



# Type III hypersensitivity reaction with immune complex deposition in 2 critically ill dogs administered human serum albumin

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## Abstract

**Objective** – To describe 2 cases of vasculitis that were attributed to a type III hypersensitivity reaction in critically ill dogs occurring 8–16 days postadministration of human serum albumin (HSA).

**Case or Series Summary** – Skin biopsies were obtained from 3 different sites in 2 critically ill dogs that developed vasculitis 8–16 days following treatment with HSA. Histopathological findings from both dogs indicated epidermal pallor, widespread edema and hemorrhage, degenerative neutrophilic perivascular infiltrates, and multifocal areas of neutrophilic or leukocytoclastic vasculitis. Immunohistochemical staining using an anti-human serum albumin rabbit antibody suggested that the antigen-antibody complexes seen in the dermis were associated with the administration of HSA.

**New or Unique Information Provided** – In this case series, we documented a leukocytoclastic vasculitis and probable antigen-antibody complexes to human albumin in the dermis of 2 critically ill dogs after administration of HSA. Previously, type III hypersensitivity reactions had only been reported in healthy dogs that had received HSA. This report also describes the potential use of immunohistochemical staining to detect the HSA antigen in tissue sections through the use of specifically labeled antibodies.

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**Keywords:** canine, complication, drug-reaction, immunohistochemistry, vasculitis

## Introduction

Administration of human serum albumin (HSA) has been used in the treatment of hypoalbuminemia in critically ill canine and human patients.<sup>3,4,15,16</sup> Critically ill dogs commonly have a significant hypoalbuminemia secondary to their underlying disease process, which can lead to severe life-threatening complications.<sup>1,3,4</sup> Hypoalbuminemia has been shown in human patients and veterinary patients with critical illness to be a poor prognostic indicator.<sup>1,5,7</sup> With severe hypoalbuminemia, patients are not able to maintain colloid osmotic pressure causing leakage of fluid out of the capillaries. Other important functions of albumin include its role as a trans-

## Abbreviations

HSA	human serum albumin
IHC	immunohistochemical

port protein, as an antioxidant, and as a component of normal platelet function.<sup>1,5</sup>

Despite the potential benefits of administering albumin to restore depleted plasma concentrations, there is controversy about its effectiveness in both people and animals. The safety of administering HSA in canine patients has also been questioned.<sup>2,7,9</sup> The incomplete homology between human and canine albumin raises concern that the administration of commercially purified HSA between species can elicit a significant immune response.<sup>2</sup>

Type III hypersensitivity reactions are delayed reactions that can be seen after an antigenic substance is administered intravenously.<sup>8</sup> The foreign antigen binds with circulating antibody and forms immune complexes in the blood. If the immune complexes cannot be cleared

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from the circulation, deposition occurs in the vessel walls of the arteries, glomeruli, and synovia.<sup>8,11</sup> The deposited immune complexes can activate complement, release chemotactic and clotting factors, cause proliferation of the epithelial cells, and trigger neutrophils to release free radicals and enzymes causing tissue destruction.<sup>1</sup> This can lead to a vasculitis, glomerulonephritis, or arthritis. The hallmark histologic lesion associated with a type III hypersensitivity reaction is a leukocytoclastic vasculitis.<sup>23,25</sup> In people, previous studies have shown immune deposits in the vasculature in the early stages of vasculitis with differing immunoglobulin complexes, including those with albumin. These immunoglobulins were shown to diffuse out of the vessel walls variably as the disease course progresses.<sup>25</sup> The systemic signs typically occur hours to weeks after exposure to a foreign antigen.<sup>8</sup> The clinical signs documented in healthy dogs with suggested type III hypersensitivity reactions to HSA include but are not limited to lethargy, facial and peripheral edema, cutaneous lesions indicative of a vasculitis, echymoses, shifting leg lameness, fever, vomiting, hypovolemic shock, difficulty breathing, oliguria, and death.<sup>2,7,9</sup>

To the authors' knowledge, this is the first documented report of a type III hypersensitivity reaction occurring in critically ill dogs administered HSA. The cases also illustrate the use of immunohistochemical staining to identify HSA antigen-antibody complexes in dogs that have developed vasculitis within days to weeks of HSA administration.

## Case Summaries

### Case 1

A 6-year-old female spayed Rottweiler initially presented for severe lethargy, a decreased appetite, occasional vomiting of 2 weeks duration, and black tarry stools. The dog's clinical signs were ultimately attributed to a septic abdomen diagnosed by visualization of free fluid on abdominal ultrasound and a cytological examination of the fluid demonstrating intracellular bacteria. Prior to surgery the patient was fluid resuscitated with a crystalloid bolus. An exploratory laparotomy was performed and a perforation at the duodenal/pyloric junction was surgically corrected. During surgery there were multiple episodes of mild hypotension and tachycardia that responded to both crystalloid and colloid boluses. Recovery from anesthesia was uneventful. There was a documented postoperative hypoalbuminemia of 9.0 g/L [0.9 g/dL] (reference interval 25.0–46.0 g/L [2.5–4.6 g/dL]). There was no known prior exposure to HSA. The dog was administered 250 mL of 25% HSA<sup>a</sup> (5.6 mL of 25% human albumin/kg or 1.4 g/kg) diluted to a 5% solution in 0.9% NaCl<sup>b</sup> and administered over 4 hours

on day 2. The dog was discharged from the hospital after a total of 7 days of hospitalization.

The dog presented to the primary care veterinarian 16 days post-HSA administration for a decreased appetite and peripheral edema. The dog was rehospitalized 3 days later due to declining condition including vomiting and worsening peripheral edema. Physical exam parameters at the time of this admission included normothermia of 100.9°F (38.3°C), a heart rate of 150/min, and panting. There was moderate scleral hemorrhage in both eyes. There was severe peripheral limb edema, and severe erythema of the ventrum.

Skin biopsies were obtained from 3 different sites (ventral trunk, limb, and face). A 6-mm punch biopsy instrument<sup>c</sup> was used to obtain the biopsy samples. The biopsies were submitted for histopathology and immunohistochemical staining. The histopathologic diagnosis was epidermal pallor, widespread edema and hemorrhage, mixed suppurative perivascular dermatitis, and multifocal areas of neutrophilic vasculitis. These changes are consistent with a primary vasculopathy.

Initial treatment included a 1-liter bolus of 0.9% NaCl<sup>b</sup>. The patient was administered prednisolone acetate<sup>d</sup> (1 mg/kg SQ q 12 h × 36 h). The dog was also treated with pentoxifylline<sup>e</sup> (10 mg/kg PO q 8 h), diphenhydramine<sup>f</sup> (2 mg/kg PO q 8 h), misoprostal<sup>g</sup> (3.4 mcg/kg PO q 8 h), sucralfate<sup>h</sup> (22 mg/kg PO q 8 h), amoxicillin/clavulanic acid<sup>i</sup> (11.5 mg/kg PO q 12 h), metronidazole<sup>j</sup> (11.5 mg/kg PO q 12 h), and continued on omeprazole<sup>k</sup> (0.5 mg/kg PO q 24 h). The patient continued to have a decreased appetite and an esophagostomy tube was placed on day 3. The dog was tolerating esophagostomy tube feedings and was discharged from the hospital for continued care at home 4 days after presentation. Medications included all previously mentioned and in addition ondansetron<sup>l</sup> (0.2 mg/kg PO q 8–12 h), prednisone<sup>m</sup> (0.5 mg/kg PO q 12 h) with instructions to taper, metoclopramide<sup>n</sup> (0.2 mg/kg PO q 8 h), and mirtazapine<sup>o</sup> (0.5 mg/kg PO q 24 h). The peripheral edema resolved and the patient's appetite returned to normal following discharge. The patient had no further known sequela to the administration of the HSA, but was euthanized approximately 2 months later for progressive hind limb lameness and a diagnosis of osteosarcoma.

### Case 2

A 10.5-year-old male neutered Norwegian Elkhound initially presented for acute lethargy, anorexia, and weakness/tetraparesis. The patient's clinical signs were ultimately attributed to an intestinal foreign body diagnosed by abdominal ultrasonography. Prior to surgery the patient was fluid resuscitated with boluses of both crystalloids and colloids. An exploratory laparotomy

was performed and the foreign body was removed via an enterotomy. There were no anesthetic complications during surgery and recovery from anesthesia was uneventful. There was documented postoperative hypoalbuminemia of 10 g/L [1.0 g/dL] (reference interval (25.0–46.0 g/L [2.5–4.6 g/dL])). There was no known prior history of receiving HSA. The dog was administered 100 mL of 25% HSA<sup>a</sup> (5.2 mL of 25% human albumin/kg or 1.3 g/kg) diluted to a 5% HSA solution in 5% dextrose and water<sup>b</sup> and given over 3 hours on day 2. The dog was discharged after a total of 7 days in the hospital.

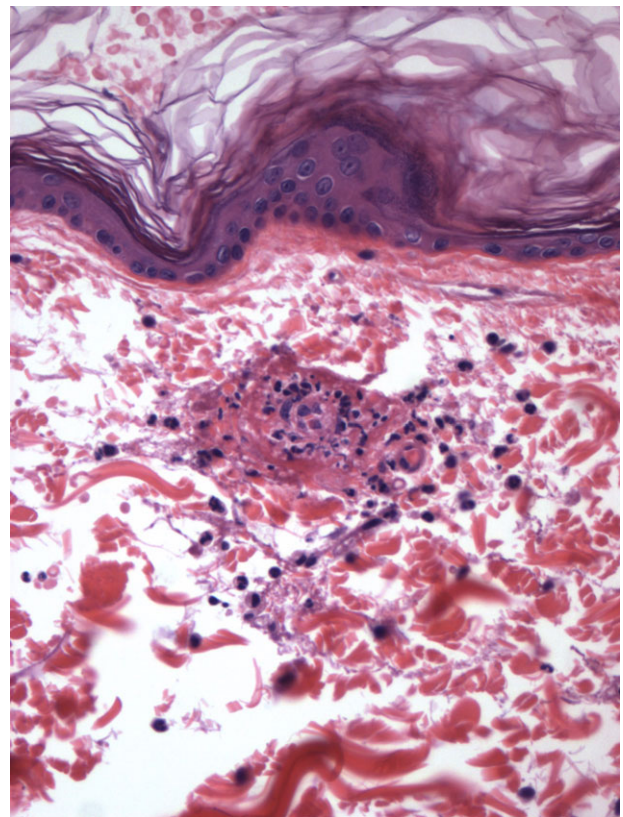
The dog represented 8 days post-HSA administration for a decreased appetite, petechiations, peripheral edema, and lethargy. Physical exam findings at the time of admission included hyperthermia at 104.2°F (40.5°C), a heart rate of 120/min, and panting. The left mandibular and left prescapular lymph nodes were mildly enlarged. Petechiation was noted on the ventral abdomen and left lateral thorax extending to the proximal hock region.

Skin biopsies were obtained from 3 different sites (ventral trunk, limb, and face). A 6-mm punch biopsy instrument was used to obtain the biopsy samples. The biopsies were submitted for histopathology and immunohistochemical staining. The histopathologic diagnosis was a leukocytoclastic vasculitis. There was epidermal pallor, dermal edema with areas of hemorrhage, and degenerative neutrophilic perivascular infiltrates. The changes are consistent with a primary vasculopathy (Figure 1).

Initial treatment consisted of administration of IV fluids with 1.25% dextrose, diphenhydramine<sup>f</sup> (2.5 mg/kg IM once, then 2.5 mg/kg PO q 8 h), dexamethasone sodium phosphate<sup>p</sup> (0.4 mg/kg IV once), and pentoxifylline<sup>e</sup> (20 mg/kg PO q 8 h). All previous medications were discontinued. The following day the patient was discharged with diphenhydramine<sup>f</sup> (2.5 mg/kg PO q 8 h), prednisone<sup>m</sup> (1.25 mg/kg PO q 12 h) with instructions to taper, and continued on pentoxifylline<sup>e</sup> (20 mg/kg PO q 8 h). The peripheral edema, petechiations, and lethargy resolved and the patient's appetite returned to normal following discharge. The patient had no further sequela noted at 1-year post-HSA administration, but was lost to follow-up after that time.

### Immunohistochemical procedure

The immunohistochemical (IHC) stain was developed by a commercial laboratory<sup>q</sup> to confirm that the areas of vasculitis were secondary to the administration of HSA. The tissue included skin biopsies from the 2 critically ill dogs given HSA, a skin biopsy from a healthy dog, and a skin biopsy from a dog with peripheral edema that had not received HSA. The preparation included cutting paraffin sections at 4 microns and affixing to posi-



**Figure 1:** Histopathology of a section of a skin biopsy from Case 2. Neutrophilic infiltrate in the vessel wall and in the interstitium, with nuclear dust (leukocytoclastic vasculitis). Hematoxylin and eosin staining (200 × magnification).

tively charged slides.<sup>t</sup> The slides were baked for 1 hour at 60°C. Deparaffinization was performed with the following washes: 3 changes of 100% xylene<sup>s</sup> (5 minutes each), 2 changes of 100% alcohol (5 minutes each), then 1 change of 95% alcohol (5 minutes), to a final 1 change of deionized water rinse.

Immunohistochemical stain protocol included placing the tissues in a casein protein block<sup>t</sup> for 5 minutes. No wash was performed prior to immersion into F0117 polyclonal rabbit anti-Human Albumin IgG<sup>u</sup> concentrate 10 g/L at a 1:4,000 and 1:8,000 dilution for 30 minutes each. This antibody reacts with human albumin. The specificity has been ascertained by crossed immunoelectrophoresis, only the albumin precipitation arch appears when using unconjugated antibody corresponding to 50 μL F 0117 per square cm gel area against 2 μL human plasma (Coomassie Brilliant Blue staining). A negative reagent control was performed with rabbit IgG isotype,<sup>v</sup> which was matched to the concentration of the primary antibody using the dilution of 1:8,000 and was immersed for 30 minutes each.

Three buffer washes were performed with tris-buffered saline with Tween, 2 minutes each, before

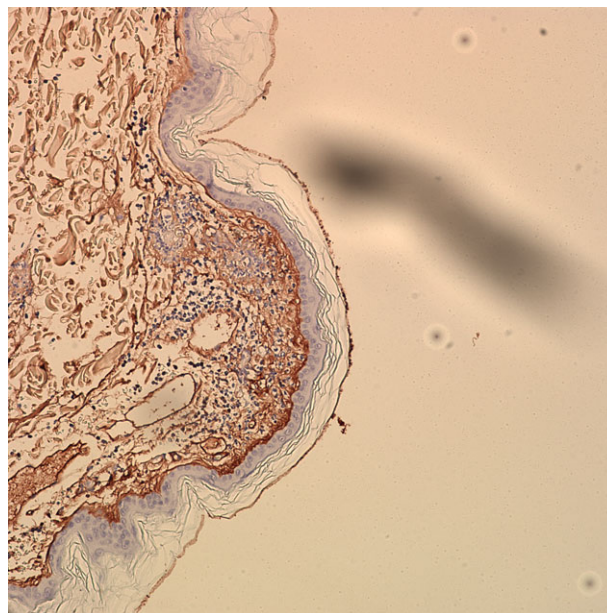


the tissue was placed in a horseradish peroxidase anti rabbit-labeled polymer detection reagent<sup>w</sup> for 10 minutes. The sections went through 3 more buffer washes with tris-buffered saline with Tween, 2 minutes each. A 10-minute incubation step using 3% hydrogen peroxide was performed. The tissue was then placed in a 3,3'-Diaminobenzidin<sup>x</sup> brown substrate/chromogen for 3–5 minutes. The tissues were immersed for 2 minutes in hematoxylin nuclear counterstain and then washed with distilled water. The slides were then dehydrated in an ascending gradient of alcohol and cleared with xylene before cover slipping with permanent mounting media.

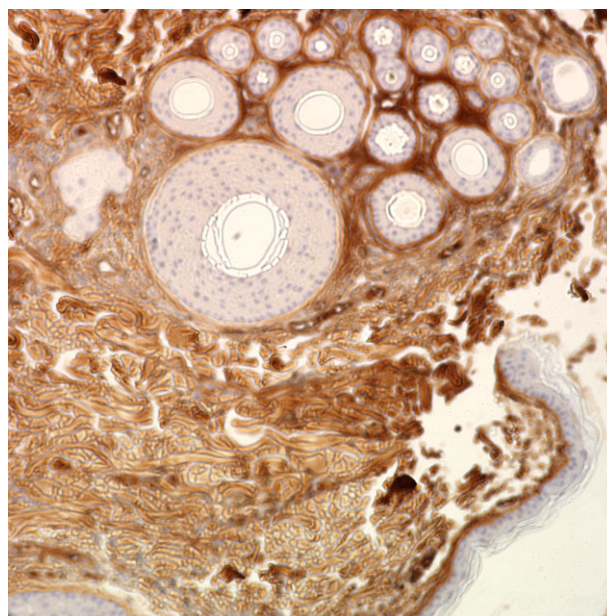
The immunohistochemical staining for HSA at the 1:4,000 and 1:8,000 dilution showed dark brown staining diffusely in the area of the dermis of both dogs that had received HSA. (Figures 2 and 3). These findings are in accordance with the leukocytoclastic vasculitis and clinical signs are highly suggestive of a type III hypersensitivity reaction and suggest the presence of HSA antibody complexes in the dermis associated with the areas of vasculitis in both patients. The negative reagent control did not show any IHC staining in the tissue of either patient using Rabbit IgG isotype at a 1:8,000 dilution (Figure 4). The negative tissue controls did not show IHC staining in the skin sections from a healthy dog and a dog that had peripheral edema with a histopathologic diagnosis of marked congestion and a mild perivascular lymphohistiocytic-plasmacytic inflammation. A Western blot was performed on the anti-human albumin antibody, which showed cross-reactivity between canine and human albumin.

### Discussion

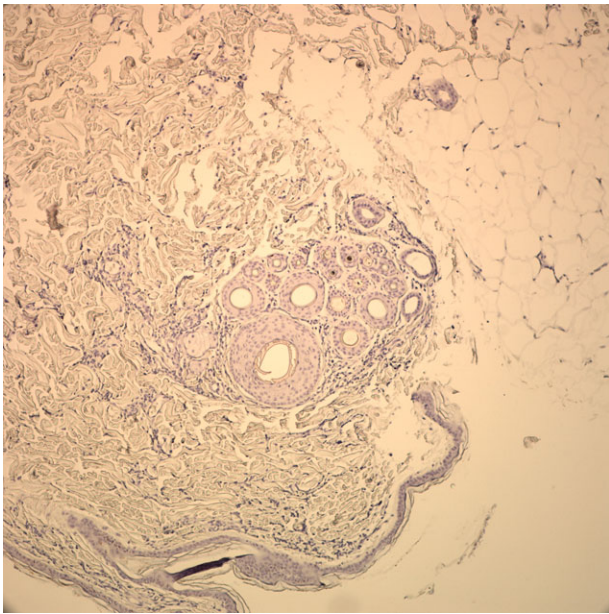
Type III hypersensitivity reactions are characterized by the formation and deposition of antibody/antigen complexes in tissues, which activates the complement cascade, causing inflammation and tissue destruction. The hallmark cutaneous finding is a small vessel vasculitis also known as leukocytoclastic vasculitis. Changes involve the arterioles, venules, and capillaries, and histopathologic findings include endothelial cell swelling, inflammatory infiltrates in the vessels and adjacent connective tissue (predominantly neutrophils), karyorrhexis of the neutrophils, and extravasation of red blood cells.<sup>23</sup> The clinical signs and histopathology from the skin biopsies in both dogs in this study confirmed the presence of a cutaneous vasculitis. Immunohistochemical staining suggests the presence of human albumin in the leukocytoclastic vasculitis lesions of the dermis and the presence of these antigen-antibody complexes support a type III hypersensitivity reaction secondary to the administration of HSA in these 2 critically ill dogs. The dogs' response to treatment and recovery with no known



**Figure 2:** Immunohistochemical staining of a section of a skin biopsy from Case 2. The section was incubated with polyclonal rabbit anti-Human Albumin Ig at a 1:4,000 dilution. Diaminobenzidin is the chromogen. Positive staining is indicated by the chocolate brown deposits in the area of the dermis (100 × magnification).



**Figure 3:** Immunohistochemical staining of a section of a skin biopsy from patient 1. The section was incubated with polyclonal rabbit anti-Human Albumin Ig at a 1:8,000 dilution. Diaminobenzidin is the chromogen. Positive staining is indicated by the chocolate brown deposits in the area of the dermis (200 × magnification).



**Figure 4:** Negative reagent control for patient 2 using Rabbit IgG isotype at a 1:8,000 dilution. No uptake of stain is seen (100 × magnification).

further sequela is consistent with a cutaneous type III hypersensitivity reaction to HSA administration.

To determine if there was specific staining of the antibody for human albumin in the tissue, a negative tissue control was performed on both healthy dog skin and skin from a dog with peripheral edema and inflammation. Approximately 60% of endogenous albumin is found extracellularly, with the highest concentration of interstitial albumin in the skin.<sup>24</sup> In a dog with an inflammatory response and peripheral edema or vasculitis there would be an even larger percentage of endogenous canine albumin in the interstitial space. Albumin will extravasate secondary to increased vascular permeability or increased capillary net filtration pressure.<sup>1,5,13,23</sup> The Western blot of the human serum albumin antibody did show an interaction of the antibody with human albumin and canine albumin. If the antibody were cross-reacting to a significant amount of canine albumin in the tissue it would be expected to also see staining in the extravascular space of the negative tissue controls, which was not seen. To further validate this observation a species-specific antibody for human albumin would need to be developed and investigated further.

HSA administration in people is associated with rare occurrence of side effects. A study on the safety of albumin administration in people found that serious adverse effects occurred with an incidence of 1.29 per 10<sup>6</sup> doses.<sup>20</sup> Despite the species-specific use of HSA and the rare report of serious adverse effects, its use re-

mains controversial in human medicine.<sup>16,20</sup> In 1998, the Cochrane Injuries Group Reviewers published a meta-analysis of 30 randomized trials that found the relative risk for death was greater with albumin versus crystalloid administration.<sup>16</sup> There have been several other meta-analyses since, which have not been able to confirm the Cochrane findings but have found no benefit in the use of albumin over crystalloids.<sup>6,19</sup> A large multi-center prospective study, known as the SAFE study, found that 4% albumin was equally as effective as normal saline for fluid resuscitation.<sup>21</sup> A follow up to this study found that in a subset of patients with traumatic brain injury there was a higher mortality rate associated with the administration of albumin.<sup>14</sup> The majority of the older studies on HSA administration evaluated the use of albumin as a resuscitation fluid, but newer literature is beginning to focus on specific indications for albumin administration. Results of more recent studies have favored the use of albumin in specific disease states including sepsis and acute respiratory distress syndrome.<sup>15,18,19,21</sup> The administration of albumin in human patients appears to be safe, but further studies are currently being undertaken to determine the benefits of albumin administration in specific subgroups.

Previous studies have documented significant type III hypersensitivity reactions to HSA in healthy dogs.<sup>2,7</sup> One study demonstrated that the administration of HSA to healthy dogs caused suspected type III hypersensitivity reactions in all six dogs, and fatal complications in 2 of the 6 dogs.<sup>2</sup> A second study documented the development of anti-HSA antibodies in nine healthy dogs receiving HSA, with 2 dogs developing signs of a type III hypersensitivity reaction.<sup>7</sup> Two of the 9 dogs that did not have adverse reactions to the initial infusion were administered a second infusion and had severe life-threatening anaphylactic reactions.<sup>7</sup> It has been suggested that the severity of the immune reaction in healthy dogs is due to either their ability to elicit an appropriate immune response or the presence of normal serum albumin concentration prior to administration of HSA.<sup>2,7,9</sup>

There have been multiple studies where critically ill, hypoalbuminemic canine patients had been administered HSA with no documented delayed adverse hypersensitivity consequence.<sup>3,9,10</sup> One study documented the presence of IgG antibodies to HSA in 14 critically ill dogs, but no type III hypersensitivity reactions were reported.<sup>9</sup> In critically ill animals, it is not uncommon to see suppression of the immune system secondary to the underlying disease process and/or unfavorable nutritional status.<sup>2,9</sup> It is thought that the compromised immune system makes it difficult for these dogs to mount a significant immune response to the highly antigenic HSA and as a result the reactions are not observed or nonexistent. It has been shown that there are lower



levels of anti-human albumin IgG in critically ill dogs compared to healthy dogs, and this study hypothesized that this could be due to gastrointestinal loss of antibodies secondary to their underlying disease.<sup>9</sup> It is also plausible that delayed hypersensitivity reactions are under-diagnosed due to lack of patient follow-up data or because patient death either occurred prior to the onset of a delayed hypersensitivity reaction or death was falsely attributed to the underlying disease process.

Critically ill hypoalbuminemic dogs are administered many medications, including HSA, to treat their underlying disease process. Clinical signs consistent with a type III hypersensitivity reaction after administration of HSA in conjunction with other treatments have been reported.<sup>4</sup> The cases in the present report suggest that these reactions can be secondary to the administration of HSA versus reactions to other medications or effects of ongoing disease. HSA administration to critically ill hypoalbuminemic dogs has not been uncommon at this emergency hospital and we have seen similar delayed reactions prior, but they have been infrequent and unpredictable in occurrence. One recent study suggested that administration of 5% human serum albumin is safe in critically ill dogs and they did not see severe hypersensitivity reactions in their patient population of 418 dogs during administration of HSA.<sup>10</sup> This study was retrospective, and the authors acknowledged that follow up data was unavailable to evaluate for delayed reactions.

In veterinary medicine, it has been found that HSA can provide oncotic support, an increase in blood pressure, and an increase in serum albumin, but it is unclear if its use effected the clinical outcome in these studies.<sup>3,4</sup> Although albumin's major role is to provide oncotic support, it has other important physiologic roles in the body. Due to the structural difference between canine and human albumin it is unlikely the human albumin molecule functions identically to canine albumin *in vivo* in dogs. Although endogenous albumin has both anti-inflammatory and anti-coagulant effects, it is thought that HSA may promote inflammation and hypercoagulability in dogs secondary to its antigenicity.<sup>5</sup> The recent availability of canine albumin may be a safer and more physiologic option in dogs for albumin supplementation. Lyophilized canine albumin is made from controlled fractionation of pooled canine donor plasma.<sup>7</sup> Future studies are needed to test the safety of species-specific canine albumin and to determine if there is a survival advantage in certain disease states.

In summary, type III hypersensitivity reactions related to the administration of HSA occur not only in healthy dogs but also in critically ill canine patients. Although type III hypersensitivity reactions may be self-limiting in critically ill dogs, human serum albumin should be used with caution. Patient selection should be carefully

considered prior to the use of an albumin solution. Further investigations are needed to evaluate the incidence of type III hypersensitivity reactions in critically ill dogs receiving human serum albumin. Therapeutic goals should be aimed at correcting the underlying cause of the decreased serum albumin, and exogenous albumin supplementation for colloid oncotic support should only be considered after exhausting other treatment options. Clients with patients where HSA administration is a consideration should be advised of the potential for type III hypersensitivity reactions. The recent availability of canine albumin provides a species specific option for albumin supplementation, but further studies are needed to determine its safety and efficacy.

### Acknowledgments

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### Footnotes

- <sup>a</sup> Plasbumin-25, Bayer Healthcare, Elkhart, IN.
- <sup>b</sup> 0.9% Sodium chloride and 5% dextrose in water, Hospira Inc, Lake Forest, IL.
- <sup>c</sup> 6 mm biopsy punch, Miltex Inc, York, PA.
- <sup>d</sup> Prednisolone acetate, Watson Laboratories, Corona, CA.
- <sup>e</sup> Pentoxifylline, Hoechst Marion Roussel, Kansas City, MO.
- <sup>f</sup> Diphenhydramine, Parke-Davis Pharmaceuticals, Ann Arbor, MI.
- <sup>g</sup> Misoprostal, G.D. Searle LLC, Peapack, NJ.
- <sup>h</sup> Sucralfate, Nostrum Laboratories, Inc, Kansas City, MO.
- <sup>i</sup> Clavamox, Pfizer Animal Health, New York, NY.
- <sup>j</sup> Metronidazole, TEVA Pharmaceuticals, Sellersville, PA.
- <sup>k</sup> Omeprazole, Kremers Urban Pharmaceuticals, Princeton, NJ.
- <sup>l</sup> Ondansetron, GlaxoSmithKline, Research Triangle Park, NC.
- <sup>m</sup> Prednisone, Qualitest, Huntsville, AL.
- <sup>n</sup> Metoclopramide, TEVA Pharmaceuticals.
- <sup>o</sup> Mirtazapine, Merck and Co Inc, Whitehouse Station, NJ.
- <sup>p</sup> Dexamethasone SP, Butler AHS, Dublin, OH.
- <sup>q</sup> IHCtech, LLC.
- <sup>r</sup> Positively charged slides, StatLab Medical Products, McKinney, TX.
- <sup>s</sup> Mallinckrodt Baker Inc, Center Valley, PA.
- <sup>t</sup> Protein Block, Open Biosystems, Lafayette, CO.
- <sup>u</sup> F0117 Polyclonal rabbit anti-Human Albumin Ig, Dakocytomation, Glostrup, Denmark.
- <sup>v</sup> Rabbit IgG isotype, Southern Biotech, Birmingham, AL.
- <sup>w</sup> Powervision poly-HRP anti-Rabbit IgG, Leica Microsystems, Buffalo Grove, IL.
- <sup>x</sup> Stable 3,3'-Diaminobenzidin (DAB), Invitrogen, Grand Island, NY.
- <sup>y</sup> Canine Albumin, lyophilized, Animal Blood Resources International, Stockbridge, MI.

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