

Confirmed Trapped Neutrophil Syndrome in a Border Collie Puppy in Israel

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ABSTRACT

The case report describes a Border collie puppy diagnosed with trapped neutrophil syndrome (TNS), an autosomal recessive inherited neutropenia. The report describes the clinical presentation, the course of the disease and diagnosis. Chronologically it appeared that that vaccination may have been a potential initiating factor in instigating the TNS in the puppy. The puppy also developed clinical signs of hypertrophic osteodystrophy (HOD). It was not possible to determine whether either vaccination and/or the development of HOD were coincidental. To the best knowledge of the author this is the first genetically confirmed case of TNS described in Israel and the first case documented outside the U.K or Australia. It is recommended that TNS should be weighed as a possibility in any Border collie puppy which presents with symptoms of a vague illness and confirmed neutropenia.

Keywords: Persistent Neutropenia; Hypertrophic Osteodystrophy; Trapped Neutrophils Syndrome; Bone Marrow Hypercellularity; Border Collie.

INTRODUCTION

Trapped neutrophil syndrome (TNS) is an autosomal recessive inherited neutropenia known in Border Collies since the 1990's (1). The clinicopathologic presentation of the disease is an immuno-deficiency characterized by peripheral neutropenia and retention of neutrophils in the bone marrow. Clinical signs often appear by 16 weeks of age. Persistent or recurrent infections usually result in death or euthanasia at less than one year of age (1, 2, 3). The causative mutation has been identified in the canine VPS13B gene and a DNA-based diagnosis is available (4). Long term prognosis is usually poor although 2 cases of successful long term management have been described (3).

CASE DESCRIPTION

A two month old Male Border Collie born in a litter of five to working farm dogs without any known medical conditions.

The breeder reported that the dog was in good health since its delivery but was about 25% smaller than its littermates.

The dog received its first puppy DA2LPP vaccination and was adopted out to a family 5 days later. The new owners changed the diet and presented the dog to the referring veterinarian two days after adoption, reporting occasional vomiting and watery yellowish diarrhea, weakness, partial anorexia and reluctance to walk. On examination the dog was quiet yet responsive, vitals normal but it was unwilling to stand. The dog ate Hill's A/D canned diet (Hill's Science Diet, USA) at the clinic and was discharged with metronidazole 15mg/kg PO BID for 7 days.

Two days later, on re-examination the dog was ambulatory, more alert with fewer signs of diarrhea. The dog continued to improve and presented for its second DALPP vaccination. One day later the dog was brought into the clinic in a depressed, weak and anorexic. The dog was given an injection of dexamethasone SP (dose unknown) and I/D canned

food (Hill's Science Diet, USA). Two days later the owners reported mild improvement followed by a deterioration of its overall condition.

At the next visit, the dog was weak and was hospitalized overnight with Lactate Ringer's Solution IV, doxycycline suspension 10mg/kg PO SID and amoxi-clavulonic acid 12mg/kg PO BID. The next day the dog was discharged eating and drinking, with instructions to feed high quality puppy food and physical therapy exercises. Eight days after discharge the owners reported continued improvement followed by an acute deterioration. On presentation the following day the dog was weak, unwilling to stand, anorexic, with painful forelimbs on palpation.

At that point, 25 days after adopting the dog it was returned back to the breeder. A complete blood count (CBC) and chemistry profile revealed the following abnormal findings: Leukopenia, neutropenia, anemia (Hematocrit = 29%), thrombocytopenia, and low blood urea nitrogen and albumin concentrations.

Thrombocytopenia and neutropenia were not confirmed on a blood smear evaluation. The dog was hospitalized with LRS IV, amoxi-clavulonic acid 12mg/kg PO BID, enrofloxacin 5mg/kg SQ BID, Vitamin B12 500mg IM, and iron inj (dose/brand unknown). After 2 days of supportive therapy without significant clinical change the dog was referred to "Knowledge Farm Emergency and Specialty Center".

Upon arrival to the Knowledge Farm hospital the dog was quiet but responsive. Auscultation was normal for heart and lungs, abdominal palpation was normal and the body temperature was 38.3°C. The dog was reluctant to stand or walk but was able to stand assisted. Neurological examination revealed normal reflexes and normal cranial nerves without conscious proprioception deficits in all 4 limbs. Both elbows were swollen and painful and marked pain was also noted on palpation of both forelimbs proximal to the carpi. A complete blood count was repeated (Procyte Dx, IDEXX Diagnostics, Westbrook, Maine, USA): The dog was anemic (Hematocrit=23.5%) with a severe leukopenia and neutropenia and a mild thrombocytopenia. The results were confirmed on blood smear evaluation.

The dog was treated with Lactated Ringer's Solution (Hartman's Solution, Teva Medical Ltd., Ashdod, Israel) IV overnight. The appetite returned but the puppy was still painful and reluctant to stand the next day. Radiographs of the fore- and hindlimbs demonstrated mild soft tissue swelling

around both elbows and radiolucent lines in the metaphyseal areas of the distal radius, ulna, tibia and fibula of all 4 limbs, consistent with hypertrophic osteodystrophy (HOD).

The neutropenia along with the swollen elbows raised a concern for septic arthritis. The owner declined additional diagnostics and Metronidazole (Flagyl, Sanofi-Avetnis, Israel) was added to the dog's treatment as well as Carprofen (Rimadyl, Zoetis) 2.2mg/kg PO SID-BID as was required by pain assessment.

Due to the likelihood that the dog could not become a working dog, it was donated to the hospital and continued to receive supportive care both at the hospital and at one of the veterinarian's home for the next month and a half. During that time, the dog exhibited cyclic clinical signs which included fever (up to 41.6°C), anorexia and reluctance to walk – all intermittently, with apparently "normal days in between". During hospitalization CBC's and blood smear evaluations were performed on multiple occasions and marked neutropenia was always present. The lowest neutrophil count was found to be $0.73 \times 10^3/\mu\text{L}$ and the highest $3.99 \times 10^3/\mu\text{L}$.

Serologic analysis for *Neospora caninum* and *Toxoplasma gondii* were sent to the laboratory of the Kimron Veterinary Institute, Beit Dagan and was found to be negative. Cobalamine levels were checked (A.M.L Veterinary Department, Herzlia, Israel) and found to be elevated 989pg/mL (Reference Interval 300-800 pg/mL). A blood sample was sent for genetic testing (VHL Genetics, Wageningen, The Netherlands) for Trapped Neutrophil Syndrome (TNS) evaluation. Repeated radiographs exhibited resolving HOD of the fore- and hind limbs.

Approximately 1.5 months after its arrival at the referral hospital, the dog became once again febrile and anorexic and developed bloody diarrhea. Its blood glucose was 57 mg/dL and abdominal ultrasound scan revealed severe enteritis, mesenteric lymphadenopathy and mild ascites. Blood smear demonstrated marked neutropenia with neutrophils appearing toxic. The assessment was of sepsis and the dog was humanely euthanized.

Immediately after the euthanasia a femur was removed and sent for histological evaluation (PathoVet Ltd., Rehovot, Israel). The marrow examination revealed hypercellularity with a high myeloid:erythroid ratio and predominance of hypersegmented neutrophils. Megakaryocyte numbers were estimated to be in the normal range showing orderly maturation. The myeloid granulocytic reserve was observed to be high.

The results of the genetic testing confirmed the diagnosis of TNS. This dog was homozygously affected for the mutation, indicating that both parents were carriers. The breeder of this dog declined further genetic testing of the dog's parents or siblings.

DISCUSSION

The main differentials for this dog's persistent neutropenia included TNS, familial cobalamin deficiency (described in this breed) (5) and cyclic neutropenia which has been described in Gray collies (6). Vitamin B12 levels were normal and the dog's neutrophil counts did not improve with cobalamin treatments. Repeated CBC's and blood smear evaluations did not reveal normal neutrophil counts at any point of time, making both of these diagnoses unlikely. Parvoviral enteritis was not considered a likely diagnosis due to the chronicity of the neutropenia and lack of significant gastrointestinal signs for the most part of the course of the disease.

The swollen joints in addition to the marked neutropenia raised a concern for septic arthritis. Arthrocentesis was not performed primarily due to financial consideration but was also a concern for iatrogenic introduction of infectious agents into the joints due to the neutropenia. Radiographs of multiple joints were more consistent with HOD than septic arthritis. Broad spectrum antibiotics were given in case a septic agent was after all present.

HOD is a syndrome seen mostly in large breed growing puppies 3-4 months of age. The etiology of HOD is not clear, but an auto-immune etiology has been suggested (7, 8). HOD is also a major feature in "Weimaraner Immunodeficiency Syndrome" in which neutrophil dysfunction or failure to produce sufficient IgA/IgG antibody levels have been suggested as possible causes (9, 10). It was interesting to find two apparently disconnected conditions in this dog, one involving a neutrophil disorder and the other HOD, which may suggest some connection between the two syndromes.

Another interesting observation is that vaccination has been implicated as a potential instigating cause of HOD and may also have been a factor in preceding the clinical symptoms in this dog with TNS. It is difficult to determine whether this is a temporal coincidence or a true cause and effect.

This dog initially responded to supportive therapy but eventually developed severe gastroenteritis with hypoglyce-

mia. Furthermore the toxic changes in the neutrophils were suggestive of sepsis resulting in the decision for euthanasia at only 5 months of age. Immunomodulatory therapies as well as corticosteroids were not used to manage this dog's condition.

Genetic testing definitively confirmed a diagnosis of TNS and histological evaluation of the bone marrow revealed a hypercellular bone marrow, a high myeloid:erythroid ratio, and a high myeloid granulocyte reserve.

The clinical presentation and clinical course of disease in this dog is consistent with previously published reports, consisting of intermittent pyrexia, lethargy, HOD like bone lesions, joint pain and gastrointestinal signs (1, 2, 3).

It is possible that in cases when owners do not want to wait for genetic testing results in a sick puppy (which may take several weeks), a bone marrow aspirate may be a rapid method to differentiate between TNS and other similar syndromes based on the presence of a hypercellular marrow.

Interviews with several Border collie breeders in Israel revealed "Parvoviral" related death cases in well vaccinated puppies, suggesting the possibility that some of this puppies were actually TNS cases rather than truly parvoviral infections.

TNS is a relatively newly recognized disease entity, which has been identified in Britain and Australia since the 1990's but relatively little has been published about it and its existence is not widely known in the practicing veterinary community. In addition, to the best of my knowledge, no confirmed cases outside the U.K or Australia have been documented and certainly not in Israel. It is recommended that TNS should be weighed as a possibility in any Border collie puppy in Israel (and likely worldwide) which presents with symptoms of a vague illness and confirmed neutropenia.

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