

# Graft-versus-Host Disease in a Dog After Reduced-intensity Hematopoietic Stem Cell Transplantation from a DLA-identical Littermate

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**Abstract.** *Background: Graft-versus-host disease (GvHD) is an adverse effect following hematopoietic stem cell transplantation (HSCT) in humans. Dogs represent a key model organism for the development of treatment protocols for HSCT. However, detailed descriptions of canine GvHD and its treatment are rare. Herein we describe the development of canine GvHD and therapeutic intervention. Materials and Methods: A female Beagle received an allogeneic HSCT from a dog leukocyte antigen-identical littermate (conditioning with 4.5 Gy total body irradiation; immunosuppression with cyclosporine A). Results: GvHD developed at day +52 and was treated with methylprednisolone, cyclosporine A, antibiotics, antiviral medication and analgesics. The dog initially responded to the treatment but GvHD relapsed twice. Within one week after discontinuation of glucocorticoid, GvHD recurred resulting in inevitable euthanasia of the animal. Conclusion: GvHD represents a life-threatening disease after HSCT in canines. Immediate therapeutic treatment is indicated and even a successful initial treatment response does not necessarily prevent GvHD recurrence.*

Humans and dogs can be affected by several hematopoietic diseases such as refractory anemia, leukemia or lymphoma. Common therapeutic regimens addressing hematopoietic

malignancies include chemotherapy and irradiation, as well as hematopoietic stem cell transplantation (HSCT) in humans. In contrast to the human setting, HSCT is currently not yet employed as a common treatment option in veterinary medicine. However, dogs are used as a default model for human transplantation research. Considering their genetic identity, dogs and humans have a greater similarity than do humans and rodents (1). Accordingly, dogs are considered a valuable biological model for clinical research in humans. Further comparable aspects include size, common metabolic pathways, and cardiovascular similarities, as well as high transferability of canine experimental data to clinical applications. Numerous studies using dogs as model organisms for human HSCT document different transplantation protocols (2-5). Recently, autologous HSCTs were successfully performed in dogs suffering from lymphoma and this was consequently considered to be a novel potential treatment option for dogs (6). Additionally, a curative allogeneic HSCT was also performed in a canine case of T cell lymphoma (7).

In general, major allogeneic transplant associated complications include infections as well as graft-versus-host disease (GvHD). Both are associated with high morbidity and mortality rates (8-10). They also appear similarly as in humans as life-threatening side-effects following canine HSCT (11, 12).

Donor T cells play a key role in GvHD (13). In human allogeneic GvH effects are mediated by activation and proliferation of alloreactive donor T cells that attack the recipient tissues, mainly the skin, bowel and liver (9). Prevalence of acute GvHD is described affecting 35-50% of human HSCT recipients (14). Chronic GvHD is seen in approximately 50% of patients after allogeneic HSCT (13).

The traditional classification defined acute GvHD as occurring until day 100 after myeloablative HSCT and chronic GvHD as manifestation after day 100 (15). The

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National Institutes of Health revised the classification in 2005, focusing on clinical symptoms rather than on the historical strict temporal separation by the day 100 cut off: acute GvHD (features of chronic GvHD are absent) is thereby divided into classic acute GvHD (within 100 days) and late acute or persistent, recurrent GvHD (after day 100 but with features of acute GvHD) (16). Typical skin, liver or gastrointestinal abnormalities should be classified as acute GvHD without relation to the time of occurrence. Chronic GvHD is divided into classic chronic GvHD (features of acute GvHD are absent) and overlap syndrome (combined features of chronic and acute GvHD) (16, 17).

The most commonly affected organs in acute GvHD are the skin, the liver and the gastrointestinal tract. Earliest and most common symptoms in humans are maculopapular skin rashes, often starting with palm and sole involvement. The hepatic symptoms are characterized by elevated liver enzymes and hyperbilirubinemia. Diarrhea, nausea and vomiting are also typical symptoms. In chronic GvHD, the most commonly affected organs are the skin (with lichenoid and sclerotic rash), eyes, mouth, joints, liver, gastrointestinal tract and sometimes also the lungs (14). Due to the rare therapeutic application of allogeneic HSCT in dogs, detailed data regarding canine GvHD are limited and recent studies describing development and treatment of canine GvHD in detail are rare.

This case report describes the course of GvHD and the treatment in a dog after an allogeneic HSCT from a dog leukocyte antigen (DLA) identical littermate during an HSCT study consisting of 22 dogs in total. No other dog in this study showed any signs of GvHD.

## Materials and Methods

A 2-year-old female Beagle dog received a reduced-intensity allogeneic HSCT as part of an HSCT study using the following experimental setting. Conditioning consisted of 4.5 Gy total body irradiation (TBI). Within 24 h after TBI, HSCs from a DLA identical sibling were infused intravenously. The stem cells were harvested under general anesthesia from the femur, iliac spine and humerus. The dog received the unmodified graft containing  $7.2 \times 10^6$  CD34+ cells/kg bodyweight. The DLA identical sibling donor was selected by matching for highly polymorphic DLA-associated class I and class II microsatellite markers by polymerase chain reaction amplification and subsequent capillary electrophoresis (18, 19). The day of HSCT was designated as day 0. For pre- and post-transplant immunosuppression, the dog received 30 mg/kg cyclosporine A orally, from day -1 to day +35 post HSCT.

All procedures were performed in accordance with national and international guidelines. The initial study was approved by the National Animal Protection Board Mecklenburg-Vorpommern (State Institute for Agriculture, Food Safety and Fishery Mecklenburg-West Pomerania, Germany; LALLF M V/TSD/7221.3 1.1-028/13) in accordance with the EU Directive 2010/63/EU for animal experiments.

## Results

The dog developed full donor chimerism (defined as >95% of cells of donor origin) in the granulocyte and peripheral blood mononuclear cell (PBMC) compartment within 7 and 21 days, respectively, and remained a stable full donor chimera until euthanasia at day +168. The hematopoietic recovery occurred rapidly. Leukocyte count  $>1 \times 10^9/l$  and platelet count  $>20 \times 10^9/l$  were recorded at days +9 and +15, respectively. The dog was in good physical condition without any signs of discomfort until day +50 post HSCT. On day +50, the first clinical symptoms of acute GvHD occurred. The physical examination revealed purulent discharge of both eyes. The dog was therefore treated with 1% chloramphenicol eye ointment for two days. On day +52, the dog showed lameness, skin and ear erythema, extreme salivation, dry and encrusted nose with mucopurulent discharge (Figure 1) and an increased body temperature of 40.0°C. In addition, blood analyses showed highly elevated liver enzymes: alanine transaminase: 1,507 U/l [upper normal limit (UNL)=70 U/l]; aspartate transaminase: 270 U/l (UNL=50 U/l), gamma-glutamyl transpeptidase: 30 U/l (UNL=5 U/l), alkaline phosphatase: 1,315 U/l (UNL=108 U/l) (Figure 2). Based on these symptoms and findings, acute GvHD of the skin as well as the liver was diagnosed. The dog was immediately treated with 14 mg methylprednisolone (PRED) (1 mg/kg/d) for immunosuppression, metamizole as analgesic, and antiviral (acyclovir) and antibiotic (sulfamonomethoxazole) medication.

On day +55, cyclosporine A (30 mg/kg/d) was added to the medication as steroid-sparing strategy so the PRED was tapered by 3% that day. Due to reduced general health condition, fluids (500 ml) were given the same day. During the course of disease, the liver enzymes increased sharply (Figure 2). The dog showed no diarrhea. Considering the clinical situation, antibiotics were switched during the course of disease (details indicated in Figure 3). During the whole episode of disease, the dog's skin was also treated with antibacterial shampoo and zinc cream whenever open skin lesions occurred in order to reduce bacterial skin infection. Dimetinden (2.5 mg/d, *s.c.*) was used to treat generalized itching. Posatex ear ointment (orbifloxacin, mometasone furoate, posaconazole) was applied because of Malassezia ear infection and antibiotic eye drops were given to treat purulent eye infection. After five days of treatment, the exanthema improved. Thereafter, a gradual dose reduction of PRED to 7 mg/d was started and combined with a reduction of cyclosporine A to 15 mg/kg/d at day +60. Further reduction of the PRED dose by 1 mg every third day was started from day +57. Beginning on day +69, the dog lost hair, especially on the head but also on the body.

The first GvHD recurrence occurred when PRED was reduced to 3 mg/d on day +73. Erythema of the skin and skin



Figure 1. Photographic documentation of graft-versus-host disease (GvHD) after hematopoietic stem cell transplantation. Upper panel: Eye redness during the early phase of GvHD on day +53 (left) and axilla with open skin lesions during the final, steroid refractory phase of GvHD on day +166 (right). Middle and lower panel: Development of skin erythema and skin lesions over time inside the pinna of the ear and on the abdomen.

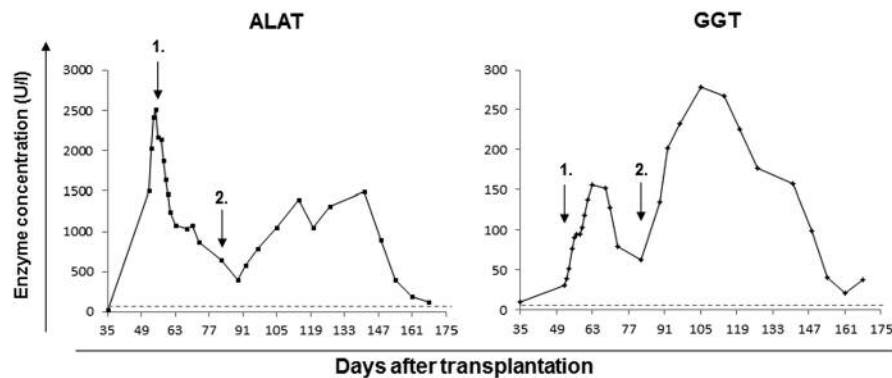


Figure 2. Liver enzyme concentrations. Alanine transaminase (ALAT) and gamma-glutamyl transpeptidase (GGT) before the onset and during the time course of graft-versus-host disease (GvHD). The horizontal dashed lines show the upper normal limits for the enzymes. 1: Onset of GvHD; 2: first recurrence of GvHD.

lesions recurred (Figure 1) and the GvHD became severe again. On day +82, the skin condition continued to worsen, resulting in weep wounds and purulent abscess. Consequently PRED was raised to the initial dose of 1 mg/kg/d on day +82 and cyclosporine A was also increased to the initial dose a day later. Furthermore, the dog developed a purulent abscess

located near the left anterior superior iliac spine on day +84 and on the contralateral side on day +111 that was flushed daily with a povidone iodine solution (betaisodona) resulting in abscess reduction over time. The skin healed within a few days. The immunosuppression was tapered even more slowly this second time. The PRED dose was reduced to 0.7 mg/kg/d

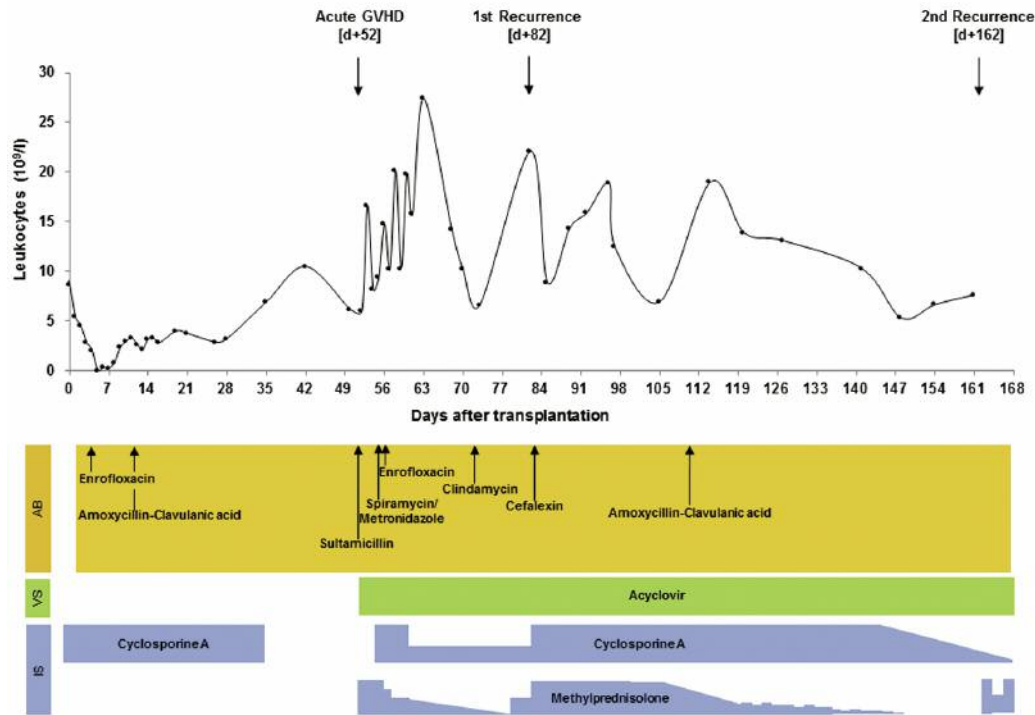


Figure 3. Schematic representation of leukocyte count and treatment timeline following hematopoietic stem cell transplantation (HSCT). Upper panel: The leukocyte count decreased immediately after HSCT as a consequence of the conditioning but had reached normal levels by day +50. Starting with the onset of graft-versus-host disease (GvHD) on day +52, large fluctuations in the leukocyte count were detected as a result of infections during the GvHD. Arrows indicate the time points of GvHD onset and the first and second recurrence. Lower panel: Specification antibiotics (AB), antivirals (VS) and immunosuppressants (IS) that were applied as scheduled during the first 35 days post transplantation and that were administered for treatment of GvHD starting on day +52. Changes in dosing of the immunosuppressants are reflected in the magnitude of the blue bars.

on day +100. Compared to the first reduction course, the second tapering phase was intended to be longer. The treatment plan was to taper PRED slowly by 10% reduction every 3 days. However, variations were allowed as of physician's direction based on clinical findings. At a PRED dosage of 1 mg, a gradual reduction of cyclosporine A by 10% every second day was concurrently initiated. When cyclosporine A was down to 20 mg/kg/d, the skin became lightly reddish and the abscess became purulent again. At 15 mg/kg/d cyclosporine A, the skin became more reddish and the dog started losing hair around its eyes and mouth. Despite the GvHD signs, tapering was continued due to the infection.

The GvHD eventually recurred severely when the cyclosporine A dose was reduced to 12 mg/kg/d on day +162. The skin showed erythema again and there were open wounds, especially in the ears (Figure 1). Signs of strong itching recurred. In order to attenuate the GvHD, the dog was immediately treated with increasing doses of PRED up to 14 mg/d during the next three days. Despite the intervention, the skin condition deteriorated and lesions also occurred on the body and paws. The eyes still showed

purulent discharge. The dog was treated with metamizole again to relieve pain. Since it was not possible to keep the dog in an acceptable health condition without medication and the second recurrence was of increasing intensity, the dog was euthanized on day +168.

## Discussion

Major limitations of allogeneic HSCT are based on immunological complications such as GvHD, rejection of the graft, or delayed immune reconstitution, all of which are accompanied by a high risk of infection (8). Dogs are well established model animals for preclinical HSCT research (2, 3, 5, 11, 20, 21). HSCT related side-effects such as GvHD and its effective treatment in canines have rarely been described in detail (11, 20, 21).

This study presents a case of GvHD in a dog following reduced-intensity allogeneic HSCT. Canine GvHD was mainly characterized here by skin erythema, skin lesions and highly increased liver enzymes and therefore demonstrated a high degree of similarity to GvHD in humans (14).

In a previous study, Storb *et al.* investigated treatment options for canine GvHD in dogs receiving a myeloablative conditioning and HSCT from non-DLA identical unrelated donors (11). The treatment of these dogs consisted of either methotrexate or cyclophosphamide. In the current study, the dog received reduced-intensity conditioning with 4.5 Gy and received the HSCT from a DLA-matched sibling. A comparison between both studies is therefore difficult as different T-cells might be GvHD effector cells. The occurrence of GvHD in haplo-identical transplantation is even higher since the leukocyte antigen matches only 50%. Chen *et al.* showed initial engraftment without developing GvHD in a study of dogs receiving a haplo-identical transplantation with nonmyeloablative conditioning and cytotoxic T lymphocyte antigen 4 Ig in combination with a donor PBMC infusion (22).

In humans, the gold standard treatment for acute GvHD is a glucocorticosteroid-based immunosuppressive therapy (23). Concordantly, in the present study, the glucocorticosteroid PRED was applied as first-line therapy, resulting in an initial successful improvement of GvHD symptoms.

Attentive supporting care is necessary due to the reduced immune status as a result of treatment with immunosuppressive medication of long duration and potential synergistic effects of multiple medications (24). Herein, the dog received glucocorticoids for a total of 97 days. The duration of application rather than the total dose increases the risk of adverse effects (25). Infections in dogs after HSCT are documented, as in humans (12). Therefore the dog received several antibiotics as extensive infectious prophylaxis and treatment. In addition, the dog was treated with topical antibiotics. Overall, GvHD resulted in intensive care treatment for more than 90 days.

GvHD itself but in particular GvHD treatment triggers the development of opportunistic infections. We applied several prophylactic measures, however, the dog developed severe infections and subsequently was euthanized due to infection in combination with steroid-refractory GvHD.

Steroid-refractory GvHD is described in humans and associated with mortality rates of up to 90% (26). It is defined as no improvement of the skin within 5 days of treatment or if the manifestation in any organ worsens over 3 days (23). Our HSCT recipient initially responded to high dose treatment with PRED and cyclosporine A. However, it was not possible to taper off the two drugs completely. Even the use of a prolonged tapering schedule after the first recurrence of GvHD was not sufficient to eventually control the disease and to prevent the second recurrence of GvHD. At that stage, GvHD was no longer treatable even with high dose immunosuppressive therapy, and was therefore defined as steroid-refractory.

## Conclusion

Severe canine GvHD is a possible life threatening side-effect following allogeneic HSCT. The occurrence and symptoms are comparable with those of human GvHD. Immediate treatment at first signs of severe GvHD seems advisable. However, in cases of steroid-refractory GvHD, alternative therapeutic options need to be identified.

## Conflicts of Interests

The Authors declare that they have no competing interests in regard to this study.

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