

Steroid-Responsive Cold-Agglutinin Disease with Hematological and Dermatological Manifestations in a Dog

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ABSTRACT

An 8-year old, spayed female mixed-breed dog presented with necrotic, ulcerated lesions in the body extremities. Both pinnae showed extensive ulceration and necrosis with full-thickness fissures, extending horizontally through most of the pinnae. Crust-covered ulcerations, with variable degrees of necrosis and sero-sanguineous discharge were noted in the paw-pads, the dorsal aspect of the muzzle and the tail-tip. The mucous membranes were pale. A complete blood count revealed a regenerative anemia. Direct whole blood in-saline slide agglutination test showed mild agglutination (1 on a scale of 4) at room temperature, which was markedly increased (4 of 4) following a 15-minute incubation at 4°C (39.2°F). Histopathology of biopsies from the pinnae, paw-pads and the muzzle demonstrated non-inflammatory, obliterative arteriopathy, with thrombosis, necrosis and exudative dermatitis. The acral distribution of the lesions, the histopathological findings and the accentuation of in-saline slide hemagglutination following cold incubation, were supportive of cold agglutinin disease (CAD). Imaging studies, biochemistry as well as serology and polymerase-chain reaction tests for endemic blood parasites were negative. Immunosuppressive prednisone treatment led to marked improvement of the skin lesions and resolution of in-saline slide agglutination and anemia. To the best of the authors' knowledge, this is the first report of a favorable response to prednisone treatment in a dog with concurrent hematological and dermatological manifestations of CAD and the first report of CAD in Israel.

Key words: Cold; Agglutinin; Prednisone; Anemia; Thrombosis; Hemolytic

CASE PRESENTATION AND TREATMENT

An 8-year old, spayed female mixed miniature Pinscher dog was referred to the Hebrew University Veterinary Teaching Hospital (HUVTH) due to necrotic, ulcerated lesions in body extremities. The dog resided in a mountainous area (767 meters above sea level), with mean ambient temperature highs and lows during the preceding month of 16° to 8°C. Two weeks prior to presentation, a bleeding lesion on the muzzle and exudative lesions on the pinnae were noted. The

dog had no prior medical problems and was not vaccinated or medicated during the preceding six months. The referring veterinarian prescribed amoxicillin (dose and duration unknown), with no improvement.

At presentation, the dog was quiet, alert and responsive, with a body condition score of 5/9, normal vital signs, pale mucous membranes and a mild generalized lymphadenopathy. Both pinnae showed alopecia, necrosis and ulcerated lesions, sero-sanguineous exudate and full thickness fissures, with almost complete tissue disconnection. The distal, dorsal

Figure 1: (a) Left pinna; Note the alopecia, necrosis and ulcerations, with sero-sanguineous exudate and full thickness fissure, extending horizontally throughout most of the pinna (arrow); (b) Note the alopecia and ulcerations with crusts on the dorsal aspect of the distal muzzle and nasal planum hyperkeratosis; (c) Ulcerations of variable severity in the paw-pads of both hindlimbs; (d) An ulcerated lesion with sero-sanguineous secretions at the distal tail.



aspect of the muzzle showed alopecia, extensive crust-covered ulcerations, and nasal planum hyperkeratosis. The footpads of both hindlimbs and the left frontlimb showed ulcerations of variable severity. Ulceration on the tip of the tail was also noted (Figures 1a-d).

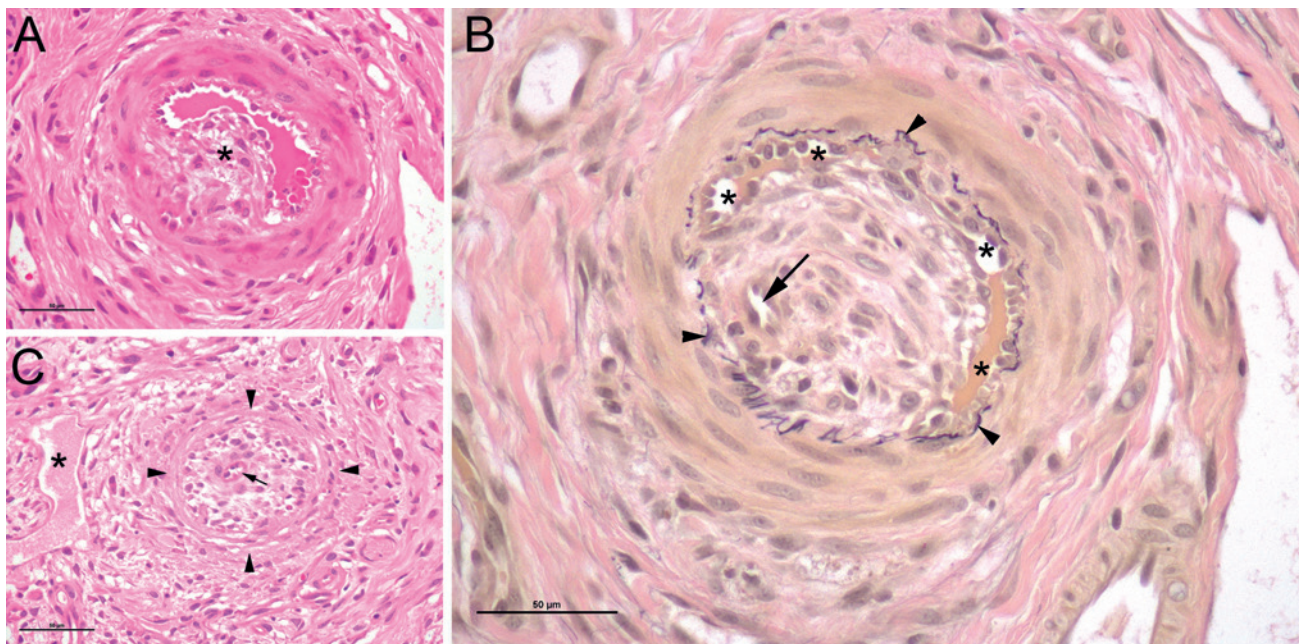
Hematological abnormalities included a macrocytic hypochromic, regenerative anemia (RBC $3.26 \times 10^6/\mu\text{L}$; reference interval [RI] $5.7\text{--}8.8 \times 10^6/\mu\text{L}$; hematocrit 28.3%; RI 37–57%), mature neutrophilia ($16.15 \times 10^3/\mu\text{L}$; RI $3.9\text{--}8.0 \times 10^3/\mu\text{L}$), anisocytosis, mild spherocytosis and a mild polychromasia (2–4%). Direct in-saline slide agglutination test of whole blood collected in EDTA showed mild hemagglutination (1 on a scale of 4) at room temperature (approximately 25°C). After 15 minutes of incubation at 4°C , marked in-saline slide hemagglutination (4 of 4) was noted. The whole blood in the EDTA tube was then incubated at

room temperature and the test was repeated, demonstrating markedly decreased microscopic in-saline hemagglutination (1 of 4). An osmotic fragility test (in 0.54% saline) was positive. Serum biochemistry abnormalities included mild hyperglobulinemia (5.4 g/dL; RI 1.8–3.9 g/dL), hypoalbuminemia (2.42 g/dL; RI 3.0–4.44 g/dL) and hypocholesterolemia (122 mg/dL; RI 135–362 mg/dL). Urinalysis was unremarkable.

Fine needle aspirates from the popliteal and pre-scapular lymph nodes showed evidence of reactive lymphadenopathy, with no evidence of neoplasia or infection. Serology (enzyme-linked immunosorbent assay) (1) for *Leishmania infantum*, and polymerase chain reaction (PCR) tests for *Babesia canis* and *Ehrlichia* spp. (Karnieli Laboratory, KiryatTivon, Israel) were negative. Thoracic survey radiographs and abdominal ultrasonography were unremarkable.

In light of the history of exposure to cold ambient tem-

Figure 2: (A) A medium sized artery from the pinna with focal damage and proliferation of the wall (asterisk) causing partial occlusion. (B) A serial section of the artery stained with an elastin stain demonstrates subtotal occlusion of the lumen by very cellular tissue within which there is recanalization (arrow). The residual lumen is marked with asterisks. Arrowheads: fragmented internal elastic lamina. (C) The wall of the vessel in the center of the field has undergone extensive damage (“ghost vessel”). Its approximate outer margin is marked by arrowheads. There is full occlusion by cellular tissue with recanalization (arrow). In the surrounding tissue there is edema and fibroplasia. A dilated lymphatic vessel is marked (asterisk). (H&E X40)



peratures, the acral distribution of necro-ulcerative skin lesions and the marked in-saline slide agglutination at 4° C, the dog was tentatively diagnosed with CAD (2-5). Additional differential diagnoses for the skin lesions included vasculitis, vaso-constrictive toxin-associated ischemic necrosis, systemic lupus erythematosus, disseminated intravascular coagulation, cutaneous adverse drug reaction, and cryoglobulinemia (2). The regenerative anemia was presumed secondary to immune-mediated hemolysis, in light of the positive direct in-saline agglutination test and presence of spherocytosis.

In pursuit of a definitive diagnosis, biopsies were obtained from lesions on the pinnae, affected paw-pads and muzzle. The necrotic, severely lacerated distal parts of the pinnae were surgically excised. Pending histopathology results, the dog was treated initially with prednisone (1.66 mg/kg, PO, q12h), clindamycin (12.5 mg/kg, PO, q12h) and omeprazole (1.66 mg/kg, PO, q12h).

Microscopic examination of biopsies from the pinnae demonstrated obliterative arteriopathy (Figure 2a-c), widespread vasculopathy, extensive fibrosis and deep necrosis. The most striking lesions were in medium sized arteries which showed degenerative changes ranging from loosening and

separation of the intima to diffuse and transmural cellular loss (“ghost vessels”). Partial to full obliteration of the arterial lumen by cellular tissue, in some cases with recanalization, was common. Veins showed loosening and fragmentation of their walls. Throughout the entire thickness of the pinna there was extensive fibrosis of variable maturation accompanied by marked dilation of lymphatic vessels and variable edema. Multifocal necrosis frequently extended to and involved the aural cartilage. Necrotic tissue was covered by thick serocellular crusts. Away from these, inflammatory infiltration was mild. In the paw pads and muzzle there was vasculopathy, dermal fibrosis, ulceration, exudative dermatitis and hyperkeratosis. The histological findings corroborated the clinical diagnosis of CAD (2).

The dog was evaluated again 18 days after initiation of treatment. The owners reported normal behavior and appetite. The skin lesions on the muzzle and both pinnae had either completely healed or were covered with epithelium, with no discharge. In the left frontlimb, all paw-pads ulcers had healed. Several ulcers were still noted in the paw-pads of both hind-limbs, albeit smaller than at first presentation. CBC showed near resolution of the anemia (RBC 5.48 X10⁶/

μL ; RI 5.7-8.8: hematocrit 41.7%; RI, 37-57), with mild polychromasia (2-4%). Spherocytes were absent. In-saline slide agglutination was negative, both at room temperature and after a 15 minute incubation at 4°C. Treatment with prednisone was continued, unchanged, and azathioprine (2.1 mg/kg, PO, q24h) was added to facilitate a more rapid prednisone tapering, while maintaining immunosuppression, once skin lesions would have completely resolved. Unfortunately, the dog was hit by a car and died three weeks later. According to the owners, during that period, the remaining ulcers had completely healed. Residual skin lesions included mild crusting in the paw-pads and muzzle, in addition to mild non-ulcerating erythema in both pinnae.

DISCUSSION

The diagnosis of CAD in the present dog was based on classical historical, clinical, laboratory and pathological features of the disease, with ischemic tissue necrosis secondary to arterial thrombosis in the extremities, and concurrent microscopic hemagglutination that was markedly accentuated following cold incubation (2-5).

Cold agglutinins are defined by their ability to agglutinate erythrocytes at temperatures below normal body temperature (4-6). While a wide range of such temperatures may allow cold-hemagglutination cold agglutinins (CA) characteristically reacting at optimal temperatures of 0° to 4° C (32°F-39.2°F) (4, 5). The thermal amplitude, defined as the highest temperature at which CAs react, is far more important in determining the clinical outcome than the actual CA titer (5, 6). Higher thermal amplitudes allow hemagglutination, complement fixation, thrombosis and hemolysis at distal body parts, even at relatively mild ambient temperatures (4-6). The cold-agglutinin-erythrocyte interaction initiates the classical complement cascade (3, 4). Due to the presence of erythrocyte surface regulatory proteins, the terminal phase of the complement cascade, namely the membrane attack complex formation, rarely occurs (3-5). Therefore, intravascular hemolysis is uncommon in CAD (3-5). However, while erythrocytes-bound CAs in body extremities elute from the erythrocytes upon their return to the central circulation, at core body temperature, complement proteins remain erythrocyte-bound, facilitating extravascular phagocytosis of opsonized cells, resulting in extravascular hemolysis and spherocytosis (3, 4). The regenerative nature

of the anemia of the dog, the positive in-saline slide agglutination and presence of spherocytosis are consistent with extravascular immune-mediated hemolytic anemia (7). Microangiopathy secondary to peripheral microthromboses might have contributed to the hemolysis, notwithstanding the lack of schistocytosis in the blood. Blood loss through the necrotic skin lesions, albeit mild, is an additional possible contributing reason for the anemia in this dog. The moderate anemia nearly resolved following 2 weeks of immunosuppressive prednisone treatment. Furthermore, while Coomb's test may have proved more sensitive and specific in detecting hemagglutinins compared to the in-saline slide agglutination test (8), the negative in-saline slide agglutination test at follow-up indicated an apparent response to the immunosuppressive treatment.

In humans, CAD may be benign or pathologic, primary or secondary, and the latter is mostly associated with malignancies or acute infections (4, 5). Polyclonal, low-titer CAs are relatively common, benign and unassociated with clinical signs or hemolysis (4). Monoclonal CAs, on the other hand, are pathogenic, and are commonly associated with variable clinical disease and hemolysis (4). Similar associations have never been established in dogs. The dog described herein had mild hyperglobulinemia, but electrophoresis to determine clonality was not performed. In dogs, immune mediated hemolysis or CAD, as well as hyperglobulinemia, might result from vaccination, drugs, infections and malignancies (7). Neither recent vaccination nor drug exposure were reported herein. Common endemic parasitic and rickettsial infections were ruled out. Imaging studies of both the chest and the abdomen, blood tests and lymph node aspirates were not supportive of neoplasia.

Case reports of CAD in dogs are rare, and include few, older reports of idiopathic CAD (9-11), and a case of lead-associated CAD in a puppy (12). In another report, CAD was described in two newborn puppies, presumably resulting from ingestion of maternally-derived CAs in the colostrum (13). Two recent reports described asymptomatic, non-hemolytic cold-reactive hemagglutinins in 3 dogs (14,15). In one, CAD was associated with *Mycoplasma cynos* pneumonia, and resolved with resolution of the infection (14), similarly as in the case of *Mycoplasma* pneumonia in humans (4, 5). The other 2 dogs were serendipitously diagnosed with cold-reactive agglutinins, with no apparent underlying cause, or clinical and hematological consequences (15).

While exposure to cold environment is associated with clinical CAD and exacerbation of hemolysis, higher antibody thermal amplitudes mean that even in temperate environments, clinical signs may be severe when the ambient temperature exceeds the antibody thermal amplitude (4-6). In the present dog, CAD became apparent clinically under mild weather conditions (mean ambient temperature highs and lows of 16° to 8°C, respectively). *In vitro* agglutination was only assessed at room temperature and at 4°C, and we have no data of in-saline slide agglutination at the temperature range of 4°C to 25°C and >25°C. The strong positive agglutination such as had occurred at 4°C might have occurred also at temperatures higher than 4° C *in vivo*, in light of the occurrence of CAD at higher ambient temperatures.

Notwithstanding the apparent favorable response to treatment, with disappearance of the in-saline agglutination and resolution of skin lesions, firm conclusions should be made cautiously as long-term follow-up could not be made. Experience with glucocorticoid therapy in human primary CAD cases has been poor, with low, short-term response rates, and other commonly prescribed immunosuppressive drugs (i.e. chlorambucil, azathioprine and cyclophosphamide) proving to be similarly unrewarding (4, 5). However, human primary CAD disease is often associated with clonal B-lymphocyte proliferation, which has not been established in dogs, limiting the comparison across species. Of the two single previous cases describing glucocorticosteroid use in dogs with CAD, one with hematological manifestations of CAD and the other with dermatological manifestations, successful response was observed only in the latter, which was exposed to mild environmental temperatures, similar to the present dog (9, 10). The present case is the first report of glucocorticoid-responsive CAD in a dog presenting both dermatological and hematological manifestations. Despite the rarity of clinical CAD in dogs, reflected by the paucity of publications, larger studies are essential to establish the prognosis and treatment recommendations, particularly in light of current human recommendations, which discourage prednisone use.

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