Clinical findings, treatment, and outcome of trapped neutrophil syndrome in Border Collies: 12 cases (2011-2022)

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Structure Summary

Objectives: This study aimed to evaluate clinical signs, diagnostic findings, treatment administered and short (survival to 28 days) and long-term prognosis (survival > 6 months) in dogs diagnosed with trapped neutrophil syndrome.

Methods: Medical records of 12 dogs (ten Border Collies and two Border Collie Crossbreeds) homozygous for VPS13B gene mutation causing trapped neutrophil syndrome from 7 veterinary institutions between January 2011 and June 2022 were evaluated retrospectively.

Results: The most common clinical signs at time of diagnosis were pyrexia, abnormal gait, and gastrointestinal signs. Concurrent metaphyseal osteopathy and immune-mediated polyarthritis were common. Seven dogs had a segmented neutrophil count below, four dogs within and one dog above the analyser reference interval at presentation. Two dogs had a septic source identified and both were additionally identified to be homozygous mutant positive on DNA testing by PCR for canine cyclic neutropenia. All dogs received at least one antimicrobial agent and ten dogs received treatment with prednisone or prednisolone (median starting dose 1 mg/kg/day; range 0.5-2.5 mg/kg/day). Nine dogs were alive at 28 days and six dogs were alive at six months post diagnosis.

Clinical Significance: Trapped neutrophil syndrome should be suspected in young Border Collies with pyrexia, lameness, and gastrointestinal signs. Neutropenia may not always be present and long-term survival is possible. A septic focus was not commonly identified in our population however, our results suggest that if identified, testing for concurrent canine cyclic neutropenia should be considered.

Introduction

Trapped neutrophil syndrome (TNS) is an autosomal recessive inherited disease caused by a mutation of the VPS13B gene that affects Border Collies (Shearman & Wilton 2011). The protein VPS13B is found within the outer member of the Golgi apparatus and plays an essential role maintaining its structural and functional integrity (Seifert *et al.* 2011). First reports of TNS were from New Zealand in the 1990s (Allan *et al.* 1996), but since then cases have been reported worldwide, including Australia (Wouda *et al.* 2010), United Kingdom (Mason *et al.* 2014), Japan (Mitzukami *et al.* 2012), Israel (Gans 2015) and United States of America (Hegler *et al.* 2020).

Clinical signs of TNS are typically first apparent by 4 months of age and may include pyrexia, lameness, joint swelling, and reluctance to walk (Allan *et al.* 1996, Wouda *et al.* 2010, Shearman & Wilton

2011, Gans 2015, Hegler *et al.* 2020). Facial dysmorphism (characterised by a narrow, elongated skull) and stunted growth have also been described in affected dogs (Allan *et al.* 1996, Shearman & Wilton 2011, Mitzukami *et al.* 2012, Mason *et al.* 2014, Gans 2015, Zoto *et al.* 2022). The disease is characterised by a persistent peripheral neutropenia despite evidence of myeloid hyperplasia within the bone marrow (Allan *et al.* 1996, Mitzukami *et al.* 2012, Mason *et al.* 2014). In one case report, multiorgan neutrophilic inflammation and increased number of sinusoidal neutrophils in the liver were reported (Zoto *et al.* 2022).

A mutation of the VPS13B is associated with Cohen syndrome in people (Shearman & Wilton 2011). Patients with Cohen syndrome are characterised by microcephaly, typical facial features, childhood hypotonia and joint hyperextensibility, retinochoroidal dystrophy and myopia by 5 years of age, and periods of isolated neutropenia (Kivitie-Kallio & Norio 2001). Some of the features present in Cohen syndrome are also identified in dogs with TNS including facial dysmorphism and neutropenia (Shearman & Wilton 2011).

To date, clinical recommendations for TNS are based on a small number of published case reports, the largest of which included 3 dogs (Mason *et al.* 2014). Current treatment protocols typically include use of glucocorticoids and antibiotics and are aimed at controlling clinical signs, treating secondary bacterial infections, and improving quality of life. Long term prognosis for these dogs is thought to be poor, with many dogs dying or being euthanised before 1 year of age due to persistent neutropenia, recurrent bacterial infections, sepsis, or recurrent polyarthritis (Mizukami *et al.* 2012, Mason *et al.* 2014, Gans 2015, Zoto *et al.* 2022).

This retrospective, multicentre study was undertaken to improve the understanding and management of TNS by aiming to identify a larger TNS dog population and evaluating their clinical signs, diagnostic findings, treatment administered, and short and long-term prognosis.

Materials and Methods

Full ethical approval for this study was obtained and granted by the University of Edinburgh's Veterinary Ethical Review Committee. A veterinary clinical diagnostic laboratory offering genetic testing for TNS (Laboklin UK & Ireland, Manchester, UK), was contacted and assisted in the identification of cases. This diagnostic laboratory identified 23 dogs diagnosed with TNS. The veterinary practices which submitted genetic testing for these TNS dogs were contacted by the diagnostic laboratory for consent to include the patient details within our study. Consent for six dogs to be included within the study was obtained. Additionally, referral hospitals were directly contacted by email by study investigators for dogs diagnosed with TNS. Dogs diagnosed with TNS from seven institutions based either in North America or Europe were identified and their medical records were reviewed. The seven institutions were: Hospital for Small Animals, The Royal (Dick) School of Veterinary Studies, University of Edinburgh, UK; Langford Vets Small Animal Practice, University of Bristol, UK; Queen Mother Hospital for Animals, Royal Veterinary College, UK; Colorado State University, Veterinary Teaching Hospital, USA; Anderson Moores Veterinary Specialists, UK; University Animal Hospital, Norwegian University of Life Sciences, Norway and Dryfe Vets Ltd., Lockerbie, UK. Diagnosis of TNS was defined as a positive homozygous mutant phenotype result on DNA testing by PCR from a buccal swab or whole blood in EDTA.

An electronic spreadsheet (Microsoft Office Excel 2013, Microsoft Corp.) was developed for data collection and distributed by email to all participating institutions. Data was extracted from each institution's medical record database and included signalment, age at diagnosis of TNS, clinical signs prior to diagnosis of TNS, clinical signs on examination, diagnostic results including haematology, biochemistry, urine analysis and culture, synovial cytology and culture, bone marrow cytology and core biopsy, diagnostic imaging findings, and results of blood cultures, infectious disease testing and canine cyclic neutropenia (CCN) PCR testing, when available. Data collection was in accordance with General Data Protection

Regulation (GDPR) guidelines (https://gdpr-info.eu/) to include anonymisation of data collection and client consent to utilise data.

For the purpose of this study, pyrexia was defined as a temperature greater than 39.2°C (Ettinger 2017). Haematology values obtained within the first 24 hours to a referral hospital were recorded. For cases which were not referred and remained under the care of the primary veterinarian, the first available haematology values were used. Absolute cell count values were recorded along with whether they were within, above or below the individual laboratory analyser reference interval.

The time period between first seeking veterinary attention and reaching a diagnosis of TNS was determined by recording the date the client first sought veterinary attention and the date of sample submission for TNS genetic testing.

Additional data extracted from clinical records included treatment administered and outcome. If glucocorticoids were administered, the starting dose (mg/kg/day) was recorded. All doses recorded are for prednisone/ prednisolone. If glucocorticoids other than prednisone/ prednisolone were administered, the potency of the glucocorticoid in question (e.g., dexamethasone) relative to prednisone/ prednisolone was determined to enable dose conversation.

Short-term survival was defined as survival to 28 days and long-term survival was defined as survival of at least 6 months post diagnosis. The reason for non-survival was recorded when available. Further information was obtained from the primary veterinarian +/- client for dogs surviving at least 12 months post diagnosis.

All data was amalgamated into one spreadsheet and analysed by one study investigator (AS).

Literature search

The published veterinary literature was reviewed in March 2022 and October 2023 using the following databases (PubMed and Google scholar) using the keywords “trapped neutrophil syndrome”, “neutropenia” and “canine cyclic neutropenia”.

Statistical Analysis

Data was collected and summarised using an electronic spreadsheet (Microsoft Office Excel 2013, Microsoft Corp.). Owing to the retrospective nature and small number of dogs included within this study, results are presented in a descriptive manner only as number (percent). Where appropriate data is presented as median (range).

Results

Medical records of dogs that met the inclusion criteria from seven institutions based either in North America or Europe between January 2011 and June 2022 were analysed. A total of 12 dogs met the inclusion criteria, being positive for TNS on PCR evaluation. Case 1 has been partially described in a previous case series (Mason *et al.* 2014). The median age at diagnosis was 4.62 months (range 0.75 to 9 months) The population included two males and ten females, all intact, from which ten dogs were Border Collies and two Border Collie crossbreeds. Eleven dogs were referred to a referral centre for investigations and treatment, whilst one dog remained under the care of a primary veterinary practitioner.

Clinical Signs

All 12 dogs (100%) were pyrexic at time of presentation with a median temperature of 40.3°C (range 39.3°C - 41.4°C). Nine dogs (75%) had a history of and/ or evidence of an abnormal gait or lameness on physical examination, with the thoracic limbs most commonly affected. Four dogs (44%) had more than one limb affected. Three dogs (25%) had overt joint effusion on physical examination. Six dogs (50%) had

a history of gastrointestinal signs, four dogs (33%) were underdeveloped and known to be smaller than their littermates, four dogs (33%) were lethargic, four dogs (33%) were deemed ataxic on examination, three dogs (25%) had a peripheral lymphadenopathy, and one dog (8%) was painful on palpation of the neck and temporomandibular joint. One dog (8%) (Case 3) had evidence of an upper respiratory tract obstruction on presentation requiring emergency tracheostomy tube placement followed by mechanical ventilation for suspected non-cardiogenic pulmonary oedema. Severe laryngeal oedema along with submandibular lymphadenopathy were noted in this dog.

Median duration of clinical signs prior to seeking veterinary attention could not be determined due to the retrospective nature of the data. The mean number of days between first seeking veterinary attention and reaching a diagnosis of TNS was 68.3 days (standard deviation = 53.91).

A total of 8/12 dogs received treatment prior to referral. Two dogs were administered both corticosteroids and antibiotics before referral, three dogs were administered corticosteroids and two dogs had antibiotic therapy prior to referral.

Clinicopathologic Analyses

Haematology results for the 12 dogs can be found in Table 1. Six dogs (50%) had haematology results confirmed by blood smear examination. C-reactive protein, blood ammonia and serum cobalamin concentration results, where available, can also be found within Table 1. Biochemistry results for the 12 dogs are shown in Table 2. Eight dogs (66%) had more than one haematology performed during treatment (Table 3). The number of occasions the neutrophil count fell below, within and above the analyser reference interval can be found in Table 3.

Five dogs (42%) had arthrocentesis performed on a single or multiple joints (including the three dogs with overt joint swelling). Synovial fluid cytology was performed by a board-certified clinical pathologist and was consistent with neutrophilic or mixed inflammation in all dogs without evidence of

bacteria, suggestive of immune-mediated polyarthritis (IMPA). Synovial fluid culture was negative for all 5 dogs (100%).

Six dogs (50%) had a urinary culture performed with only one dog having a positive culture (Case 8). This dog cultured *Escherichia coli*, but the method of urine collection could not be confirmed.

Blood cultures were performed in two dogs (17%) and were negative in both dogs. No other cultures were performed for any of the patients. A bone marrow aspirate was performed in two dogs (17%) with myeloid hyperplasia and concurrent erythroid hypoplasia noted in both cases.

Six dogs (50%) were tested for at least one infectious disease: *Toxoplasma gondii* serology (n=3), distemper virus PCR (n=2), antibodies for *Anaplasma* spp, *Borrelia burgdorferi*, *Ehrlichia* spp and antigens against *Dirofilaria immitis* (IDEXX SNAP 4Dx Plus) (n=3), *Bartonella* spp PCR (n=2), *Rikettsia* spp PCR (n=2), *Neorikettsia* spp PCR (n=2), *Wolbachia* spp PCR (n=2), *Blastomyces dermatidis* serology (n=1), *Histoplasma* spp serology (n=1), *Aspergillus* spp and *Coccidioides Immitis* serology (n=1). No infectious agents were identified in any of the six dogs tested.

Six dogs (50%) were DNA tested for CCN by PCR. Two dogs were positive (homozygous mutant) for this condition (Cases 5 and 8).

Diagnostic Imaging Findings

Radiographs of at least one site were performed in 11 dogs (92%). All cases had their imaging findings reviewed by a board-certified radiologist or resident under the supervision of a board-certified radiologist, except for Case 12 which remained under the care of the primary veterinarian. Eight dogs (67%) had limb radiographs performed. Changes consistent with metaphyseal osteopathy were identified in 6/8 dogs (75%) with the distal ulna and radius being the most commonly affected. Thoracic radiographs were performed in nine dogs (75%). A patchy pulmonary alveolar pattern, predominantly affecting the caudodorsal lung fields and consistent with non-cardiogenic pulmonary oedema was identified in the dog

presenting with an upper respiratory tract obstruction (Case 3). A diffuse interstitial opacity affecting the caudodorsal lung fields with unknown significance was identified in another dog (Case 5). No other abnormalities were identified in the remaining seven dogs. Abdominal radiographs were performed in 3 dogs (25%) with no significant findings identified.

Seven dogs (58%) had an abdominal ultrasound performed. Findings were unremarkable except for Case 5 which had findings suggestive of a renal abscess and Case 8 which had focal severe gastric submucosal thickening identified. The nature of this thickening was not investigated further.

Two dogs (17%) had a CT performed. Case 3 had a CT of the head and thorax, and findings included marked laryngeal oedema and swelling causing complete upper airway obstruction with marked secondary non-cardiogenic pulmonary oedema. Case 11 had a CT of the thorax, abdomen and musculoskeletal system, and findings included moderately increased amount of joint fluid in both scapulohumeral joints. One dog (8%) had an MRI of the T3-L3 performed, and findings included bilateral iliac lymphadenopathy and bilateral focal myositis adjacent to the coxofemoral joints.

Treatment and Outcome

All dogs received at least one antimicrobial agent within the first 28 days following diagnosis, with amoxicillin-clavulanic acid being the most commonly administered (8/12 dogs [67%]). A total of 7/12 dogs received more than one antimicrobial agent (three dogs received two antimicrobial agents, two dogs received three antimicrobials and two dogs received four different antimicrobials). The antimicrobial treatment median course length was 12.5 days (range 5 – 28 days). Other antimicrobial agents used were ampicillin-sulbactam, cefalexin, cefuroxime, cefazolin, clindamycin, marbofloxacin, metronidazole.

Ten dogs (83%) received glucocorticoid treatment (prednisone or prednisolone), median starting dose 1 mg/kg/day; range 0.5-2.5 mg/kg/day. No dog had a second immunosuppressive agent added.

Two dogs (Case 5 and Case 10) did not receive glucocorticoid treatment. Of the 10 dogs that received glucocorticoid treatment, two dogs received one glucocorticoid treatment course of less than 28-days duration only and eight dogs received either one glucocorticoid course lasting more than 28 days or more than one glucocorticoid course each lasting less than 28 days (Table 4).

Nine dogs (75%) were alive at 28 days post diagnosis. One dog was euthanised nine days post discharge due to marked musculoskeletal pain and no obvious response to supportive treatment (Case 4). One dog was euthanised 17 days post discharge due to recurrence of pyrexia and limb lameness after stopping antibiotics (Case 7). Case 5 was euthanised after five days of hospitalisation due to a perceived guarded to poor long-term prognosis following diagnosis of a presumptive renal abscess. A post-mortem examination was later performed. Histopathology confirmed the presence of a renal abscess, which cultured *Escherichia coli*. Pyogranulomatous ulcerative colitis with intralesional fungal structures was also identified on postmortem examination.

Six dogs (50%) were alive at six months post diagnosis and six dogs (50%) were alive at 12 months post diagnosis (Table 3). Two dogs were lost to follow up at six months post diagnosis (Cases 6 & 9). Case 10 was euthanised approximately 6 months post diagnosis due to concern for a repeated infection, however, the exact location or source of infection is not known or definitively documented. No septic source was identified during initial investigations and the dog's neutrophil count had remained within normal limits each time a haematology profile had been performed.

During data analysis, five dogs (42%) were identified as being alive at more than 12 months following diagnosis. Further information relating to the treatment of these five dogs is stated below.

Case 1 was diagnosed in 2011 and lived for 10 years post diagnosis of TNS. Over this period, the dog was seen at the referral hospital on 32 occasions. Eight of these visits were due to recurrence of pyrexia and lethargy. This dog received glucocorticoids during the first few months of treatment, but these were subsequently discontinued due to no impact on neutrophil count. This dog received numerous antibiotic

courses throughout the ten years, with the most commonly administered antibiotic being amoxicillin - clavulanic acid.

Case 2 was diagnosed with TNS in January 2021 and glucocorticoid treatment has been continued since diagnosis (1.7mg/kg/day). This dog has presented several times to its primary veterinarian for episodes of pyrexia, which responded to antibiotic treatment and a temporary increase in glucocorticoid dose (typically up to 2.6mg/kg/day). Review of all available haematology results has found that this dog has remained neutropenic since diagnosis.

Case 3 was diagnosed with TNS in November 2020 and glucocorticoid treatment has been continued since diagnosis (dose range 0.22 – 0.7mg/kg/day). Since diagnosis this dog has had a suspected flare up of metaphyseal osteopathy, intermittent episodes of a superficial dermatitis when the glucocorticoid dose was increased and multiple episodes of urinary tract infections which have been treated with amoxicillin-clavulanic acid. Since diagnosis, this dog has remained neutropenic.

Case 11 first started receiving glucocorticoid treatment soon after development of clinical signs in December 2021. When glucocorticoids were discontinued, the dog's clinical signs relapsed (lethargy, lameness). Consequently, glucocorticoid treatment was restarted and continued long term. At his last re-assessment, in September 2022, this dog was doing well on glucocorticoid 0.5 mg/kg every other day.

Case 12 was diagnosed with TNS in October 2021 and has been receiving glucocorticoid therapy since. In September 2023 this dog was stable on a glucocorticoid dose of 0.5 mg/kg every 12 hours.

Discussion

Based on our literature search, this is the largest study to date describing the clinical signs, clinicopathologic results, diagnostic imaging, treatment, and outcome in a population of dogs with TNS. The results of our study suggest that pyrexia and lameness are the two most common clinical signs associated with this condition, severe neutropenia is not always present at the time of diagnosis and long-term survival can be achieved.

Trapped neutrophil syndrome is caused by a mutation of the VPS13B gene, most commonly associated with the Border Collie breed. Two dogs included within our study were Border Collie crossbreeds. Acknowledging that TNS is associated with an autosomal recessive mode of inheritance, this suggests that both sire and dam must have had at least one mutant gene (i.e. carrier status) for TNS. This suggests that TNS is possible in non-Border Collie dog breeds. A recent study by Donner *et al.* screened over 1 million dogs to examine the prevalence and distribution of a total of 250 genetic disease-associated variants amongst a general canine population. Within this population, two crossbreed dogs were identified as being autosomal recessive for the mutant VPS13B gene and had clinical signs compatible with TNS. One of these dogs was genetically identified as being predominantly Border Collie (62%), whereas the second dog's predominant genetic breed type was German Shepherd (33.8%). The authors of this study therefore speculated that TNS has a greater prevalence within the canine population than initially thought and suggested that TNS should be considered in any young dog with compatible clinical signs, regardless of breed type (Donner *et al.* 2023).

The average age at time of diagnosis of TNS was approximately 4 months for dogs within this study. This finding is consistent with veterinary literature with most dogs showing clinical signs by 4 months (Allan *et al.* 1996, Wouda *et al.* 2010, Shearman & Wilton 2011, Mizukami *et al.* 2012, Mason *et al.* 2014, Ganz 2015, Hegler *et al.* 2020, Zoto *et al.* 2022). The majority of dogs within our study were female, suggesting a possible sex predisposition, however, this has not been previously reported. Pyrexia

was the most common clinical sign identified on examination, being present in all dogs in our study, which is consistent with previous reports of dogs with TNS (Mizukami *et al.* 2012, Mason *et al.* 2014, Hegler *et al.* 2020, Zoto *et al.* 2022). Persistent neutropenia in these dogs resulting in an impaired innate immune response with secondary infection affords the most likely explanation for the high prevalence of fever in these dogs. However, not all TNS dogs within this study were documented to have a neutropenia at the time of investigations and only 2/12 (17%) dogs had a possible septic focus identified. Fever is not typically associated with Cohen syndrome in people, unless it develops in response to an infection (Kivitie-Kallio and Norio 2001, Chandler *et al.* 2003). In this study population, only a low number of potential/ confirmed septic foci were identified. The non-standardised diagnostic approach for each dog in this retrospective study, raises concern that septic foci may have been missed, however, our results do raise concern for a different pathophysiology of fever. Pyrexia in TNS dogs may be associated with activation of a systemic inflammatory response not associated with a septic focus. Even though inflammatory cytokine profiles from TNS dogs are not known, both metaphyseal osteopathy and immune-mediated polyarthritis, two conditions which were commonly diagnosed in our study population, are known to be associated with upregulation of a proinflammatory response and pyrexia (Hegemann *et al.* 2005, Stull *et al.* 2008, Robertson *et al.* 2023). Pyrexia was reported to be the most common clinical sign in a population of dogs diagnosed with metaphyseal osteopathy in one study (Robertson *et al.* 2023) and current evidence suggests that metaphyseal osteopathy may be an immune-mediated disease as affected dogs have a cytokine profile similar to that of children with autoimmune bone conditions (Safra *et al.* 2016). C-reactive protein, an acute phase protein and biomarker for systemic inflammation, was only measured in three (25%) dogs and was elevated in two dogs which did not have evidence of infection. Further studies are required to assess the inflammatory cytokine signature in dogs with TNS and the utility of inflammatory biomarkers.

The majority of the dogs in this study were neutropenic on presentation (58%). However, it was not unusual for dogs in this study to have a neutrophil count within or above the analyser reference interval either at presentation, or at some point during ongoing management for TNS, indicating that neutropenia is

not a consistent feature of TNS as was previously believed. An episode of mild neutrophilia has been recently reported, in a dog diagnosed with TNS in a report by Zoto et.al. Similar inconsistencies in neutrophil count have been reported by Zoto *et al.* and in Cohen syndrome, suggesting that heterogeneity within both syndromes exists (Zoto *et al.* 2022, Kivitie-Kallio *et al.* 1997, Chandler *et al.* 2003, Kolehmainen *et al.* 2003). The cause of neutropenia in TNS has not yet been confirmed but given genetic similarities between TNS and Cohen syndrome, a shared pathophysiology could be presumed. Neutropenia has been observed both in TNS and Cohen syndrome despite normal or increased bone marrow cellularity with normal granulocyte morphology and development. It is therefore speculated that neutropenia in both syndromes results from inappropriate myeloid maturation, increased neutrophil margination, premature clearance and increased neutrophil apoptosis leading to their reduced survival (Kivitie-Kalliio *et al.* 2001, Duplomb *et al.* 2019, Zoto *et al.* 2022). Additionally, it appears that in, certain cases, an increased demand, such as severe infection or inflammation, may stimulate release of "trapped" neutrophils from the bone marrow resulting in circulatory neutrophilia, which could explain the neutrophilia seen with Case 3. In view of this, the term "trapped neutrophil syndrome" might be misleading. Furthermore, renal abscess formation and pyogranulomatous colitis were found in Case 5 further supporting the hypothesis that neutrophils are released from the bone marrow and are able to reach sites of infection. This finding is in accordance with findings of a recent case report by Zoto *et al.* who found multiple suppurative lesions on necropsy (Zoto *et al.* 2022). Presence of a septic focus was confirmed or suspected only in two cases (16.6%) in this study. However, the retrospective nature of this study relying on review of clinical notes, differences in diagnostic approaches and use of broad-spectrum antibiotics in all cases may have led to an underestimation of this number. The authors of this study would therefore recommend that a thorough diagnostic work up is instituted to identify any source of infection which, if left untreated, could result in life-threatening complications.

Interestingly, the two dogs within this study which had a septic focus identified or suspected, were positive (homozygous mutant phenotype) for both TNS and CCN on PCR testing. Based on literature

search, this is the first study to document presence of concurrent TNS and CCN. Canine cyclic neutropenia, also known as Gray Collie Syndrome, is associated with a 1 base pair deletion mutation within the AP3B1 gene. This defect results in impaired intracellular trafficking and misdirection of proteins to membranes rather than granules. Consequently, dogs with CCN have markedly reduced mature neutrophil elastase and instead accumulate enzymatically inactive neutrophil elastase precursors (Benson *et al.* 2003). Affected dogs have cyclic fluctuations in haematopoietic cells and humoral regulators of haematopoiesis resulting in neutropenic episodes lasting 3 – 4 days every 8 – 14 days with neutrophil counts often near zero during the nadir (Lund *et al.* 1967, Dale *et al.* 1972). Neutropenic episodes are linked to increased susceptibility to infections, with death being frequent within the first few months of life due to secondary infections (Lund *et al.* 1967, DiGiacomo *et al.* 1983). The aetiology of neutropenia in dogs with TNS and CCN is different given that a marked depletion of neutrophil precursors is identified within the bone marrow of dogs with CCN, in contrast to the presence of myeloid hyperplasia in dogs with TNS (Lund *et al.* 1967, Allan *et al.* 1996, Mizukami *et al.* 2012, Mason et al 2014). The two dogs which were positive for CCN were also the two dogs which had a source of infection identified. It would be therefore prudent to consider genetic testing for both TNS and CCN in dogs with suggestive historical and clinical findings.

Lameness or abnormal gait is a common clinical sign associated with TNS (Allan *et al.* 1996, Wouda *et al.* 2010, Shearman & Wilton 2011, Mizukami *et al.* 2012, Mason *et al.* 2014, Ganz 2015, Hegler *et al.* 2020, Zoto *et al.* 2022). Neutrophilic or mixed inflammation of at least one joint was confirmed in 42% dogs in our study, to include 100% dogs who had arthrocentesis performed. Our results are in concordance with previous literature reports, which have suggested an association between immune-mediated joint disease and TNS in dogs although the cause of this is unknown (Mason *et al.* 2014, Hegler *et al.* 2020, Zoto et al 2022). Juvenile rheumatoid arthritis has been reported in people with Cohen syndrome but the pathophysiology of it remains unclear (Rodrigues *et al.* 2018). Metaphyseal osteopathy was confirmed in 50% of the cases in the current study and has been reported in other publications (Mason *et al.* 2014, Hegler *et al.* 2020). Metaphyseal osteopathy is a rare condition with unknown aetiology,

affecting young dogs with the most common clinical signs being ostealgia, pyrexia, and lethargy (Safra *et al.* 2016, Robertson *et al.* 2023) and has been associated with increased concentrations of inflammatory cytokines (Safra *et al.* 2016). The optimal treatment for metaphyseal osteopathy has not been determined with both non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids being used (Robertson *et al.* 2023). In one study, treatment with glucocorticoids was superior to treatment with NSAIDs (Safra *et al.* 2013). In the current study, six dogs had evidence of metaphyseal osteopathy, but they were not tested for inflammatory markers. Furthermore, not all of those dogs had synovial fluid culture performed, therefore presence of concurrent septic arthritis cannot be fully excluded. Given the high occurrence of orthopaedic abnormalities, Border Collies or their crossbreeds presenting with metaphyseal osteopathy or immune-mediated polyarthritis should be tested for TNS, especially if concurrent neutropenia or gastrointestinal signs are present. Additionally, arthrocentesis with bacterial culture should be considered for dogs with joint pain or effusion and suspected TNS.

Optimal treatment for TNS is not known. The use of antibiotics when neutropenia and pyrexia are both present is reasonable. Although the absolute neutrophil count cut-off for antimicrobial prophylaxis in veterinary medicine is not known, one study showed that a cut-off value of 0.75×10^9/L for antimicrobial prophylaxis is well tolerated in canine cancer chemotherapy patients and minimizes the prescription of antimicrobials (Bisson *et al.* 2019). Future studies regarding the initiation of antimicrobial therapy in patients with TNS are needed.

The small number of cases within our study meant that the role of glucocorticoids as a treatment for TNS could not be fully assessed. However, the authors suggest glucocorticoid administration should be considered in dogs affected by TNS with concurrent metaphyseal osteopathy or IMPA. The efficacy and dosing of glucocorticoid therapy required in the absence of IMPA or metaphyseal osteopathy is uncertain. Glucocorticoids may be beneficial given their known role in promoting neutrophil maturation, stimulating neutrophil release from the bone marrow, reducing tissue accumulation and extravasation of neutrophils through reduced expression of binding proteins (e.g. L-selectin) and inhibiting neutrophil apoptosis

(Ronchetti *et al.* 2018). Further studies are required to assess the utility of glucocorticoids in this patient population. In people, congenital and acquired neutropenia, including those in Cohen syndrome, can be treated using granulocyte colony stimulating factor (G-CSF), a cytokine regulator responsible for stimulation of granulopoiesis (Seow *et al.* 1998, Mehta *et al.* 2015). Use of G-CSF, although not yet described in TNS, could be considered in animals not responding to other supportive treatment.

The retrospective nature of this study meant that there are some inherent limitations, including missing data and lack of standardised work up of cases, as previously mentioned. The treatment administered prior to referral consistent of corticosteroids and antibiotics, may have impacted the findings of this study (e.g. negative cultures). As this was a multicentre study, a number of different haematology analysers were used to assess automated white blood cell and neutrophil counts. The authors tried to limit the effect of this by displaying the data as automated neutrophil count being within, above or below the analyser reference interval and not comparing absolute numbers obtained by different machines. Moreover, not all haematology profiles had a concurrent blood smear assessment for confirmation. As such, inaccurate values cannot be ruled out. There is also the possibility of false negative results when investigating for an infectious focus (e.g. urine culture, synovial fluid culture) in these dogs. The varied treatment approaches within this study along with the small number of cases make it difficult to draw firm conclusions relating to treatment recommendations for dogs with TNS. Glucocorticoids were the only immunosuppressant administered to dogs in this study, therefore it is unknown if there is role for alternative immunosuppressants (e.g. cyclosporine) or GM-CSF with these dogs. However, to be able to determine optimal treatment protocols for this condition, a better understanding of the immune response and neutrophil kinetics of dogs with TNS will be required. Future studies are needed to determine optimal treatment strategies and investigate factors correlating with long-term survival and prognosis.

Conclusions

Trapped neutrophil syndrome should be suspected in any young Border Collie or Border Collie crossbreed with pyrexia, lameness, and gastrointestinal signs. A neutrophil count within the reference interval should not decrease the index of suspicion of TNS and DNA testing should be performed to confirm the disease. There are no clear guidelines regarding the treatment, but antibiotics should be considered when there is marked neutropenia or overt infection. Glucocorticoid use is suggested if there is evidence of immune-mediated polyarthritis and potentially metaphyseal osteopathy. TNS carries a guarded prognosis, but long-term survival is possible.

Acknowledgements

We would like to thank Dr. Mansour Makki at Laboklin Veterinary Diagnostic Laboratory (UK) for assisting in case recruitment.

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