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TOPICAL AUTOLOGOUS PLATELET-RICH PLASMA (PRP) IN MANAGEMENT OF PERIANAL FISTULAS IN A GERMAN SHEPHERD DOG

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Abstract

Platelet-rich plasma (PRP) is the processed liquid fraction of autologous peripheral blood with high concentration of platelets. Thanks to its ability to speed up the healing process, PRP is used in the treatment of many diseases in which tissue regeneration is required. Canine perianal fistulas disease (PAF) is a painful and chronic disease of the perianal tissues that affects medium to large breed dogs, predominantly German shepherd dogs.
The aim of this report is to describe the clinical efficacy of autologous PRP as an adjuvant therapy in the treatment of multiple perianal fistulas in a nine year old German shepherd dog.
Autologous PRP (3.5 mL) containing $8x10^5$ platets/ml was administered directly into fistulas by 2 injections at weekly intervals. Complete healing of the lesions occurred four weeks after the first treatment with PRP.
This case report hypothesizes that autologous PRP could be considered as an excellent adjuvant to conventional therapies for the treatment of canine perianal fistulas.

Key words: *Platelet-rich plasma, perianal fistulas, dog.*

INTRODUCTION

Platelet-Rich Plasma is a product made of autologous plasma with a higher concentration of platelets (PLT) than is found in normal whole blood (Marx, 2001).
Platelets contain storage pool of growth factors which promote tissue repair and influence the reactivity of vascular and other blood cells in angiogenesis and inflammation. In recent years the application of PRP has been widely extended in diverse medical and surgical procedures (Anitua et al., 2004). Thanks to its regenerative features, in human medicine autologous PRP is used predominantly in the fields of maxillofacial surgery (Marx et al., 1998), orthopedic surgery (Savarino et al., 2006), periodontic surgery (Hanna et al., 2004), plastic surgery (Powell et al., 2001), thoracic surgery (Englert et al., 2005), vascular surgery (Crovetti et al., 2005) and ophtalmology (Korobelnic et al., 1996).
Interest in the use of PRP is also growing in veterinary medicine, as shown by several studies conducted on its therapeutic use expecially in musculoskeletal, tendon (Torricelli et al., 2011; Bosch et al., 2011; Sample et al., 2018) and soft tissue (Farghali et al., 2017; Farghali et al., 2019; Perego et al., 2021) injures in horses and dogs.
Canine perianal fistula disease (PAF), also known as anal furunculosis, is a painful and chronic disease in which sinus tracts or ulcers spontaneously occur in the skin and soft tissues around the anus (Cain, 2019).
The disease affects predominantly German shepherd dogs (Budsberg et al., 1985), however other purebred and mixed-breed dogs also are affected (Day et al., 1992).
Inflammation and ulcerations around the anal region lead to tenesmus, constipation, hematochezia, self-mutilation, anal stenosis and severe discomfort. Dogs can show systemic signs like anorexia, weight loss, diarrhea and lethargy (Jamieson et al., 2002).
PAF must be distinguished from other conditions that can lead to perianal fistulization

such as anal sacs rupture, perianal ulcerated neoplasms (perianal adenoma or adenocarcinoma) or mucocutaneous lupus erythematosus (MCLE). Furthermore, glandular tissue left behind after an anal sacculectomy can result in chronic perianal draining tracts that can be misdiagnosed as PAF.
Usually the diagnosis of PAF is based on anamnesis, clinical signs and findings of the physical examination, but, in doubtful cases, performing histopathology can be necessary to exclude neoplasia or MCLE (Cain, 2019).
Although development of PAF was once believed related to anatomic conformation, the condition is now recognized as immune mediated, although the pathogenesis has not been fully delineated. Medical management based on immunosuppressive agents is the current standard of care for dogs with perianal fistulas (Cain, 2019). Few data are available on the possible clinical use of PRP (Perego et al., 2017), although its regenerative properties may be extremely helpful in treating the disease.
This report wants to describe the therapeutic efficacy of autologous PRP as an adjuvant therapy in the treatment of multiple PAF in a German shepherd dog.

CASE DESCRIPTION

A nine years old German shepherd dog, neutered female, with multiple perianal fistulas was treated for at least 9 months with cycles of prednisolone (Deltacortene®, Bruno Farmaceutici S.p.A, Italy) and various antibiotics orally. No significant improvement was noted after this period of time, so the dog was referred for clinical evaluation at the University Veterinary Teaching Hospital (OVUD) of the University of Perugia.
The dog was fully vaccinated against canine distemper virus (CDV), canine parvovirus CPV, leptospirosis, and infectious canine hepatitis (ICH) and received regular prophylaxis against ectoparasites.
On physical examination the dog presented poor coat and nutrition (3/9 BCS). Examination of perianal region revealed multiple fistulas in the dorsal and right lateral portion of perineum (Figure 1). These lesions were accompanied by erythema, serous/blood exudate, anal itching, pain on defecation leading to dyschezia.
A complete blood count (CBC) and biochemical profile revealed no abnormalities.

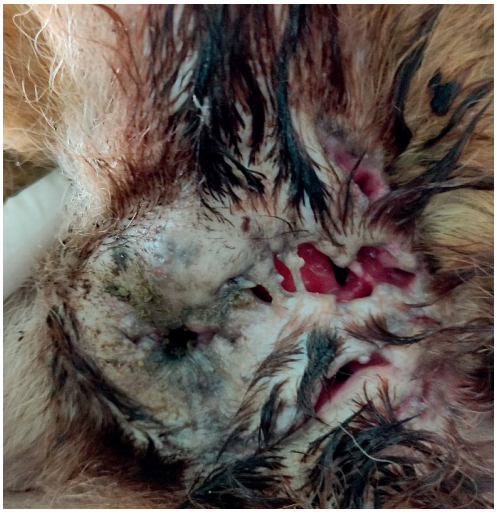

Figure 1. Multiple perianal fistulas on first physical examination

Due to the refractoriness to medical management, treatment with PRP infiltrations was instituted to promote tissue healing and regeneration.
At day 0 (D0) autologous PRP was obtained from whole blood collected from the jugular vein in citrate-dextrose solution vacutainer (Becton–Dickinson-Vacutainer®) according to Bianchini (2016). The platelet pellet, obtained after two centrifugations, was resuspended at final concentration of 8×10^5 PLT/µL and immediately injected into fistulas.
After placing the animal under general anesthesia, trichotomy, cleaning and washing of the perianal region was performed (Figure 2). During the cleaning of the lesions, a small and fresh vegetal foreign body (grass awn) was removed from the path of a fistula. A swab specimen of the fistulas was collected sterile for microbiological culture. With the help of a probe, 8 fistulas with a depth between 1.5 and 5 cm were recognized. A total amount of 0.5 ml of PRP were administrated in for fistulas longer than 2.5 cm whereas 0.25 ml were used for fistulas less than 2.5 cm in length (Figure 3).

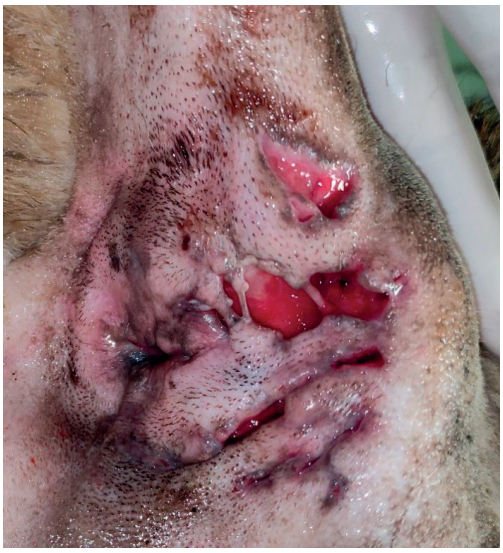

Figure 2. Perianal region after trichotomy, cleaning and washing at D0

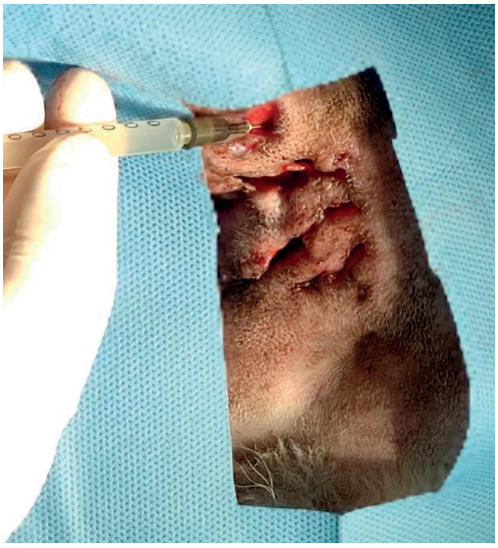

Figure 3. Perianal region during treatment with PRP (D0)

Seven days later (D7) the dog was checked and only three fistulas were found, ranging in depth from 0.7 to 1.5 cm (Figure 4). Therefore, the treatment with PRP (8×10^5 PLT/µL) was repeated by administering 0.25 mL in each fistula. In the meantime, the result of the microbiological culture had given a negative result.

At day 14 (D14) follow up no fistulas were found. Only a small superficial wound remained that was not treated with PRP (Figure 5).

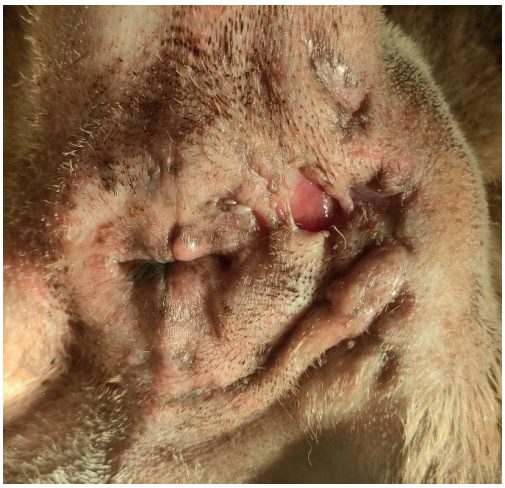

Figure 4. Clinical stage after 7 days (D7). A marked reduction of the fistula openings is observed

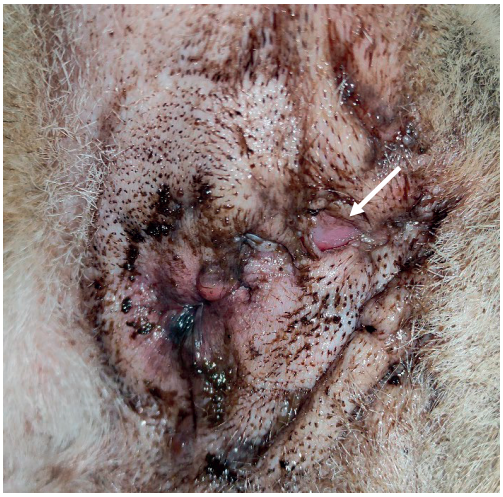

Figure 5. Clinical stage after 14 days (D14). Only a small superficial wound is left (white arrow)

The dog was then checked every two weeks in the following two months to evaluate the clinical improvement.

Medical therapy, starting from D0, involved the administration of 20 mg/kg metronidazole (Metrobactin®, Le Vet Beheer B.V., Italy) for 14 days, 1mg/kg prednisone (Deltacortene®, Bruno Farmaceutici S.p.A, Italy) for 1 week, then reduced to 0.75 mg/kg for five weeks and to 0.5 mg/kg for two weeks.

Tacrolimus ointment was applied topically for 4 weeks (PROTOPIC® 0,1%, Leo Pharma, Italy). From D0 the diet of the dog was restricted to a novel-protein (fish) diet.

Lesions improved significantly, with a marked reduction of the fistula openings observed starting one week after the first PRP application. Total disappearance of anal pruritus, serum exudate and dyschezia occurred in 14 days, while complete healing of the lesions occurred 4 weeks from the first treatment with PRP (Figure 6).

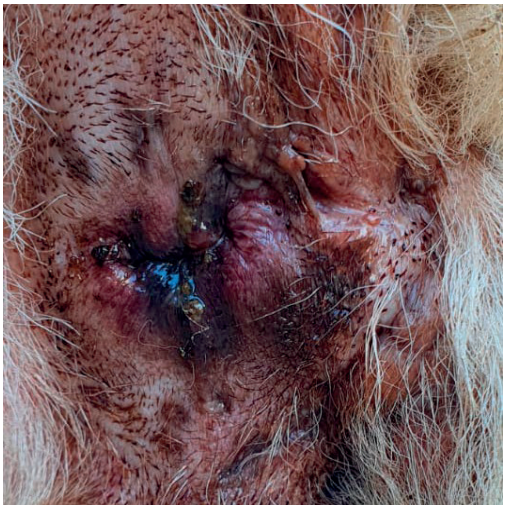

Figure 6. Complete healing of the lesions 4 weeks after the first application of PRP (D28)

DISCUSSIONS

Canine perianal fistulas disease is an extremely debilitating condition in affected dogs and can result in severe illness, up to euthanasia, if not effectively managed.

Treatment of PAF is challenging due to the lack of a complete awareness about its etiopathogenesis.

It was once believed that the development of PAF in the German shepherd dog was linked to anatomic features, such as low tail carriage and a higher density of perianal apocrine sweat glands (Budsberg et al., 1985; Killingsworth et al., 1988), but the consciousness that several breeds with different tail carriage can develop the condition and the lack of clinical response to antimicrobial therapy alone, led to investigate new theories (Killingsworth et al., 1988).

In the case of German shepherd dog, the strong association between the breed and perianal fistulas suggested a genetic susceptibility. In recent years few studies have explored potential genetic risk factors for the disease in German shepherd breed with interesting preliminary results (Kennedy et al., 2008; Barnes et al., 2009; Massey et al., 2014). A potential shared pathogenesis has been suggested for human Crohn's disease (CD) and ulcerative colitis and canine perianal fistulas, due to a particular genetic region found in both diseases (Massey et al., 2014). Furthermore clinical (Sandborn et al. 2003; Galandiuk et al. 2005) and histological (Day et al., 1992) similarities have been recognized between PAF and perianal CD in man.

This led to think that PAF probably has an immune-mediated pathogenesis, as many studies have tried to demonstrate (Day and Weaver, 1992; Day et al., 1993; Harkin et al. 1996; Mathews et al. 1997; Mathews and Sukhiani 1997; House et al. 2003; Tivers et al. 2008; Kennedy et al. 2008), and may be related to an inflammatory colitis or other chronic inflammatory bowel conditions (Jamieson et al.,2002), some of which can be related to food sensitivity, but, in the case of PAF, it is rarely the only contributing factor (Proverbio et al., 2010).

Due to these considerations, over the years there has been a paradigm shift from surgical management to long-term medical management of canine perianal fistulas. Surgical intervention and correction of anatomic factors were once the mainstays of therapy, with varying recurrence rates and a high prevalence of complications (Vasseur, 1984; Milner et al., 2006). Today immunosuppressive drugs are the most commonly used therapies for management of canine perianal fistulas, with the best evidence of efficacy for calcineurin inhibitors (cyclosporine A or tacrolimus) (Harkin et al., 1996; Mathews and Sukhiani 1997; Mathews et al., 1997; Tisdall et al., 1999; Griffiths et al., 1999; Misseghers et al., 2000; Patricelli et al., 2002; Mouatt, 2002; Doust et al., 2003; O'Neill et al., 2004; Hardie et al., 2005; Klein et al., 2006; House et al., 2006; Harkin et al., 2007; Stanley and Hauptman, 2009).

Despite their effectiveness, finding alternative treatment strategies, which allow at least to reduce the dosages or treatment duration of immunomodulatory molecules, is very important due to the many side effects of these agents and very high costs of some of them

(Mathews and Sukhiani 1997; Tisdal et al., 1999).

On purpose, PRP is increasingly used in regenerative medicine, as evidenced by several published and experimental reports in human and veterinary medicine, and could represent a new resource in the treatment of PAF.

Autologous PRP is a cost-effective and readily available therapeutic blood derivative. It is rich in growth factors, expecially platelet-derived growth factors (PDGF) and transforming growth factor-β (TGF-β) (Kim et al., 2009), which influence cellular recruitment, proliferation and differentiation enhancing wound healing and tissue regeneration (Anitua et al., 2004).

In this clinical case, PRP was combined with prednisone, tacrolimus and metronidazole, which are conventionally used alone or combined for the treatment of PAF (Harkin et al., 1996; Tisdal et al., 1999; Misseghers et al., 2000; Stanley and Hauptman, 2009).

Tacrolimus and prednisone are both immunomodulatory agents.

Tacrolimus, such as cyclosporine, is a calcineurin inhibitor. They cause decreased growth and activation of T lymphocytes (Palmeiro et al., 2013) but, unlike cyclosporine, which is most effective when administered orally, tacrolimus is effective topically, minimizing the risk of systemic adverse effects (Lauerma et al., 1997).

Its topical application inhibits T-lymphocyte activation and cytokine elaboration in the skin and draining lymph nodes (Homey et al., 1998) and appears to be safe when applied for long periods in humans, with no systemic accumulation and minimal adverse effects (Reitamo et al., 2000). It brings considerable benefits in humans with psoriasis, atopic dermatitis, pyoderma gangrenosum, oral and perineal Crohn's disease, and vulvar lichen sclerosis (Assmann et al., 2003; Assmann et al., 2000; Assmann et al., 2001; Casson et al., 2000; Ruzicka et al., 1999; Ruzicka et al., 2003; Ruzicka et al., 1997; Schuppe et al., 1998).

To authors knowledge, there are few studies involving topical application of tacrolimus ointment in dogs with perianal fistulas (Misseghers et al., 2000; Stanley and Hauptman, 2009).

Tacrolimus and prednisone used individually for the treatment of perianal fistulas have provided encouraging results. In 1996 Harkin et al. observed that 67% of patients with PAF, treated with oral prednisone and a commercially available novel-protein diet for up to 16 weeks, improved but only the 33,3% of dogs achieved complete wound healing (Harkin et al., 1996).

In 2000 Misseghers et al. evaluated the effect of 16 weeks topical application of 0.1% tacrolimus ointment as the sole treatment for perianal sinuses in 10 dogs. In that study, 5 dogs had complete resolution of lesions, 4 dogs had a partial response and 1 dog did not improve.

A better outcome resulted from the combination of the two molecules, in association with a novel-protein diet and metronidazole, administered for 16 weeks (Stanley and Hauptman, 2009). At the end of that period, perianal sinuses resolved completely in 15 of 19 dogs treated.

For this study, the authors chose to combine them, in order to provide initial systemic immunosuppression with prednisone and to inhibit local T-lymphocyte activation with tacrolimus.

Despite the negative outcome of the microbiological culture, metronidazole was administered due to its antimicrobial activity against faecal anaerobes and immunomodulatory effects (Killingsworth et al., 1988).

Finally, a strict diet based on a new protein (fish) was included with the intent of avoiding allergens in case the perianal sinuses or any concomitant colitis may have been caused or exacerbated by a food antigen.

This drugs association led to complete wound healing after only 4 weeks from the starting of the treatment.

We can not determine to what extent every drug was responsible for the healing of the fistulas, but, based on the results of the studies conducted on their use, we can hypothesize that PRP could have reduced healing time.

The observation of the dog is still in progress, as only 8 weeks have passed from the first application of the PRP to the drafting of this case report, but in this period no recurrences of lesions and no adverse effects were observed.

Unfortunately, recurrence of disease is a common problem following cessation of immunosuppressive treatment for immuno-mediated pathologies, therefore it will be

interesting to observe if there will be a recurrence of the lesions, once the low dose of prednisolone-based therapy is stopped.

CONCLUSIONS

This case report hypothesizes that the autologous PRP obtained with a double centrifugation method could be considered as an excellent adjuvant to conventional therapies for the treatment of canine perianal fistulas.
Associating PRP with therapeutic protocols already note may speed the healing of perianal fistulas.
For this purpose, new large-scale studies are needed in order to elucidate a clear mechanism of action and identify negative effects of use.

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