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Efficacy of Romiplostim in thrombocytopenic dogs

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Abstract
A research investigation was carried out at the Veterinary Clinical Complex of KNP College of Veterinary Science, Shirwal, to examine the Signalment and frequency of thrombocytopenia in canines. The study involved the analysis of 20 thrombocytopenic dogs, where a detailed history was obtained and thorough clinical examinations were conducted to gather the necessary data. Dogs with a medical history of symptoms such as nasal bleeding, petechial haemorrhages, hematemesis, melena, fever, enlarged lymph nodes, anaemia, and lethargy underwent screening for thrombocytopenia. The common general observations in thrombocytopenic dogs were anorexia, in appetence, fever, petechial haemorrhages and melena. Affected dogs had significantly decreased platelet count. Administration of the injection romiplostim resulted in an 80% success rate in rapid recovery of platelet count and absence of any adverse effects.

Keywords: Thrombocytopenia, romiplostim

1. Introduction
Small, fragmented, nuclei-free cells known as platelets circulate within the blood and possess a highly intricate structure and appearance. Platelets play an important function in haemostasis maintenance by mediating blood clotting at sites of blood vessel injury. Furthermore, they are implicated in a variety of physiological processes that go beyond their haemostatic role, including as inflammation, angiogenesis, innate immunity and others. In thrombocytopenia, platelet transfusions and high-dose intravenous immunoglobin G for immediate management or vincristine, azathioprine, mycophenolate mofetil, cyclophosphamide, cyclosporine, danazol, leflunomide and steroids are used although most relapses have major adverse effects and lastly splenectomy for long-term management and chances of recurrence is more. These therapeutic options are not utilised in the same way and there are no universally accepted guidelines for when to utilize them. Factors influencing owner compliance have been investigated with contradicting results; the spectrum of individual customers' needs differ greatly, posing a challenge across the profession.

2. Material and Methods
The selected 20 dogs in the research were client-owned and came with clinical indications of bleeding or haemoprotozoan infection. They were assessed with a venous blood test (vena saphena and vena cephalica) for platelet count and haemato-biochemical analysis was conducted concurrently. Two millilitres (ml) of the four millilitres of blood collected was transferred to sterile EDTA vials for complete blood counts and two millilitres (ml) to sterile vials containing clot activators for serum collection. Dogs that were chosen for treatment had platelet counts below one lakh/µl. Giemsa-stained blood smears were also prepared for microscopic examination. Dogs with the signs of bleeding/petechial haemorrhages/prolonged bleeding from venous puncture/fever/enlarged lymph nodes/ splenomegaly/anaemia/lethargy were screened for thrombocytopenia and positive cases were equally divided into two groups, group I comprising 10 dogs were treated with standard treatment and group II comprising 10 dogs were treated with standard treatment and injection romiplostim @ 5 µg/kg (Romy® Intas Pharmaceuticals Ltd) SC two doses at weekly interval. Ten dogs in group I were treated with Clindamycin (25 mg/kg, PO, BID), Doxycycline (10 mg/kg, PO, OID), Metronidazole (15 mg/kg, PO, BID) with Haematinics + Liver tonics for 21 days + Tab. Prednisolone @ 2 mg/kg once daily for the first week, 1 mg/kg once daily for the next week and for last seven days @ 0.5 mg/kg on alternate days.

Ten dogs in group II were treated with Clindamycin (25 mg/kg, PO, BID), Doxycycline (10 mg/kg, PO, OID), Metronidazole (15 mg/kg, PO, BID) with Haematinics + Liver tonics for 21 days + Inj. Romiplostim @ 5 µg/kg SC two doses at weekly interval

3. Results and Discussion

Based on the clinical signs, haematological analysis and laboratory examination, the condition was diagnosed as thrombocytopenia in 20 dogs. Giemsa-stained blood smears were also prepared for microscopic examination showed pleomorphic piroplasms that were either oval or ring-shaped and had unipolar or bipolar chromatin condensation at the periphery [1, 2]. All dogs were positive for babesia spp. under microscope. Dogs having platelet clumps were excluded from the study. Cases with platelet issues on automated analyzers, such as platelet clumps, abnormal platelet distribution or giant platelets or cases displaying platelet clumps on peripheral smears were excluded [3].

The mean temperature measured in the thrombocytopenic dogs was 103.71 ± 0.15 oF, higher than the temperature measured in the healthy control group (101.09 ± 0.25 oF) [11].

Table 1: Platelet count (x 10^5/µl) in group I before and after treatment (n=10)

Case No.	Group I (x 10^5/µl)		
	BT	AT	
		Day 10	Day 21
P01	0.8	1.16	1.09
P02	0.95	0.69	1.65
P03	0.72	0.66	0.99
P04	0.86	1.01	1.21
P05	0.8	1.5	4.81
P06	0.38	0.71	0.69
P07	0.67	1.99	0.9
P08	0.86	1.5	2.9
P09	0.84	0.9	2.25
P10	0.14	1.64	4.81
Mean ± SE	0.70 ± 0.80	1.17 ± 0.14	2.13 ± 0.49

**p<0.01 *p<0.05

Table 2: Platelet count (x 10^5/µl) in group II before and after treatment (n=10)

Case No.	Group II (x 10^5 /µl)		
	BT	AT	
		Day 10	Day 21
P11	0.95	1.23	3.95
P12	0.27	0.54	2.21
P13	0.59	0.90	2.89
P14	0.45	1.33	1.25
P15	0.30	0.71	1.80
P16	0.32	0.79	1.67
P17	0.41	0.79	0.93
P18	0.22	0.85	2.01
P19	0.14	0.90	1.90
P20	0.85	1.16	1.64
Mean ± SE	0.45 ± 0.08	0.92 ± 0.07**	2.02 ± 0.27**

It was observed that anorexia, inappetence, fever, petechial haemorrhages and melena were the most prevalent clinical findings in thrombocytopenic dogs. Anorexia, inappetence, petechiae, ecchymoses and mucosal bleeding such as gingival bleeding, epistaxis, urinary and gastrointestinal haemorrhage could be seen in primary or secondary thrombocytopenia [4]. Three most common presenting issues in dogs with thrombocytopenia were melena, anorexia and lethargy or depression which is in accordance with the present study [5]. All thrombocytopenic dogs showed significant (p<0.01) decrease in Hb, PCV, Platelet count and TEC with significant (p<0.01) increase TLC count. Thrombocytopenia was the prominent feature of babesiosis caused by *Babesia gibsoni*. The low platelet count might be due to elevation of body temperature, immune mediated destruction of thrombocytes or splenic sequestration or coagulatory consumption of platelets from haemolytic or vascular injury. The breakdown of erythrocytes might be caused by mechanical damage from replicating babesia parasites, as well as damage from antibodies, the complement system and oxidizing factors [6, 7]. Thrombocytopenia in dogs infected with *Anaplasma phagocytophilum*, *Ehrlichia canis* and *Babesia gibsoni* has been reported in association with antiplatelet antibodies [23]. Anaemia with severe thrombocytopenia was observed in babesiosis [8]. These studies are in accordance with present findings. Platelet sequestration in the spleen, immune-mediated platelet destruction, and the development of disseminated intravascular coagulation were all possibilities for thrombocytopenia.

Serum biochemical estimation of thrombocytopenic dogs with babesiosis revealed significant (p<0.05) increase in total bilirubin, direct and indirect bilirubin as against healthy dogs. There was a non-significant rise in the mean values of ALP, ALT and BUN in thrombocytopenic patients as compared to healthy controls and there was non-significant decrease in serum protein. Canine babesiosis with decrease in mean albumin values while significant rise in mean values of ALP, ALT, BUN and creatinine as compared to healthy dogs. Elevated liver enzymes, hypoproteinaemia with hypoalbuminemia and elevated blood levels of SGPT, creatinine and BUN observed in canine babesiosis [9, 11]. Considerable alterations in hepatic enzymes and reduced albumin levels were found in centrilobular hepatitis with hypoxic liver injury [10]. Kidney injury or cardiac injury might have been a major cause of elevated serum AST activity in dogs. In affected dogs, hypoalbuminemia was found, which might be caused by inflammation, hepatic dysfunction, ascites or malnutrition [11, 9]. On day 10, non-significant increase in platelet count was recorded in eight dogs out of ten positive cases of thrombocytopenia in treatment group I. Two dogs exceeded 1.5 lakh/µL platelet count but it was non-significant compared to before treatment values. On 21st day seven cases had increased platelet count as compared to day 10 but only five dogs had platelet count more than 1.5 lakh/µl with non-significant change in platelet count indicating that prednisolone had 50% efficacy in treating thrombocytopenic dogs. In treatment group II on day 10, all positive cases of thrombocytopenia resulted in highly significant increased values of platelet count. On 21st day, 8 cases showed highly significant (p<0.01) increase in platelet count with more than 1.5 lac/µl count indicating 80% efficacy of injection romiplostim @ 5µg/kg in treatment of thrombocytopenic dogs.

On day 21, all the dogs in groups I and II showed clinical improvement. However, in group I all dogs experienced polyuria, polydipsia and one dog had vomition during the whole course of therapy [12]. These side effects were likely to be caused by the impact of glucocorticoids on antidiuretic hormone (ADH), resulting in excessive fluid loss through

urine and increasing the sensation of thirst to compensate for fluid loss [13, 14]. Vomiting is associated with unpleasant nausea sensations in steroidal therapy [15]. Steroid use cause polydipsia and polyuria, by increasing bladder fill beyond the normal capacity, resulted in promoting urinary incontinence. This urinary incontinence was considered to have had a negative impact on the welfare of affected dogs [13].

Dogs under treatment in Group II did not show any adverse effects during and after treatment. In one study 4 of 5 treated dogs, romiplostim administration resulted in rise in platelet count within 3 to 6 days of therapy. All the dogs not only handled the medicine well, but also promptly responded with a rise in platelet counts [16]. Romiplostim was completely (100%) effective in treating acute cases of thrombocytopenia in human beings, with a 96.2% effectiveness in chronic cases of thrombocytopenia [17].

On injection romiplostim administration there were no after effects and the platelet count recovered to its normal level. There were no negative incidents [18]. Pharmacodynamic studies of romiplostim in various animal species including mice, rats, rabbits, monkeys and dogs showed good tolerability and a dose-dependent increase in platelet counts [19]. FDA (2009) authorized the use of romiplostim on August 22, 2008 to treat persistent ITP in patients who had an unsatisfactory response to corticosteroids, immunoglobulins or splenectomy. Romiplostim was an Fc-peptide fusion protein (peptibody) that bound to the thrombopoietin receptor and activated intracellular transcriptional pathways, stimulating the synthesis of more platelets. Romiplostim imitated human thrombopoietin and was made in Escherichia coli using recombinant DNA technology. It promoted the formation of megakaryocyte colony-forming cells in the bone marrow and increased platelet synthesis through the JAK2 and STAT5 kinase pathways by binding to thrombopoietin receptors [20, 21]. Canine and human TPO-R protein sequences were found to have a significant amount of conservation at the C-terminus, where the potential TPO binding site (EpoR-lig-bind domains) was located. Given the substantial conservation of canine and human protein sequences and the fact that romiplostim interacts with the extracellular portion of TPO-R, this was believed to be the molecular cause of the therapeutic effect on canine ITP. Romiplostim was used to treat ITP in five dogs, who appeared to handle the medication well. Four of the five dogs had a significant increase in platelet levels immediately after treatment, and a higher dosage of romiplostim helped one dog with secondary ITP who had not responded to prednisolone. Within two days of the initial treatment, the platelet counts of the treated dogs appeared to start to rise. Romiplostim was well-tolerated and was associated with an increase in platelet counts in dogs, with four of the five dogs going into recovery and no relapses observed over the three to ten-month follow-up period [16].

4. Conclusions

The therapeutic study revealed that standard treatment with prednisolone had 50% efficacy in treating thrombocytopenia in canine babesiosis while standard treatment with injection romiplostim had 80% efficacy with early raise in platelet count in recovered dogs.

5. References

1. Mahalingaiah MKC, Asoor M, Thimmaiah RP, Narayanaswamy HD, Mukartal SY, Elattuvalappil AM, *et al*. Prevalence of canine babesiosis in different breeds of dogs in and around Bengaluru. Advances in Animal and Veterinary Sciences. 2017;5(3):140-144.
2. Miro G, Checa R, Paparini A, Ortega N, Gonzalez-Fraga JL, Gofton A, *et al*. Theileria annae (syn. Babesia microti-like) infection in dogs in NW Spain detected using direct and indirect diagnostic techniques: Clinical report of 75 cases. Parasitology and Vectors. 2015;8:217.
3. Sahu N, Mohanty M, Adhya AK. Estimation of Total Platelet Count From Peripheral Blood Smear Needs a Correction Factor. Cureus. 2022;14(8):e28327.
4. Cortese L, Christopherson PW, Pelagalli A. Platelet Function and Therapeutic Applications in Dogs: Current Status and Future Prospects. Animals 2010;10(2):201.
5. Scuderi MA, Snead E, Mehain S, Waldner C, Epp T. Outcome based on treatment protocol in patients with primary canine immune-mediated thrombocytopenia: 46 cases (2000–2013). The Canadian Veterinary Journal. 2016;57(5):514.
6. Boozer AL, Macintire DK. Canine babesiosis. Veterinary Clinics of North America: Small Animal Practice. 2003;33:885-904.
7. Shah SA, Sood N, Tumati S. Haemato-biochemical changes in natural cases of canine babesiosis. Asian Journal of Animal Science. 2011;5(6):387-392.
8. Almendros A, Burchell R, Wierenga J. An alternative combination therapy with metronidazole, clindamycin and doxycycline for *Babesia gibsoni* (Asian genotype) in dogs in Hong Kong. Journal of Veterinary Medicine Science. 2020;82(9):1334-1340.
9. Reddy BS, Sivajothi S, Reddy LSV, Solmon Raju KG. Clinical and laboratory findings of Babesia infection in dogs. Journal of Parasitic Diseases. 2016;40(2):268-72.
10. Taboada J, Lobetti RG. Infectious diseases of dog and cat, Edn 3, W.B. Saunders, Greene C, St. Louis; c2006. p. 722-735.
11. Kumar P, Kumar A. Haemato-biochemical changes in dogs infected with Babesiosis. International Journal of Chemical Studies. 2018;4:25-28.
12. Grundy SA, Barton C. Influence of drug treatment on survival of dogs with immune-mediated hemolytic anemia: 88 cases (1989-1999). Journal of the American Veterinary Medical Association. 2001;218:543-546.
13. Coit VA, Gibson IF, Evans NP, Dowell FJ. Neutering affects urinary bladder function by different mechanisms in male and female dogs. Europe Journal Pharmacology. 2008;584:153-8.
14. O'Neill DG, Riddell A, Church DB, Owen L, Brodbelt DC, Hall JL. Urinary incontinence in bitches under primary veterinary care in England: prevalence and risk factors. Journal of Small Animal Practice. 2017;58(12):685-693.
15. Hay Kraus BL. Effect of dosing interval on efficacy of maropitant for prevention of hydromorphone-induced vomiting and signs of nausea in dogs. Journal of American Veterinary Medicine Association. 2014;245(9):1015-20.
16. Kohn B, Bal G, Chirek A, Rehbein S, Salama A. Treatment of 5 dogs with immune-mediated thrombocytopenia using Romiplostim. BMC Veterinary Research. 2016;12:96.
17. Reiser M, Josten KM, Dietzfelbinger H, Seesaghur A, Schill M, Hippenmeyer J, *et al*. Romiplostim for primary

immune thrombocytopenia in routine clinical practice: results from a multi-centre observational study in Germany. Acta Haematologica. 2022;145(4):394-403.
18. Bussel JB, Buchanan GR, Nugent DJ. A randomized, double-blind study of romiplostim to determine its safety and efficacy in children with immune thrombocytopenia. Blood. 2011;118(1):28-36.
19. Kuter DJ, Rummel M, Boccia. Romiplostim or standard of care in patients with immune thrombocytopenia. New England Journal of Medicine. 2010;363(20):1889-1899.
20. Rodeghiero F, Stasi R, Gernsheimer T. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113(11):2386-2393.
21. Cooper KL, Fitzgerald P, Dillingham K, Helme K, Akehurst R. Romiplostim and eltrombopag for immune thrombocytopenia: Methods for indirect comparison. International Journal of Technology Assessment Health Care. 2012;28(3):249-5.
22. Susan M. Cotter. The Merck Veterinary Manual, 11th Edition, MERCK & CO, USA; c2017, 7.
23. Wilkerson MJ, Shuman W, Swist S, Harkin K, Meinkoth J, Kocan AA. Platelet size, platelet surface-associated IgG and reticulated platelets in dogs with immune-mediated thrombocytopenia. Veterinary Clinical Pathology. 2001;30(3):141-149.