Canine Sterile Steroid-Responsive Lymphadenitis in 50 dogs

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Abbreviations used:

ALP: alkaline phosphatase

CSSRL: canine steroid-responsive lymphadenitis

CRP: C-reactive protein

CT: computed tomography

FNAs: fine needle aspirates

FNAC: Fine needle aspiration cytology

FUO: fever of unknown origin

IMHA: immune mediated haemolytic anaemia

IMPA: immune mediated polyarthritis

ITP: immune mediated thrombocytopenia

MRI: magnetic resonance imaging

NSAIDs: Non-steroidal anti-inflammatories

PAS: periodic acid Schiff

PCR: polymerase chain reaction

TTR: time to referral

UK: United Kingdom

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Structured Summary

Objectives: To report clinical and laboratory features, treatment responses and outcome in dogs diagnosed with canine sterile steroid-responsive lymphadenitis in the United Kingdom.

Methods: Medical records of dogs diagnosed with canine sterile steroid-responsive lymphadenitis from 2009 to 2016 at six specialist referral centres were evaluated retrospectively.

Results: The study included 50 dogs. Springer Spaniels appeared to be over-represented (16/50 dogs). Young dogs (median age 3 years and 9 months) and females (31/50) were typically affected. Clinical presentation was variable, with pyrexia (39/50), lethargy (35/50) and anorexia (21/50) being the most commonly reported clinical signs. Lymph node cytology and/or histopathology demonstrated neutrophilic, pyogranulomatous, granulomatous or necrotizing lymphadenitis without a detectable underlying cause in all cases.

As a sterile immune-mediated aetiology was suspected, all dogs received prednisolone with a subsequent rapid resolution of clinical signs and the lymphadenopathy in most of the cases.

Clinical significance: Canine sterile steroid-responsive lymphadenitis should be considered in dogs with pyrexia of unknown origin with inflammatory lymphadenopathy when no underlying cause can be found and often responds well to therapy with immunosuppressive corticosteroids.

Keywords: Canine sterile steroid-responsive lymphadenitis, Lymphadenomegaly, Fever of unknown origin, Corticosteroids

Introduction

Lymph node enlargement or lymphadenopathy is often encountered during physical examination in canine patients (Thangapandiyan *et al*, 2010). Lymph node enlargement is categorised into solitary (single lymph node), regional (chains of lymphatic nodes draining a specific anatomic region) or generalised (multicentric lymph node enlargement affecting multiple anatomic regions). The causes of lymph node enlargement include oedema, reactive hyperplasia, inflammation, infection and neoplasia (Sapierzynski *et al*, 2009). Fine needle aspiration cytology (FNAC) is a valuable diagnostic test to investigate the cause of lymph node enlargement due to its low cost, simplicity and rapid results (Cowell *et al*, 2003).

The normal cell distribution on cytological evaluation of the lymph node is reported to be 85-90% of small lymphocytes, <10% of medium-sized and large lymphocytes, <3% of plasma cells, and rare neutrophils, eosinophils and macrophages (MacNeill, 2011).

Lymphadenitis is defined as an infiltration of one or more non-lymphoid inflammatory cells in a lymph node (Teske, 2014). Neutrophilic lymphadenitis, also called purulent or suppurative lymphadenitis, is characterised by a neutrophil population exceeding 5% of the cellular population within a lymph node. It may be associated with bacterial, neoplastic or immune-mediated diseases. Granulomatous lymphadenitis is diagnosed when the percentage of histiocytic cells is increased above 2% of the total cell population in a lymph node. Pyogranulomatous lymphadenitis is considered when lymph nodes contain mixed inflammation comprised of increased numbers of neutrophils and macrophages (McNeill, 2011). Pyogranulomatous lymphadenitis can be associated with fungal, mycobacterial and neorickettsial infections, leishmaniasis, bartonellosis, prothotecosis, juvenile cellulitis, vasculitis and idiopathic lymphadenitis (Ishida, 2017; Raskin *et al*, 2016). There is a small number of cases reported with sterile lymphadenitis but this disease is currently poorly understood. These cases can often present with pyrexia.

Pyrexia, or fever, is defined as increased body temperature due to an elevation of the thermal set point in the anterior hypothalamus secondary to pyrogen release (Ramsey *et al*, 2017). Fever of unknown origin (FUO) is a major diagnostic challenge in both human and veterinary medicine (Chervier *et al*, 2012). Although the human literature is relatively complete regarding FUO, there are few studies in the veterinary literature to explore the more common causes of canine FUO (Battersby *et al*, 2006; Chervier *et al*, 2012; Dunn *et al*, 1998).

The aim of this study was to report the clinical presentation, diagnostic testing, treatment response and outcome of canine sterile steroid-responsive lymphadenitis (CSSRL), which is not well described in the veterinary literature.

Materials and Methods

The medical records of dogs diagnosed with canine sterile steroid-responsive lymphadenitis from 2009 to 2016 at six specialist referral centres in the United Kingdom (UK) were retrospectively evaluated. The data from each institution was retrieved via searches of practice management systems with computerised and paper-based records. Collaboration between institutions was achieved by completing a standardised spreadsheet. Data collected included signalment, history (including time to referral and pre-referral treatment), physical examination findings (including lymph node size and distribution), clinical pathology data (including results of lymph node cytology and/or histopathology and infectious disease screening), diagnostic imaging results, treatment and outcome (including time to relapse, repeat treatment). Dogs with incomplete medical records were excluded. The study was approved by the ethics committee of the School of Veterinary Medicine and Science, University of Nottingham.

Case inclusion criteria required a diagnosis of lymphadenitis either with cytology, histopathology or both in which no underlying cause was discernible and a positive response to treatment with glucocorticoids was seen. Neutrophilic lymphadenitis was diagnosed when the neutrophil population in the lymph node was >5%; granulomatous lymphadenitis was diagnosed when histiocytic cells comprised >2% of the lymph node population and pyogranulomatous lymphadenitis was diagnosed when there was a mixed inflammatory infiltrate with increased numbers of neutrophils and macrophages within the lymph node; necrotizing lymphadenitis was diagnosed when there was neutrophilic or histiocytic inflammation accompanied by necrosis within the lymph node; reactive hyperplasia was diagnosed when there were increased numbers (15-30%) of medium and large lymphocytes with increased numbers of plasma cells. Diagnostic investigations in each case excluded other potential causes of lymphadenomegaly such as infectious, inflammatory and neoplastic.. In all

the cases, haematology, biochemistry, urinalysis, urine culture, thoracic radiographs, abdominal ultrasound were performed. When appropriate, echocardiography, abdominal radiographs, arthrocentesis with synovial fluid analysis and culture, cerebrospinal fluid analysis, tests for arthropod borne diseases including *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Borrelia burdorferi*, *Leishmania infantum* and *Bartonella henselae*, lymph node culture, Ziehl Neelsen and Periodic acid-Schiff (PAS) staining of lymph node FNAC aspirates, bronchoscopy and bronchoalveolar lavage cytological analysis and culture, computed tomography (CT), magnetic resonance imaging (MRI), C-reactive protein (CRP), pleural or peritoneal fluid cytological analysis, FNAC of liver or spleen, skin biopsies, faecal analysis, exploratory laparotomy and haemoculture were also performed.

All the cases were treated with glucocorticoids, with gradual dose decreases over the following weeks depending on response. Clinical reassessment was performed regularly and response to treatment assessed on the basis of owner's perception of clinical signs, physical examination (resolution of the pyrexia if present, resolution or improvement of the lymphadenopathy by more than a 50% reduction of the lymph node size if assessable or improvement of the dog's demeanour). In some cases diagnostic imaging was repeated to assess for resolution of lymphadenopathy (if not externally assessable) or measurement of C-reactive protein if it was measured initially and was elevated.

Statistics were performed using a chi square test and the result was considered significant at $p<0.05$ and outcome was defined as being dead or alive three months after diagnosis.

Results

Canine sterile steroid-responsive lymphadenitis was diagnosed in fifty dogs enrolled in this study. Twenty breeds featured 7 mixed-breed dogs. English Springer spaniels (16/50) were the most common breed followed by Cocker spaniels (4/50), Border collies (3/50), German Shepherds (2/50) and Beagles (2/50).

The age at presentation ranged from 6 months to 10 years (median, 3 years and 9 months). Thirty-one of the dogs were female (62%; 40% neutered) and 19 were male (38%; 20% neutered). There were no significant differences between English Springer spaniels and other breeds with regard to age (median 43.8 months *versus* 44.8 months) and sex (female 58.82%, 60% neutered *versus* 68.75%; 72.7% neutered).

Previous history included idiopathic epilepsy in 2 dogs, intervertebral disc disease in one dog, previous septic peritonitis in one dog, hamartoma in the right hip and otitis in one dog and protein losing nephropathy and spontaneous (resolved) haemothorax in another dog.

Prior to referral most dogs received antimicrobial and/or anti-inflammatory therapy without a significant clinical response. Forty-one dogs were treated with antimicrobials which included co-amoxiclav (31/41) metronidazole (7/41), enrofloxacin (6/41), doxycycline (6/41), marbofloxacin (5/41), 4 cases with cephalexin (4/41), clindamycin (1/41) and pradofloxacin (1/41). Twenty-eight dogs received non-steroidal anti-inflammatories (NSAIDs) which included meloxicam (20/28), carprofen (7/28) and firocoxib (1/28). Five dogs were treated with anti-inflammatory dose of glucocorticoids (0.5-1mg/kg/once a day) including 4 cases (80%) treated with prednisolone, and 1 case (20%) treated with methylprednisolone. Nine of the 45 dogs that received treatment prior to referral presentation had a partial clinical response, this included 3 dogs treated with antimicrobials and NSAIDs, 3 dogs receiving antimicrobials and glucocorticoids, 2 dogs only receiving antimicrobials and 1 dog receiving glucocorticoids. Five

of the fifty dogs did not receive any medication prior to referral. Median time to referral (TTR) was 29.8 days (range from 2 to 90 days).

Clinical presentation varied widely between animals but the most common complaints were clinical signs such as pyrexia (39/50), lethargy (30/50) and anorexia (21/50). Other clinical signs are summarised in table 1. Thirty-three animals were pyrexic at presentation, with a median rectal temperature of 39.9°C (range of 39.1°C-40.9°C).

Clinical sign	Number of dogs
Pyrexia	39
Lethargy	35
Anorexia	21
Cough	7
Tachypnoea	1
Dyspnoea	1- inspiratory 1- mixed
Vomiting	3
Diarrhoea	4
Haematochezia	1
dysphagia	2
Neck pain	3
Spinal pain	2 – thoracolumbar 2 - lumbosacral
Joint pain	2

Abdominal pain	3
Joint effusion	2 – carpus 1- stifles 1 - Tarsus
Arrhythmia (accelerated idioventricular rhythm)	1
Dermatological signs	1 – gingival ulcerations 1 – pustules 1- ulcers in pinnae and face
Ventral cervical swelling	7
Facial swelling	2
Epistaxis (bilateral)	2

Table 1: Summary of clinical signs including numbers of dogs.

Although lymphadenomegaly was grossly palpable in most cases, eleven animals presented without any external sign of lymphadenomegaly, but thoracic and intraabdominal lymphadenopathy was later diagnosed through further investigation. The mandibular (32/50), superficial cervical (23/50 and popliteal (21/50) lymph nodes were most commonly affected. Objective measurements of the lymph nodes were not available in many cases; however subjectively lymphadenopathy ranged from mild to marked. Intra-thoracic and intra-abdominal lymphadenomegaly was documented with diagnostic imaging (thoracic radiographs, abdominal radiographs, abdominal ultrasound, CT or MRI) performed or interpreted by boarded radiologists. Intrathoracic lymphadenopathy was noted in 4 of the 50 cases affecting the sternal (2/50) and tracheobronchial (2/50) lymph nodes. Other changes on thoracic imaging included the presence of a mild to moderate bronchointerstitial pattern in 3 dogs, focal alveolar

infiltrate in 2 dogs and nodular pattern in one dogs. Bronchoalveolar lavage cytological analysis included mixed inflammation with a negative culture in all dogs that presented with radiographic changes on thoracic imaging. Intraabdominal lymphadenopathy was documented in 25 of the 50 dogs affecting the mesenteric (15/25), medial iliac (9/25) and sublumbar (1/25) lymph nodes. Other changes on abdominal imaging included the presence of minimal volume abdominal effusion in 5 dogs, mild splenomegaly in 4 dogs and hepatomegaly in 3 dogs. In 2 dogs analysis of the peritoneal fluid revealed the presence of a neutrophilic transudate with negative culture. Splenic FNAC revealed reactive hyperplasia in 3 of the 4 dogs with splenomegaly and hepatic FNAC documented mild vacuolar change and mild neutrophilic inflammation in one dog.

Main clinicopathological findings included mild non-regenerative anaemia (haematocrit 0.31-0.35L/L; RI: 0.37-0.55) in 5 cases (10%), mild to moderate neutrophilic leucocytosis (neutrophil count 20-35x10^9/L; RI: 3-11.5x10^9/L) in 11 cases (22%), monocytic leucocytosis (monocyte count 1.7-6.7x10^9/L; RI: 0.2-1.4) in 4 cases (8%) and neutrophilic and monocytic leucocytosis in 4 cases (8%) and moderate regenerative anaemia (HCT: 0.17L/L; RI: 0.37-0.55) and severe thrombocytopenia in one case (2%). Main biochemical abnormalities included mild to moderate elevation in alkaline phosphatase (ALP: 154-600IU/L; RI: 14-105) in 8 cases (16%), mild to moderate hypoalbuminaemia (albumin values 16-21g/l; RI: 25-40) in 4 cases (8%) and mild hyperglobulinaemia (globulin values 47-52g/l; RI: 23-45) in 2 cases (4%).
Arthropod-borne diseases were tested in 37 of the cases (74%) and of these, 100% of the cases were tested for *Borrelia burgdorferi* with serology, 34 cases (91.9%) were tested for *Bartonella henselae* with PCR from blood, 9 cases (24.3%) were tested for *Anaplasma phagocytophilum* with PCR from blood, 4 cases (10.8%) were tested for *Ehrlichia canis* with PCR of blood and 1 case (2.7%) was tested for *Leishmania infantum* with serology. All the results were negative.

Arthrocentesis and subsequent synovial fluid cytological analysis and culture was performed in 9 out of 50 cases (18%) from which 4 (44.44%) were considered normal, 4 (44.44%) showed marked neutrophilic inflammation and 1 (11.11%) showed mild neutrophilic inflammation. All the cultures were negative.

Cerebrospinal fluid analysis was performed in 7 out of 50 cases (14%) from which 6 (85.71%) was cytologically normal and 1 (14.28%) showed neutrophilic and lymphocytic inflammation.

CRP was assessed in 6 out of 50 cases and was elevated in all of them (range 84.1-689mg/L; reference interval >10mg/L).

In all dogs, a diagnosis of lymphadenitis was reached with cytology and/or histopathology. Cytological assessment was performed in 45 of the 50 dogs, histological assessment in 28 of the 50 dogs and both in 22 dogs. The predominant type of lymphadenitis diagnosed on cytology was neutrophilic (29/45), followed by pyogranulomatous (6/45), granulomatous (5/45) and reactive hyperplasia (5/45). Conversely, the predominant type of lymphadenitis diagnosed on histology was pyogranulomatous (13/28) followed by neutrophilic (9/28), necrotizing (4/28) and granulomatous (2/28). In the cases in which both cytology and histopathology was performed, good agreement was found in seven of the 22 cases, whereas in the remaining 15 cases cytological diagnosis differed from histological diagnosis. In eight cases with a cytological classification of neutrophilic lymphadenitis, five were classified as pyogranulomatous lymphadenitis and three as necrotizing lymphadenitis on histology. In the five dogs classified as reactive hyperplasia based on cytology, two were classified as neutrophilic lymphadenitis, one as pyogranulomatus lymphadenitis, one as granulomatous lymphadenitis and one as necrotizing lymphadenitis on histology. In one case classified as having pyogranulomatous lymphadenitis on cytology was classified as having neutrophilic lymphadenitis on histology and one dog with granulomatous lymphadenitis on cytology was

classified as having pyogranulomatous lymphadenitis on histology. Culture of lymph node tissue or aspirates was performed in 28 dogs and was negative in all instances.

Four of the 50 cases were diagnosed with other concurrent immune mediated diseases. One dog had Evans syndrome one dog had concurrent immune mediated polyarthritis (IMPA), one case was diagnosed with concurrent IMPA and meningitis and one case was diagnosed with concurrent IMPA and pyogranulomatous skin nodules.

All the animals were treated with corticosteroids. Prednisolone was the first line treatment chosen in 47 of the 50 dogs, of which 34 dogs commenced 1mg/kg dose per day (dose range 0.5-3mg/kg per day). Two of the 50 dogs were started with dexamethasone (dose range 0.2-0.3mg/kg per day) and later were transitioned to prednisolone. Only one of the 50 dogs initially responded to antimicrobial therapy (co-amoxiclav), but it relapsed four weeks after stopping therapy, and was subsequently started on prednisolone, which improved immediately it's clinical signs.

Forty-seven of the fifty animals (94%) showed marked improvement in clinical condition, decrease in pyrexia and decrease in lymphadenomegaly within 12-48 hours of initiation of corticosteroid administration. The treatment protocol followed in each case was different due to the multi centre retrospective nature of this study, but overall, a decrease of 25-50% of the prednisolone dose was scheduled every 2-4 weeks, continuing treatment for at least 3-6 months. In some of the cases, CRP concentration was used for monitoring response to the treatment and the values normalised when there was clinical improvement.

In nine of the 50 dogs, additional immunosuppressive treatments where used in combination with prednisolone. Of these nine dogs cases, four received azathioprine, two ciclosporin, one

cyclophosphamide, one mycophenolate and one chlorambucil. Of these dogs, there were two beagles, two English springer spaniels, one Border collie, one German shepherd dog, one Cavalier King Charles Spaniel and one Pointer. In five of the cases, additional immunosuppressives were used at the time of recurrence of clinical signs, whereas in four of the cases they were used initially to decrease the side effects related to the corticosteroids. The most common adverse effects of corticosteroids reported were those commonly attributed to this medication, including polyuria, polydipsia, polyphagia and lethargy. Other less common adverse effects included alopecia, muscle atrophy, gastrointestinal clinical signs and wound infections.

In terms of outcome, 22 of 50 dogs were not receiving medication and had no clinical signs three months after stopping medication. Eight of 50 dogs were still receiving tapering doses of prednisolone without a relapse detected three months after diagnosis. One of 50 dogs remained on 0.35mg/kg of prednisolone every other day. Due to the multi centre nature of the study, and the fact that many dogs continued their care at their primary veterinary clinic, 13 dogs were lost to follow-up whilst receiving decreasing doses of prednisolone. Six of 50 dogs were euthanized due to deterioration or lack of response to the treatment and one died acutely while the medication was being reduced. The cause of death was unknown and no post-mortem examination was available.

Eighteen dogs had a recurrence of their clinical signs during the study period of which 13 were Springer Spaniels. The average time to return of clinical signs was 19 weeks after diagnosis. In 12 of the 18 cases prednisolone had been withdrawn at the time of recurrence of clinical signs whereas the rest were still on tapering doses of corticosteroids. Two dogs were monitored without adding further treatment and they did not show further progression of signs. Fourteen

dogs recommenced increased doses of prednisolone, which resulted in resolution of the clinical signs and the lymphadenopathy. Two other dogs had two episodes of return of clinical signs of which one responded well to re-treatment with prednisolone on each occasion while the other responded well on the first occasion but not the second. In one of the 18 cases with recurrence of clinical signs there was a rapid decrease in prednisolone dose over 3-4 weeks the rest has a reduction over 3-6 months.

Relating outcome with diagnosis, of the dogs that were clinically well without treatment (3 months after treatment withdrawal), 10 out of 22 had neutrophilic lymphadenitis, 10 out of 22 had pyogranulomatous lymphadenitis, one out of 22 had granulomatous lymphadenitis and one out of 22 had necrotizing lymphadenitis. Of the cases that were euthanized or died, three out of 6 had neutrophilic lymphadenitis and three out of 6 (50%) had pyogranulomatous lymphadenitis. There wasn't a statistically significant relationship between the type of lymphadenopathy and the outcome (p-value 0.13).

Twelve of the 22 dogs that were well three months after discontinuing treatment had external lymphadenopathy, six dogs had internal lymphadenopathy and four had both internal and external lymphadenopathy. Of the six dogs that were euthanized or died, five had external lymphadenopathy and 1 had documented internal and external lymphadenopathy. There wasn't a statistically significant relationship between the location of the lymphadenopathy and outcome (p-value 0.27).

Discussion

This study describes canine sterile steroid-responsive lymphadenitis (CSSRL) as a cause of lymphadenopathy and FUO in dogs, its medical management and treatment outcomes. To the

authors' knowledge, primary sterile lymphadenitis without evidence of other diseases has not been well described in the veterinary literature.

Dogs in this study were mainly presented for pyrexia, lethargy, inappetence and varying degrees of peripheral or internal lymphadenopathy were subsequently documented. Lymphadenopathy is encountered in many diseases processes and determining the cause of lymphadenopathy can require time-consuming and expensive investigations. Thorough diagnostic investigations were performed in all the patients that were recruited for this study; however several diagnostic evaluations performed were different between cases due to the different clinical presentations and clinicians involved in the study. Investigations in all the cases failed to find an underlying infectious (bacterial, protozoal or fungal), inflammatory or neoplastic condition. All the animals that had tissue samples submitted for culture (lymph node, blood, urine, bronchoalveolar lavage fluid, synovial fluid or cerebrospinal fluid) showed no bacterial growth; however this particular point is difficult to fully characterise, as many animals were pre-treated with antimicrobials, which could preclude the growth or bacterial organisms. On the other hand, the fact that many of these animals were treated with antimicrobials and showed no clinical improvement could suggest that the disease process was unlikely to be bacterial in origin.

In these groups of dogs with CSSRL it appears more common in females compared to males (31 females and 19 males). This finding is similar to findings in other immune mediated diseases such as IMHA or ITP being also overrepresented in female dogs in some studies (Carr *et al*, 2002; O'Marra *et al*, 2011; Putsche & Kohn, 2008; Weinkle *et al*, 2005).

Median age at initial presentation was 3 years and 9 months, with ages ranging from 6 months to 10 years old. Anecdotal evidence had suggested that CSSRL occurred more frequently in younger animals and the findings of this research support this hypothesis. This is similar to the age incidence of other primary immune mediated diseases, for example IMPA, being more prevalent in young adult dogs (Johnson & Mackin, 2012)

The most frequent clinical signs, documented were lethargy, pyrexia and inappetence. Immune-mediated disease is frequently responsible for causing vague and non-specific signs. The pyrexia, which was present in the 78% of the cases, may have been a contributing factor causing lethargy and inappetence. Other factors that may also have contributed to animals being unwilling to eat may have resulted from lymphadenopathy in the area of the neck and head, which was preventing them from eating. In addition, a small number of dogs presented with neck pain and abdominal pain, both of which these conditions could account for anorexia. Respiratory signs were present in several cases, 7 animals presented with cough and 2 animals were dyspnoeic. Thoracic radiographs were obtained in all the cases and sternal or tracheobronchial lymphadenopathy, diffuse bronchointerstitial pattern, focal or diffuse alveolar pattern and nodular lung patterns were present in some animals. A few animals developed severe respiratory complications soon after initiating treatment with corticosteroids but in most of them the thoracic abnormalities resolved after starting treatment. This also remains uncertain, but some of the changes noted could be vasculitis-related or potentially a secondary sequelae of the underlying primary immune-mediated disease process. Therefore, even if pyrexia, inappetence and lethargy are the more common clinical signs according to the cases studied here, a variety of other clinical signs can be present with this condition. Additionally, concurrent immune mediated conditions such as IMHA, ITP, IMPA and meningitis were detected in 4 cases. It is uncertain if this could be part of a reactive process

due to the primary immune mediated that we would consider being the lymphadenitis or part of a multi-systemic immune mediated condition. This would be further supported by the fact that these dogs had generalised external and even internal lymphadenopathy rather than local lymphadenopathy from the affected areas.

Regarding the lymphadenopathy, it was not restricted to peripheral lymph nodes, and in certain cases there were no signs of peripheral or external lymphadenopathy. From the results we obtained, mandibular, superficial cervical and popliteal lymph nodes were the lymph nodes that were most frequently affected. This finding itself could highlight some controversy with the argument that these are the lymph nodes that are immunogenically challenged the most due to the anatomic regions from which they drain. Also, these are the lymph nodes more readily palpated on general physical examination. Regarding outcome, there was no relationship noted between the number of nodes affected or their location as to outcome or response to treatment.

In all dogs, a diagnosis of lymphadenitis was reached with lymph node cytology and/or histopathology. Based on cytology, the predominant type of lymphadenitis was neutrophilic, whereas the predominant type of lymphadenitis that was documented from the histopathology samples was pyogranulomatous. The discrepancy between cytology and histopathology may be attributable to the fact that sections obtained for histopathology may have been more representative samples, particularly as they would have preserved the architecture of the lymph node. The type of inflammation present did not appear to alter overall outcome for dogs in this study.

Prednisolone was the first line immunosuppressive treatment chosen most dogs, of which 34 dogs commenced a 1mg/kg dose per day (dose range 0.5-3mg/kg per day). Due to the inherent

difficulties with a retrospective study from a multi-centre database, the reasoning for the starting doses and protocol of continuation of treatment was difficult to establish. Most of the animals showed marked improvement in clinical condition, decrease in pyrexia and decrease in lymphadenomegaly within 12-48 hours of initiation of corticosteroid administration. In some of the cases, CRP concentration was used for monitoring response to the treatment and the values normalised when there was clinical improvement. Animals had previously been subjected to intravenous fluid therapy, non-steroidal anti-inflammatories, and antimicrobials of varying classes, all of which had showed minimal improvement and when started on corticosteroids their clinical signs improved dramatically within 12-48 hours. Only one of the cases did not exhibit a good clinical response and was euthanized 24h after starting treatment due to clinical deterioration; another case initially responded to antimicrobial therapy, but it relapsed four weeks after stopping the therapy, and was subsequently commenced prednisolone therapy, which immediately improved it's clinical signs.

Eighteen dogs had recurrence of clinical signs during the study period, of which 13 were English Springer Spaniels. In 12/18 dogs, prednisolone had been discontinued at the time of recurrence, whereas the rest were still on tapering doses of corticosteroids. Only one dog that relapsed had a shorter treatment period before relapse (3-4 weeks) compared to the other cases (3-6 months), making unlikely that this was the cause of relapse in the other cases. Most of the cases responded well to increasing the prednisolone dose without addition treatment. This could suggest that particularly in ESS, with over 70% of animals relapsing within the time period of the study, a more progressive or slow process tapering of corticosteroids could be necessary.

A small number of animals (9/50) required a second line immunosuppressive medication in order to either control the lymphadenitis (5/9) when they relapsed or reduce the adverse effects

446 of corticosteroids (4/9). The adverse effects of corticosteroids reported were those, which are
447 commonly attributed to prednisolone, including mainly polyuria, polydipsia, polyphagia and
448 lethargy and were not classified as severe.
449

450 Sixteen of the fifty cases in this study were English Springer Spaniels, which could suggest a
451 breed predisposition. A case of sterile neutrophilic-macrophagic lymphadenitis associated with
452 nodular panniculitis in a Springer Spaniel has been previously reported (Dandrieux *et al*, 2011).
453 Indeed, a journal letter published in 2002 also reported a number of Springer Spaniels
454 presenting with generalised lymphadenopathy consistent with granulomatous necrotising
455 lymphadenitis and pyrexia with or without pyogranulomatous dermatitis (Hoffman *et al*, 2002).
456 Moreover, ESS (among other breeds) have also been reported to be affected by a rare form of
457 mineral-associated lymphadenopathy (Day, 1996). Twenty breeds were represented in this
458 study, three of which were Spaniel breeds (English springer spaniel, Cocker spaniel and
459 Cavalier King Charles spaniel). It has been well documented that there is a breed predilection
460 for immune-mediated haemolytic anaemia (IMHA) in Springer Spaniels and Cocker spaniels
461 (Weinkle *et al*, 2005; Reimer *et al*, 1999), whether any links to susceptibility to immune-
462 mediated disease could be extrapolated from this study remain to be evaluated and could
463 provide an area for future work.

464 This study was limited by issues inherent to most retrospective studies, including mainly a lack
465 of uniformity of the diagnostic investigations and the treatment plans. The diagnostic work-up
466 was not always the same because the cases were seen during different periods of time and by
467 different clinicians from different referral centres. Also, the varied presentations of the cases
468 initially guided investigations based on the clinical signs presented. For the same reason, some
469 of the cases were lost in follow-up, which makes difficult to interpret the long-term response
470 or outcome of the dogs suffering this condition.

To the authors' knowledge, primary sterile lymphadenitis without evidence of other diseases has not been well characterised in dogs. Diagnosis of canine sterile steroid-responsive lymphadenopathy involves extensive investigations to rule out any detectable underlying infectious, inflammatory or neoplastic causes. Most of the cases responded to prednisolone therapy and the rapid resolution of clinical signs was associated with normalisation of the lymphadenopathy. In addition, some of the cases relapsed after discontinuation of the treatment or while decreasing the dosage of the medication, being also suggestive of a primary immune-mediated disease process.

In conclusion, idiopathic or primary sterile steroid-responsive lymphadenitis should be considered a differential diagnosis in young-adult dogs (especially female Springer Spaniels) presenting with pyrexia and peripheral and/or internal lymphadenopathy. The apparent breed predisposition in Springer Spaniels warrants further study.

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