Evans Syndrome:
Breaking down IMHA and ITP

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Introduction

Immune-mediated haemolytic anaemia (IMHA) is the most common cause of haemolytic anaemia in dogs and immune-mediated thrombocytopenia (ITP) is the most common cause of severe thrombocytopenia in dogs.[1,2] Both can occur in cats, but are less common.[1] IMHA and ITP may occur simultaneously or separate from each other. When they occur together they are known as Evans syndrome, an autoimmune disease in which an individual's antibodies attack their own red blood cells and platelets.[1]

Pathophysiology

IMHA can be primary (idiopathic), or secondary (viral, bacterial, parasitic, drug or vaccine induced).[2] Approximately 60-75% of IMHA in dogs is primary IMHA rather than secondary.[2] Vaccines and some medications (cephalosporin's, trimethoprim-sulfa) have been reported as a secondary causes of IMHA.[1] In both primary and secondary IMHA, the destruction of red blood cells (RBCs) occurs because antibodies are produced against the red blood cell membrane antigens.[2] An antibody is a specialized immune protein which is produced in response to an antigen in the body and works to destroy the antigen. It is unknown what triggers this inappropriate antibody production in primary IMHA. Antibody attachment to cell membranes triggers RBC destruction by a number of different mechanisms. With high levels of antibody attachment the RBC membranes may become damaged so that water leaks into the cytoplasm.[4] This causes swelling and rupture of the RBC thus causing hemolysis.[4] Antibodies may bind to two different RBCs, which, in turn, causes the RBCs to clump together, also known as agglutination.[4] This clumping of RBC will slow down the passage of other RBCs through the vessels.

There is an increased rate of extra vascular haemolysis within the spleen and liver because of the removal of the antibody-coated RBCs by the macrophages.[3] While generally the antibodies attack the antigens of mature RBCs, in some patients, they may attack the precursors of RBCs in bone marrow.[4] In most IMHA patients regeneration of RBCs is apparent, but if the antibodies attack the bone marrow the patient may develop a non-regenerative IMHA.[4]

ITP, also written with the acronym of IMT, occurs also because of primary (idiopathic) or secondary (drug induced, neoplasia) reasons.[1] Similarly to IMHA, ITP occurs because antibodies are produced against platelet antigens which ultimately destroy platelets.[3] The major organ of immune-mediated platelet destruction is the spleen, which is also a major source of anti-platelet antibodies.[5] Platelet life span is correlated to platelet-associated antibody levels. In ITP patients, platelet life span is reduced from the normal four to eight days to usually less than one day.[5] In patients with high antibody levels platelets may survive less than one hour.[5] Platelets that survive usually function normally.

Immune-mediated haemolytic anaemia

IMHA can affect both dogs and cats, but is typically seen in middle aged dogs.[2] Dogs that have secondary IMHA due to disease are commonly spayed females.[3] There is some evidence of genetic predisposition in Cocker Spaniels and Miniature Schnauzers, but it is also prevalent in Poodles, Irish Setters, Dobermans, Lhasa Apsos, English Springer Spaniels and Old English Sheepdogs.[1,2,3] Roughly 60% of dogs with IMHA will also experience concurrent ITP (Evans Syndrome).[6]
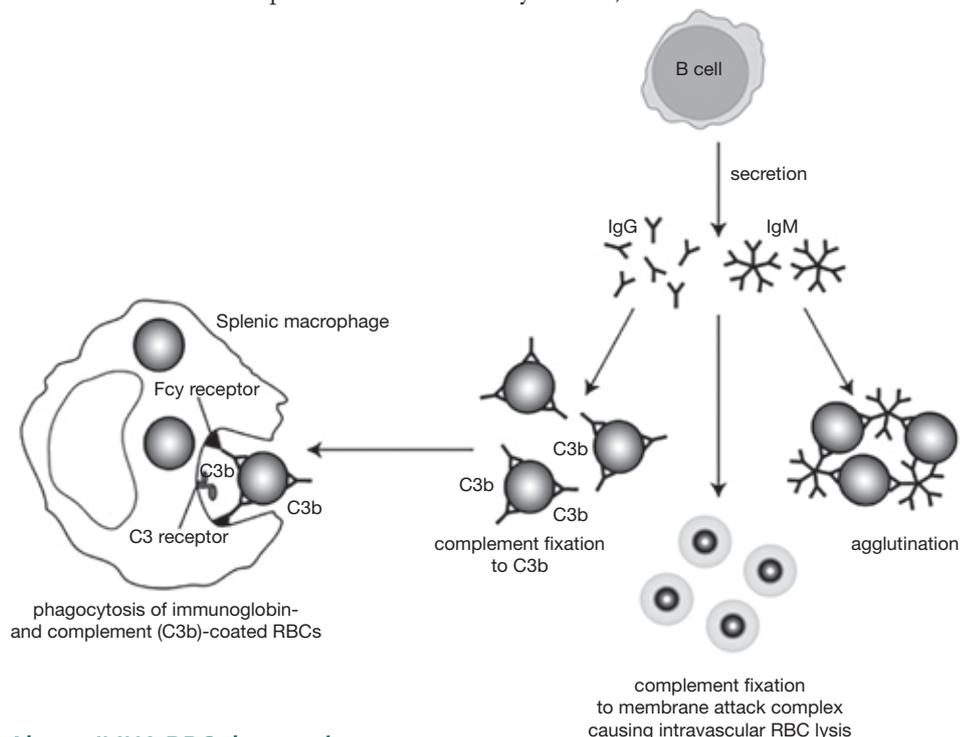
Above: IMHA RBC destruction
Image sourced from http://www.eclinpath.com/wp-content/uploads/IMHA-rbc-destruction.jpg

Because the animal's body is destroying its RBCs, it typically presents with signs of anaemia. Anaemic signs include weakness/collapse, lethargy, dull/depressed mentation, pale/white mucous membranes, bounding pulses, heart murmur and tachycardia.[1] Some patients may present with a fever due to the immune and/or inflammatory response while others may be hypothermic due to the anemia.[1, 4] The spleen and, less commonly, the liver may be enlarged since they are the major organs where RBC destruction takes place. As large quantities of RBCs are broken down, bilirubin is released into the blood stream which may overwhelm the liver causing some patients to become icteric.[5]

Diagnosis of IMHA

The *diagnosis* of IMHA relies heavily on blood work. On a complete blood count (CBC) 95% of dogs will have spherocytes (small, spherical RBCs).[9] Agglutination, also known as clumping, of the RBCs usually occurs.[1] There are several reported methods on how to perform a slide agglutination test, but all involve anticoagulated blood and 0.9% NaCl. One reference suggests taking one drop of anticoagulated blood (from a purple or capillary tube) and mixing with 10 drops of 0.9% NaCl on a slide.[1] Rouleax, which causes the RBC's to look like 'stacked coins' under the microscope, will disperse while agglutination will clump.[1] Another reference suggests taking equal parts of 0.9% NaCl

and anticoagulated blood, placing them on a slide and rocking it back/forth for a minute.[7] It is not possible to differentiate between rouleax and autoagglutination using this method. [7] Lastly there are other references that recommend one drop of anticoagulated blood should be mixed with "several" drops of 0.9% NaCl on a slide and rocked back/forth.[8] It is recommended to look at the slide using a microscope to identify rouleax versus agglutination. No matter the method used, a positive agglutination test does not confirm or rule out IMHA, but it does mean the condition is acute and severe.[4]

A CBC should always be submitted to an outside laboratory to check for reticulocytes. Reticulocytes are immature red blood cells. The presence of them indicates a functioning bone marrow and often a better prognosis for the pet. Reticulocytes will circulate for about a day in the blood stream before developing into mature red blood cells. Reticulocytes are found in approximately 60-70% of IMHA patients and the number of reticulocytes is relative to the degree of anemia.[2]

The Coombs' test, also known as a direct antiglobulin test (DAT), offers a more conclusive diagnosis for IMHA.[9] The Coombs' test detects antibodies that are attached to the RBCs[9], and consists of running a series of dilutions until agglutination occurs. False

positives can occur, but they are rare. False negatives can occur because of a low amount of circulating antibodies.[9] If the patient receives a blood transfusion or if there is a large amount of agglutination a direct Coombs' test will not be accurate.[1, 9]

Flow cytometry is a newer test for evaluating antibodies in dogs.[10] It allows for the detection of red cell surface bound immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies.[10] Anaemic dogs are more likely to be positive for antibodies (IgG, IgM, or both) than nonanaemic dogs.[11] However, dogs with IMHA have significantly higher percentages of the antibodies than other dogs that are anaemic for non-IMHA causes.[11] Flow cytometry for the detection of IgG on RBCs is highly sensitive and, therefore, very specific for the diagnosis of IMHA.[11]

In cats it is important to test for feline leukaemia and *Mycoplasma haemofelis* as they are often the cause of IMHA.[3] The organism will fall off red blood cells in samples that are greater than 24 hours old. Therefore it is best to make a blood smear with a fresh sample.[12] In an acute infection the organism is only visible 50% of the time.[12] In cats the anaemia is often regenerative and therefore offers a better prognosis.[3]

Complications of IMHA

The *complications* of IMHA are vast. These include thrombocytopenia, disseminated intravascular coagulation (DIC), thromboembolism, gastrointestinal (GI) ulceration, renal failure and refractory anemia.[2] Thrombocytopenia can occur because of ITP (Evans Syndrome) or because of platelet consumption.[2] The exact physiology of why ITP occurs concurrently with IMHA is unknown.[13]

DIC can be triggered for a variety of reasons including thromboplastic substances released from the RBC membranes or tissue ischemia from hypoxia,[2] Ultimately coagulation times should be checked and patients should be monitored for signs of excessive bleeding, petechiae and ecchymoses.

Thromboemobolism, particularly pulmonary thromboembolism, can occur in patients with IMHA, but it is unknown how frequently this occurs. The exact pathogenesis remains unknown, but the release of thromboplastic substances from RBCs, high volume of circulating bilirubin, venipuncture,
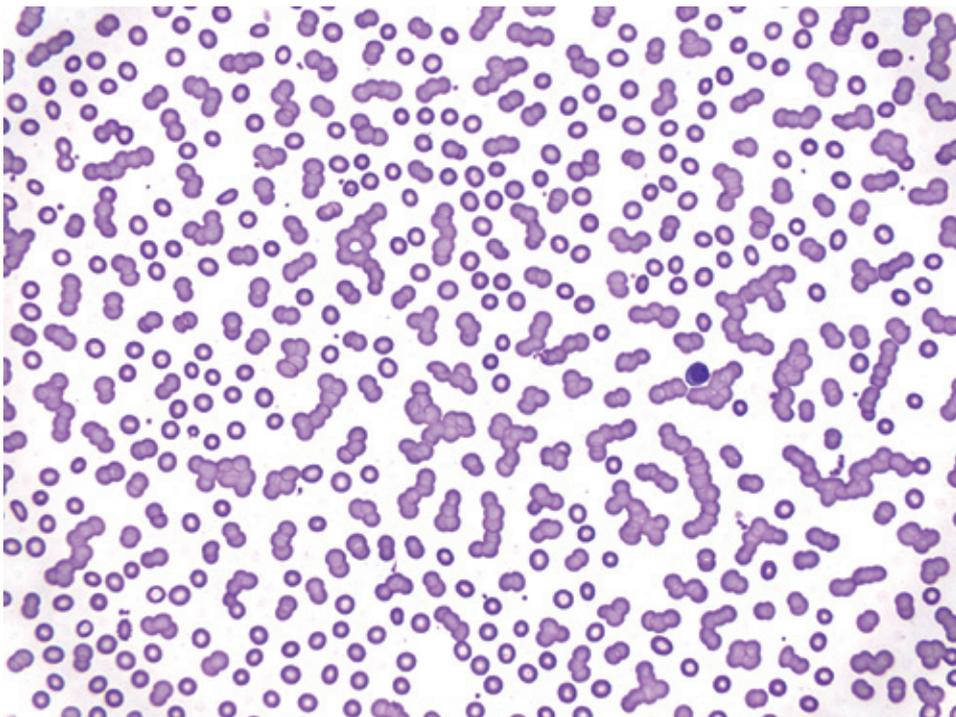
Above: Note the stacking appearance of the RBC's
Image sourced from http://www.myelomapennstate.net/images/DrBayerl/Rouleaux50x.jpg.

catheters, and immunosuppressive agents may be contributing factors. [2,14] Pulmonary thromboembolism should be suspected in patients that suddenly develop respiratory distress. [4]

Gastrointestinal ulceration and bleeding can occur secondary to DIC, the use of glucorticosteroids, ischemic injury to the GI mucosa and thrombocytopenia. [2] GI protectants are frequently used in these patients for these reasons. Renal injury can occur because of hypoperfusion, vasoconstriction, ischemia and dehydration. [2] Patients may experience refractory anaemia because of a direct immune injury to erythroid precursors, bleeding from GI tract or suppressed erythropoiesis by immunosuppressive agents. [2]

Treatment of IMHA

Treatment first involves dealing with the anaemia. This may include oxygen supplementation and/or red blood cell transfusion. There are valid concerns that transfusing a patient may worsen the IMHA, thus the recommendation that patients should not be transfused until they have a packed cell volume (PCV) less than 20–22%. [4] If patients with a PCV greater than 22% are transfused there is an increased risk of thromboembolism. [4] Haemoglobin based oxygen carriers are no longer available, but should they be available they offer greater oxygen carrying capacity then red blood cell transfusion and quickly alleviate signs of hypoxia. [4]

Fluid therapy is important. Stabilization of patients is usually achieved through the use of crystalloids. Maintenance of tissue perfusion is equally important, even when it results in further lowering of the hematocrit. [14] Fluids are also important in maintaining renal perfusion and helping to deal with the high levels of circulating bilirubin. [10] Patients experiencing DIC should receive fresh frozen plasma.

Treatment may involve dealing with any underlying diseases that may have caused the IMHA (rickettsia, *Mycoplasma*, etc.). If the pet is experiencing primary IMHA, immunosuppressive drugs are given along with GI protectants to prevent GI bleeding.

Corticosteroids (prednisone, prednisolone) are the primary drugs used for helping to suppress the immune system. [13] High doses of corticosteroids help to reduce phagocytosis of RBCs, decrease production of cytokines and decrease the production of

immunoglobulin IgG. [3,16] Steroids are not as beneficial when dealing with intravascular haemolysis that is mediated by IgM, so in those cases other immunosuppressive agents (cyclosporine, cyclophosphamide) can be used. [3] Unfortunately, the studies involving other immunosuppressive agents are limited and have shown they are not any more effective then corticosteroids. [16] The most common corticosteroids are prednisone or prednisolone. [16] The difference between prednisolone and prednisone is how they are metabolized. Prednisone is metabolized by the liver while prednisolone is not. Therefore prednisolone is generally considered easier in patients with liver disease. Response to corticosteroids includes rising haematocrit, adequate reticulocytes, reduced spherocytes, and reduced agglutination of the RBCs. [2]

Azathioprine is a purine (mimics DNA and RNA) analogue immunosuppressive drug that is commonly combined with prednisone therapy. [16] Since it is commonly used with prednisone, its efficacy alone is unknown. Reports and studies are variable when it comes to its effectiveness when used with prednisone. It is thought to have a synergistic effect which allows for a faster reduction of prednisone. [6] Azathioprine is low-cost and well tolerated in dogs which is why it's an attractive choice as an additional line of defense. [17] Unfortunately it can take up to six weeks for the medication to take full effect so it is used in conjunction with other medication. [17]

In humans intravenous immunoglobulin (IVIG) is considered a first line treatment and is used in cases where prednisone doses are dangerously high or are ineffective. [13] There are several studies showing evidence that IVIG competitively inhibits the binding of canine IgG making it effective for IMHA. [17] The mechanism of action of IVIG is not completely understood, but it may work on the cytokine network and help to neutralize autoantibodies. [16] There have been no studies with regards to only IVIG being used by itself, so it is recommended that it is used in conjunction with prednisone or after an attempt with prednisone has occurred. [13] Unfortunately due to expense, periodic limited availability, potential increased risk of thromboembolic disease and the concern of the pet building a hypersensitivity reaction to the human product it is usually not the first choice of treatment. [16]

Lastly both cyclosporine (Atopica™, Novartis) and danazol (Danocrine, Winthrop Pharmaceuticals) have both been used to treat IMHA as secondary line medications. [17] Cyclosporine has been widely used in people for IMHA as well as for transplantation surgery to help decrease factors that stimulate an inappropriate autoimmune response. [17] Cyclosporine is generally well tolerated in dogs, but rare gastrointestinal signs can occur as a side effect which resolve after the drug is discontinued. [17] The efficacy of danazol is not supported by any published reports and is rarely used perhaps because it can be hepatotoxic in dogs. [17]

A splenectomy can be performed, but is recommended as a last attempt in treatment due to the numerous risk factors of putting the pet under a general anaesthesia who is anemic. [17] A splenectomy may help to decrease RBC destruction in patients with IMHA. [16] It is not recommended if the pet also has ITP as the pet will likely bleed from the surgery and die as a result of its inability to clot. In humans where a splenectomy was performed on patients with Evans sydrome, the patient's response was poorer than those just receiving medical management. For patients who experience reoccurrences of Evans syndromes a splenectomy may be an option.

Prognosis

Prognosis depends on numerous things, but is more favourable in the cat than the dog. The number of blood transfusions required is a negative prognostic factor for IMHA. [4] This could be because of the severity of the disease or because of the transfusion complications themselves. Dogs with Evans syndrome have a worse prognosis than those that have IMHA or ITP alone. [18] Patients who experience DIC have a worse prognosis. [14]

Immune-mediated thrombocytopenia

While not as common as IMHA, ITP occurs in dogs and less commonly in cats. [19] There are numerous similarities between IMHA and ITP. It is more common in middle aged females. [19] Breeds that are predisposed include Airedales, Dobermans, Old English Sheepdogs, Cocker Spaniels and Poodles. [3]

Clinical Signs of ITP

Clinical signs include petechial haemorrhage, ecchymoses, melena, haematuria, retinal haemorrhage and epistaxis. A worsening of the signs occurs in patients with severe thrombocytopenia (<10,000 platelets/μL). [6]

Diagnosis of ITP

A *diagnosis* is performed by obtaining a platelet count. An in-house count can be performed by counting 10 high power fields, and divide by 10 to get the average per field.[1] Then multiple by 15,000 to obtain the total estimated platelet count.[1] A normal patient should have between 200,000-800,000 cell/μL.[1, 20] While a low in-house platelet test may suggest ITP, it is best to send a CBC for a manual platelet count to be read. A platelet count of less than 30,000 in addition to a low mean platelet volume (MPV) is highly suggestive of ITP.[1] MPV (the average size of platelets) is increased when bone marrow has an active response to an insult (like an immune response). The bone marrow will release a high number of immature platelets which will increase the average size of the platelets, thus increasing MPV. Conversely the number of megakaryocytes in the bone marrow will be reduced. A 2008 study showed MPV to be almost 100% reliable when differentiating ITP from other causes of thrombocytopenia.[21] An ELISA test for the presence of antiplatelet antibodies can be performed though it is not very specific to ITP.[10] A negative results will rule out ITP, but a positive result just indicates thromocytopenia.[10] ITP is ultimately a disease of rule-outs.

Complications of ITP

The biggest *complication* of ITP is DIC. Coagulation times should be checked and patients should be monitored for signs of excessive bleeding, petechiae and ecchymoses.

Treatment of ITP

Treatment is similar to that of IMHA. While transfusing with red blood cells is done to prevent life threatening anaemia in IMHA patients, the transfusion of platelet rich plasma or whole blood is not as common. Unfortunately platelets are extremely fragile and, if transfused, the patient's immune system will often destroy new platelets within hours.[10] One application of transfusing is for those patients where a splenectomy has been chosen as a method of treatment.[10] Transfusing patients with whole blood right before surgery may decrease the rate of bleeding.

Patients with ITP will be put on corticosteroids (prednisone, prednisolone) and other adjunctive therapies may also be added (azathioprine, cyclophosphamide, danazol, cyclosporine).[20] Vincristine may be added to the therapy because it interferes with some immune system effects on the platelets and also helps to mature megakaryocytes into functional platelets faster.[20] Several studies have shown that the addition of vincristine along with prednisone helped dogs reach 40,000 cells/μL faster than prednisone alone.[20]

Nursing care

Patients with Evans syndrome often require intensive veterinary nursing care which is dependent on their degree of illness. Upon initial presentation these patients may require oxygen supplementation. Oxygen should be provided initially by the least-stressful route.[22] Oxygen hoods made from elizabethan collars tend to be well tolerated. Oxygen hoods tend to provide quick relief and FiO_2 levels can get up to 60% oxygen very quickly.[23] Small oxygen cages can be utilized for cats and FiO_2 levels can get up to 40%, but take anywhere from 25 minutes for the FiO2 to reach a level greater than 40%.[23] You also cannot work with your patient if they are in a cage. Flow-by oxygen efficacy is still debated since it is unknown how much of the oxygen the animal actually inhales. The oxygen tubing must be inches away from the animal's nose in order for it to be effective.[23] Long term oxygen therapy can include the use of oxygen cages or nasal oxygen lines. Patients may need arterial blood gas or pulse oximetry performed to monitor overall oxygenation ability.

Blood transfusions may be needed and will require diligent monitoring. Patients should be monitored for any signs of a blood transfusion reaction including urticaria, vomiting, collapse, fever, shaking or panting.

Patients will also need to be monitored for blood transfusion illness, which can take up to five days to occur.

Physical exams should occur minimally every eight hours and include a heart rate, pulse rate, respiratory rate and effort, mucous membrane colour, capillary refill time, rectal temperature and neurological status. If there is any change from normal parameters, the veterinarian should be notified. Besides a stethoscope and thermometer to monitor vitals there are a couple other tools which can be utilized to improve patient monitoring: blood pressure, lactate and central venous pressure (CVP). Because patients are at risk for DIC it is important to look for early signs, which include excessive bleeding after venipuncture and/or petechiae on the gums, pinna or abdomen.

It is important that IMHA patients have their blood pressure monitored minimally every eight to twelve hours. If the mean arterial pressure (MAP) falls below 60 mmHg, the kidneys and other organs are not appropriately perfused putting the patient at risk for organ failure. Normalization of blood pressure, defined by a of MAP of 80-120 mmHg or systolic between 110-160 mmHg, is the goal in any Evans syndrome patient.[25]
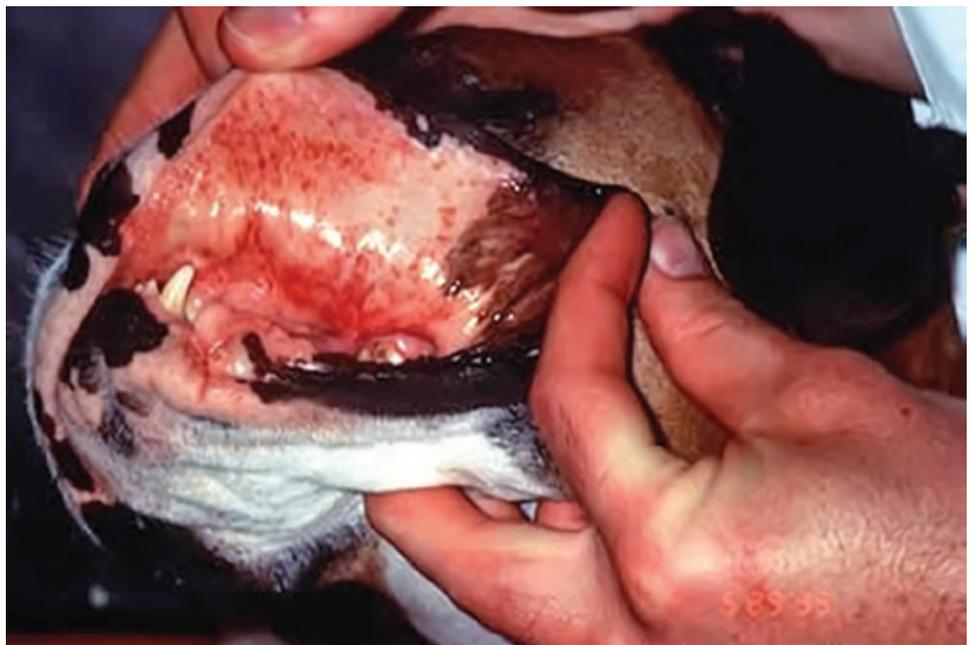
Above: Note the petechial haemorrhage on the gums
Image sourced from http://www.hazeldeananimalhospital.ca/happy-tails/

Lactate accumulates in the tissues and blood as a result of inadequate oxygen availability, caused by tissue hypoperfusion, and can occur in Evans syndrome patients. Lactate can be measured using a simple hand-held device similar to a blood glucose machine. It is important to normalize lactate concentrations through fluid therapy, blood pressure normalization, and providing adequate oxygen delivery to the tissues.

Since IMHA patients often require frequent blood draws, a central line should be placed. While the internal and external jugular veins are usually used for central catheter placement, it is not recommended for patients at risk of thrombus. The lateral saphenous vein is an alternative for use in dogs. While this can be used in cats also, the medial saphenous is more commonly used. If the patient is experiencing Evans syndrome then venipuncture should be avoided on large vessels unless the veterinarian feels the patient has adequate platelets.[1] If a central line is placed, CVP can be performed. CVP is generally monitored when a patient is prone to changes in blood pressure or when aggressive fluid therapy is being utilized.

Urine output should be monitored and recorded. Quantifying urine output is key in monitoring fluid therapy as well as in patients with renal disease. In both dogs and cats, one to two ml/kg/hr of urine should be produced (if the patient is not on fluids) or the volume out should equal the volume given.[24]

Lastly, nutritional support must be considered in Evans syndrome patients that are hospitalized. Providing nutritional support to these patients early is essential in order to minimize weight loss and to provide adequate energy for metabolic support.[26] In both human and veterinary patients a better outcome is seen in those that receive nutritional support earlier.

Since some of these patients may present collapsed and unable to walk at all, it is important to ensure the patient is kept clean, dry and on adequate bedding. Patients who do not turn themselves should be turned to prevent atelectasis of lung lobes.

Ultimately the patient's condition may change quickly depending on the progression of the disease. It is imperative that appropriate veterinary nursing care is provided to them to allow for the best outcome.

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