Association of azathioprine and prednisone in the control of immunomediated hemolytic anemia in dogs

Associação de prednisona e azatioprina na terapêutica de cães portadores de anemia hemolítica imunomediada

DOI: 10.34188/bjaerv4n2-098

Recebimento dos originais: 04/01/2021
Aceitação para publicação: 31/03/2021

Rodrigo Prevedello Franco
Doutor na área de Clínica Médica de Pequenos Animais - Cardiologia Veterinária, pela FCAV-UNESP/Campus Jaboticabal
Universidade de Marília/UNIMAR
Instituto Qualittas de Pós-Graduação
Specialli Inst. Veterinário
Endereço: Av. Higino Muzi Filho, 1001 - Mirante, Marília - SP, 17525-902
E-mail: vetrpf@yahoo.com.br

Ana Flávia Mendonça
Médica Veterinária pela Universidade Paulista
Instituição: Hospital Veterinário Unimar
Endereço: Avenida Itu,271, bairro cascata, Marília - SP, Brasil, 17515-030
E-mail: mendoncaanaf@gmail.com

Thaís Yuri Nakamura
Médica Veterinária pela Universidade de São Paulo/Faculdade de Zootecnia e Engenharia de Alimentos
Instituição: Hospital Veterinário Casa do Criador
Endereço: Rua Almirante Barroso, 204 -Palmital, Marília - SP, Brasil
E-mail: thais.tyn@gmail.com

Thainá Pires dos Santos Sauniti
Aprimoramento em Clínica Médica de Pequenos animais - Universidade de Marília
Instituição: Hospital Veterinário Casa do Criador
Av. Higino Muzi Filho, 1001 - Mirante, Marília - SP, 17525-902
E-mail: thaai.sauniti@hotmail.com

Beatriz Teixeira Martuchi
Aprimoranda em Clínica Médica de Pequenos animais - Universidade de Marília
Instituição: Hospital veterinário da unimar
Endereço: Avenida Itu,271, bairro cascata, Marília - SP, Brasil, 17515-030
E-mail: martuchibeatrizteixeira@gmail.com

ABSTRACT
Immune-mediated hemolytic anemia (IMHA) is an immunological disease, in which the immune-mediated destruction of erythrocytes occurs, leading to a prominent drop in globular volume (GV). The diagnosis of the disease must be made through the association of clinical manifestations with specific complementary exams, with therapy aimed at inhibiting intravascular hemolysis in the
control of GV. The present study aims to report the therapeutic association of prednisone and azathioprine in the control of IMHA in two dogs, demonstrating its evolution and the clinical effects of the pharmacological association. At the Veterinary Hospital of UNIMAR, a canine, female, Blue Heeler breed, seven years old and a canine, male, Border Collie breed, seven months old, both showing apathy, hyporexia, progressive weight loss, regenerative anemia, leukocytosis, and test of positive saline autoagglutination. After IMHA was diagnosed, polytherapy with prednisone (2mg/kg/SID) associated with azathioprine (2/mg/kg/SID) was instituted. Initially, the first animal showed liver damage due to the side effects of the drugs. As for the control of IMHA, the two animals described showed improvement and stabilization of the clinical and hematological condition after the association, making it possible to reduce the dosages of both drugs without worsening the conditions and ceasing the side effects caused by the chronic use of the drugs. Through this report it is possible to conclude that the use of the therapeutic combination of prednisone and azathioprine for the control of IMHA has greater benefits, being possible to use lower doses of both drugs, reducing their side effects and, consequently, resulting in a better survival.

**Keywords:** Azathioprine, Immune-mediated hemolytic anemia, Prednisone.

---

### Introduction

Immune-mediated hemolytic anemia (IMHA) is a disease characterized by a prominent drop in globular volume (GV) due to an immune response, being primary or idiopathic, when there is no apparent cause, or secondary to infectious, neoplastic diseases or even in iatrogenic cases through medications or vaccinations (MCCULLOUGH, 2003, GARDEN et al., 2019). IMHA can be shown
in dogs of any breed. As for the age, young or middle-aged dogs are more common and do not have sexual predisposition (MILLER et al., 2004). The clinical signs are not pathognomonic, being more frequent to present: weakness, mucosal pallor, jaundice, anorexia, vomiting, dyspnea, tachycardia, fever, hepatomegaly, hemoglobinuria and bilirubinuria (BALCH; MACKIN, 2007).

According to the ACVIM guidelines, for the diagnosis of IMHA in dogs and cats, there is no a test considered to be the gold standard. Therefore, it is necessary to associate clinical manifestations of hemolysis such as hyperbilirubinemia and hemoglobinuria with complementary tests such as blood count showing anemia, spherocytes and leukocytosis; agglutination test in positive saline; coomb test. In addition, the positive response to immunosuppressive treatment is also a great indicator of the disease (GARDEN et al., 2019).

Therefore, the goal of treatment is to inhibit hemolysis so that the GV is stabilized. Immunosuppressive treatment is recommended with drugs that have this mechanism of action. The drugs of choice are glucocorticoids, such as prednisone (2 to 3 mg/kg/SID) (SWANN et al., 2019). This drug has anti-inflammatory and immunosuppressive effects, however its chronic use can trigger a series of side effects (PEREIRA et al., 2011). At the beginning of treatment for IMHA, there are greater recommendations for the use of corticosteroids as monotherapy, however, another immunussuppressive drug may be associated in cases of immediate risk of the animal's life; when there is no maintenance or increase in GV during the first week of treatment or due to the deleterious effects of high doses of corticosteroids. The drugs that can be used in combination with prednisone are azathioprine, cyclosporine, mycophenolate and leflunomide (SWANN, et al., 2019). Azathioprine is used in the veterinary routine as an immunosuppressive drug. They are widely used in association with corticosteroids, with the aim of reducing their dose so that there are less deleterious effects. However, it is known that the use of azathioprine can also lead to side effects such as bone marrow suppression and hepatotoxicity (NETO et al., 2008). Swann and Skelly (2013) reported that studies on the association of prednisone with azathioprine for the treatment of IMHA show good results in terms of response to treatment and longer animal survival.

2 OBJECTIVE

The present study aims to report the therapeutic association of prednisone and azathioprine in the control of IMHA in two dogs, demonstrating its evolution and the clinical effects of the pharmacological association.
3 DEVELOPMENT

CASE 1

A canine, female, Blue Heeler breed, seven years old, weighing 19.85 kg, arrived for clinical care showing apathy, hyporexia, progressive weight loss and emesis with evolution of 20 days; receiving previous treatment based on human doxycycline and vitamin supplement according to veterinary guidance, but without clinical improvement. On physical examination, pale and slightly jaundiced mucous membranes, reactive popliteal lymph nodes, moderate dehydration, splenomegaly and severe abdominal pain were observed. In complementary exams, hypochromic macrocytic anemia (GV: 17%), thrombocytopenia (35x10³ / µl) and leukocytosis by lymphocytosis and monocytosis (33.6x10³ / µl), increased ALT enzyme (99 IU / L) and direct bilirubins (0.9 mg / dL), indirect (0.5 mg / dL) and total (1.4 mg / dL). PCRs for Babesiosis and serology for Leptospirosis were also requested, with negative results. Due to the patient's anemic and hemodynamic condition, blood transfusion was performed, but with a subsequent clinical picture of transfusion reaction. The transfusion was stopped and the clinical picture was controlled based on promethazine (0.3 mg / kg / IM), hydrocortisone (50 mg / kg / IV) and ondansetron (0.5 mg / kg / IV), together with with the institution of maintenance and hepatoprotective therapy. The saline agglutination test was performed 72 hours after the transfusion was performed, being positive and leading to clinical suspicion of IMHA. Thus, prednisone-based therapy (2 mg / kg / BID) was instituted with the presence of significant improvement of the patient in two days, presenting hypochromic macrocytic anemia (GV: 22%), neutrophilia leukocytosis and lymphocytosis (26.2x10³ / µl). After seven days, the prednisone dosage reduction (2 mg / kg / SID) started and with the inclusion of azathioprine (2 mg / kg / SID), both orally. After seven days of association, the animal continued to show progressive improvement, with no anemia (GV: 39%) and an improvement in leukocytosis (22x10³ / µl). However, there was impairment of liver function (ALT: 654 IU / L and alkaline phosphatase: 2032 IU / L). Dosage adjustments were made, with the current maintenance values at 0.5 mg / kg / 72 hours of prednisone and 1 mg / kg / 48 hours of azathioprine, together with hepatoprotective therapy. After three months of therapy, the patient's hematological values are within the normal range (GV: 40%; total leukocytes: 12.3x10³ / µl) and with an improvement in serum biochemical values of liver function (ALT: 178 IU / L) and Alkaline phosphatase (267 IU / L). In addition, the animal is clinically stable, including an increase in body weight (24 kg).
CASE 2

A canine, male, Border Collie breed, seven months old, weighing 8.10 kg, arrived for clinical care showing apathy, emesis, and progressive weight loss with evolution of 30 days, receiving previous treatment based on enrofloxacin, omeprazole, metoclopramide and domperidone, but with no clinical improvement. Physical examination revealed pale mucous membranes, hyperthermia, reactive submandibular lymph nodes, moderate dehydration, hepatosplenomegaly, abdominal pain, lean body score (3/9) and ixodidiosis. Complementary examinations identified hypochromic microcytic anemia (GV: 18%), leukocytosis due to neutrophilia and monocytosis (22.2x10³/µl). First, treatment was performed for the clinical diagnosis of hemoparasitosis with omeprazole, ondansetron and doxycycline, however after four days of treatment, there was no improvement in the animal's clinical condition. The blood count was repeated, finding changes similar to the first exam, showing hypochromic microcytic anemia (GV: 17%) and neutrophilia leukocytosis (22.3x10³ / µl). In addition, a reticulocyte count classified as mild regeneration (186,660 / µl) and a positive saline autoagglutination test were requested. Due to the suspicion of IMHA, dexamethasone (0.25 mg / kg / IV) was initially performed as an outpatient treatment. Subsequently, it was replaced by prednisone (2 mg / kg / BID). After seven days of treatment, the animal showed partial improvement of the clinical picture showing normophagy and absence of emesis, however the same did not occur with the hematological profile, presenting hypochromic microcytic anemia (GV: 19%) and reticulocyte count compatible with marked regeneration (556,920 /µl). Thus, therapy with an association of prednisone (2 mg / kg / BID) and azathioprine (2 mg / kg / SID) was instituted, both orally. After fourteen days of association, there was a significant improvement in the condition, although the animal continued to present hypochromic microcytic anemia and with a marked rate of regeneration (636,640 / µl), there was a significant increase in GV (30%) and absence of leukocytosis (6.9x10³ /µl). Then, the prednisone dosage reduction (2 mg / kg / SID) started and the azathioprine dosage (2 mg / kg / SID) was maintained for fourteen days. With the stabilization of the condition, dosage adjustments were made, with the current maintenance values at 0.5 mg / kg / SID of prednisone and 1.5 mg / kg / SID of azathioprine. After three months of therapy, the patient's hematological values are close to normal values (GV: 36% and total leukocytes: 9x10³ / µl). In addition, the animal is clinically stable, showing an increase in body weight (16.3 kg).

4 DISCUSSION

According to Balch and Mackin (2007), immune-mediated hemolytic anemia (IMHA) is common and has a high frequency in the veterinary routine. The precipitous classification of hemolytic anemia as autoimmune (primary) should be used with caution, as there are other factors
that can lead to secondary immune-mediated hemolytic symptoms (BRANDÃO et al., 2003). Garden et al. (2019) reported that infectious diseases are the main factors that trigger AHIM, followed by cancer, inflammatory conditions, medications and vaccinations. They also showed that among hemoparasitosis, babesia has a greater chance of leading to IMHA. Both patients described had a suspicion of previous infectious diseases.

According to Balch and Mackin (2007) in laboratory tests, anemia that is generally regenerative and leukocytosis can be found. Laboratory tests in both cases demonstrated the findings described above and in cytology, marked regeneration was confirmed through reticulocyte counts. The diagnosis of both cases was performed as recommended by the ACVIM guidelines for the diagnosis of IMHA (GARDEN et al., 2019), that is, through the association of clinical manifestations with the results found in complementary tests such as blood count, serum biochemistry and test agglutination solution in saline. Furthermore, in both cases, good responses to treatment with immunosuppressants were found, which is a strong indicator of IMHA.

According to Balch and Mackin (2007), prednisone at a dose of 1-2 mg / kg, is the drug of choice for the therapy of IMHA, due to its immunosuppressive effect, and an increase in hematocrit is expected to occur in 24 to 96 hours after start of treatment. In both animals, initial immunosuppressive therapy was performed using monotherapy with prednisone (2 mg/kg/BID) due to the strong recommendation of the ACVIM guidelines for treatment. (SWANN et al., 2019). The first animal described responded to prednisone administration in 48 hours, as can be seen by the increase in the percentage of GV and by the reduction of leukocytosis. The second reported patient, on the other hand, presented the same hematological profile even after the prednisone was instituted.

Swann et al. (2019) reported that there is the possibility of opting for the association with other immunosuppressive drugs in cases of immediate risk of the animal's life; when there is no maintenance or increase in the GV during the first week of treatment or due to the deleterious effects of high and chronic doses of corticosteroids, such as: endocrine diseases, arterial hypertension, liver diseases, among others (PEREIRA et al., 2011).

Like corticosteroids, azathioprine is used due to its immunosuppressive effect (NETO et al., 2008). The use of prednisone with azathioprine was carried out in both animals in order to reduce the dose of corticosteroids to avoid greater adverse effects caused by their chronic use. In addition, in the second animal described, the association was also made due to the lack of response to steroid monotherapy. Burgees et al., (2000) carried out a retrospective cohort study in which it was shown that the association of azathioprine with prednisone and cyclophosphamide in dogs with IMHA resulted in increased survival of these animals. Another study carried out with 21 dogs diagnosed with IMHA, found that patients treated with the association of azathioprine and other
immunosuppressive drugs had a higher probability of survival when compared to dogs in which polytherapy was not instituted (GOGGS et al., 2008). After the association of prednisone with azathioprine in both animals in this study, it was possible to observe an exponential improvement in the hematological condition, with an increase in the GV (%) close to the reference values and absence of leukocytosis. However, as observed in the first patient described, the use of these two drugs worsened the hepatic condition, represented by the exorbitant increase in liver enzymes (ALT and alkaline phosphatase). According to Pereira et al., (2011) corticosteroids can cause hepatomegaly and degeneration of hepatocytes. In addition, azathioprine may also have a hepatotoxic character. As the doses of both drugs were reduced in the first patient described, associated with hepatoprotective therapy, the deleterious effects were regressed, as well as Neto et al. (2008) reported, the toxic effects presented by the drug, are dose dependent.

With stabilization of the clinical and hematological condition, it was possible to reduce the dosages of both drugs without worsening the symptoms. Swann and Skelly (2013) when analyzing studies referring to the association of prednisone with azathioprine for the treatment of IMHA reported that they presented good results in terms of response to treatment and longer animal survival. The positive result of the association of immunosuppressive drugs in this study corroborates the effectiveness of the treatment cited by the authors.

5 CONCLUSION

Through this report it is possible to conclude that the use of the therapeutic combination of prednisone and azathioprine for the control of IMHA presents greater benefits when compared to the institution of glucocorticoid monotherapy. With the association, it is possible to use lower doses of both drugs, reducing their side effects and, consequently, resulting in better survival.
REFERENCES


