**Supporting information 3: Evidence evaluator instructions for medical and transfusion management of ITP PICOs**

You have been assigned on average 3 PICO questions. Evidence evaluation consists of 3 main initial steps:

1. Selection of the literature to answer your assigned PICO questions.
2. Evaluation of the literature to answer your PICO questions.
3. Drafting of summary statements answering your PICO based on your reviewed literature.

*Following PICO question completion, we will also request input via a survey on approximately 20 non-PICO questions based on your literature reading and expert opinion. These questions couldn't utilize the PICO format due to lack of comparator, insufficient veterinary literature, or both. All PICO and non-PICO questions are listed at the end of these instructions. Please keep the non-PICO questions in mind as you read your literature for any information that might be relevant to these questions.*

**Overview of PICO**
Questions have been set up as PICO questions:
*P: patient population*
*I: intervention*
*C: comparator*
*O: outcomes of interest*

- These PICO questions have been divided into the following categories: Diagnosis, Medical Treatment, and Transfusion Treatment. You have been assigned to one of these categories based on your interest/expertise. There is also a group assessing the evidence for co-morbidities associated with secondary ITP; performed via a different process.
- Each evidence evaluator is assigned on average 3 PICO questions. We have grouped PICO questions we expect will have overlapping literature to try to streamline the process.
- Each PICO question has been assigned to 2 separate evidence evaluators.

1. **Selection of the literature to answer your assigned PICO questions**
    a. Your first task is to select the appropriate literature to answer your assigned PICO questions from the 273 manuscripts that met our strict initial inclusion criteria. You will be invited to access the Box folder containing these articles. If literature you are aware of is missing from these 273 papers that can help answer your PICO question, please add it to your list. We are only utilizing *canine/feline primary literature* in this part of the process. Human literature and review articles are excluded.
        i. If you find >20 papers that are applicable to your PICO, you may wish to be more selective in your literature review. If you find that very few papers match your PICO question, you may need to be more liberal for instance by considering more papers that don't exactly match the PICO question but are "close enough" to provide useful information.
        ii. If you find what you think is a useful foreign language article in a language you cannot read, please contact your Domain Chair.
    b. Send the list of your selected titles to your Domain Chairs for approval.
        i. Medical Treatment Domain Chairs: Barbara Kohn (barbara.kohn@fu-berlin.de) and Andrew Mackin (Mackin@cvm.msstate.edu).
        ii. Transfusion Domain Chair: Robert Goggs (r.goggs@cornell.edu).
    c. From your selected articles, select 1 key reference ≥ 5 years old (a paper that you feel would almost invariably be cited by subsequent papers on the subject) and send that title to Andrew Mackin (Mackin@cvm.msstate.edu) who will perform a quick search for more recent literature that has cited this manuscript. This is just a fail-safe mechanism to make

sure we are not missing any important papers that we did not identify during our initial screening process.

d. Based on your article list, any added references identified by Dr. Mackin's search, and the literature search of your evidence evaluator partner, you will be sent final lists of articles to use for answering your PICO questions.
e. As you evaluate your articles, please peruse their references. If you find a useful reference that has been missed, please alert your Domain Chairs! This has occurred during some trial runs.

**2. Evaluation of the literature to answer your PICO questions**

a. You will utilize a Treatment excel sheet to extract data from each article relating to each PICO. Ideally, you should carefully read the paper before you start to extract data or to assess the strength of evidence.
b. For each article you are evaluating, first select the appropriate PICO question, and then the outcome the article assessed from the spreadsheet's drop-down lists. A given paper may assess only 1 outcome or several different outcomes.
    i. Please fill out a separate line in excel for each OUTCOME for each PICO question. For example, if a given paper addressed both of the following outcomes relating to a given PICO,
        1. Time needed for the patients to reach an adequate platelet count
        2. Percentage of patients that survived to discharge

    Each outcome would have its own Excel line. Much of the data you entered for one outcome can be copied/pasted into the other outcomes, or you can simply leave boxes in each Excel line blank if the answer is identical to answers in the above line, but some questions will not have the same answer for each outcome.
c. Only select outcomes evaluated by the manuscript, not something simply mentioned in the manuscript. For example, only select the outcome "Received RBC-containing transfusion?" if there is data in the manuscript that allows comparison between treatment groups regarding the receipt of RBC-containing transfusions. If the paper simply mentions that 2 dogs received pRBCs, but this cannot be compared between groups, then skip this outcome.
d. Throughout the spreadsheet you will find links to guide you in your decision process. For example, if you have forgotten the difference between a cohort study and case-control study, a hyperlink will provide guidance.
e. Next, you will initially be asked to evaluate each manuscript's level of evidence based on GRADE criteria (the first set of questions on the spreadsheet). You will do this for each PICO and each outcome for the same paper (copy/paste is fine or leaving blank works if the answers don't change from outcome to outcome).
    i. Only fill out the GRADE questions for the type of study described in the paper (prospective vs. observational vs. experimental). Skip the rest.
    **ii.** The GRADE system then provides a quality of evidence rating system (high, moderate, low, or very low) for each outcome reported across all relevant studies. These ratings are the endpoints of a series of upgrading or downgrading steps that result from the presence or absence of certain study characteristics. For example, for clinical trials (CTs), the initial quality of evidence rating starts high and can be modified from there based on the study's characteristics. Observational studies start low but can be upgraded. See https://gdt.gradepro.org/app/handbook/handbook.html for more information. **Domain Chairs will use this for final evidence weighting, but we wanted you to understand how all your efforts answering GRADE questions will be utilized.**
    iii. We have created links to a GRADE manual throughout the spreadsheet. Use it as you progress through evaluation of each manuscript or read it in advance to familiarize yourself with the process. You can find it at: https://gdt.gradepro.org/app/handbook/handbook.html (*N. B. that evidence

evaluators were provided access to an abbreviated ITP-specific guide derived from the derived from the GRADE Working Group resources: https://www.gradeworkinggroup.org/).

iv. For added information, as desired, a video overview of bias, indirectness, and imprecision can be found here: https://cornell.hosted.panopto.com/Panopto/Pages/Viewer.aspx?id=2bc5dec0-253e-4037-8196-abd4010a438a&start=0.
N.B. This was written for the RECOVER CPR guidelines. We are using a different data entry system, but the concepts are the same.

v. N.B. If you are working with a case series/report, the GRADE questions will not apply, and you should select – NA – case series/report.

f. On the spreadsheet you will see prompts to enter animal numbers in treatment and control groups and animal numbers with the selected outcomes in each group. Please enter this information if it is available. Domain Chairs will then use this information to calculate relative risk, standard error, and 95% CI for that outcome for that treatment. If you would like those RR, SE, and CI statistics for your PICO summaries, please ask.

**3. Summary of PICO Question Answer**

Once you have completed the evidence evaluation process you should summarize your findings in a short conclusion statement on the template provided. This statement is extremely important and will be the foundation for the ITP Consensus Statement and your essential written contribution as co-author.
This summary consists of (1) a consensus of science statement, (2) a treatment recommendation and (3) a list of knowledge gaps that you identified.

**a. Consensus on Science Statement**

In this section you will summarize the body of evidence for that PICO question. This should reference specific papers that you feel most strongly support your conclusion and include brief summaries of any data from those studies that helped you reach your conclusion. **Ideally divide your summaries into sections on dogs vs. cats.** The generic format for the statement is as follows:

*We found x studies in dogs that supported the intervention, x studies in dogs that were neutral, and x in dogs that opposed the intervention.*

Here is an example (hypothetical) for the question "In dogs with non-associative IMHA (P), is treatment with unfractionated heparin (I) compared with use of any other antithrombotic agent (C) associated with different primary or secondary outcomes (O)?"

*We identified 4 papers in dogs that either directly (Weinkle 2005) or indirectly (Mellett 2011, Morassi 2016, Helmond 2010) addressed this question.*
*Based on the veterinary literature alone, there is insufficient evidence is available to make strong recommendations on selection of anticoagulant vs. antithrombotic in dogs with IMHA. The strongest evidence supports the use of individually dose-adjusted UFH (Helmond 2010), but this study only compared dogs receiving a fixed dose of UFH to those receiving an individually adjusted dose of UFH. UFH was not compared to other antithrombotics. One retrospective study of dogs with IMHA suggested those receiving ultra-low-dose aspirin had a survival benefit over those dogs receiving heparin (Weinkle 2005). However, this study is limited by less rigorous inclusion criteria and is confounded by lack of control for illness severity. Other retrospective data (published only as an abstract Orcutt 2009) suggest that individualized heparin dosing may provide superior thromboprophylaxis compared to aspirin. One further RCT found no difference in survival in dogs treated with rivaroxaban compared with dogs treated with clopidogrel and ultra-low-dose aspirin, but this study was under-powered and does not demonstrate equivalency of interventions (Morassi 2016).*

*Other anticoagulants including enoxaparin and rivaroxaban appear to be safe and may be efficacious, (Morassi 2016, Panek 2015), but RCTs are lacking. In a prospective study of UFH use in dogs with IMHA, dosages of 300 U/kg SC q6h generated anti-Xa activities below the target ranges in more than half of the dogs (Breuhl 2009). A subsequent RCT of dogs with IMHA compared UFH treatment administered at constant dose with individually dose-adjusted UFH treatment based on anti-Xa monitoring (Helmond 2010). This study demonstrated significantly longer survival in dogs given individually adjusted doses, with only 1/8 non-survivors compared to 6/7 non-survivors in the constant dose group. Dosages of UFH of 150-566 U/kg q6h were required to achieve anti-Xa activities between 0.35 and 0.7 U/mL (Helmond 2010). However, this study enrolled a small number of dogs and provided incomplete information on masking and randomization.*

*Clopidogrel may be efficacious for arterial thromboprophylaxis in dogs (Brainard 2010, Borgarelli 2017, Hasa 2001, van Giezen 2009, Bjorkman 2013). However, insufficient evidence is available to judge the efficacy of clopidogrel for prevention of venous thrombosis in dogs. Aspirin is a safe and effective drug for prevention of arterial thrombosis in dogs (Dyken 1973), but insufficient evidence is available to judge the efficacy of aspirin for the prevention of venous thrombosis in dogs. Thirty percent or more of healthy dogs fail to respond to low dose aspirin (Dudley 2013, Sharpe 2010), and a minimum dosage of 2.0-5.0 mg/kg PO q12-24h is required for reliable platelet inhibition in responders (Brainard 2007, Nielsen 2007, McLewee 2017). Studies have demonstrated the failure of 1.0 and 3.5 mg/kg PO q12h of aspirin to reliably inhibit canine platelets (Hoh 2011, Grauer 1992). Although aspirin at a dosage of 0.5 mg/kg q24h in combination with glucocorticoids appears safe (Graham 2009) aspirin dosages ≥2 mg/kg administered to dogs receiving concurrent prednisolone may be associated with increased gastrointestinal bleeding (Whittemore 2017). Among the antiplatelet drugs, 1 RCT detected no difference in mortality in dogs treated with ultra-low-dose aspirin, clopidogrel, or both (Mellett 2011). However, this study appears to have been underpowered and may have used an inappropriate statistical test for comparison of survival between groups.*

**b. Treatment Recommendation**

After reviewing all the relevant literature and based on your overall assessment of the evidence, provide a statement on your treatment recommendation based on your evidence evaluation and include your assessment of the strength of evidence (strong, moderate, weak, none). For our hypothetical example:

*We suggest the administration of unfractionated heparin (UFH) with individual dose adjustment (using an anti-Xa assay) in preference to other drugs. This drug should not be used without individual dose adjustment. If this is not available or feasible, we suggest administering injectable low-molecular-weight heparins or direct oral Xa inhibitors. When using injectable low-molecular-weight heparins, we suggest individual dose adjustment (using an anti-Xa assay) may be useful to achieve a therapeutic dose. If antiplatelet drugs are administered, we suggest that clopidogrel be used in preference to aspirin.*
*Strength of recommendation: Weak (Select from None, Weak, Moderate, Strong)*

*The only randomized controlled trials were underpowered, had moderate risks of bias, and only compared subsets of the different antithrombotic options such as UFH fixed dose vs. UFH adjusted dose or clopidogrel vs. aspirin or clopidogrel vs. rivaroxaban.*

The Domain Chairs will compare the objective findings from the evidence analyses of both evaluators assigned to each PICO question and will then communicate out potential consensus statements for feedback and approval.

c. **Knowledge Gaps**

Provide a statement on important gaps in knowledge that hinder a more confident

recommendation. For our example:

*There are no studies that directly compare all of many of the different anti-thrombotic medications in a prospective randomized clinical trial. Such a large clinical trial is needed before any strong recommendations about the ideal antithrombotic approach can be made. Furthermore, more research is required in determining the ideal targets for antithrombotic therapy. The original derivation of UFH aPTT prolongation targets was performed using thrombotic models in dogs (Wessler 1955). Subsequently, these aPTT targets were demonstrated to correlate with recommended 0.35-0.70 U/mL anti Xa activity targets in humans (Basu 1972, Chiu 1977). These activity targets protect against thrombosis in people (Guyatt 2012) and are the basis for the currently recommended targets for anti-Xa activity in dogs. However, we recognize that there is limited evidence of efficacy for these targets against patient-centered outcomes (eg, prevention of documented thrombosis, mortality) in dogs with clinical disease. Higher dosages than the initial starting dosages listed above may be necessary to attain these anti-Xa activity targets in dogs with diseases that predispose to thrombosis (Helmond 2010, Lynch 2014). Initiating antithrombotic treatment at these dosages and increasing the dose incrementally based on individual monitoring may provide a margin of safety for patients against hemorrhagic complications.*

***References in summary statements**: Please add any references in (Author Year) format without using a reference manager. We will add references via a reference manager when we integrate summaries prior to submission to JVIM.

**Never be shy! Ask for help as needed! We appreciate your time, help, and expertise.**

**Medical Treatment PICO questions**

**P - Population**
**I – Intervention** (treatment, therapy…)
**C - Comparison or Control**
**O - Outcome** (desired or of interest)

***T- Time period*** *(ie. "Over six month period.." or "In three years...")*

**Population:**

Patients with primary ITP

**Outcomes:**

***Primary:***

- Time needed for the patients to reach an adequate platelet count (approximately 40,000-60,000/uL or more, please note specific count utilized in paper in comments)
- Time needed for a patient to reach a normal platelet count
- Percentage of patients that survived to discharge
- Percentage of patients that survived at least 1 month after diagnosis
- Percentage of patients that survived 3-6 months after diagnosis
- Percentage of patients that survived greater than 6 months after diagnosis
- Percentage of patients that survived X time after diagnosis (if doesn't fit the above timepoints, make sure to denote in comments what X is)

***Secondary:***

- Duration of hospitalization
- Received RBC-containing transfusion (Y or N)
    - (Note: fresh whole would be both RBC and platelet-containing transfusion)
- Received platelet-containing transfusion (Y or N)
- Number of RBC-containing transfusions received
- Number of platelet-containing transfusions received
- Rate of relapse after initial response
    - Platelet count at least halving after initial response
    - Platelet count dropping below normal after normalizing
- Complete remission rate (normal platelet count without therapy)
- Cost to client during initial hospitalization

***Other:***

- Adverse reactions to therapy
- Thrombosis

**PICO QUESTIONS:**

1. In patients with primary ITP (P), is treatment with combined glucocorticoids and vincristine (I) compared with use of glucocorticoids alone (C) associated with different primary or secondary outcomes (O)?

2. In patients with primary ITP (P), is treatment with combined glucocorticoids and IVIg (I) compared with use of glucocorticoids alone (C) associated with different primary or secondary outcomes (O)?

3. In patients with primary ITP (P), is treatment with combined glucocorticoids and IVIg (I) compared with use of glucocorticoids and vincristine (C) associated with different primary or secondary outcomes (O)?

4. In patients with primary ITP (P), is treatment with combined glucocorticoids and a 2nd immunosuppressive agent (I) compared with use of glucocorticoids alone (C) associated with different primary or secondary outcomes (O)?

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

6. In patients with primary ITP (P), is treatment with IVIg alone (I) compared with use of prednisone or prednisolone (C) associated with different primary or secondary outcomes (O)?

7. In patients with primary ITP (P), is initial treatment with a high-dose of short-acting prednisone or prednisolone or dexamethasone IV (I) compared with use of oral prednisone or prednisolone (C) associated with different primary or secondary outcomes (O)?

8. In patients with primary ITP (P), is initial treatment with high doses of prednisolone or prednisone (> 2 mg/kg/day) (I) compared to more conservative dosages (2 mg/kg or dosing based on $m^2$ as in IMHA) (C) associated with different primary or secondary outcomes (O)?

9. In patients with primary ITP (P), is maintenance treatment with glucocorticoids and a 2nd immunosuppressive agent (I) superior to glucocorticoids alone (C) in order to prevent a relapse (O)?

10. In patients with primary ITP (P), is treatment with glucocorticoids and any second agent (I) compared to treatment with glucocorticoids and any other second agent (C) associated with different primary or secondary outcomes (O)?

11. In patients with primary ITP (P), is treatment with melatonin (I) compared with no melatonin therapy (C) associated with different primary or secondary outcomes (O)?

12. In patients with primary ITP (P), is treatment with thrombopoietin-receptor agonists (I) compared with no thrombopoietin-receptor agonist therapy (C) associated with different primary or secondary outcomes (O)?

13. In patients with primary ITP (P), is treatment with splenectomy (I) compared with no splenectomy (C) associated with different primary or secondary outcomes (O)?

14. In patients with primary ITP (P), is treatment with therapeutic plasma exchange (TPE) (I) compared with no TPE (C) associated with different primary or secondary outcomes (O)?

15. In patients with primary ITP (P), is ITP requiring ongoing therapy (I) as opposed to remaining disease free (C) associated with the development of thrombosis (O)?

16. In patients with ITP undergoing treatment (P), does development of coagulation test abnormalities (NOT just thrombocytopenia) (I) versus normal coagulation test results (C) worsen any outcomes (O)?

17. In patients with ITP undergoing treatment (P), does administration of an antithrombotic (I) as opposed to no antithrombotic therapy (C) improve any outcomes (O)?

18. In patients with primary ITP (P), is the use of proton pump inhibitors and/or sucralfate and/or other gastroprotectants (I) compared to no gastric protectant therapy (C) associated with different primary or secondary outcomes or less evidence of gastric erosion/ulceration/GI bleeding (O)?

19. In patients with primary ITP (P), are vaccinations after ITP diagnosis (I) compared with no vaccinations (C) associated with a higher rate of ITP relapse (O)?

20. In patients with secondary ITP (P), is antimicrobial/viral treatment with combined glucocorticoids (I) compared with use of antimicrobials/virals alone (C) associated with different primary or secondary outcomes (O)?

(*Note that some of the PICO questions were merged for brevity and clarity during the guideline development*).

**NON-PICO QUESTIONS:**

1. In patients with primary ITP, what should we do with emergency patients, beyond transfusion, in terms of supportive therapy (strict rest, duration of hospitalization, procedures to avoid, medications to avoid)?

2. In patients with primary ITP, when should a second agent beyond glucocorticoids be added?

3. In patients with primary ITP, what dosage of vincristine should be used?

4. In patients with primary ITP, is there rationale to use vincristine alone vs. in combination with prednisone or prednisolone?

5. In patients with primary ITP, what dosage of IVIg should be used?

6. In patients with primary ITP, how should we monitor therapy, both short term and long term?

7. In patients with primary ITP, what is the role of measuring platelet/megakaryoyte-related antibodies in monitoring therapy?

8. In patients with primary ITP, how often do relapses occur/what are the reasons for relapses?

9. In patients with primary ITP, what are the best disease severity predictors?

10. In patients with primary ITP, should we individualize therapy using bleeding scoring systems such as DOGiBAT?

11. In patients with primary ITP, how is remission defined? (no/partial/complete remission?)

12. In patients with primary ITP, what are recommendations for tapering of prednisolone or prednisone if patients are in remission?

13. In patients with primary ITP, what are recommendations for tapering of second immunosuppressive drug if patients are in remission?

14. In patients with primary ITP, what monitoring for adverse effects is recommended in patients on immunosuppressive treatment?

15. In patients with primary ITP, what monitoring is recommended for patients in remission?

16. In patients with a relapse, what tests should be performed/diagnostic-work-up?

17. In patients with primary ITP, what recommendations should be made about vaccinations in patients that have had ITP?

18. What is the difference in outcomes in patients with primary ITP compared to outcomes in patients with megakaryocytic hypoplasia or aplasia?

19. Is there rationale to employ probiotics in treatment of patients with primary ITP?

**Transfusion PICO questions**

**Outcome measures:**

- Post-transfusion platelet count
- Change in (Δ) platelet count [pre to post]
- Clinical bleeding (subjective)
- Clinical bleeding (scoring system e.g., DOGiBAT)
- Duration of bleeding (h or d)
- Post-transfusion hematocrit
- Change in (Δ) hematocrit [pre to post]
- Survival to discharge or to 28d
- Duration of hospitalization
- Days to event (death or euthanasia)
- Received additional blood products (binary Y/N)
- Received RBC-containing transfusion (Y or N) (FWB would be RBC and platelet-containing)
- Received platelet-containing transfusion (Y or N)
- Volume (mL/kg) of additional blood products administered
- Number of RBC-containing transfusions received
- Number of platelet-containing transfusions received

**PICO QUESTIONS:**

In dogs with primary ITP (P), does treatment with any platelet-containing transfusion product (I), compared to no platelet-containing products (C), improve any outcomes (O)?

- Cryopreserved platelets (DMSO-stabilized)
- Lyophilized platelets (Formaldehyde-stabilized)
- Lyophilized platelets (Trehalose-stabilized)
- Fresh whole blood
- Fresh platelet concentrates
- Platelet-rich plasma
- Vincristine-loaded platelets EXCLUDED

In dogs with primary ITP (P), does treatment with any RBC-containing transfusion product (I), compared to no RBC-containing products (C), improve any outcomes (O)?

- Packed red blood cells
- Fresh whole blood
- Stored whole blood
- Hemoglobin based oxygen carrying solutions (HBOCS) EXCLUDED

In dogs with primary ITP (P), does treatment with one platelet-containing product (I), compared to any other platelet-containing products (C), improve any outcomes (O)?