Il seguente lavoro è stato in parte presentato come poster al ECVIM-CA Congress, tenutosi a Goteborg (Svezia) il 8-10 settembre 2016.

Clinical efficacy of autologous platelet-rich plasma (prp) in treatment of perianal fistulas in a german shepherd dog

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SUMMARY
Platelet-rich plasma (PRP) derived from whole blood is characterized by platelet concentrations above baseline in a small volume of plasma and can stimulate cell proliferation accelerating the healing process. Canine perianal fistula disease (PAF) is a chronic and painful disease of the anus, perianal skin, anal sacs, and adjacent tissues that affects predominantly German shepherd dogs.
This report describes the clinical efficacy of autologous PRP obtained with an in-house double centrifugation validated method in the treatment of multiple perianal fistulas in an eleven year old German Shepherd dog.
Autologous PRP (0.5ml) was obtained from a citrated blood sample of 8 ml by in-house double centrifugation and administered directly into fistulas by 3 injections at weekly intervals.
Complete healing of the lesions occurred one month after the first treatment with PRP without the use of any drugs. No recurrences were observed in a one year follow up period.
This case report demonstrates that autologous PRP obtained with an in-house double centrifugation method could be an effective, minimally invasive and easy to perform topical therapy in the treatment of canine perianal fistulas.

KEY WORDS
platelet rich plasma, perianal fistulas, dog, in-house method

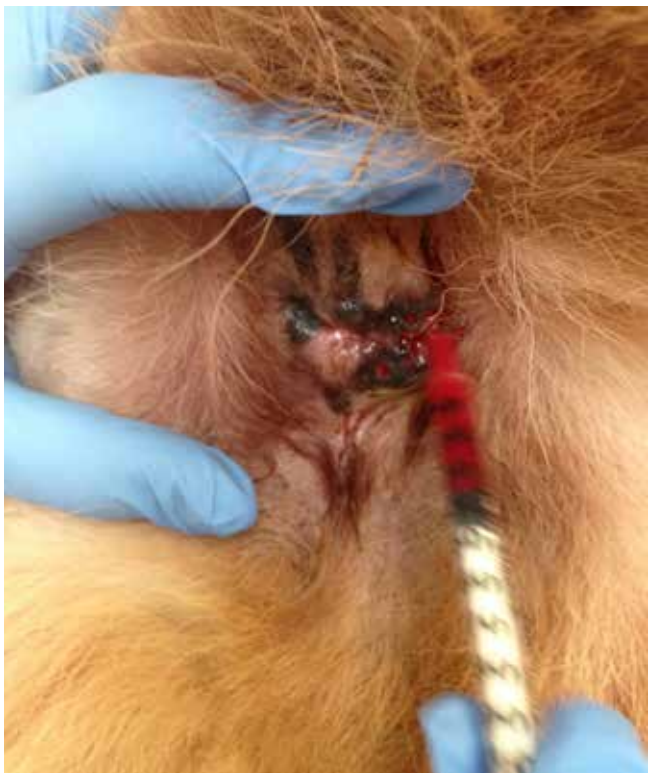
INTRODUCTION

Platelet-rich plasma (PRP) derived from whole blood, is characterized by platelet concentrations above baseline in a small volume of plasma (20) that can accelerate the healing process (1). PRP provides increased concentration of platelet-derived growth factors (30) which can stimulate cell proliferation angiogenesis, wound healing, production of fibroblasts, collagen, osteoblasts, and decrease the inflammatory reaction.
In human medicine the regenerative features of autologous PRP are used predominantly in orthopaedic surgery, maxillofacial surgery, dentistry, medicine, cosmetic surgery and in dermatology (19). Most studies of the use of PRP in animals report the animal treatment as a model for human medicine (4,12).
Studies of the use of PRP in veterinary medicine have concentrated on the therapeutic use of PRP in musculoskeletal, tendon and soft tissue injuries in horses (2,35). There are few reports of the therapeutic use of PRP in dogs (17,28).
Canine perianal fistula disease (PAF) is a chronic and painful disease of the anus, perianal skin, anal sacs, and adjacent tissues (22) The disease affects medium- to large-breed dogs—predominantly German shepherd dogs (3). However, other breeds can be affected including Labrador retrievers, Irish setters, Old English sheepdogs, Bulldogs, and Collies. (6)
PAF is characterized by inflammation and ulcerations with draining fistulous tracts around the anal region, occasionally involving the rectal lumen. Clinical signs include tenesmus, hematochezia, constipation, self-mutilation, anal stenosis, and severe discomfort; these can lead to systemic signs of illness such as lethargy, anorexia, and weight loss. (15) Medical mainly lifetime treatment with immunomodulatory therapy has achieved some clinical success, while surgical options have been reported with no long-term results. Historically, medical management of canine PAF has been directed at altering the local environment of the perineum through use of tail braces, regular cleansings of the affected area, and controlling infection (8). Surgical treatments are associated with varying recurrence rates and a high prevalence of complications. (8)
No data are available on the possible therapeutic role of PRP in the treatment of fistulas in dog but its reported properties may be beneficial in treating this syndrome.
This report describes the clinical efficacy of autologous PRP obtained with an in-house double centrifugation validated method in the treatment of multiple perianal fistulas in a German Shepherd dog.

CASE DESCRIPTION

A 10 year old German shepherd, neutered female, with multiple perianal fistulas had been treated with 25 mg/kg metronidazole plus 150,000 iu/kg spiramycin (Stomorgyl®, Merial) orally per day and local disinfections with 10% povidone-iodine solution. No improvement was noted over

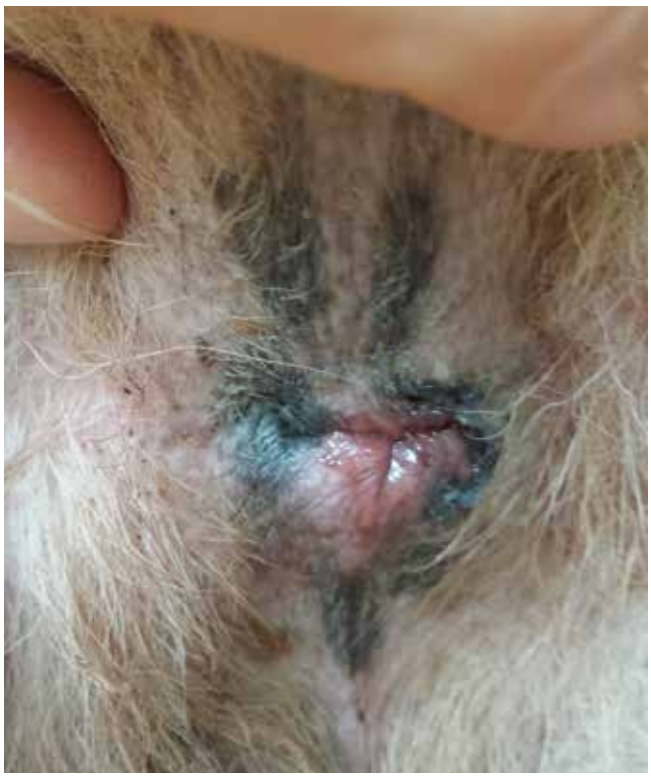
Picture 1: Multiple perianal fistulas before the treatment at D0



Picture 2: Anal region during the treatment with PRP at D0

two months and the dog was presented for clinical evaluation at the dermatological clinic of Department of Veterinary Medicine, University of Milan.
The dog was fully vaccinated against canine distemper virus (CDV), canine parvovirus CPV, leptospirosis, and infectious canine hepatitis (ICH) with regular prophylaxis against endo- and ectoparasites and had a history of canine leishmaniasis. The dog had not shown clinical signs of leishmaniasis for many years, the last treatment with meglumine antimoniate had been seven years previously and the last immunofluorescence antibody test (IFAT) titer from 6 months previously was 1:40.
On the day of the first visit (D0) no significant clinical abnormalities were seen on general physical examination. Examination of perianal region revealed 5 fistulas, four of which were confluent, in the right dorsal portion of perineum, with erythema, serum/hematic exudate, anal pruritus, diarrhea and dyschezia. (Picture 1).
A complete blood count (CBC), biochemical profile and complete urine analysis revealed no abnormalities. The IFAT title for *Leishmania infantum* was 1:40.
Immunosuppressive therapy was not considered appropriate due to the past medical history of the dog and treatment with PRP was instituted to promote tissue healing and regeneration. The owner gave informed consent for treatment, measurements, and for data recording. The treatment was carried out in accordance with the Institutional Ethical permission for "Evaluation of clinical effects of the use of autologous platelet-rich plasma (PRP) in treatment of dogs with different skin diseases" dated 13 January 2015.
At D0 a 0.5 ml of autologous PRP was obtained from a blood sample following a previously validated in-house double centrifugation method (26). Briefly, 8 ml of blood was collected from the cephalic vein using a 10 ml syringe (Sterile syringe 10 ml PIC, Italy) and a large gauge (21 gauge) needle (Hypodermic needle, 21G x 1 1/2" PIC, Italy) in order to minimize platelet activation. The blood was immediately placed into a test tube with a conical bottom (CELLSTAR® Centrifuge Tubes, Polypropylene, Sterile, 15 ml, graduated conical bottom, blue screw cap Greiner Bio-One, Germany) (Tube A) containing 1 ml of sodium citrate 3.8 %. All samples were maintained at room temperature (18-25 °C), on a laboratory blood rocker and processed and analysed within 30 minutes of collection. Tube A was centrifuged at 610 g for 10 minutes at room temperature using a laboratory centrifuge (EBA 20, Hettich, Germany) to produce a blood cell component (BCC) in the bottom of the tube and *sine* erythrocyte components (SEC) in the upper fraction of the tube. The entire SEC, comprising buffy coat (rich in young large platelets), was transferred to another 10 ml graduated conical tube without anticoagulant (Tube B) (CELLSTAR® Centrifuge Tubes, Polypropylene, Sterile, 15 ml, graduated conical bottom, blue screw cap Greiner Bio-One, Germany) and centrifuged at 1600 g for 15 minutes. This centrifugation resulted in two new components: platelet poor plasma (PPP) in the upper fraction and a platelet pellet in the lower fraction (visible as a red button on the bottom of the tube). After removing PPP, the platelet pellet was resuspended in approximately 25% of the PPP volume to obtain PRP. The obtained PRP (concentration 731000 platelet/µL) was immediately injected directly into fistulas (0.1 ml for each fistula) (Picture 2).
The same treatment was repeated after 7 days (D7) (0.5 ml of autologous PRP, concentration 905000 platelet/µL) and after 14 days (D14) from the first treatment (0.5 ml of autologous PRP, concentration 1131000 platelet/ µL).
The dog was checked every 2 days for the first week to monitor for any adverse reaction, then twice a month for 2 months and finally every 3 months for 1 year to evaluate the clinical improvement and for photographic documentation.
No other treatment was given to the dog throughout the follow up.
Lesions improved well, with a marked reduction of the fistula openings and total disappearance of anal pruritus, serum/hematic exudate, diarrhea and dyschezia after 14 days (D14) from the first PRP application (Picture 3).

Picture 3: Clinical stage after 14 days (D14)
Complete healing of lesions had occurred after one month (D30) from the first treatment with PRP (Picture 4), without the use of any other drugs. No recurrences were observed in a 1 year follow up period (Picture 5).



Picture 4: Complete healing of the lesions one month after first application of PRP (D30)

DISCUSSION

PRP is increasingly used in therapeutic tissue regeneration, as evidenced by several published clinical and experimental reports in human and veterinary medicine. However, studies of clinical efficacy in dogs and cats are very rare and furthermore, in dogs many different manual or semi-automated methods for PRP production are described (32, 17,9,29,25) producing wide ranges of PLT concentration. Few studies provide information about platelet activation and the values of Platelet-Derived-Growth-Factor (PDGF) concentration (30,32,29,26).

Autologous PRP is a cost-effective and readily available therapeutic blood derivative. It is rich in growth factors and cytokines and increases tissue regeneration by affecting cell recruitment, proliferation, and differentiation. Topical PRP application may be particularly effective in tissue regeneration because of the high leucocyte concentration which results in local debridement and antibacterial activity. (5)

The in-house double centrifugation method used in our study is inexpensive and requires no special equipment. It allows final product to be produced in 1 hour and achieves an adequate platelet and PDGF concentration in the PRP (26). It has been reported that leukocyte concentrations in PRP should be controlled to minimize inflammation after PRP injection but high WBC counts are acceptable in PRP preparations used for autologous topical application (33) and other authors believe that WBC are important regulatory cells contained in PRP and necessary for wound healing (23). The only limitation of this manual method is that it is operator dependent and requires experienced technicians. Despite the relatively small amount of whole blood initially collected in this study a sufficient volume of autologous PRP for clinical use in PAF was obtained.

PAF is a debilitating and progressive clinical condition that requires lifelong monitoring and, potentially, lifelong treatment. Although PAF is a well-documented disease, the cause is poorly understood.

Reports on the etiopathogenesis have attempted to link an immunopathological, bacterial, hormonal, endocrine, or anatomical basis for the disease; however, none have been conclusive. (16,34,8)

Canine PAF have similar histological lesions to severe Crohn's disease of the perineum in human patients (7), and is noticeably difficult to treat, frustrating both veterinarians and owners. However, some success has been achieved utilizing immunomodulatory therapy such as cyclosporine (21,13) with or without ketoconazole (24) azathioprim (34), topical tacrolimus (31), and prednisone (11).

Successful use of immunosuppressive drugs in dogs with PAF supports the theory that PAF has an immunological basis. However, treatment with immunosuppressive agents is associated with an increased risk of complications, significant costs, and frequent relapses when therapy is discontinued, (34,21) making alternative treatment strategies desirable.

Recently perianal fistulas in dogs have been successfully treated with human embryonic stem cell-derived mesenchymal stem cells in a canine model of human fistulizing Crohn's disease (10).

In human beings perianal fistula has been associated with an allergy to cow's milk (14). When a group of dogs (mostly German shepherd dogs) suffering from perianal fistulas was exclusively fed a fish and potato diet for an extended period after surgery, there was a lower rate of recurrence with less frequent and severe complications relative to previous studies, in which only surgical treatment was performed (18). A recent study hypothesized an association between perianal fistulas and adverse food reaction (AFR) in German Shepherd dog (27)

In this clinical case the aetiology of the perianal fistulas was not clear, but PRP was applied to these nonhealing lesions in an attempt to enhance healing, especially since

immunosuppressive drugs were contraindicated given the past history of the dog.
Complete healing of the lesions was seen within one month from the first treatment with PRP without using other drugs and no adverse effects were reported. Interestingly, the main signs of pain, erythema, dyschezia, disappeared only 14 days after the first application.
The dog was kept under observation for 1 year and no recurrences were observed. Recurrence of disease is a common problem following cessation of immunosuppressive treatment for immune-mediated diseases, although an immune-mediated basis for canine PAF has not been established. The fact that no recurrence was seen in the dog in this study encourages further studies on larger number of subjects.

Picture 5: No recurrences were observed in 1 year of follow up

CONCLUSION

This case report demonstrates that autologous PRP obtained with an in-house double centrifugation method appears to be an effective, minimally invasive and easy to perform topical therapy for the treatment of canine perianal fistulas. Large-scale studies are required to elucidate a clear mechanism of action and identify any complication associated with its use.

ACKNOWLEDGMENTS

The work presented in this manuscript was supported by the Department of Veterinary Medicine of University of Milan, Italy. The authors thank the owner for giving consent for enrollment of his dogs in this clinical study.

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