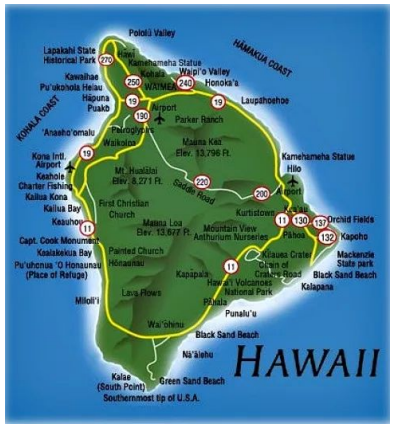
# Immune-mediated disease and Immunosuppressives

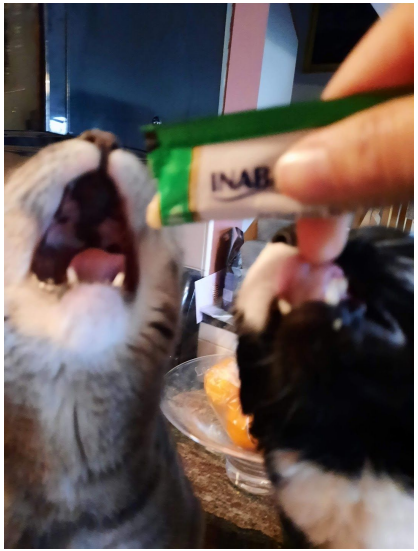
Lauren Carvalho, DVM, DACVIM (SAIM)
Small Animal Internist
Cape Cod Vet Specialists

# Who am I?

- Originally from Hilo, Hawaii
- Currently living in Sagamore Beach area with husband, 2 golden retrievers, and 2 cats







# Who am I?

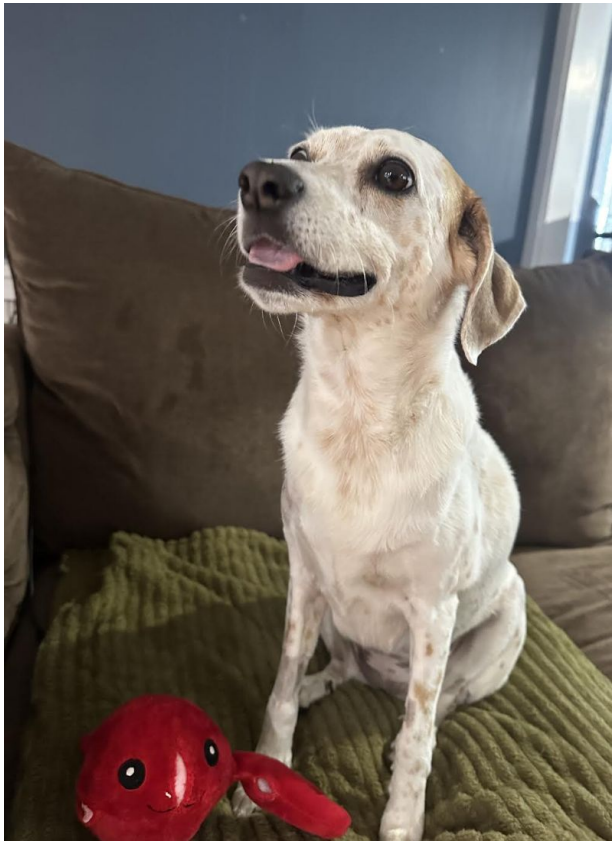
- Tufts University,
  Cummings School of Vet Medicine
  - Veterinary school, 2015-2018
  - SA rotating internship, 2018-2019
  - SA IM residency, 2019-2022
- CCVS, Buzzards Bay
  - Internist, 2022-present
- Special interests:
  - Nephrology and urology
  - Minimally invasive procedures
  - Immune-mediated disease

Tufts University | Cummings School of Veterinary Medicine

CCVS
CAPE COD VETERINARY SPECIALISTS

# Plan

- Mechanisms of IM disease
- General approach to IM disease
- Immunosuppressives
- Disease overviews
  - IMHA
  - ITP
- Case study



# Immune-mediated disease

- Clinical syndrome caused by overactivation of the immune system in the absence of an infection or other discernible cause.
- Getting a diagnosis can be challenging!
- According to a study by the American Autoimmune Related Disease Association:
  - Average time for a diagnosis of a serious autoimmune disease is 4.6 years.
  - During that period, the patient will have seen an average of 4.8 doctors.

**Think of how much harder in our patients who can't speak!**

CCVS
CAPE COD VETERINARY SPECIALISTS

# MOAs - hypersensitivity reactions

| | Type I | Type II | Type III | Type IV |
|---|---|---|---|---|
| **Nickname** | Immediate | Antibody mediated | Immune complexes | Delayed or cell-mediated |
| **Antibody** | IgE | IgG or IgM | IgG or IgM | |
| **Main cells responsible** | Mast cells, basophils | 1.Complement<br>2.Marcrophages/lymphocytes(IgG)<br>3.None | Local cell proliferation, neutrophils | 1.Delayed: CD4+ Th1 cells<br>2.Cytotoxic: CD8+ Th1 cells NK cells |
| **Mechanism** | Vasodilation<br>Vasopermeability | 1.Osmotic lysis<br>2.Lysis by macro/lympho<br>3.Blocking receptor (Ach) | Deposition of immune complexes, complement activation | Apoptosis |
| **Example of diseases** | Anaphylactic shock<br>Food allergies, reactions to vaccines, asthma, rhinitis, $EBP_{\text{eosinophilic bronchopneumopathy}}$ | Transfusion reactions, IMHA, ITP, pemphigus, MG | Anterior uveitis (blue eye) MPGN, polyarthritis, vasculitis, SLE (II and III), Dirofilaria, borrelia, leishmania, ehrlichia, serum sickness | Granulomatous reactions (blasto, histo, coccidioido, FIP), SJS, contact allergy, thyroiditis, Addison, KCS |
| **Cytokines** | IL-4 (Th2 activity), TNF-α | Membrane Attack Complex (C5b-9) | C3a, C5a<br>TGF-β (MPGN) | IFN-γ (Th1 activity), IL-2, TNF |
| **Mediators** | Histamine, heparin, leukotriene, prostaglandins, PAF | | Proteases, oxidants | Perforin, granzymes |
| **Treatment** | Epinephrine, steroids | Steroids, other immunosuppressant | Removal of antigen, +/- immune suppressive therapy | Removal of the allergen |

# General Approach

1. Identify and address any trigger factors
2. Organ-specific care (eg. transfusions for IMHA & ITP, pain control for IMPA, pyridostigmine for MG, etc.)
3. Immunosuppression with steroid +/- second agent
4. After durable remission is attained, tapering of immunosuppression

# Immunosuppressives

- Glucocorticoids
- Secondary agents:
  - Cyclosporine (modified)
  - Azathioprine
  - Mycophenolate
  - Leflunomide
  - Chlorambucil
- IVIG

CCVS
CAPE COD VETERINARY SPECIALISTS

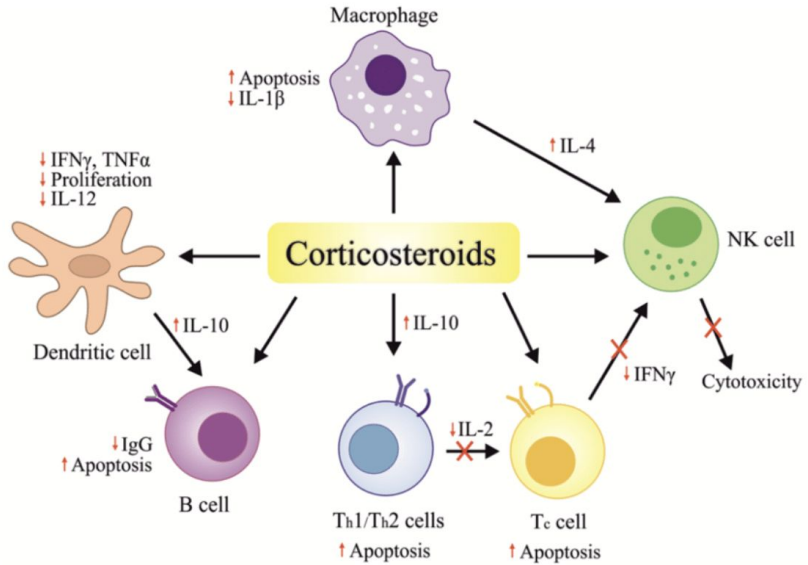
# Glucocorticoids


- **First-line treatment for immune-mediated disease starts working in 24-48 hours!**
- Stabilize the cell membrane of endothelial cells and **inhibit production of local chemotactic factors,** decreasing infiltration of neutrophils, monocytes, lymphocytes.
- Inhibits release of arachidonic acid from membrane phospholipids, **preventing synthesis of prostaglandins, thromboxanes, leukotrienes, which are mediators of inflammation.**

# Glucocorticoids

- **Redistributes monocytes and lymphocytes** from the peripheral circulation to lymphatics and bone marrow, primarily affecting T cells.
- Reduces **T cell** activation and cytotoxicity.
- Suppress **cytokine activity** and alter **macrophage function.**

**Affects macrophages, complement, lymphocytes, everything!**




Ordonez et al, 2014

# Glucocorticoids

## Prednisone / prednisolone

- 2mg/kg/day or 50mg/m2 in large dogs
- Always prednisoLONE for cats

## Dexamethasone SP (injectable)

- In hospital use or for PLE / malabsorptive cases
- Label is 4mg/mL = 3mg/mL dex + 1mg/mL SP
- 7x potency as prednisone
- Biologic half life 36-48 hours

## Budesonide

- ONLY GI/liver cases
- Cats with heart disease, steroid intolerance

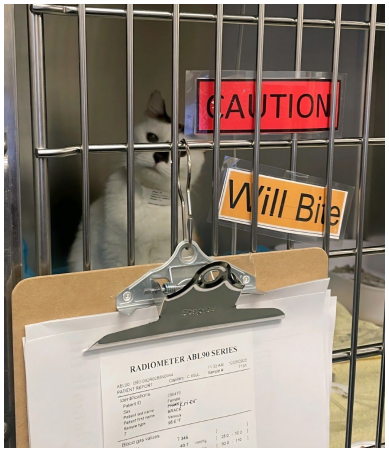
CCVS
CAPE COD VETERINARY SPECIALISTS

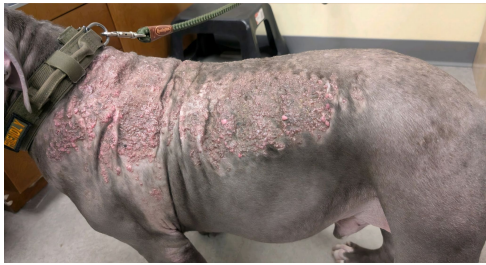
# Glucocorticoids

- Adverse effects:
  - PU/PD to the point of affecting QOL
  - Panting
  - Cachexia
  - Ravenous appetite / inc risk for FB


# Glucocorticoids

- Adverse effects:
  - Ligament weakness, CCL tears
  - Increased risk for DM
  - Increased risk for CHF
  - Dermatologic (pyoderma, calcinosis cutis)

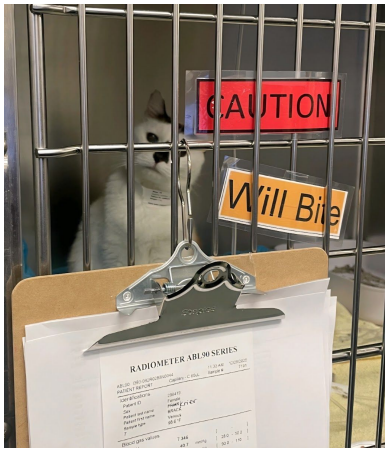



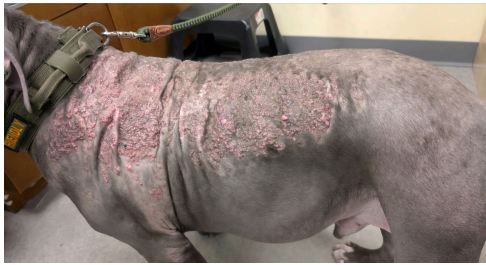



# Glucocorticoids

- Adverse effects:
  - Ligament weakness, CCL tears
  - Increased risk for DM
  - Increased risk for CHF
  - Dermatologic (pyoderma, calcinosis cutis)

So what can we do to minimize this?

Received: 3 February 2021 | Accepted: 5 August 2021

DOI: 10.1111/jvim.16250

STANDARD ARTICLE

Journal of Veterinary Internal Medicine
Open Access
ACVIM
American College of Veterinary Internal Medicine

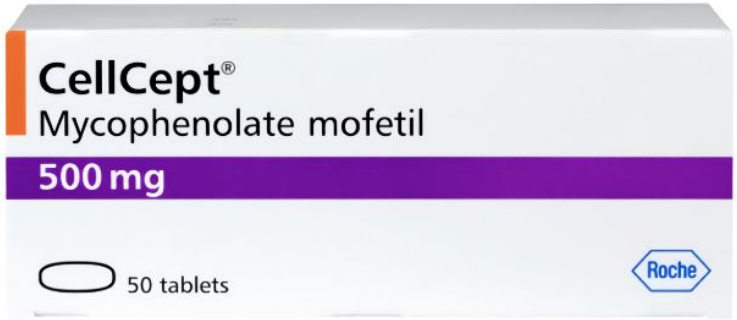
# Effects of desmopressin acetate administration in healthy dogs receiving prednisolone

Pamela Galati | Todd Archer | Robyn Jolly | Alyssa Sullivant | Robert Wills | Patty Lathan

- 5 ug/kg SQ q12 administered to healthy dogs receiving prednisolone reduced PU/PD and increased USG
- CAUTION: Serum sodium significantly reduced, requires careful monitoring

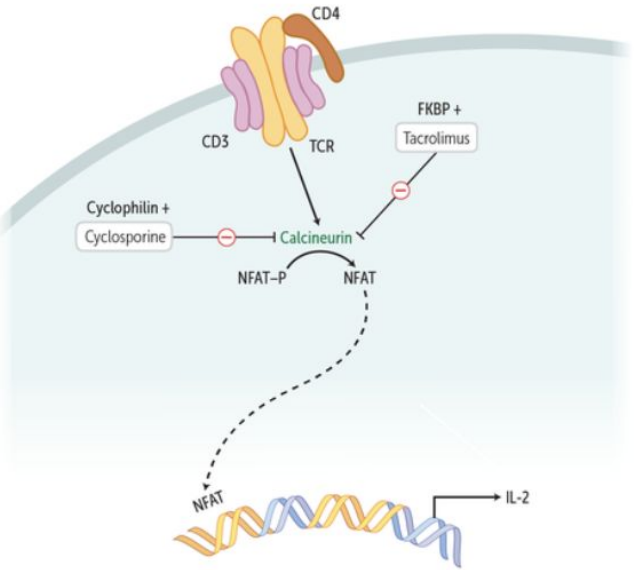
# Secondary Immunosuppressive Agents!






# Cyclosporine (modified)


- Calcineurin inhibitor
- Calcineurin normally dephosphorylates NFAT, which goes to the nucleus and causes cytokine gene activation.
- Cyclosporine (bound to cyclophilin) inhibits calcineurin and therefore inhibits early T cell activation preventing synthesis of several cytokines, in particular IL-2.
- Without stimulation by IL-2, further T-cell proliferation is inhibited, and T-cell cytotoxic activity is reduced.

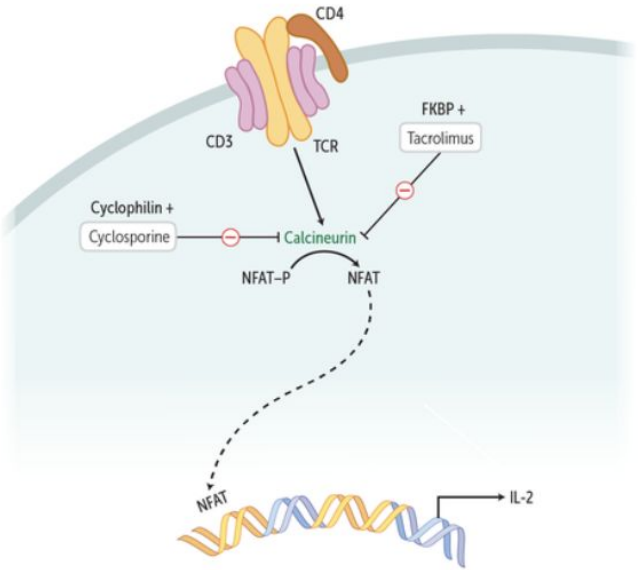



https://quizlet.com/932935121/calcineurin-inhibitors-proliferation-inhibitors-tacrolimus-cyclosporine-sirolimus-flash-cards/

# Cyclosporine (modified)

- Cyclosporine also stimulates cells to secrete TGF-B, which is a potent inhibitor of IL-2 stimulated T-cell proliferation and generation of antigen-specific cytotoxic lymphocytes.

- Not cytotoxic or myelotoxic, action is specific for lymphocytes.

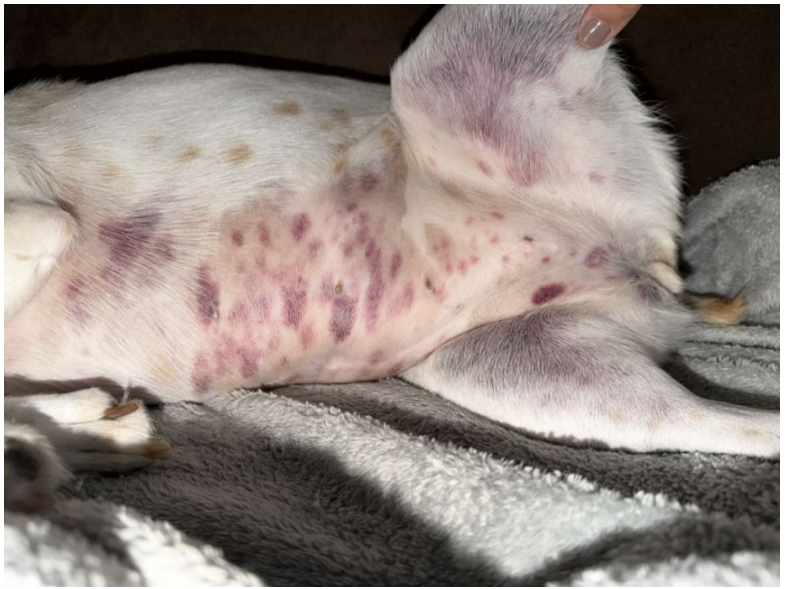



https://quizlet.com/932935121/calcineurin-inhibitors-proliferation-inhibitors-tacrolimus-cyclosporine-sirolimus-flash-cards/

# Cyclosporine (modified)

- For immune-mediated disease, dose at 5mg/kg BID, temporarily up to 7mg/kg BID for life-threatening cases

- **Starts to take effect within 72 hours, which makes this the secondary agent of choice in life-threatening disease when time is of the essence!**

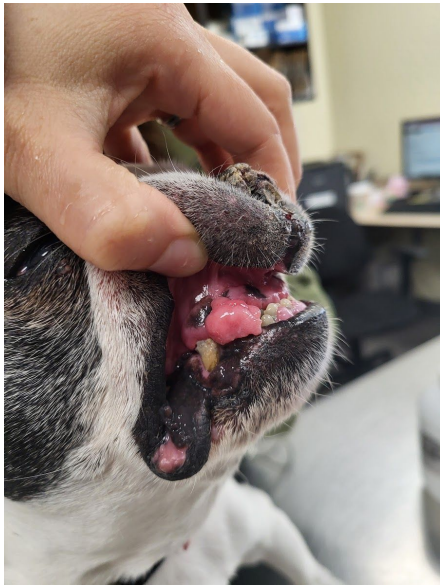


# Cyclosporine (modified)

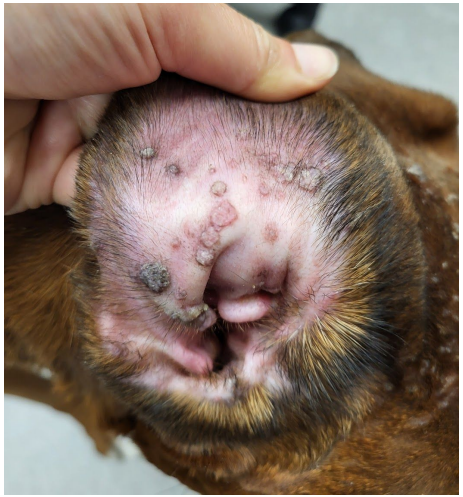
- Many potential drug interactions:
  - Inhibits P-glycoprotein pumps, can cause accumulation of various drugs in tissues
  - Ketoconazole inhibits P450 enzyme, which is responsible for breakdown of cyclosporine -> can combine with cyclo with ketoconazole to reduce drug requirements for perianal fistulas:
    - CsA 0.5 - 2.5mg/kg BID
    - Keto 5 - 10mg/kg q24
- Drug monitoring of blood concentrations if concerned!
  - Peak, trough

# Cyclosporine (modified)

- Adverse effects: GI (keep frozen), gingival hyperplasia, fibropapillomatosis, pyoderma, hirsutism, secondary infections, use with caution in regions where fungal disease is common



Vet Record CaseReports

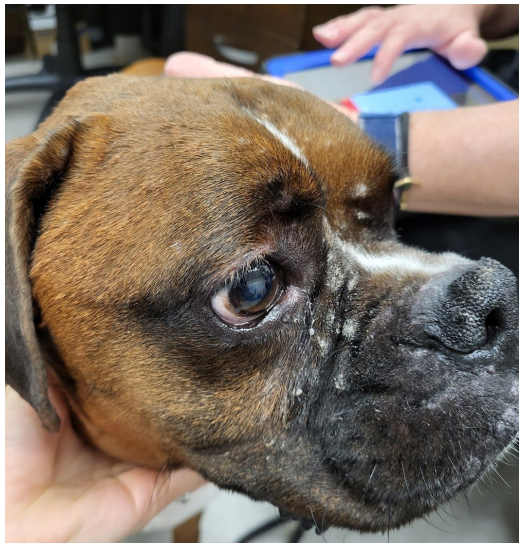


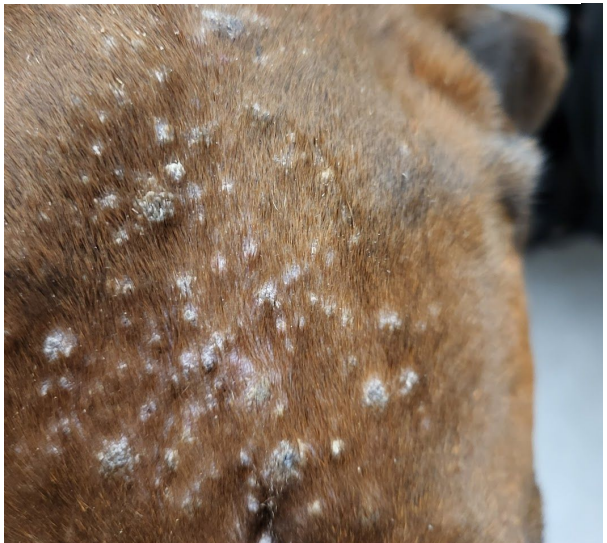
CASE REPORT | Open Access

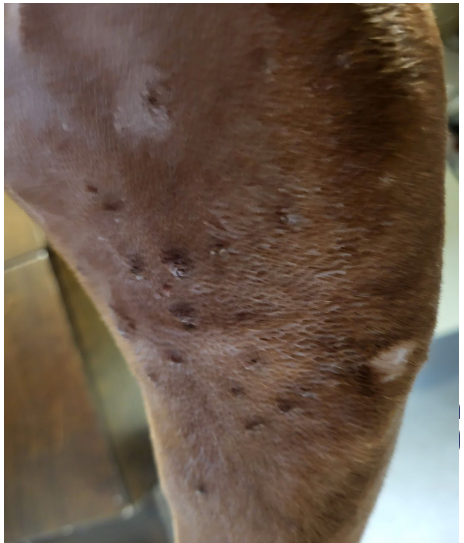
# Cyclosporine-induced psoriasiform-lichenoid dermatosis in four dogs: Successful treatment with antibiotics and cyclosporine dosage reduction

Junko Ike, Ryosuke Ueda, Takeshi Mukosaka, Maiko Sekiguchi, Toshiroh Iwasaki

First published: 26 August 2024 | https://doi.org/10.1002/vrc2.984 | VIEW METRICS

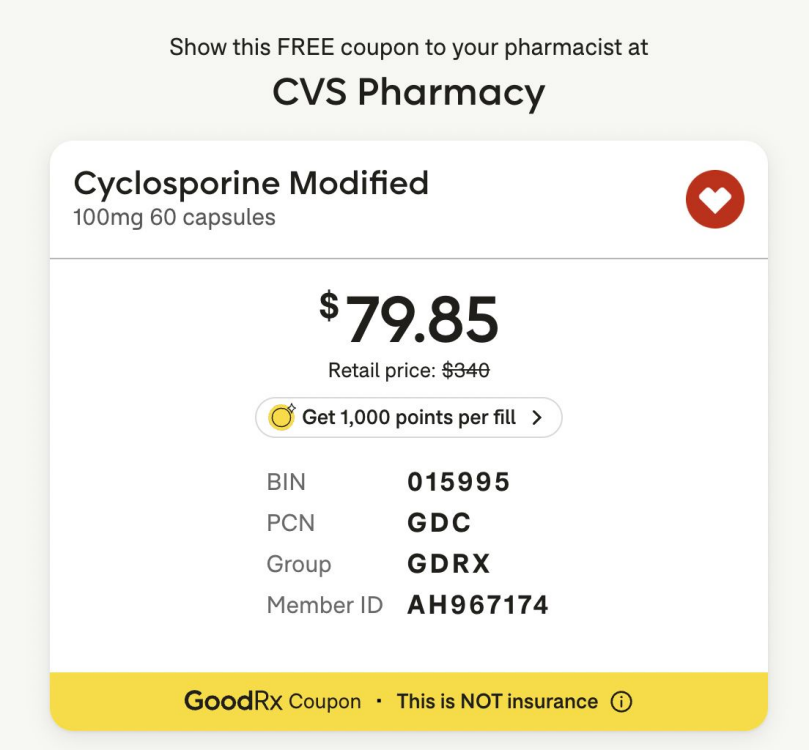






# Cyclosporine (modified)

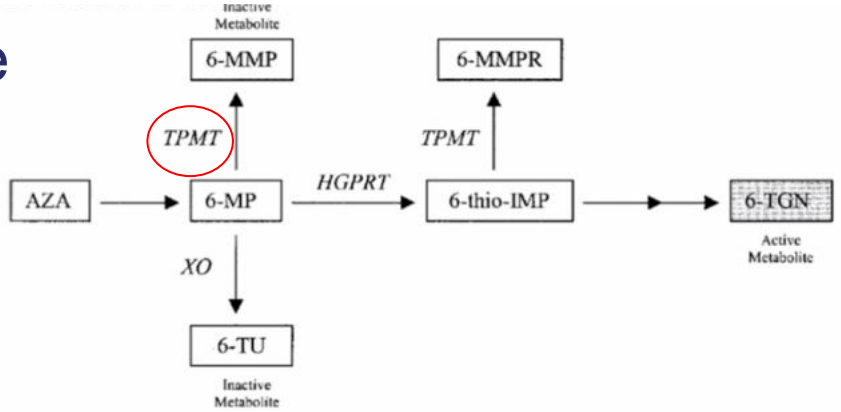
- Atopica very expensive, but modified generic cyclosporine is affordable!
  - Atopica 100mg BID for 1 month = ~$500
  - Modified generic cyclosporine 100mg BID for 1 month, using GoodRx coupon = ~$80



# Azathioprine

- **Purine antagonist,** purines are needed by lymphocytes for mitogen response

- Converted to active metabolite **6 mercaptopurine (6-MP)**, metabolized by **thiopurine methyltransferase (TPMT)**

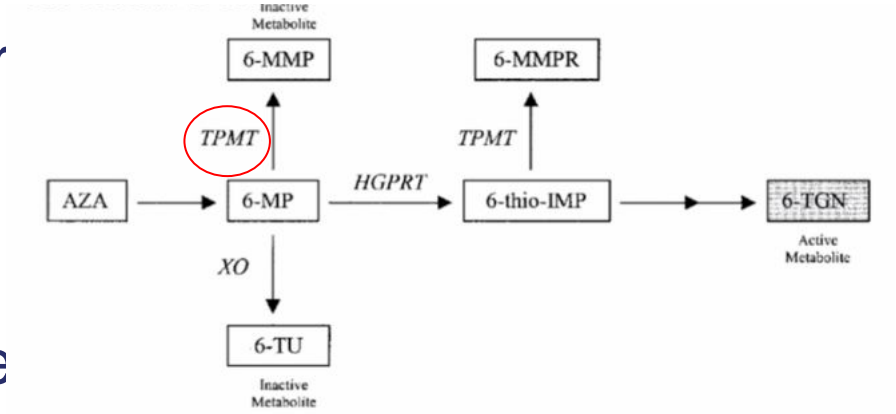


# Azathioprine

- **Purine antagonist,** purines ar needed by lymphocytes for mitogen response

- Converted to active metabolite **6 mercaptopurine (6-MP)**, metabolized by **thiopurine methyltransferase (TPMT)**

- TPMT is high in malamute, low in giant schnauzer and cats

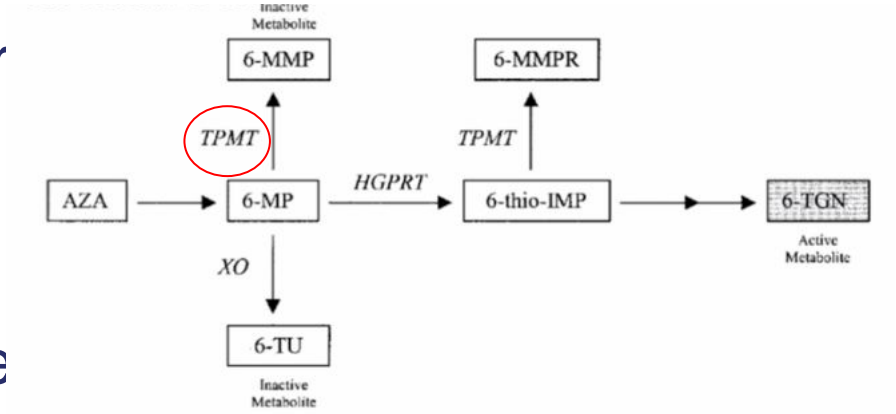


# Azathioprine

- **Purine antagonist,** purines ar needed by lymphocytes for mitogen response

- Converted to active metabolite **6 mercaptopurine (6-MP)**, metabolized by **thiopurine methyltransferase (TPMT)**

- TPMT is high in malamute, low in giant schnauzer and cats




Do not use in cats due to concern for toxicity!

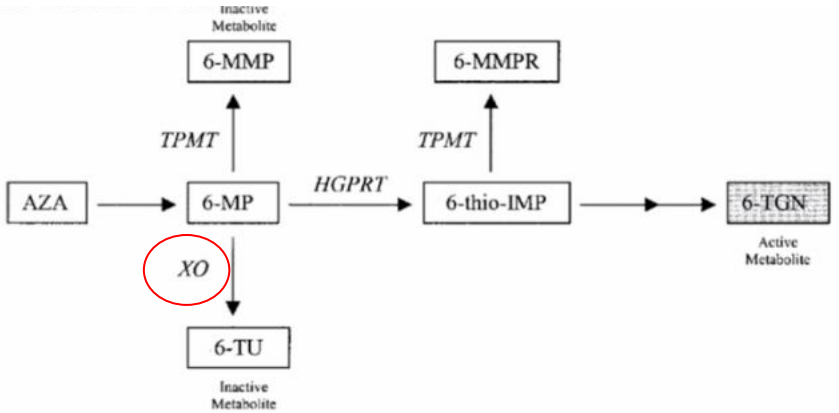
Caution in giant schnauzers!

May not be as effective in malamutes

# Azathioprine

- Potential drug reaction:

Allopurinol inhibits xanthine oxidase breakdown of 6-MP, do not use in combination due to concerns for toxicity!



# Azathioprine

- **Cytotoxic, myelotoxic**
- Dose at 2mg/kg (or 50mg/m2 in large breeds) q24 for 1-2 weeks, then q48 longterm
- 2-6 weeks for max effect
- Adverse effects: Bone marrow suppression, hepatotox, acute pancreatitis

CCVS
CAPE COD VETERINARY SPECIALISTS

# Azathioprine

Journal of Veterinary Internal Medicine
Open Access
*J Vet Intern Med* 2015;29:513–518

**Incidence, Timing, and Risk Factors of Azathioprine Hepatotoxicosis in Dogs**

K. Wallisch and L.A. Trepanier

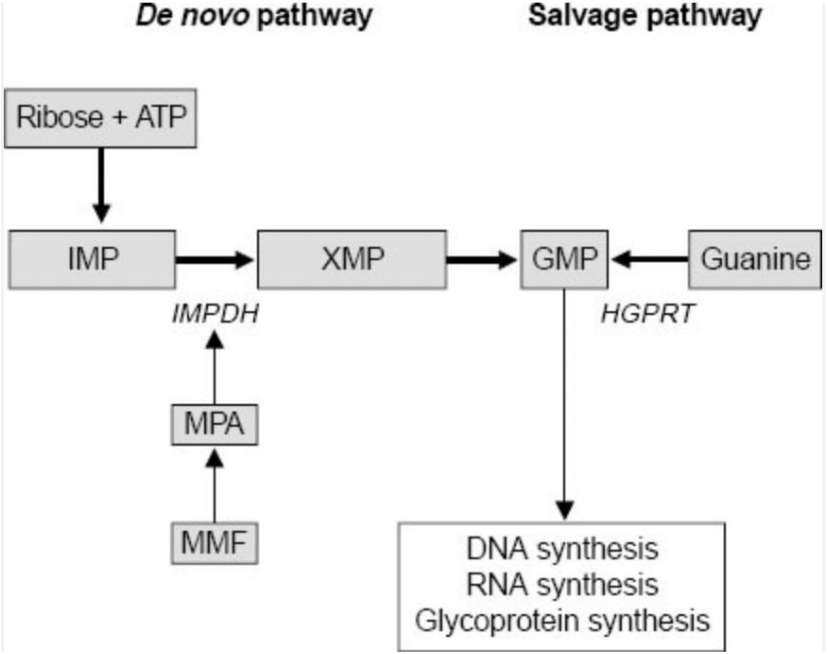
- In dogs, hepatotox in 15% with median onset 14 days, GSD overrepresented.
- Thrombocytopenia/neutropenia in 8% with median onset 53 days.

## Monitoring protocol:

CBC and liver enzymes every 2 weeks for first 2 months, then reduce to every 3 months longterm

# Mycophenolate

- Mycophenolate is a prodrug hydrolyzed by liver esterases to mycophenolic acid.
- **Purine antagonist**, cytostatic to lymphocytes via inhibition of **inosine monophosphate dehydrogenase (IMPDH)**, an enzyme necessary for purine biosynthesis.
- Selective inhibitor of T- and B-cell proliferation during the S phase of the cell cycle.

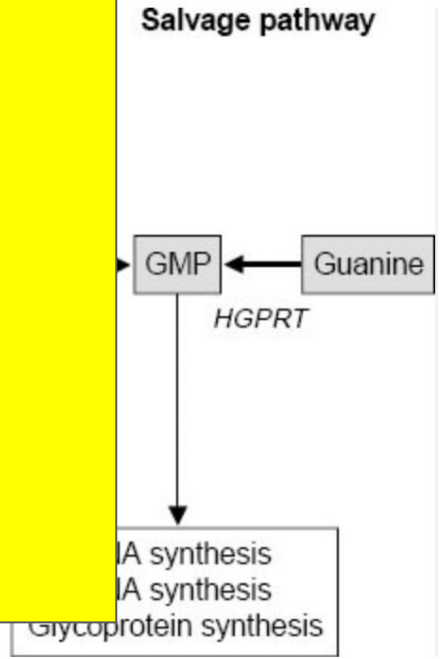



Gallagher et al, 2001

# Mycophenolate

- Mycophenolate is a prodrug
  hydro
  to my
- **Purin**
  to lym
  of **ino**
  **dehy**
  enzyn
  biosyn
- Selective inhibitor of T- and B-cell proliferation during the S phase of the cell cycle.

**What other secondary immunosuppressive is a purine antagonist?**

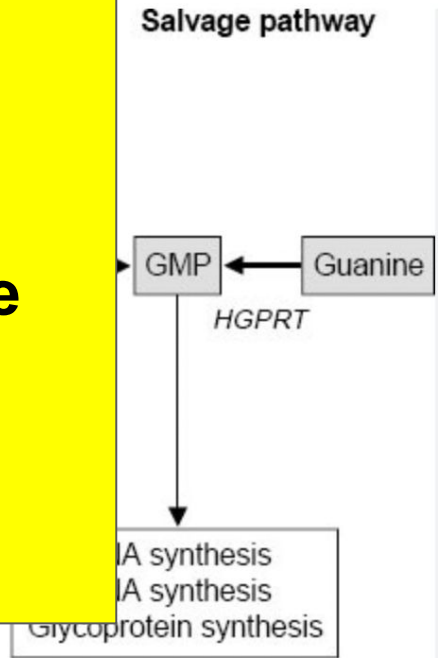



Gallagher et al, 2001

# Mycophenolate

- Mycophenolate is a prodrug
  hydro
  to my
- **Purin**
  to lym
  of **ino**
  **dehyc**
  enzyn
  biosyr
- Selective inhibitor of T- and B-cell proliferation during the S phase of the cell cycle.

**Since mycophenolate and azathioprine are BOTH purine antagonists, you would never use them together in triple immunosuppressive protocols!**



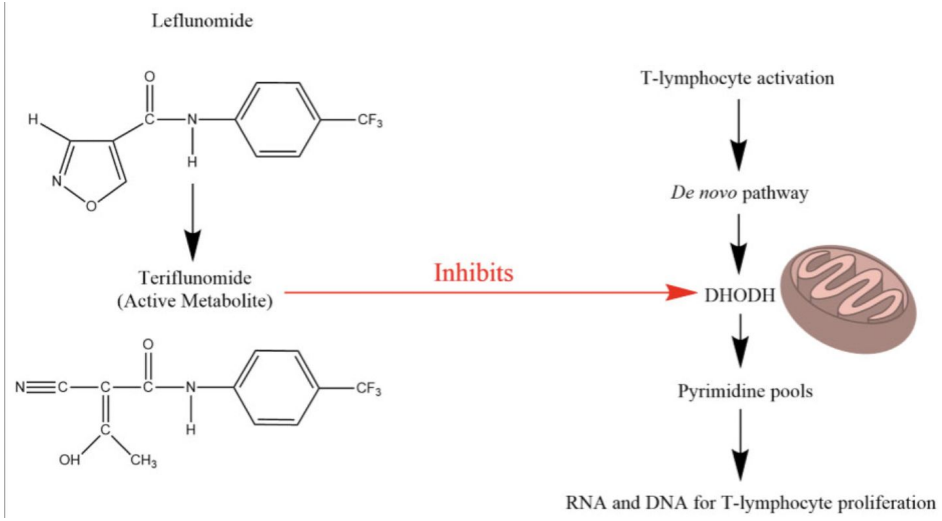

Gallagher et al, 2001

# Mycophenolate

- 8-15mg/kg BID
- Starts to take effect within 72 hours, full effect not for weeks-months
- Dose reduce for renal disease, important because this is drug of choice for immune-complex glomerulonephropathy (ICGN)!
- Very unlikely to be myelotoxic
- Adverse effects: GI, particularly diarrhea

# Leflunomide

- **Pyrimidine antagonist**, synthetic organic isoxazole that the intestinal mucosa metabolizes to its active form **teriflunomide** (A77 1726), target is **dihydroorotate dehydrogenase** in the pyrimidine biosynthesis pathway.

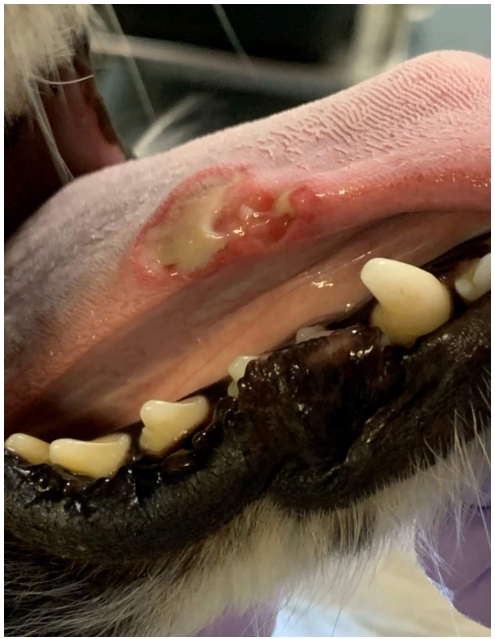


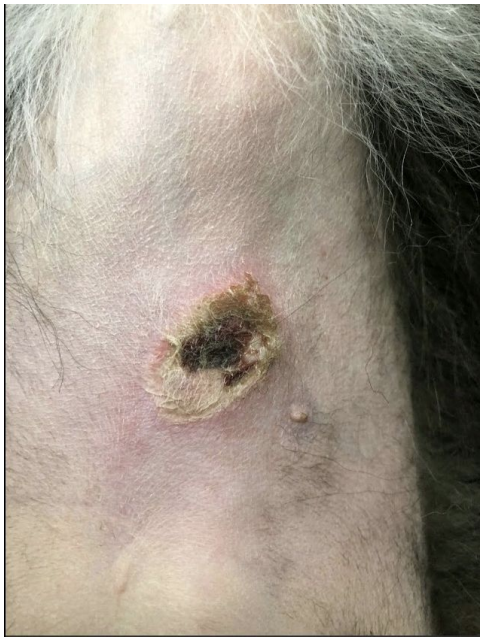

Ku et al, 2022

# Leflunomide

- Adverse effects: Hepatotoxicity, myelosuppression, GI, lethargy, cutaneous drug reactions (oral, paw pad ulceration and crusting skin lesions), hemorrhage

**Renally eliminated, so greater risk for toxicity with renal insufficiency





Journal of Veterinary Internal Medicine
ACVIM American College of Veterinary Internal Medicine
Open Access

*Standard Article*

*J Vet Intern Med* 2017;31:1502–1507

# A Retrospective Study on the Safety and Efficacy of Leflunomide in Dogs

M. Sato, J.K. Veir, M. Legare, and M.R. Lappin

- Starting dose: 2 mg/kg/day rather than previous 3-4 mg/kg/day
- Significant dose differences between dogs with adverse events (2.9mg/kg/day) and dogs without adverse events (1.6mg/kg/day).

# Leflunomide

- Monitoring: CBC/Chem at 2, 4 weeks, then q 4-6 weeks
    - Consider PK monitoring, active metabolite teriflunomide, long half life 24h in dogs and 60h in cats, so wait at least 1w in dogs and 2w in cats
    - Trough should be sufficient, but clinical response has been documented across a wide range of blood concentrations

CCVS
CAPE COD VETERINARY SPECIALISTS

# Chlorambucil

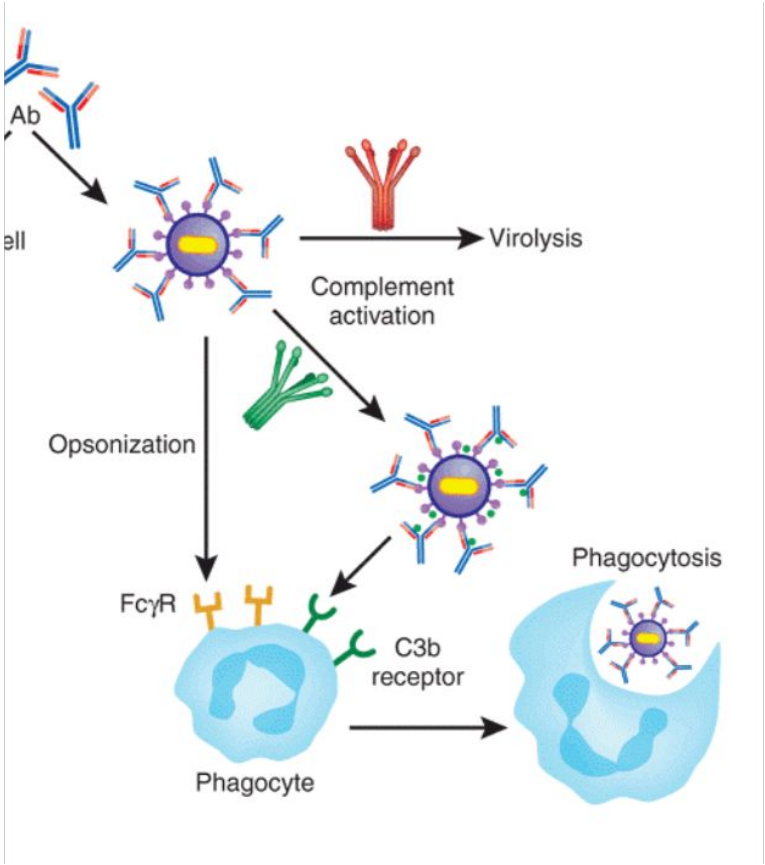
- Alkylating agent, prevents cell replication by damaging DNA -> targets B cells, cytotoxic
- Cytotoxic drug of choice in cats. Much less commonly used in dogs for this purpose.
- Up to 2 weeks for therapeutic efficacy
- Expensive, need to compound

# Chlorambucil

- Adverse effects: Myelosuppression, GI upset, acquired Fanconi, neurotoxicicty (myoclonus)
- Many dosing protocols. For cats, commonly 0.1 - 0.2mg/kg q24 OR 2mg/cat q48-72h, then extend interval when remission is attained.
- Monitor CBC/Chem/UA at least 1 month after starting, then every 2-3 months longterm. Monitoring depends on protocol.

# hIVIG

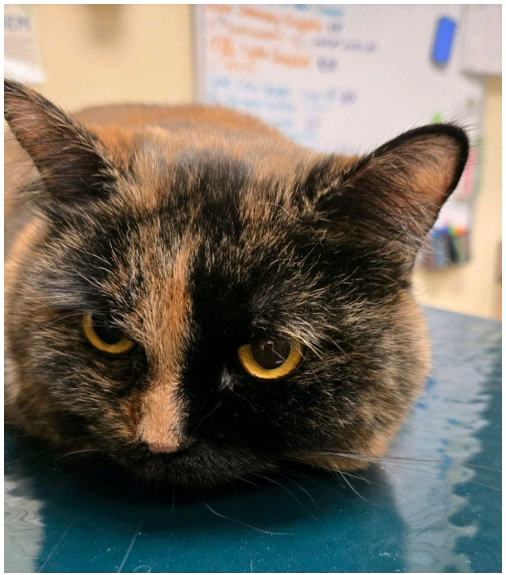
- Antibodies (immunoglobulins) from healthy donors used to modulate an overactive immune system in autoimmune crisis
- Blockade of Fc receptors on monocyte-macrophage phagocytic system, leading to suppression of antibody production/binding and decreased complement activation.



# hIVIG

- Dose: 0.5 - 1.5 g/kg IV over 6-12 hours
- Very expensive, cost for 20kg dog = ~$3500
- Can be difficult to find
- Very safe, adverse effects rare but include possible hypercoagulable state, anaphylaxis with repeated treatment

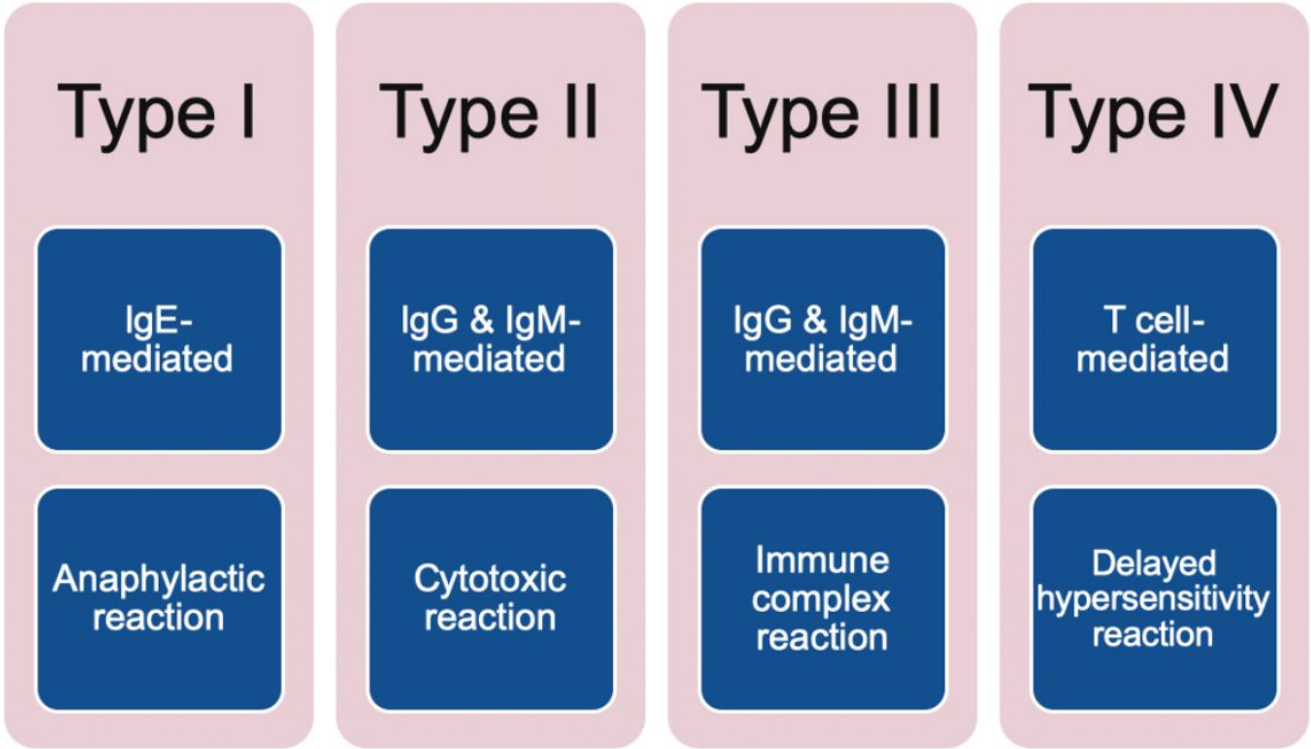
# Immune-mediated hemolytic anemia (IMHA)



# IMHA

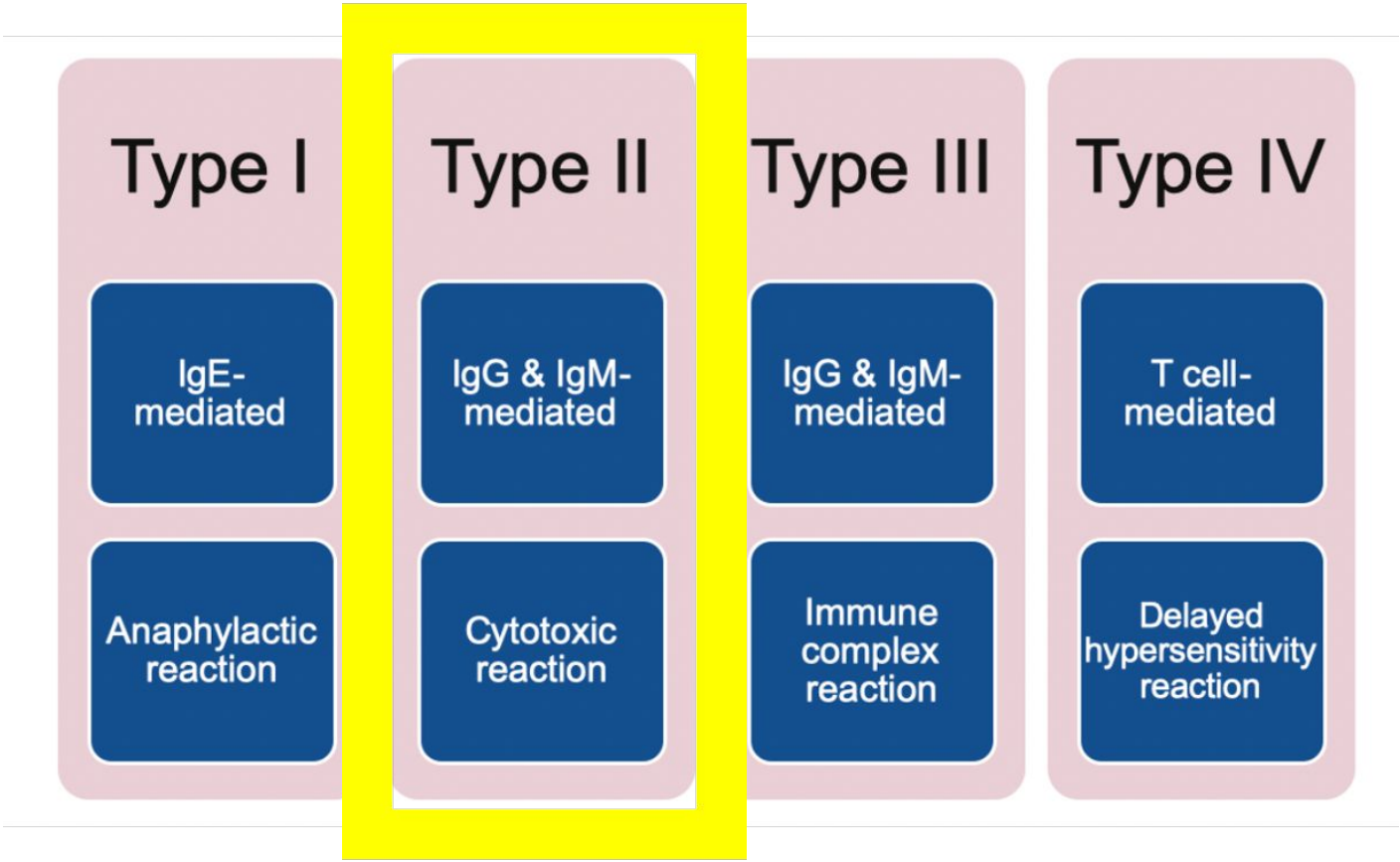
- Failure of self-tolerance. Formation of autoantibodies against RBCs, leading to destruction by phagocytic cells in the liver and spleen (extravascular hemolysis) or by complement proteins within blood vessels (intravascular hemolysis).
- Icterus, severe anemia, need for multiple transfusions, $$$$$

# IMHA - Which type of hypersensitivity reaction?




https://foamid.com/2019/10/23/a-rash-of-beta-lactam-allergies-part-2-the-education/

# IMHA - Which type of hypersensitivity reaction?




https://foamid.com/2019/10/23/a-rash-of-beta-lactam-allergies-part-2-the-education/

# IMHA

- Type II or Antibody-Mediated Hypersensitivity Reaction
- Mediated by IgM or IgG targeting membrane-associated antigens. Target cells become coated with antibodies, a process termed opsonization, which leads to cellular destruction by three mechanisms:
  - Phagocytosis
  - Complement-dependent cytotoxicity
  - Antibody-dependent cell cytotoxicity

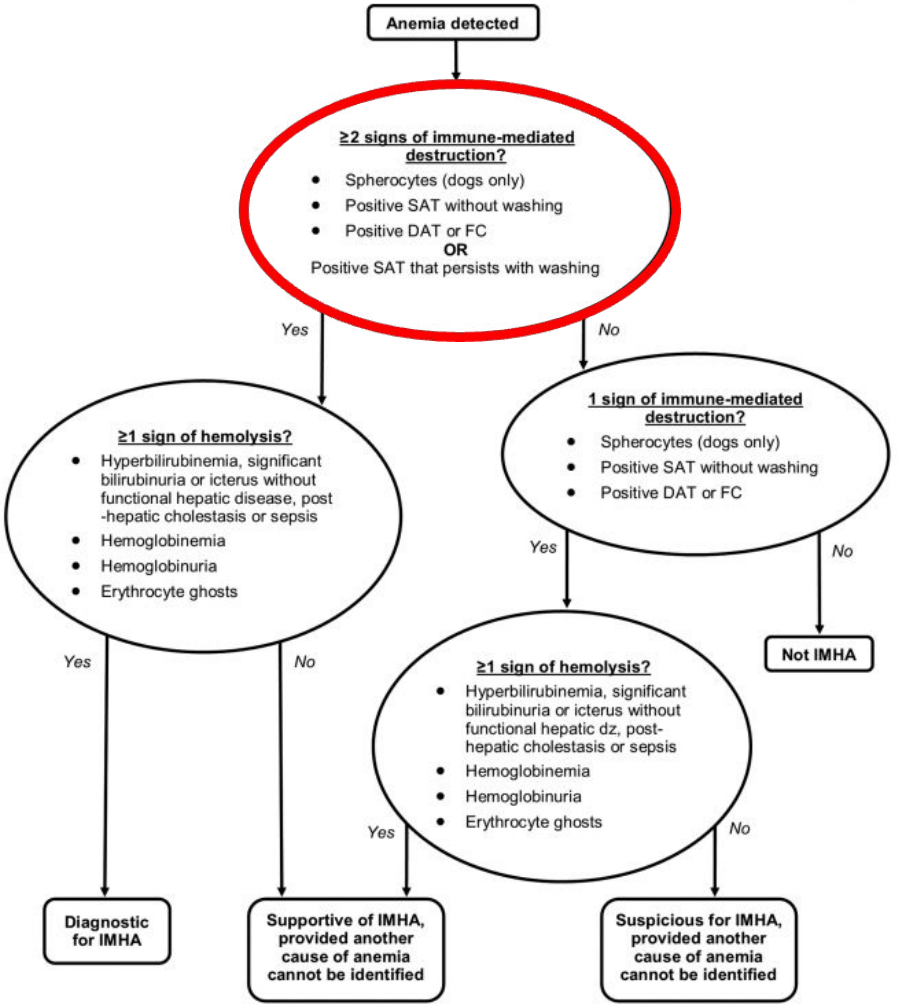
# IMHA

- **Primary (idiopathic)** if no trigger is found.
- **Secondary** when it can be attributed to an underlying disease.
  - Cancer
  - Drugs
  - Vaccines
  - Inflammatory processes
- **Prognosis:** Guarded, 50-70% survival to discharge; ~15% relapse

# ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats

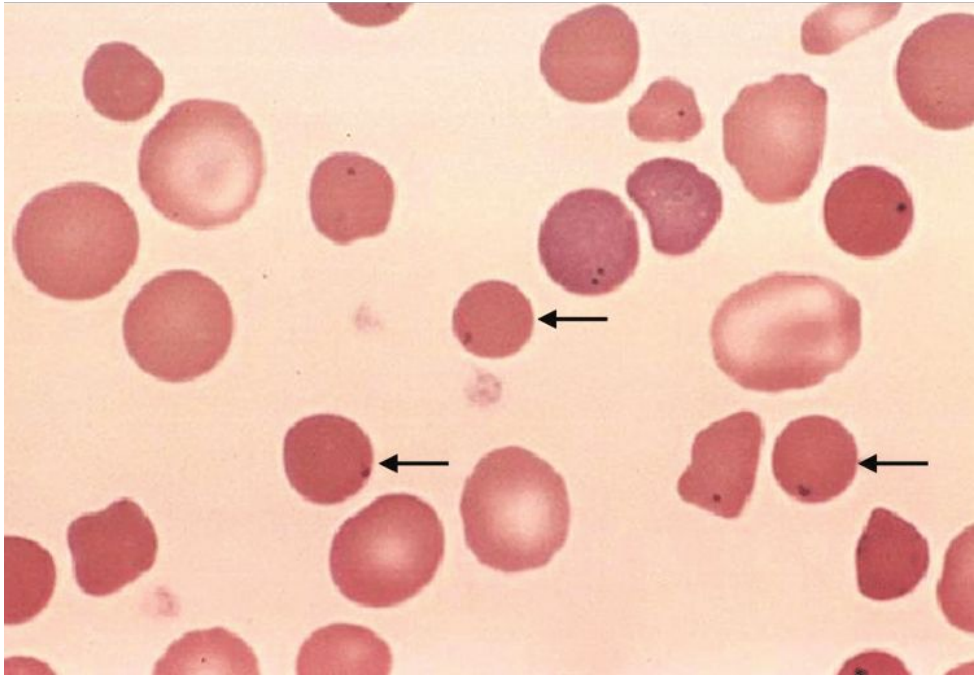
Oliver A. Garden[1] | Linda Kidd[2] | Angela M. Mexas[3] | Yu-Mei Chang[4] | Unity Jeffery[5] | Shauna L. Blois[6] | Jonathan E. Fogle[7] | Amy L. MacNeill[8] | George Lubas[9] | Adam Birkenheuer[7] | Simona Buoncompagni[10] | Julien R. S. Dandrieux[11] | Antonio Di Loria[12] | Claire L. Fellman[13] | Barbara Glanemann[4] | Robert Goggs[14] | Jennifer L. Granick[15] | Dana N. LeVine[16] | Claire R. Sharp[17] | Saralyn Smith-Carr[18] | James W. Swann[19] | Balazs Szladovits[4]

*J Vet Intern Med.* 2019;33:313–334.



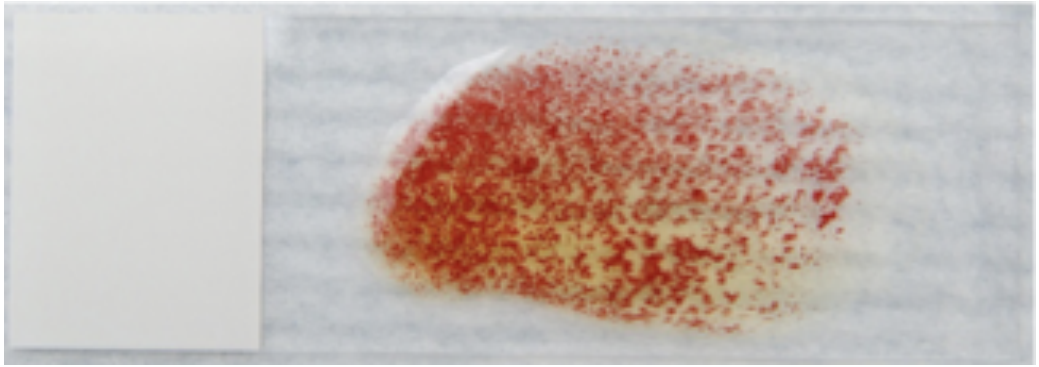

- Need both IM-destruction AND hemolysis to be IMHA
- Sometimes backed into a corner and must immunosuppress somewhat empirically

# IM-destruction: Spherocytes



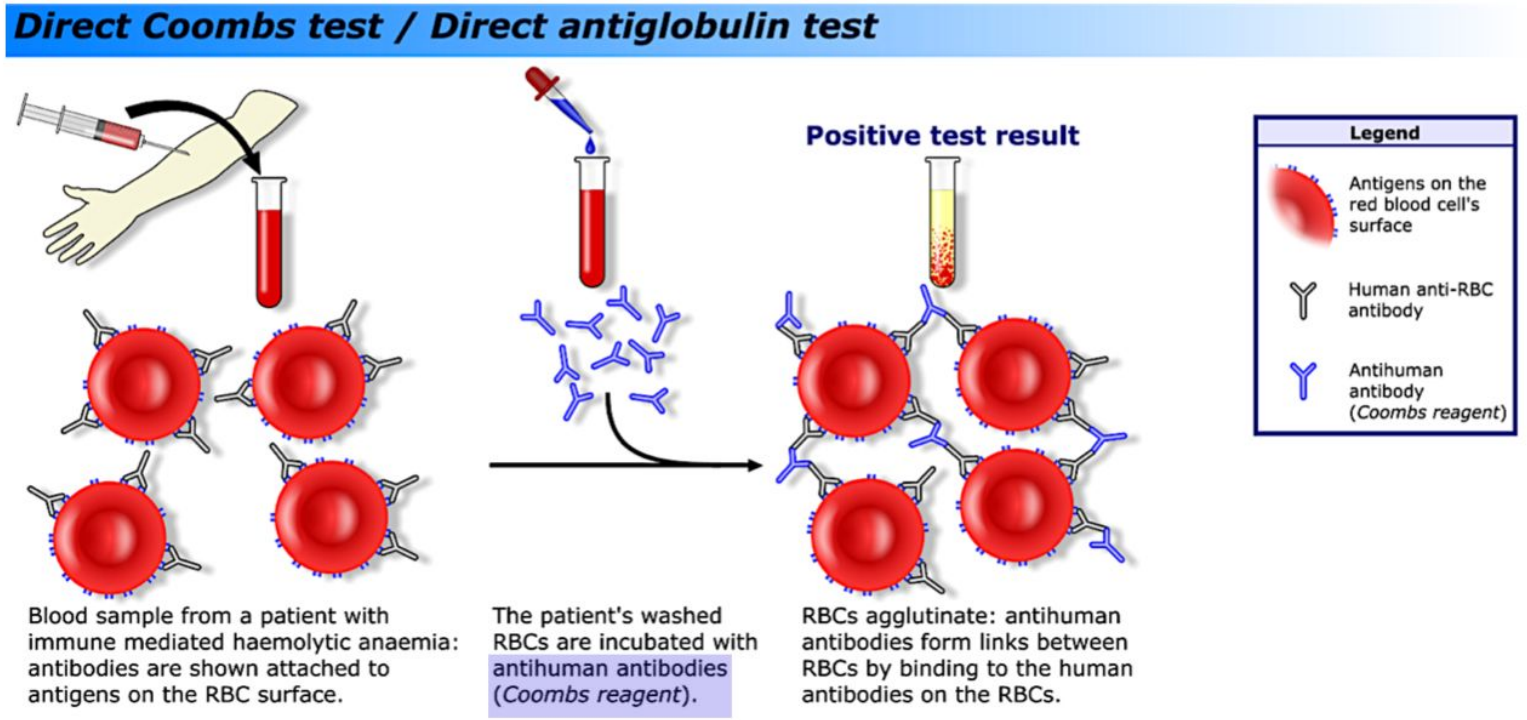
**A threshold of ≥5 spherocytes/×100 oil immersion field** therefore could be considered supportive of a diagnosis of IMHA, but 3-4 spherocytes/×100 oil immersion field also may be consistent with IMHA provided no other cause of spherocytosis is identified.

# IM-destruction: Saline agglutination

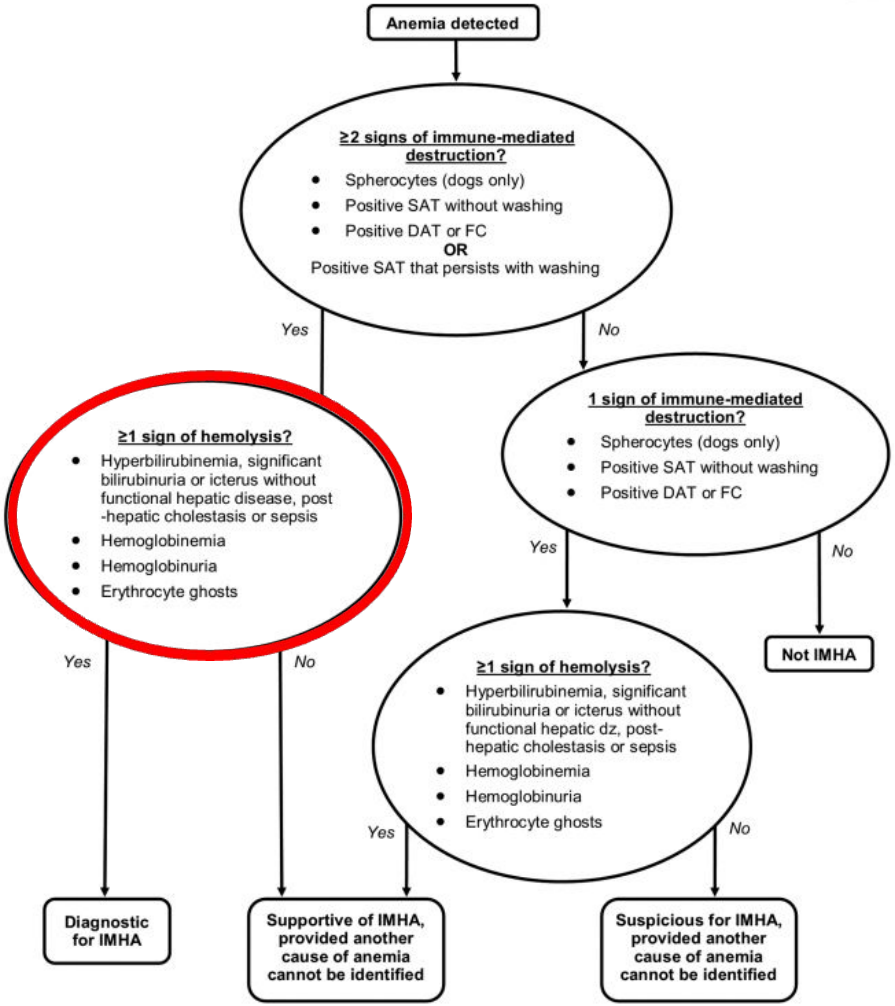


Saline agglutination testing performed by mixing **4 drops of saline with 1 drop of blood** has a reported specificity of 100% for IMHA in dogs.

# IM-destruction: Coombs/DAT

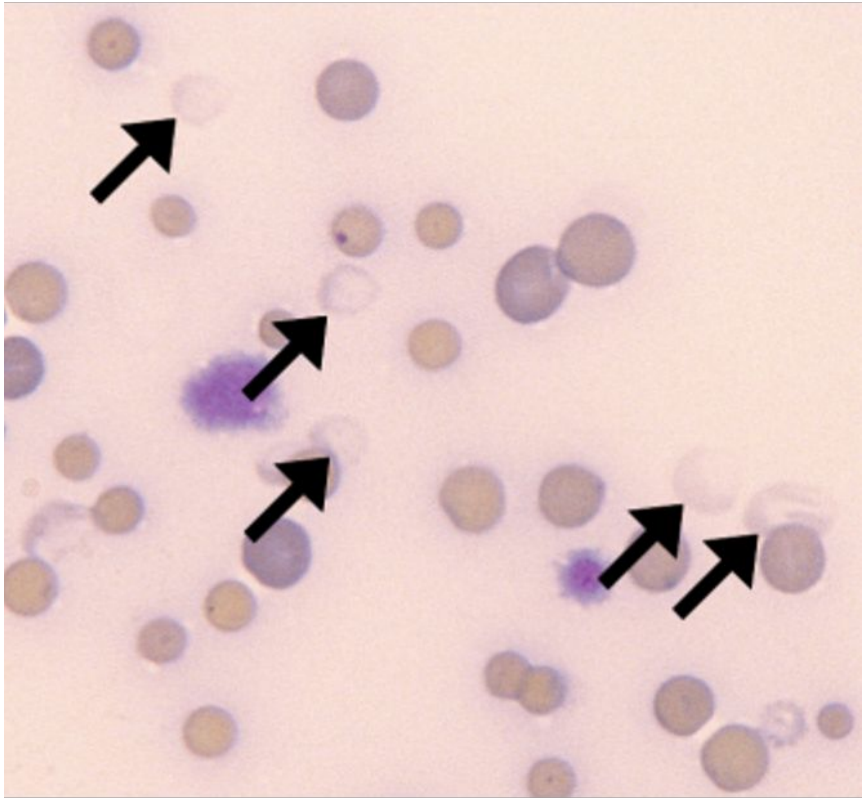



https://microbiologyinfo.com/coombs-test-principle-types-procedure-and-result-interpretation/




- Need both IM-destruction AND hemolysis to be IMHA
- Sometimes backed into a corner and must immunosuppres somewhat empirically

# Hemolysis



- Hyperbilirubinemia, hyperbilirubinuria, icterus WITHOUT functional hepatic disease, cholestasis, sepsis
- Hemoglobinemia, hemoglobinuria
- Ghost cells

# IMHA - What about regeneration?

- Don’t worry about regeneration at time of diagnosis, but should be watching for it
- How long before you’d expect to see a regenerative response?

# IMHA - What about regeneration?

- Don’t worry about regeneration at time of diagnosis, but should be watching for it
- How long before you’d expect to see a regenerative response?

***2-5 days after hemorrhage or hemolytic event**

***Feline reticulocytes remain in punctate phase longer (2-3 weeks), which are not measured**

***Bone marrow aspirate/core if persistently nonregenerative**

# IMHA - Screening

- **History -** vaccination, antibiotics, drugs, travel, flea/tick
- **PE -** include fundic exam
- **Labs -** CBC including blood smear, Chem, UA
  - Consider urine culture, fecal float (report of hookworms/IMHA)
- **Imaging -** AXR to rule out zinc, CXR/AUS
- **Infectious disease (dogs) -** Babesia PCR and serology, heartworm, anaplasma, bartonella, ehrlichia, +/- leishmania
- **Infectious disease (cats) -** B felis, M. haemofelis PCR, FeLV/FIV

# Don’t forget babesia!

**9yo SF Pittie**

- **Hospitalized Nov 2024 for suspect IMHA, reg anemia, tick PCRs neg (including babesia), AUS/CXR WNL**
- **Relapse Jan 2025, bone marrow unremarkable, recheck tick PCRs POS FOR BABESIA GIBSONI**

# IMHA - Screening

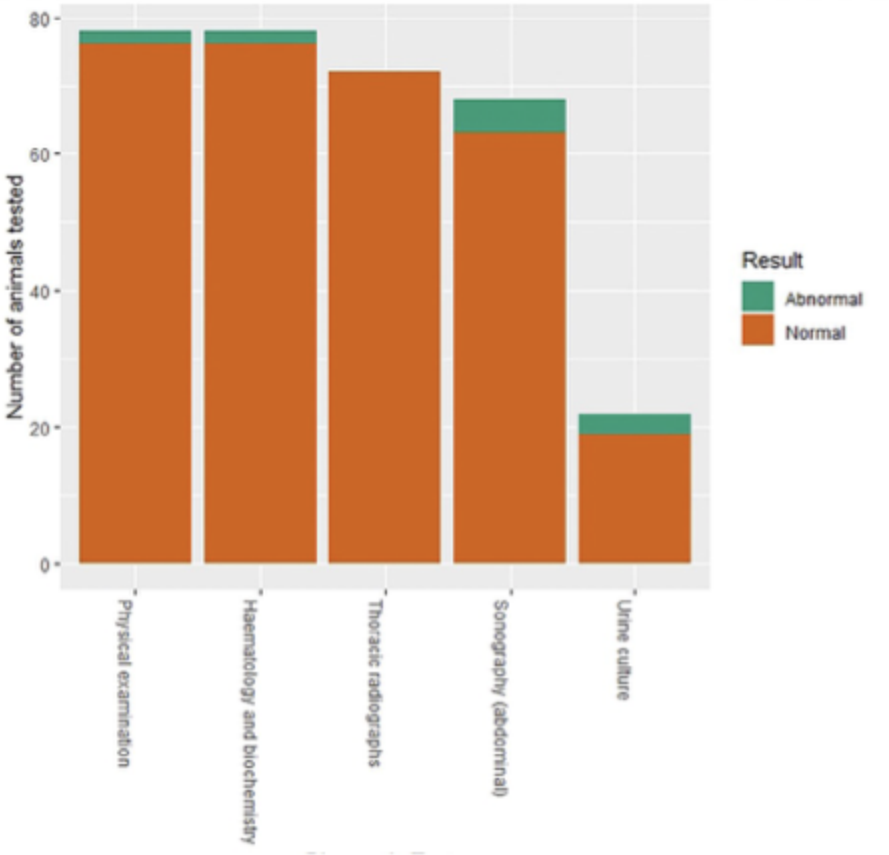
**How often would you say you identify a trigger for IMHA on abdominal ultrasound or CXR?**

ORIGINAL ARTICLE **Australian Veterinary Journal** Volume 98 No 12, December 2020

# The utility of screening diagnostic tests in identifying associative immune-mediated haemolytic anaemia in dogs

GM Woodward and JD White[x]

- 78 dogs
- Secondary/associative IMHA was confirmed (3) or suspected (7) in 10 dogs (13%, confidence interval [CI] 7.1%-22%), with 68 cases presumed to be non-associative.
- Secondary IMHA was present in 3/29 (10.3%) of dogs with criteria diagnostic for IMHA, 4/42 (9.5%) of dogs with criteria supportive for IMHA and 3/7 (42.9%) of dogs with criteria suspicious for IMHA.
- AUS was performed in 68 dogs and identified possible triggers in five (7.3%, CI 3.2% to 16%).
- CXR were performed in 70 dogs but did not identify any potential triggers (0%, CI 0% to 5.2%).
- Urine culture was performed in 22 dogs and was positive in three (14%, CI 4.7% to 33.3%).
- **Routine screening tests, particularly thoracic radiographs, have a low yield in identifying potential triggers of associative IMHA, but are more likely to be useful in dogs fulfilling less stringent diagnostic criteria of IMHA.**



# IMHA - Treatment

- Start treatment after all diagnostic samples have been collected (where possible)
- Transfuse when clinical for anemia
- **Fresh pRBC, ideally no older than 7-10 days, are recommended for use in dogs with IMHA.**
  - Increased risk of mortality in dogs with hemolysis, 90% of which had IMHA. For every 7 day increase in storage, there was a 0.79 lesser odds of 30 day survival (95% CI, 0.64-0.97; P = .024. (Hann et al, JVIM, Nov-Dec 2014;28(6):1830-7.)

# IMHA - Immunosuppressive treatment (dogs)

- Steroids as firstline, prednisone 2 mg/kg/day or 50mg/m2 for large breed
- Highly recommend second agent:
  - Modified cyclosporine, fastest acting!
  - Mycophenolate
  - Azathioprine
  - Leflunomide

# IMHA - Immunosuppressive treatment (cats)

- Steroids as firstline, prednisolone 2-3 mg/kg/day
- Highly recommend second agent:
  - Modified cyclosporine, fastest acting!
  - Chlorambucil

# IMHA - Immunosuppressive tapering

- When hematocrit stable and >30% for 2-4 weeks, begin tapering pred by 25%. If a second drug is used, pred can be tapered by 25-50%.
- Provided hematocrit remains stable and >30%, taper pred by 25% every 3 weeks. If a second drug is used, can taper by 25-33% or consider a taper every 2 weeks.
- Taper second agent after off pred.
- Steroid typically given for 3-6 months, total immunosuppressive treatment 4-8 months

# IMHA - Relapse

- Confirm the relapse – estimated relapse frequency of 11-15% in the literature
- Assess for possible triggering events/missed diagnoses (e.g. Babesia)
- If relapse on pred alone, add second drug
- If relapse during tapering, return to previous dose unless fulminant disease and then return to original dose that was effective
- Once disease control reestablished, double time to initial taper and between subsequent tapers
- If the dog relapses again, lifelong medication may be needed

# IMHA - Thromboprophylaxis

- Start clopidogrel at diagnosis and continue until patient no longer on prednisone.
- If finances allow, I like dual therapy (+ enoxaparin or rivaroxaban) while patient is actively agglutinating, then back down to clopidogrel after agglutination resolves.
    - **Generic rivaroxaban now exists!**

# IMHA - Other recommendations

- Spay intact female dogs
- Antacids not routinely recommended; when needed use a PPI
- Administer prophylactic antimicrobials (eg. doxy) while awaiting diagnostic results based on breed, lifestyle, location, etc.

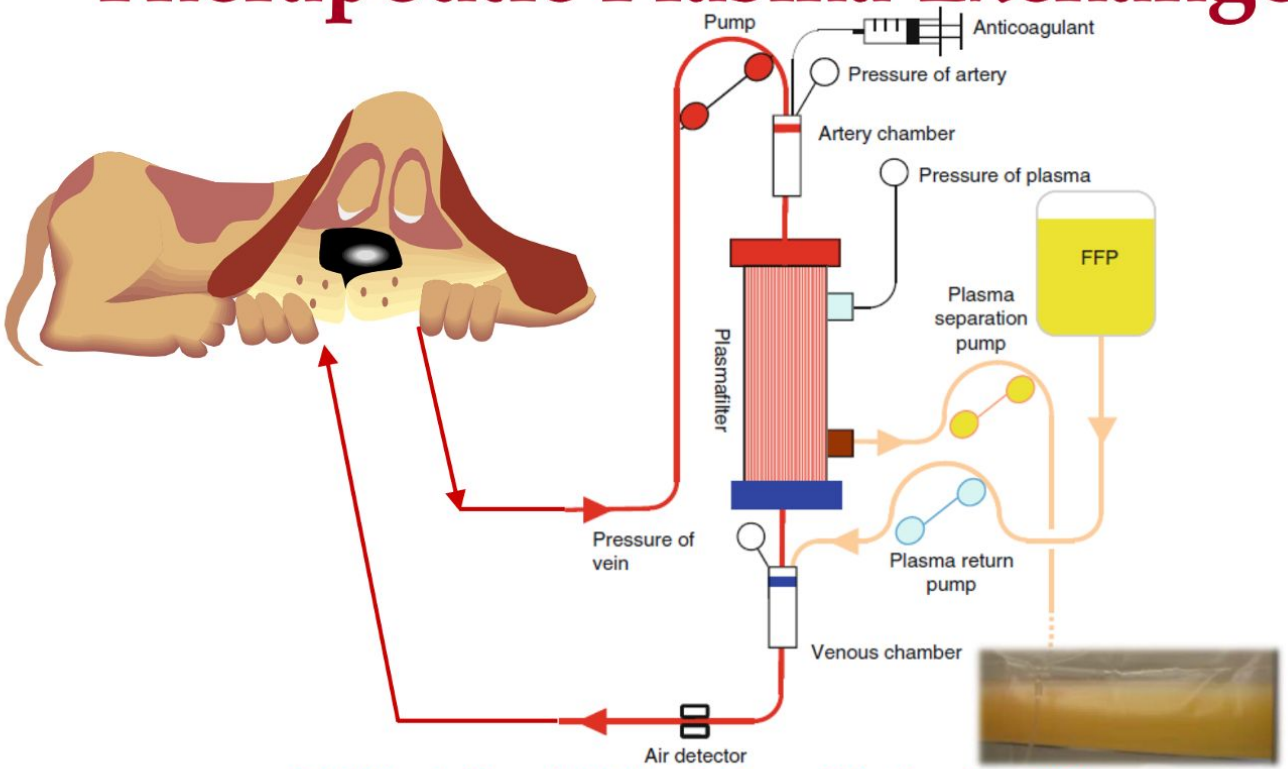
# IMHA - Refractory Cases

- If **triple immunosuppression** is needed, choose complementary MOAs
  - Eg. doesn't make sense to choose 2 purine antagonists (aza, MMF), but could consider pred + cyclosporine (calcineurin inhibitor) + purine antagonist
- **Therapeutic plasma exchange (TPE)**
- **hIVIG:** May be a "Salvage measure" when not responding to treatment with 2 agents; not recommended for routine use
- **Splenectomy** should be considered in dogs requiring continuous immunosuppression and/or with repeated relapses

# IMHA - Therapeutic Plasma Exchange

- Most promising of the adjunctive therapies for IMHA
- Extracorporeal therapy aimed at removal and replacement of plasma
- Removes large molecular weight compounds from circulation, such as **antibodies,** complement, immune complexes, inflammatory mediators, **bilirubin**
- Accelerates initial stabilization of severely affected animals, giving time for immunosuppressives to work

# Principles of Filtration (Membrane) Therapeutic Plasma Exchange




Noiri "Concise Manual of Apheresis Therapy", Springer Japan 2014


prismaflex

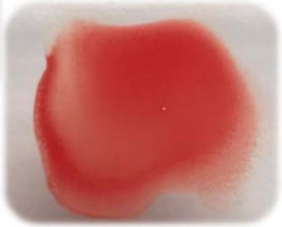
# Initial Tx Efficacy of TPE in Canine IMHA

Pre Tx

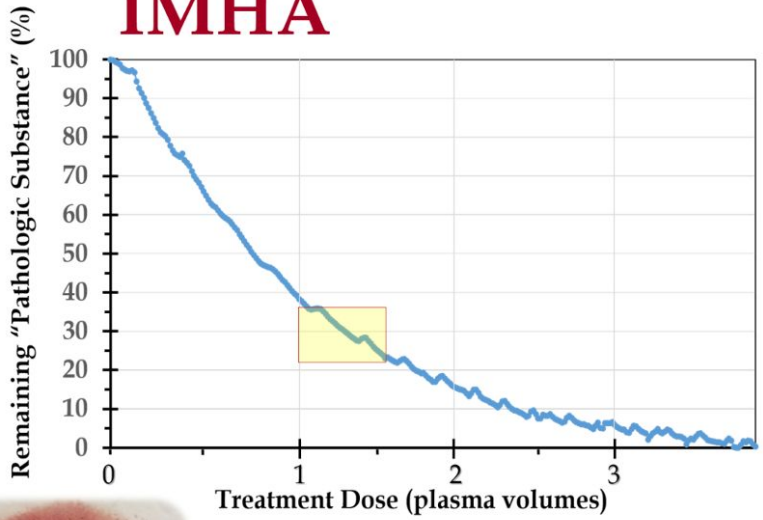
0.5 PV

0.7 PV

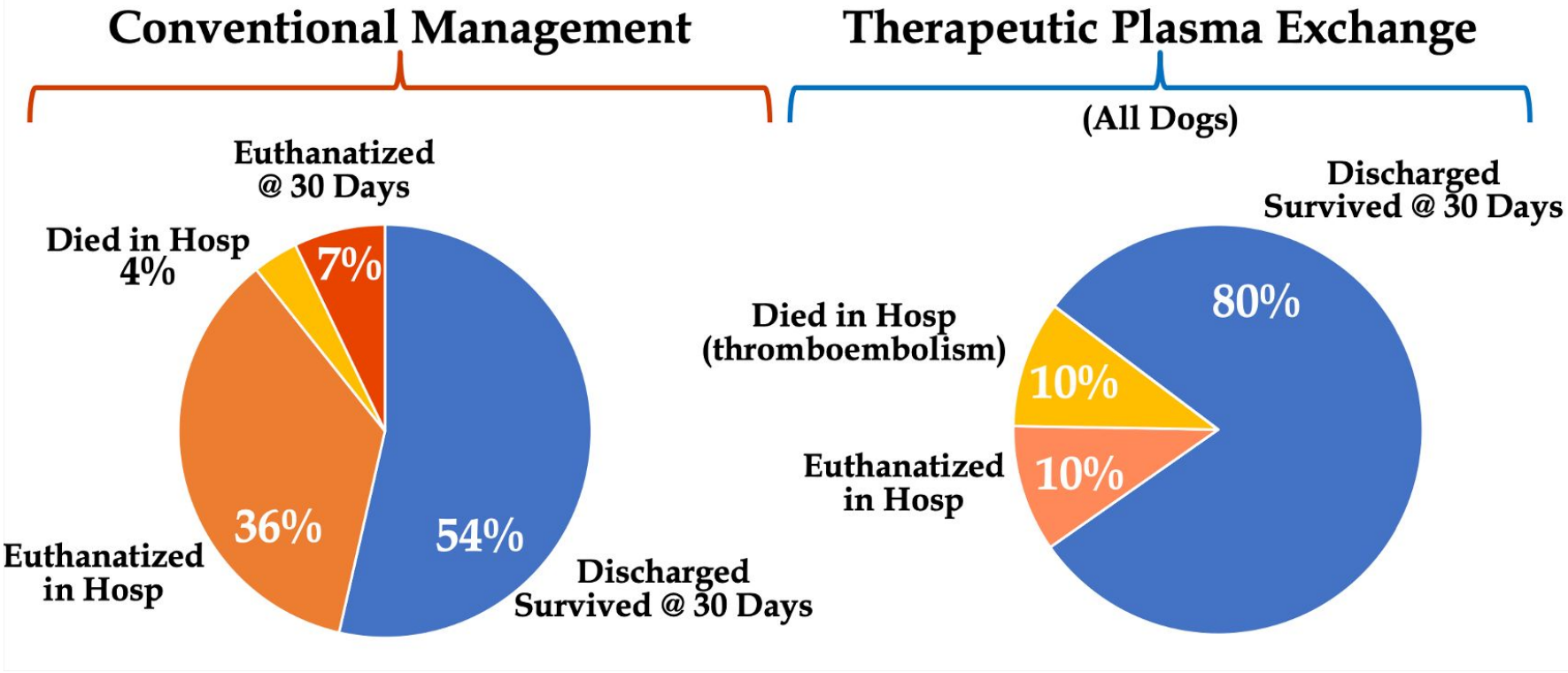
0.9 PV



1.5 PV



# Outcomes/Survival for Dogs Managed for IMHA



# IMHA - hIVIG

- Benefit is unclear / studies are lacking, but worth trying for refractory cases

Randomized Controlled Trial > J Vet Emerg Crit Care (San Antonio). 2009 Apr;19(2):158-64.
doi: 10.1111/j.1476-4431.2009.00403.x.

**Use of human immunoglobulin in addition to glucocorticoids for the initial treatment of dogs with immune-mediated hemolytic anemia**

Megan F Whelan [1], Therese E O'Toole, Daniel L Chan, Elizabeth A Rozanski, Armelle M DeLaforcade, Sybil L Crawford, Susan M Cotter

- IVIG did not improve response or shorten hospitalization

Journal of Veterinary Internal Medicine — Open Access — ACVIM

STANDARD ARTICLE | Open Access

**The use of high-dose immunoglobulin M-enriched human immunoglobulin in dogs with immune-mediated hemolytic anemia**

Jason P. Bestwick, Mellora Sharman, Nat T. Whitley, Caroline Kisielewicz, Barbara J. Skelly, Simon Tappin, Lindsay Kellett-Gregory, Mayank Seth

- Well-tolerated, but no significant advantage

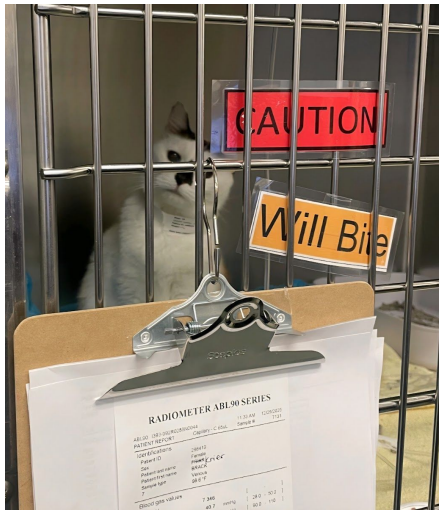
# What about PIMA?

**Clinical features of precursor-targeted immune-mediated anemia in dogs: 66 cases (2004–2013)**

(*J Am Vet Med Assoc* 2019;255:366–376)

- All received immunosuppressive therapy with steroids, 32/38 dogs that received secondary immunosuppressives got azathioprine
- 55 developed regenerative response at median of 29 days, 40 of those went into remission at median of 59 days after PIMA diagnosis
- Thromboembolic events confirmed for 9 dogs and assoc with decreased survival
- MST 913 days

# Immune-mediated thrombocytopenia (ITP)





# ITP

- Binding of antibodies to platelet surfaces, resulting in their destruction and leading to severe thrombocytopenia -> **Type II hypersensitivity reaction**

- **Primary ITP** is considered the most common cause of severe thrombocytopenia (<30K) in dogs.

- **Secondary ITP** develops as a result of an antigenic stimulus, typically drug, infectious disease, or neoplasia, leading to antibody production.

# Triggers for secondary ITP

- Retrospective, case-control study of 48 dogs failed to find association between recent **vaccination** (within 42 days) and onset of ITP
- **Drug-induced,** most commonly antibiotics eg. sulfonamides and cephalosporins. 5-7d exposure is typically required to produce sensitization and onset of thrombocytopenia, though reexposure could result in rapid thrombocytopenia.
- **Infectious:** A*naplasma phagocytophilum*, *Babesia sp*, *Ehrlichia canis*, *Leptospira sp*, *Leishmania infantum*.
- **Neoplasia:** Consumption due to bleeding tumor, DIC, splenic sequestration, decreased plt production due to myelophthisis, secondary ITP.
- **Inflammatory diseases** including chronic hepatitis, pancreatitis, SIRS have also been associated with platelet-bound antibodies in thrombocytopenic dogs.

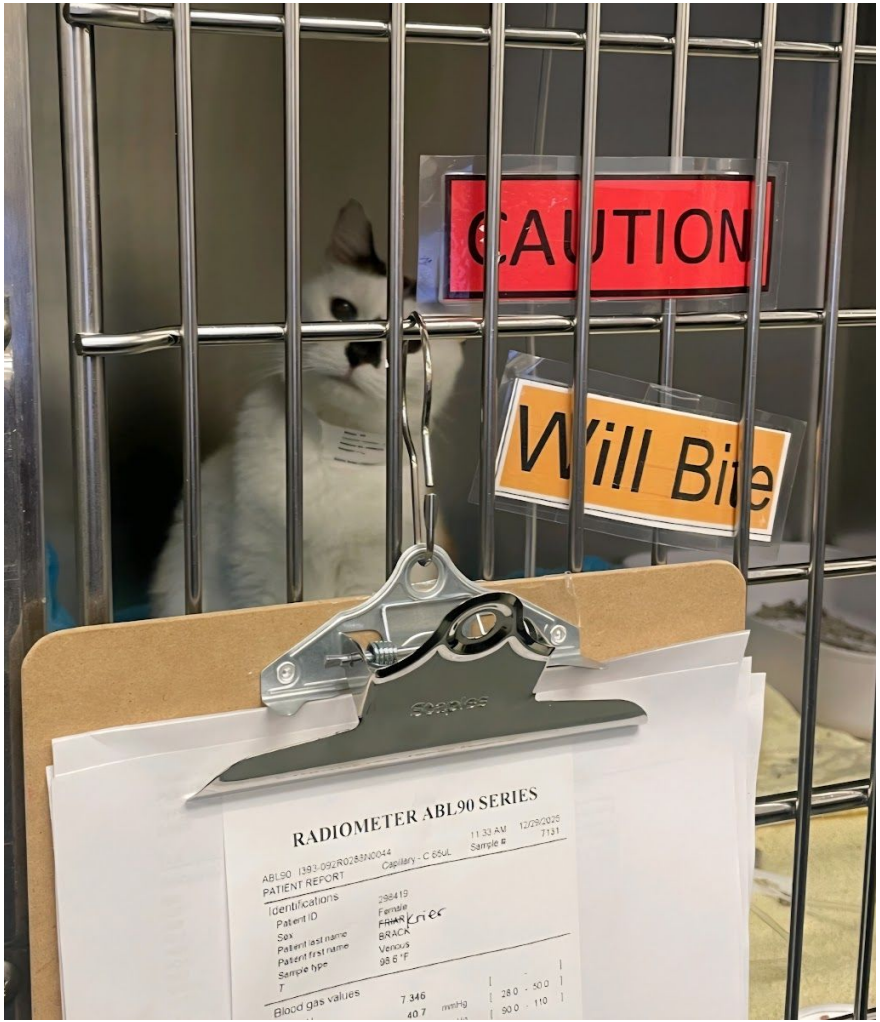
# Prevalence and disease associations in feline thrombocytopenia: a retrospective study of 194 cases

J. Ellis[1,*], R. Bell[1], D.C. Barnes[*] and R. Miller[*]

Journal of Small Animal Practice • Vol 59 • September 2018

In cats with severe thrombocytopenia (<50K plt, n 49):

- 11 unknown
- 8 cancer
- 6 primary ITP (less common than in dogs)
- 4 pos for FeLV
- 2 FIP



# ITP - Treatment

- Blood transfusions if clinical for anemia
  - Be more cautious with these compared to IMHA, can lose blood fast when GI bleeding.
- Immunosuppression with pred 2mg/kg/day (I usually dose at 50mg/m2/day in large breeds) +/- second agent. Begin tapering steroid after attaining durable remission (2-4 weeks). Total duration of immunosuppressive therapy 4-6 months.
  - Second agent options for dogs – cyclosporine, azathioprine, mycophenolate, leflunomide
  - Second agent options for cats – cyclosporine, chlorambucil

# ITP - Treatment

- Doxycycline 5mg/kg BID x 4 weeks (or until tick-borne testing is back)
- Consider vincristine 0.02mg/kg IV once
  - Stimulation of thrombopoiesis, accelerated megakaryocyte fragmentation, impaired platelet destruction.
  - For large dogs, I like to compare this dose with standard chemo dose of 0.65mg/m2 and go with whichever is lowest.
  - Not routinely used in cats
- Consider melatonin for relapsing cases
  - Aids megakaryocyte fragmentation (unlikely to hurt, might help)

# Vincristine - does it work?

**Comparison of platelet count recovery with use of vincristine and prednisone or prednisone alone for treatment for severe immune-mediated thrombocytopenia in dogs**

Elizabeth A. Rozanski, DVM, DACVECC, DACVIM; Mary Beth Callan, VMD, DACVIM; Dez Hughes, BVSc, DACVECC, Nancy Sanders, DVM, DACVIM, DACVECC; Urs Giger, Dr med vet, DACVIM

- Dogs that received prednisone and vinc 0.02mg/kg had a significantly faster increase in platelet count to >/= 40K (mean 4.9 +/- 1.1 days) than dogs that received pred alone (mean 6.8 +/- 4.5 days).

*J Vet Intern Med* 2013;27:536–541

**A Prospective Randomized Clinical Trial of Vincristine versus Human Intravenous Immunoglobulin for Acute Adjunctive Management of Presumptive Primary Immune-Mediated Thrombocytopenia in Dogs**

K. Balog, A.A. Huang, S.O. Sum, G.E. Moore, C. Thompson, and J.C. Scott-Moncrieff

- Dogs treated with either single dose hIVIG (0.5g/kg) or vinc (0.02mg/kg)
- Median platelet recovery time 2.5 days, no significant difference
- Median hospitalization 4 days, no significant difference
- No adverse effects in either group

# Vincristine - Adverse effects

Side effects:

- Nausea, vomiting, diarrhea
- Perivascular sloughing with extravasation
- Peripheral neuropathy
- Ileus in cats
- Potential for bone marrow suppression

# Vincristine - Adverse effects

Potential contraindications:

- Metabolized by the liver and excreted in bile. Avoid or dose adjust in liver failure, icterus.
- CAUTION IN MDR1 mutants!! Avoid or dose adjust.
  - P-glycoprotein efflux pumps are responsible for excretion of vincristine -> increased risk for cytopenias in MDR1 mutants.
- Increased risk for neutropenia/bone marrow suppression with cyclosporine.

Received: 10 July 2020 | Accepted: 18 December 2020

DOI: 10.1111/jvim.16029

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

# Neutropenia in dogs receiving vincristine for treatment of presumptive immune-mediated thrombocytopenia

Kathryn A. LaQuaglia[1] | James B. Robertson[2] | Katharine F. Lunn[1]

- Cyclosporine administration in combination with vincristine was associated with development of neutropenia in dogs with ITP.
- Cyclosporine inhibits P-glycoprotein pumps, which are responsible for excretion of vincristine.

Received: 10 July 2020 | Accepted: 18 December 2020

DOI: 10.1111/jvim.16029

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

# Neutropenia in dogs receiving vincristine for treatment of presumptive immune-mediated thrombocytopenia

Kathryn A. LaQuaglia[1] | James B. Robertson[2] | Katharine F. Lunn[1]

- Ideally wait a couple days to start cyclosporine after giving vincristine on admit, but in life-threatening cases, this may not be practical.
- Neutropenia is self-limiting, resolves with time.

# ITP - Prognosis

- Overall good prognosis in dogs, more guarded with GI bleeding.
  - Historic studies report 70% survival with steroids +/- second agent treatment, with improved platelets >50K by 1 week. ~30% death or euthanasia rates. Likely better than this now, with more recent studies reporting 10-15% death or euthanasia rates. (Nakamura et al, JVECC 2012)
  - Melena as a neg prognostic indicator, survival 60% compared to 90% without melena. (O'Marra et al, JAVMA 2011)
  - Relapse rates in literature range from 9-40%. (Nakamura et al, JVECC 2012)

# ITP - Prognosis

- Anecdotally more guarded in cats, more likely for severe persistent thrombocytopenia to be associated with cancer, more difficult to get them off drugs without relapse.

# ITP - Refractory Cases

- **Therapeutic plasma exchange (TPE) -** Directly remove antibodies, inflammatory mediators, immune complexes, cytokines that are causing platelet destruction.
    - Case series of 4 dogs with refractory ITP treated with 3 sequential sessions of TPE. All dogs failed medical management with pred/cyclosporine. 3 of 4 attained Plt >/= 40K after TPE. 1 dog did not respond and was euthanized. (Kopecny et al, JVIM 2019)

# ITP - Refractory Cases

- **Splenectomy -** Remove primary organ responsible for destruction of antibody-sensitized platelets.
    - Retrospective study in which 6/7 dogs with refractory ITP successfully managed with splenectomy. (Bestwick et al, JVIM 2022)

# ITP - Refractory Cases

- **hIVIG**
  - Fairly good evidence for benefit in ITP!
  - 2 randomized controlled trials showing accelerated platelet count recovery and shortened hospitalization time compared to glucocorticoids alone. Comparable in benefit to vincristine.

*J Vet Intern Med* 2013;27:536–541

**A Prospective Randomized Clinical Trial of Vincristine versus Human Intravenous Immunoglobulin for Acute Adjunctive Management of Presumptive Primary Immune-Mediated Thrombocytopenia in Dogs**

K. Balog, A.A. Huang, S.O. Sum, G.E. Moore, C. Thompson, and J.C. Scott-Moncrieff

*J Vet Intern Med* 2009;23:1071–1078

**A Prospective, Randomized, Double-Blinded, Placebo-Controlled Study of Human Intravenous Immunoglobulin for the Acute Management of Presumptive Primary Immune-Mediated Thrombocytopenia in Dogs**

D. Bianco, P.J. Armstrong, and R.J. Washabau

# ITP - Refractory Cases

- **Romiplostim -** Thrombopoietin receptor agonist, used in humans for refractory/relapsing ITP. Potential role for suboptimal platelet production.
    - 5-10 ug/kg SQ, can be repeated
    - Safe, no adverse effects in treated dog
    - Expensive! ~$6000 per 500mcg vial, not covered by Trupanion the last time we used this :(

Received: 7 August 2023 | Accepted: 31 May 2024

DOI: 10.1111/jvim.17131

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

# Romiplostim for treatment of thrombocytopenia in dogs: A retrospective assessment and clinical outcomes

Min-Ok Ryu[1] | Jin-Kyung Kim[2] | Ju-Hyun An[3] | Kyoung-Won Seo[1] | Ye-In Oh[4] | Hwa-Young Youn[1]

- Platelet count improvement in 90% of ITP dogs (18/20).
- Median time for platelet recover after administration was 4 days.
- Median time for platelet count normalization was 7 days.
- Survival to discharge for primary ITP with romiplostim was 85%.

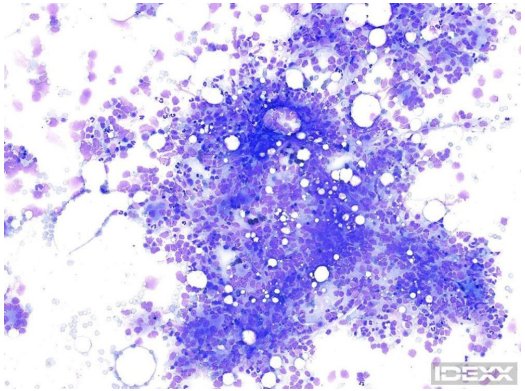
# ITP CASE: Amy, 5yo SF Mix

- Originally from Brazil, no recent vaccines or toxin exposures
- 8/4/25 Plt 0, 4DX neg, started pred
- 8/7/25 Plt 0, started leflunomide, continued to bruise/bleed, poor appetite

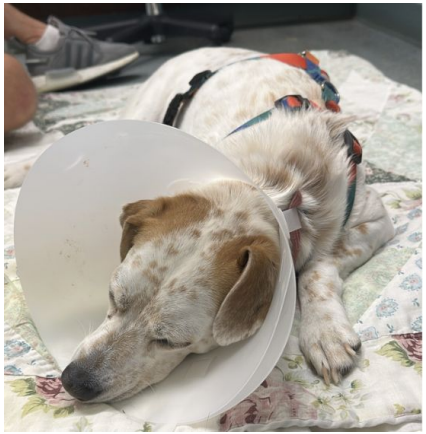
# ITP CASE: Amy, 5yo SF Mix

- 8/9/25 - 8/16/25 hospitalized at CCVS:
  - Plt 0, progressive regenerative anemia, received vincristine + started modified cyclosporine
  - CXR/AUS unremarkable; infectious disease PCRs (including leish) neg
  - 2 pRBC transfusions + fresh whole blood

# ITP CASE: Amy, 5yo SF Mix



- Bone marrow:
  - **Megakaryocyte hypoplasia** with dysplasia and atypical multinucleated cell forms
  - **Erythroid hypoplasia**
  - Myeloid hyperplasia



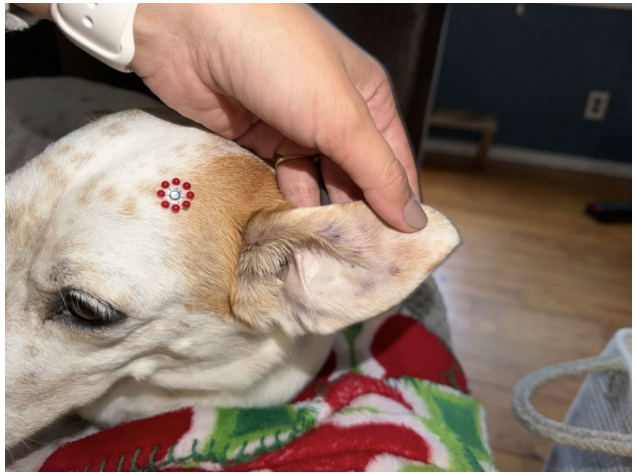
- Continuing triple agent therapy (pred, leflunomide, modified cyclosporine), also started darbepoetin

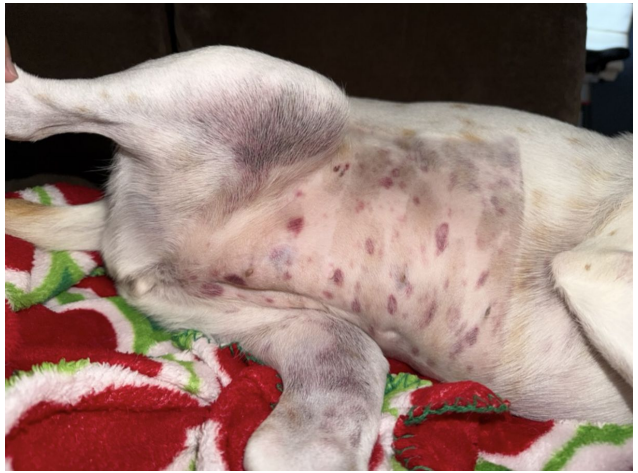
# ITP CASE: Amy, 5yo SF Mix

- Aug - Nov 2025, ITP in remission (yay!), tapered off leflunomide, maintained on slowly tapering pred +  modified cyclosporine

# ITP CASE: Amy, 5yo SF Mix

- 11/9/25 RELAPSE on modified generic cyclosporine 75mg BID (5mg/kg BID) + pred 5mg EOD; tried outpatient management with pred increased to 35mg/day





# ITP CASE: Amy, 5yo SF Mix

- 11/11/25 - 11/16/25 Plt 0, hospitalized due to failure of outpatient management
  - Received vincristine, IVIG 1g/kg IV infusion
  - 2 fresh whole blood transfusions

**Thank you Bernie!!!!**

# ITP CASE: Amy, 5yo SF Mix

- 11/11/25 - 11/16/25 Plt 0, hospitalized due to failure of outpatient management
  - Received vincristine, IVIG 1g/kg IV infusion
  - 2 fresh whole blood transfusions
  - Stabilized with modified cyclosporine 5mg/kg BID, pred 2mg/kg/day, added mycophenolate 10mg/kg BID and melatonin

# Cost of Care

- Aug 2025: $17,400
- Nov 2025 relapse: $16,700

trupanion™

Pets Best
PET HEALTH INSURANCE

# ITP CASE: Amy, 5yo SF Mix

- Amy's mom recently brought in some old meds for donation, and discovered that just prior to relapse, local pharmacy had mistakenly filled non-modified despite prescription being for modified cyclosporine

# ITP CASE: Amy, 5yo SF Mix

- **Non-modified and modified cyclosporine are NOT bioequivalent in dogs! Likely cause of relapse**


# Blood Donors Needed!

Contact: Sarah Kearney
bloodbank@capecodvetspecialists.com




# References


1. Assenmacher TD, Jutkowitz LA, Koenigshof AM, et al. Clinical features of precursor-targeted immune-mediated anemia in dogs:66 cases (2004-2013). *JAVMA.* 255(3):366-376.
2. Balog K, Huang AA, Sum SO, et al. A prospective randomized clinical trial of vincristine vs human intravenous immunoglobulin for acute adjunctive management of presumptive primary ITP in dogs. *JVIM.* 27(3):536-41.
3. Bestwick JP, Sharman M, Whitley NT, et al. The use of high-dose immunoglobulin M-enriched human immunoglobulin in dogs with immune-mediated hemolytic anemia. *JVIM.* 2022; 36(1):78-85.
4. Bianco D, Armstrong PJ, Washabau RJ. A prospective, randomized, double-blinded, placebo-controlled study of human intravenous immunoglobulin for the acute management of presumptive primary ITP in dogs. *JVIM.* 2009; 23(5):1071-8.
5. Ellis J, Bell R, Barnes DC, et al. Prevalence and disease associations in feline thrombocytopenia: a retrospective study of 194 cases. *JSAP.* 59(9):531-538.
6. Galati P, Archer T, Jolly R, et al. Effects of desmopressin acetate administration in healthy dogs receiving prednisolone. *JVIM*. 2021; 35(5):2271-2276.
7. Garden OA, Kidd L, Mexas AM, et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. *JVIM.* 2019; 33(2):313-334.
8. Hann L, Brown DC, King LG, et al. Effect of duration of packed red blood cell storage on morbidity and mortality in dogs after transfusion: 3095 cases (2001-2010). *JVIM.* 2014; 28(6):1830-7.
9. Ike J, Ueda R, Mukosaka T, et al. Cyclosporine-induced psoriasiform-lichenoid dermatosis in four dogs: Successful treatment with antibiotics and cyclosporine dosage reduction. *VetRecord. 2024;* 12:e984.
10. LaQuaglia KA, Robertson JB, Lunn KF. Neutropenia in dogs receiving vincristine for treatment of presumptive ITP. *JVIM.* 2021; 35(1):226-233.
11. LeVine DN, Goggs R, Kohn B, et al. ACVIM consensus statement on the treatment of immune thrombocytopenia in dogs and cats. *JVIM.* 2024; 38(4):1982-2007.
12. LeVine DN, Kidd L, Garden OA, et al. ACVIM consensus statement on the diagnosis of immune thrombocytopenia in dogs and cats. *JVIM.* 2024;38:1958-1981.
13. Rozanski EA, Callan MB, Hughes D, et al. Comparison of platelet count recovery with use of vincristine and prednisone or

13. 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. JAVMA.2002; 220(4):477-81.
14. Ryu MO, Kim JK, An JH. Romiplostim for treatment of thrombocytopenia in dogs: A retrospective assessment and clinical outcomes. JVIM. 2024; 38(4):2158-2164.
15. Swann JW, Garden OA, Fellman CL, et al. ACVIM consensus statement on the treatment of immune-mediated hemolytic anemia in dogs. *JVIM.* 2019; 33(3):1141-1172.
16. Viviano KR. Glucocorticoids, cyclosporine, azathioprine, chlorambucil, and mycophenolate in dogs and cats. *Vet Clin North America.* 2022; 52:797-817.
17. Wallisch K and LA Trepanier. Incidence, timing, and risk factors of azathioprine hepatotoxicosis in dogs. *JVIM.* 2015;29:513-518.
18. Whelan MF, O'Toole TE, Chan DL, et al. Use of human immunoglobulin in addition to glucocorticoids for the initial treatment of immune-mediated hemolytic anemia. *JVECC.* 2009; 19(2): 158-164.
19. Woodward GM and JD White.The utility of screening diagnostic tests in identifying associative immune-mediated haemolytic anemia in dogs. *Aust Vet J.* 2020; 98(12):586-590.

# QUESTIONS?

Feel free to email any questions to
**laurencarvalho@capecodvetspecialists.com**

CCVS
CAPE COD
VETERINARY
SPECIALISTS

# NOTICE

CE credit certificates & presentation slides will be emailed to you. If you do not receive an email with this information within a week, contact Nichole - *nicholemanfredi@capecodvetspecialists.com*

CCVS
CAPE COD VETERINARY SPECIALISTS