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TREATING IMMUNE-MEDIATED DISEASES: BEYOND PRED

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Note: Doses here are taken from relevant consensus statements or formularies.[15] It is advised that doses are reassessed with another source prior to administering to patients

TO IMMUNOSUPPRESS OR NOT

When deciding whether to immunosuppress or not, there are a set of questions to answer that can be helpful before you proceed.

- Whether a confirmed diagnosis has been achieved. This can sound simple, but as several immune-mediated diseases have part of their diagnostic criteria as being whether they respond to immunosuppressive medications or not, then it's not always the case. Reviewing the case objectively to ensure your confidence in the diagnosis and excluded pertinent differential diagnoses is worthwhile.
- Whether there are any contraindications to immunosuppression? This could be to the concept of immunosuppression, such as evidence of concurrent infectious processes or at least one that hasn't been excluded. There may be specific contraindications, or considerations, for specific medications e.g. recent NSAID history and corticosteroid use
- Have all potential samples been collected pre-treatment? Some conditions can be difficult to diagnose once immunosuppressive medications have been commenced, including neoplasia, immune-mediated conditions and endocrine disorders. Prior to administering the immunosuppressives, it can be useful to have clinical samples pre-treatment retained e.g. EDTA for Coombs test in an anaemic dog or those with a lymphocytosis, or lymph node aspirates in those with a lymphadenopathy.
- Reviewing the patient for relevant co-morbidities, for example those with heart disease which may be more likely to go into congestive heart failure when corticosteroids are commenced, or those with concurrent hepatic or renal insufficiency may have altered pharmacodynamics.

CORTICOSTEROIDS

Corticosteroids are the mainstay of immunosuppressive therapy and one of the most prescribed medications in veterinary practice. They suppress the immune system universally, by up or downregulating protein production due to their impact on transcription and translation of cellular proteins. This does mean that even with parenteral administration there is a delay between administration and effect, with early effects seen at ~ 6 hours post administration.

Dosing

Prednisolone is one of the most prescribed corticosteroids. Dosing should also be targeted at lean weight in significantly overweight animals.

We have approximate dosing for Prednisolone which we select based on our aim of the treatment.

These are commonly quoted as:

- Physiologic 0.2 mg/kg/day
- Anti-inflammatory 0.5 mg/kg/day
- Immunosuppressive 1 – 2 mg/kg/day

These are guides rather than perfect fits, and signalment and concurrent disease states often influence this. With dogs with hypoadrenocorticism, many will have signs of steroid side effects at 0.2 mg/kg/day and require lower dosages for their glucocorticoid deficient component in maintenance dosing. Equally, when they are systemically unwell then they may need a greater dose than "physiologic" to compensate for the stress on the body. There is one retrospective study that demonstrated less PUPD and biochemical changes suggestive of side effects, when given once daily rather than the dose split into twice daily.[1]

Cats are relatively resistant to the side effect of corticosteroids in comparison to dogs and tend to tolerate higher doses better. There is also a size impact of steroid dosing, with larger dogs being more susceptible to side effects at lower doses than smaller dogs. To account for this, we dose dogs above 20kg based on their body surface area at an immunosuppressive dose of 50 mg/m^2.

Dosage
There will be times when parenteral corticosteroids are necessary. The most commonly used is Dexamethasone. As it is more potent than Prednisolone, to convert your calculated dose of Prednisolone to a dose of Dexamethasone, you divide the dose by 7. This means that for Dexamethasone:

- Physiologic 0.03 mg/kg/day
- Anti-inflammatory 0.07 mg/kg/day
- Immunosuppressive 0.14 – 0.28 mg/kg/day

Dexamethasone has the additional benefit of having negligible mineralocorticoid effects. This can be of particular use when dealing with patients where volume overload is a risk, such as anaemic cats or those with pre-existing heart or kidney disease.

ANTICIPATED CLINICOPATHOLOGICAL CHANGES
Haematology
A stress leukogram is the consequence of endogenous cortisol release and can be more apparent in those receiving exogenous glucocorticoids. A helpful acronym to summarise the stress leukogram is **SMILED** – **S**egmented (neutrophils) & **M**onocytes **I**ncreased, **L**ymphocytes & **E**osinophils **D**ecreased. The neutrophilia present is not typically left shifted or made up of toxic neutrophils, and the presence of either may raise suspicion for another cause of the neutrophilia.

Serum biochemistry
Liver enzymes, particularly alkaline phosphatase (AP), but also alanine transferase (ALT), can have their production increased directly, or steroid induced alterations to the hepatocytes resulting in enzyme release from cells. The steroid induced form of AP is not seen in cats. Cholesterol production will also be increased in response to steroids, for a multitude of reasons. Hyperglycaemia can also be seen due to the antagonism of endogenous insulin. The vacuolar hepatopathy that develops, due to increased glycogen storage in the hepatocytes, leads to cell swelling and altered bile flow, with an increase in bile acids seen at times. This hepatopathy can also be appreciated on ultrasound with an enlarged and hyperechoic appearance to the liver.

Urine analysis
As steroids interfere with ADH function, there is often an isosthenuria, or hyposthenuria in response to administration. In addition, there can be increased loss of protein from the kidneys, giving rise to a proteinuria, which can be significant.

Endocrine
Corticosteroids have a clear impact by suppressing the hypothalamic-pituitary-adrenal axis. With prolonged administration, the adrenals may be small or difficult to identify on imaging. If an ACTH stimulation test is performed shortly after commencing steroids, it is unlikely to have an effect, and normal stimulation is enough to exclude hypoadrenocorticism. ACTH administration that does not result in cortisol production in a patient receiving steroids is difficult to interpret and will likely need repeated once/if the corticosteroids are discontinued.

Thyroxine (T4) can be decreased by corticosteroids, whereas the TSH should remain unaffected, and this should be borne in mind when evaluating a patient receiving steroids. Studies have shown that after three

weeks of immunosuppressive prednisolone, the T4 and fT4 both returned to normal after one week of discontinuing the steroids. [2]

Adverse effects

Steroid side effects that are commonly encountered include **polyuria with a compensatory polydipsia**, due to interference with the normal ADH signaling. This is worth bearing in mind of this confounding effect when knowing the concentration of a urine is necessary, such as differentiating causes of azotaemia. **Polyphagia** and **panting** are commonly encountered clinical signs. Longer term there will be the alteration of handling fat and muscle, with larger dogs being more susceptible to marked **muscle atrophy**, particularly around the head, as well as the abdominal musculature giving rise to the **potbellied appearance**. They will also slow **wound healing** and predispose to surgical site infections, which may be of particular concern in sites where surgical site dehiscence could be catastrophic.

Gastrointestinal ulcerations are a common finding in patients receiving prolonged steroid courses. These will not always give rise to appreciable melaena, but there can be significant enough blood loss to contribute to the anaemia.[3] There is some evidence that Omeprazole can curtail a degree of this bleeding.[4] Co-administration of Prednisolone with Aspirin increases the lesion scores of the gastrointestinal tract. **NSAIDs and steroid co-administration** increases the risk of ulcers to such a degree that it is contraindicated. Ideally a 7-day washout period between NSAID administration ceasing and steroids commencing is recommended but may not always be feasible, in which case the risks of proceeding must be considered.

Corticosteroids have an impact on all stages of the coagulation pathway, from increasing platelet activity to decreasing fibrinolysis, with the net effect of **hypercoagulability**. This should be considered particularly in patients with a tendency to thromboembolic events and may be an indication for a second line immunosuppressive being commenced to allow a more taper of the corticosteroids.

Corticosteroids are strong antagonists of insulin. Whilst insulin is anabolic, corticosteroids are catabolic. The insulin antagonism can give rise to diabetes mellitus, which is of most concern in cats where type II **diabetes mellitus** and insulin resistance are more frequently encountered. The resultant weight gain, and altered metabolism, as well as susceptibility to secondary infections can all increase diabetic instability.

Another consequence of corticosteroids is the impact on the **diagnosis and treatment of neoplasia.** Pre-treatment with steroids can make it more difficult to reach a diagnosis, due to a degree of remission with the steroids. For this reason, samples should be obtained before commencing glucocorticoids e.g. lymph node aspirates, additional blood for flow cytometry, intestinal biopsies etc. The other effect with corticosteroids is that it may lead to a decreased responsiveness to definitive chemotherapeutic protocols, particularly if they have been administered for several days.

Tapering

Our hope is that the corticosteroid course has been effective at suppressing the immune system and we have seen the desired effect. The next decision is when to taper. There are various thoughts on when to start tapering immunosuppressive medication, but no conclusive recommendations. My approach is to maintain the immunosuppressive medications at the dose which achieved control of the condition for one month before tapering, provided side effects aren't significant.

Once the disease is confirmed to be in remission, usually requiring bloodwork and clinical examination, the corticosteroid dose can be reduced by 20 – 25%. This is then repeated every 2 – 4 weeks, confirming that the disease is inactive prior to the next reduction. In those receiving multiple immunosuppressives, we tend to wean the corticosteroids first as the side effects have the biggest impact on the owner and pet's quality of life, however finances may preclude this.

If a rapid wean is needed due to significant side effects or emergence of comorbidities, then the Prednisolone dose could be dropped to physiologic (0.2 mg/kg) or higher if there is concern that there is an additional glucocorticoid need (e.g. 0.5 mg/kg).

SECOND LINE IMMUNOSUPPRESSIVES

A second agent addition can have several indications:

- Corticosteroids alone are not immunosuppressing to a satisfactory degree
- Corticosteroid side effects are, or are expected to be, significant
- Cases where a rapid wean of corticosteroids is anticipated
- Large breed dogs where steroid side effects are anticipated
- When corticosteroids are contraindicated

Generally, when choosing immunosuppressives the mechanism of action and side effect profile should be considered. When there is a similar mechanism of action, such as with Mycophenolate and Azathioprine, then their use together should be avoided. Wherever possible, having a patient receiving three immunosuppressives should be avoided, and consideration given as to whether the original second agent is having an impact or should be exchanged for another.

There is evidence in human medicine that second line immunosuppressive use chronically can predispose to neoplasia, due to the impairment of immunovigilence for the development of cellular neoplastic characteristics. This is less studied in veterinary medicine, with some select papers in the literature, but may suggest that consideration should be given the balancing the risk of relapse with prolonged courses of immunosuppressive medications.

CYCLOSPORINE

Cyclosporine's mechanism of action involves forming a complex within cells causing inhibition of the enzyme calcineurin. Calcineurin is responsible for activating the transcription factor Nuclear Factor of Activated T Cells (NFAT). The result is that T cells can no longer produce interleukin 2 (IL-2), a major driver of T Cell activation and survival. As it is one of the licensed medications, we have more information compared to other immunosuppressives.

Dosage

Recommend dosing is 5 mg/kg PO q 12 hours.[15]

Therapeutic drug monitoring is an option for cyclosporine, by measuring the amount of active drug in the blood. Sampling times are at the trough point (immediately before the next tablet is due) and peak, which is 2 hours following administration. One limitation is that there is not conclusive evidence that blood levels correlate with the degree of immunosuppression achieved. Indications for use would be when toxicity is suspected or when there is a lack of response to treatment, helping with clinical decision making as to whether dosing should be altered or not.

Adverse effects

One of the major side effects encountered with Cyclosporine are gastrointestinal signs. These tend to be transient, and dose dependent, with spontaneous resolution being common without a need for dose reductions. Cats can become anorexic as an adverse effect.

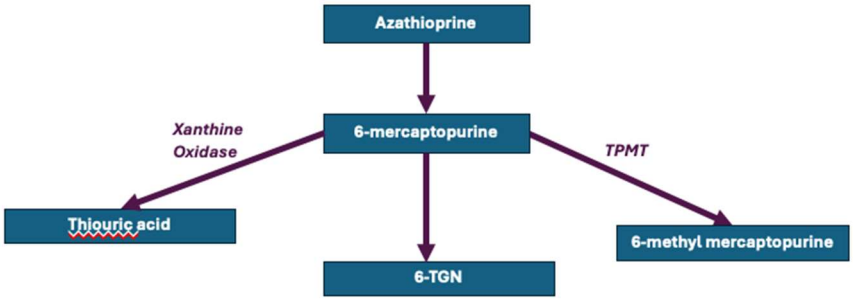
Less commonly, there can be more atypical side effects such as gingival hyperplasia, hirsutism and increased shedding.

As expected with all immunosuppressives, there can be a predisposition to secondary infections. One study identified that cutaneous fungal infections were more commonly encountered in those receiving Cyclosporine, and owners should be instructed of signs to monitor for to allow early intervention.[5] Some infections, such as Toxoplasma are combatted with cell mediated immunity, and therefore a deficit in this may predispose to infection, and consideration could be given to testing cats where latent infection is a high risk.

There is some evidence that long-term Cyclosporine can predispose to neoplastic conditions, with this predominantly demonstrated in cats that have received a kidney transplant.[6] These studies did not compare other long-term immunosuppressant medications, and it may be a reflection of an altered immune system rather than the specific agent which causes this predisposition.

A specific medication interaction to consider is that seen with Vincristine, which is commonly used to hasten the increase in circulation platelet numbers in those with immune-mediated thrombocytopenia. A retrospective study identified that those that had Vincristine and Cyclosporine co-administered were more likely to experience a neutropenia. Consideration should be given to staggered dosing, or alternative agent use.[7]

AZATHIOPRINE



Azathioprine works by competing with endogenous purines. It is a prodrug, and its downstream metabolites are similar enough to purines that it is inserted into DNA and RNA, disrupting mitosis. Lymphocytes are particularly sensitive to this as they lack a salvage pathway to synthesise purines in the way other cells can.

Enzymes convert the 6-mercaptopurine into inactive compounds by *Xanthine oxidase* and *Thiopurine methyltransferase* or an active compound, 6-TGN. This is important as a decrease in enzyme activity can result in a buildup of 6-mercaptopurine, increasing the risk of toxicity or increasing the enzyme activity which could result in fewer active metabolites and decreased efficacy as an immunosuppressive.

Cats have low TPMT activity, and therefore are at a high risk of toxicity, and therefore Azathioprine is to be avoided in this species. Drugs which interfere with *Xanthine oxidase* activity, such as Allopurinol, could also pose a risk of toxicity in dogs, and should be considered in patients with Leishmania.

Dosing

A dose of 2 mg/kg PO q 24 hours is recommended initially and then reduced to every other day dosing if the patient is responding.[15]

It can take several weeks, reported to be more than three weeks, for Azathioprine to have a therapeutic effect. Writing prescriptions as AzaTHIOprine, can help avoid accidental dispensing of Azithromycin.

Adverse effects

Gastrointestinal signs are encountered relatively commonly, as the GI tract has a high demand for purines reflecting their rapid division.

Myelosuppression and hepatotoxicity can be seen and are not typically dose dependent. Pancreatitis has been reported in humans receiving this medication, and there are some case reports in dogs; however, a definitive causation has not been proven.

With the potential adverse effects of Azathioprine known, regular haematology and biochemical assessment of liver injury and function are recommended. Discontinuation of the medication will often resolve the changes.

MYCOPHENOLATE MOFETIL

Mycophenolate mofetil is also a prodrug, becoming activated to Mycophenolic acid by gastric acid. This should be considered if the patient is receiving proton pump inhibitors, which may affect the bioavailability. The mechanism of action is the inhibition of the enzyme *Inosine monophosphate dehydrogenase* (IMPDH), which is responsible for producing purines. There are different isoenzymes of IMPDH, with Mycophenolate having a propensity to the form present in lymphocytes, making it relatively selective. As with Azathioprine, lymphocytes are particularly sensitive to the effects due to their lack of a salvage pathway to producing purines.

There are some papers where Mycophenolate mofetil was used as the sole agent, and response to treatment was seen within 2 – 6 days, confirming it can have a therapeutic effect relatively quickly.[8]

Dosing

A dosage of 8 – 12 mg/kg PO q 12 hours[15] is currently recommended in dogs. Parenteral formulations are available but need to be reconstituted very specifically.

Human formulations, typically 250mg capsules, can be used in larger dogs from 20 – 31 kg, or multiples of this weight e.g. 40 – 62 kg, which tends to be the less expensive option. For those that do not fall in these weight brackets, specifically formulated capsules should be used. Capsules should not be opened or split, and gloves worn when handling.

Mycophenolate can be used in cats, but a consistent dosing regime has yet to be established as there is inter-cat variability as to how they handle this medication.

Adverse effects

The side effect of Mycophenolate is that it results in gastrointestinal signs such as vomiting, diarrhoea and nausea. This tends to be dose dependent, and some cases will respond to lowering of the dosage to the lower end of the therapeutic range. A retrospective case series reported cytopenias were also encountered, but the numbers were small and therefore difficult to attribute causality.[9]

LEFLUNOMIDE

Leflunomide is a prodrug, converted to an active metabolite, and exerts its effect by inhibiting the enzyme *dihydro-orotate dehydrogenase*, essential in the production of pyrimidines. Again, lymphocytes lack a salvage pathway to accommodate this and are susceptible to the effects.

Dosing

In our institution we use 2 mg/kg PO q 24 hours[15]

Reported dosage rates range from 2 – 4 mg/kg PO q 24 hours. A retrospective study reported side effects to be more common at higher dosages, with no difference in those that responded or didn't at lower doses.[10]

Adverse effects

These can be variable and from a retrospective study they include gastrointestinal signs, thrombocytopenia, unexplained bleeding and lethargy.[10]

CHLORAMBUCIL

Chlorambucil is classically considered a chemotherapeutic but can be used in immunology. The mechanism of action involves cross linking DNA, preventing it being opened to allow transcription of RNA or replication of DNA. It is commonly used in cats, and they tolerate it relatively well.

Dosing
In cats, it is used at a dose of 2 mg PER CAT PO q 48 hours (or 72 hours for smaller cats)

In dogs, the recommended dosage is 0.1 – 0.2 mg/kg PO q 24 – 48 hours

Adverse effects

Myelosuppression is the main side effect and should be monitored for, particularly in the early stages of dose optimisation. The nadir is expected at 7 – 14 days, and this is when the myelosuppression is likely to be maximal.

In cats it has been reported to that Fanconi's syndrome has arisen in cats treated with Chlorambucil.[11]

INTRAVENOUS IMMUNOGLOBULIN (IVIG)

This is solution made up of human immunoglobulins pooled from multiple donors. The mechanism of action is thought to be complex, but one important aspect is the occupation of Fc receptors on phagocytic cells. With these receptors saturated, cells in circulation are spared destruction.

There is evidence that IVIG hastens the recovery of adequate platelet numbers in dogs with IMTP and should be a consideration in these patients in particular.[12]

Dosing

The dosing interval is large, reflecting the relatively few studies available, the immune-mediated thrombocytopenia ACVIM consensus statement recommends 0.5 grams/kg IV.[12]

Administration is the same as for a blood transfusion, starting with conservative rates and gradually increasing.

Adverse effects

As this is a foreign protein being administered, there is the risk of allergic transfusion reactions. Repeat doses may sensitise the patient to the antigens and whilst this has been tolerated in some, for others the patient developed anaphylaxis.

There is evidence that IVIG can predispose to a hypercoagulable state and increase the risk of thromboembolic complications.[13] For this reason, coupled with a limited effect in available studies, its use is not routinely recommended in dogs with IMHA.

The cost of this medication can be significant, particularly for larger dogs where several vials are necessary, potentially to the point of being cost prohibitive. This coupled with a relatively limited evidence base for most immune-mediated diseases in veterinary medicine means the use should be carefully considered.

THERAPEUTIC PLASMA EXCHANGE (TPE)

Therapeutic plasma exchange involves removing the patient's blood, and via either centrifuge or membrane, will separate the cellular from the plasma component. A proportion of the plasma can be discarded and replaced with balanced crystalloids and donor plasma. This has the effect of decreasing the quantity of circulating antibodies and providing time for the immunosuppressive medications to have an effect.

This does rely on having specialised equipment and facilities, as these patients are very labour and resource intense whilst the exchange is occurring.

The evidence is mainly in IMHA and IMTP, in those with disease refractory to standard care, where a positive outcome was achieved in 70 - 80% of cases. There are some case reports of other conditions being treated, such as myasthenia gravis.[14]

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