ, Day MJ. Primary immune-mediated thrombocytopenia in a cat. J Small Anim Pract. 1999;40:127-131.
13. Center SA, Randolph JF, Warner KL, et al. Influence of body condition on plasma prednisolone and prednisone concentrations in clinically healthy cats after single oral dose administration. Res Vet Sci. 2013;95:225-230.
14. Wilkins RJ, Hurvitz AI, Dodds-Laffin WJ. Immunologically mediated thrombocytopenia in the dog. J Am Vet Med Assoc. 1973;163:277-282.
15. Jans HE, Armstrong PJ, Price GS. Therapy of immune mediated thrombocytopenia. A retrospective study of 15 dogs. J Vet Intern Med. 1990;4:4-7.
16. Rozanski EA, Callan MB, Hughes D, et al. Comparison of platelet count recovery with use of vincristine and prednisone or prednisone alone for treatment for severe immune-mediated thrombocytopenia in dogs. J Am Vet Med Assoc. 2002;220:477-481.
17. Smith J, Schaer M, Chandra S. Chronic thrombocytopenia and epistaxis in a cat. Feline Practice. 1999;27:5-8.
18. Best MP, Fry DR. Primary immune-mediated thrombocytopenia and immune-mediated neutropenia suspected in a 21-week-old Maine Coon cat. Aust Vet J. 2014;92:250-253.
19. van den Broek AH, Stafford WL. Epidermal and hepatic glucocorticoid receptors in cats and dogs. Res Vet Sci. 1992;52:312-315.
20. Lowe AD, Campbell KL, Graves T. Glucocorticoids in the cat. Vet Dermatol. 2008;19:340-347.
21. Carrillo-Vico A, Guerrero JM, Lardone PJ, et al. A review of the multiple actions of melatonin on the immune system. Endocrine. 2005;27:189-200.
22. Vinther AG, Claësson MH. [The influence of melatonin on the immune system and cancer]. Ugeskr Laeger. 2015;177:V10140568.
23. Todisco M, Rossi N. Melatonin for refractory idiopathic thrombocytopenic purpura: a report of 3 cases. Am J Ther. 2002;9:524-526.
24. Hardeland R. Aging, Melatonin, and the Pro- and Anti-Inflammatory Networks. Int J Mol Sci. 2019;20.
25. Carrillo-Vico A, Lardone PJ, Alvarez-Sánchez N, et al. Melatonin: buffering the immune system. Int J Mol Sci. 2013;14:8638-8683.
26. Qin T, Feng D, Zhou B, et al. Melatonin attenuates lipopolysaccharide-induced immune dysfunction in dendritic cells. Int Immunopharmacol. 2023;120:110282.

27. Carrillo-Vico A, Reiter RJ, Lardone PJ, et al. The modulatory role of melatonin on immune responsiveness. Curr Opin Investig Drugs. 2006;7:423-431.
28. Arabacı Tamer S, Altınoluk T, Emran M, et al. Melatonin Alleviates Ovariectomy-Induced Cardiovascular Inflammation in Sedentary or Exercised Rats by Upregulating SIRT1. Inflammation. 2022;45:2202-2222.
29. Ebaid H, Bashandy SAE, Abdel-Mageed AM, et al. Folic acid and melatonin mitigate diabetic nephropathy in rats via inhibition of oxidative stress. Nutr Metab (Lond). 2020;17:6.
30. Hardeland R. Melatonin and inflammation-Story of a double-edged blade. J Pineal Res. 2018;65:e12525.
31. Peace AC, Kumar S, Wills R, et al. Pharmacodynamic evaluation of the effects of oral melatonin on expression of the T-cell cytokines interleukin-2 and interferon gamma in the dog. J Vet Pharmacol Ther. 2019;42:278-284.
32. Chen M, Stone R. Lack of Effect of Oral Melatonin on Platelet Parameters in Normal Healthy Dogs. J Am Anim Hosp Assoc. 2019;55:226-230.
33. Finlay JR, Wyatt K, North C. Recovery from Cyclophosphamide Overdose in a Dog. J Am Anim Hosp Assoc. 2017;53:230-235.