Structured Summary

OBJECTIVES: To evaluate whether screening tests used to identify infectious and neoplastic triggers for immune mediated haemolytic anaemia (IMHA), in particular a complete blood count and differential (CBC), serum biochemistry profile, urine analysis (including culture), abdominal ultrasound and thoracic radiographs, can identify triggers for steroid responsive meningitis-arteritis (SRMA).

METHODS: Retrospective descriptive review.

RESULTS: Twenty-one SRMA cases were identified in which all screening tests had been performed. All cases had changes in CBC (including neutrophilia, monocytosis, lymphocytosis, eosinopenia or anaemia); 19 had changes in biochemistry (including hypoalbuminaemia, hyperglobulinaemia, increased ALP activity, hyperphosphataemia, increased total calcium concentration, hypercholesterolaemia, hyperkalaemia, increased urea concentration and increased ALT activity); two cases had an elevated urine protein to creatinine ratio but none had positive urine culture results; no cases had abnormalities on orthogonal radiographs of the thorax; four cases had abnormalities identified on abdominal ultrasound, which following cytological examination suggested inflammation in the absence of pathological organisms.

CLINICAL SIGNIFICANCE: Screening tests used to identify infectious and neoplastic triggers in IMHA did not isolate triggers for SRMA in the population of dogs under investigation.

Keywords

Beagle pain syndrome, Necrotising vasculitis, Steroid responsive meningitis, Steroid responsive meningitis-arteritis, SRMA

Introduction

Dogs with steroid responsive meningitis-arteritis (SRMA) have inflammatory lesions of the leptomeninges and associated arteries that are responsive to corticosteroids (De Lahunta and Glass, 2009). SRMA may occur in any breed and has been reported in dogs from 3 months (Harcourt, 1978) to 9 years (Cizinauskas *et al.,* 2000), but onset is typically between 6 and 18 months of age (Tipold and Schatzberg, 2010). Cases of SRMA have a waxing-waning disease characterized by episodes of cervical hyperaesthesia, depression and pyrexia (De Lahunta and Glass, 2009). There is no definitive

ante-mortem diagnostic test for SRMA and diagnosis is based on clinical signs, laboratory findings and exclusion of other diseases (Lowrie e*t al*., 2009).

The aetiopathogenesis of SRMA is unknown (Tipold and Schatzberg, 2010), but a T helper 2 cell (Th-2) mediated immune response is suspected (Schwartz *et al.,* 2011). Given the presence of activated T cells an antigenic stimulus is believed to trigger SRMA, but no infectious agents have been identified to date (Harcourt, 1978; Meric *et al.,* 1985, Scott-Moncrieff *et al.,* 1992). Other immune mediated diseases, such as immune mediated haemolytic anaemia (IMHA), have activated T cells as part of their pathogenesis (Day and Mackin, 2008). In particular a Th-2 mediated immune response is suspected in IMHA (Whitley and Day, 2011). Twenty-five to forty percent of IMHA cases have their antigenic triggers identified and infections, neoplasms, drugs and controversially vaccinations have all been implicated (Duval and Giger, 1996; Mitchel and Kruth, 2011). There is no consensus on how best to identify these triggers in IMHA but current recommendations are to use screening tests, including a complete blood count (CBC) with differential, serum biochemistry profile, urine analysis (including culture), abdominal ultrasound and radiographs of the thorax (Miller, 2008).

Given the similarity in the immune mediated response between SRMA and IMHA it is reasonable to use the screening tests for IMHA to identify infectious or neoplastic triggers for SRMA. The aim of this study was to perform a retrospective review of SRMA cases and their screening test results to identify if any infectious or neoplastic triggers could be identified.

Materials and Methods

SRMA cases were identified by retrospectively searching clinical records of cases presenting to Langford veterinary services, University of Bristol between 2002 and 2011. Cases were included if they had a full history, record of a general physical and neurological examination; clinical signs of neck pain, depression and pyrexia; a cerebrospinal fluid (CSF) sample which documented neutrophilic pleocytosis with an absence of pathological organisms on routine cytology; CBC with differential, serum biochemistry profile and orthogonal cervical radiographs that excluded other causes for neck pain; cystocentesis urine analysis (including culture), complete abdominal ultrasound

and orthogonal inflated thoracic radiographs. Cases were excluded if they had neurological deficits in addition to neck pain and depression or were over 18 months of age at presentation.

Neck pain was defined as displaying a behavioural change (vocalisation or biting), or a decreased range of movement, when the cervical spine was manipulated in either a lateral or vertical plane. Depression was defined as a decreased response to environmental stimuli (Garosi, 2004). Pyrexia was defined as a rectal temperature greater than 39.2˚C in an unstressed dog (Miller, 2011) with persistence at 2 or more checks over 24 hours. Neutrophilic pleocytosis, in CSF, was defined as a total nucleated cell count (TNCC) > 5 cells/mm^3 and total protein >25mg/dl with >50% of the TNCC being neutrophils on a cisternal sample (Desnoyers *et al*., 2008). No cases had lumbar CSF samples taken.

Results

Inclusion criteria were met by 21 cases. The average age at presentation of the cases was 10 months (range 5-18 months). Ten were male and 11 female with 8/21 being neutered (3/11 females and 5/10 males). Twelve different pure-breeds were represented together with a single cross breed. Of the pure-breeds 4 were collies, 4 were Jack Russell terriers, 2 were Weimaraners and all other breeds (beagle, Labrador retriever, golden retriever, springer spaniel, corgi, Petit Basset Griffon Vendéen, flat coated retriever, cocker spaniel, Shih Tzu and poodle) had a single case each.

A CBC was abnormal in 21/21 cases as follows: neutrophilia in 19 cases (mean 20.24X10^9/L; range11.90-38.96; reference interval 3.00-11.50 X10^9/L); monocytosis in 19 cases (mean 2.67X10^9/L; range 1.55-4.34; reference interval 0.10-1.50 X10^9/lL; eosinopenia in 9 cases (mean 0.01X10^9/L; range 0.00-0.07; reference interval 0.20-1.40 X10^9/L); mild non regenerative anaemia in 5 cases (mean 31.6%; range 27.7-34.6; reference interval 35.0-55.0%); and lymphocytosis in 3 cases (mean 4.45X10^9/L; range 4.08-4.88; reference interval 0.70-3.60 X10^9/L). Biochemistry was abnormal in 19/21 as follows: hypoalbuminaemia in 18 cases (mean 26.1 g/l; range 17.4-31.7; reference range 32.0-38.0 g/L); hyperglobulinaemia in 10 cases (mean 40.0 g/L; range 35.9-53.9; reference interval 20.0-35.0 g/L); increased alkaline phosphatase in 16 cases (mean 188.5 IU/L; range 112.0-310.0; reference interval 0-110 IU/L); hyperphosphataemia in 15 cases (mean 1.52 mmol/L; range 1.33-2.16;

reference interval 0.75-1.25 mmol/L); increased total calcium in 7 cases (mean 2.73 mmol/L; range 2.63-2.85; reference interval 2.30-2.60 mmol/L); hypercholesterolaemia in 7 cases (mean 9.2 mmol/L; range 7.3-10.7; reference interval 3.5-7.0 mmol/L); hyperkalaemia in 4 cases (mean 4.76 mmol/L; range 4.51-5.20; reference interval 3.50-4.50 mmol/L); increased urea in 1 case (7.2 mmol/L; reference interval 2.0-7.0 mmol/L); and raised alanine aminotransferase in 1 case (236 IU/L; reference interval 20-60 IU/L). No specific triggers of SRMA were identified from the CBC with differential or biochemistry samples in any of the 21 cases. Two cases had an elevated urine protein to creatinine ratio (UP:C) of >0.5 on urinalysis (0.59 and 0.85) but none had any organisms identified on urine culture. None of the 21 cases had abnormalities on orthogonal inflated radiographs of the thorax. Four dogs had abnormalities identified on abdominal ultrasound as follows: 1 had a hypoechoic nodule within the spleen (consistent with splenitis on cytology of an aspirate); and 3 had enlarged abdominal lymph nodes (consistent with lymphadenitis on cytology of aspirates). No organisms were identified on cytology in these cases. One of the dogs with lymphadenitis had an exploratory celiotomy at the referring veterinarians prior to admission, whilst another documented gastrointestinal signs with vomiting and diarrhoea.

One dog had aspiration of a mass in the neck which was palpable on general physical examination. This documented neutrophilic inflammation on cytology but no pathological organisms were identified after culture. This case had an abscess diagnosed 3 months prior to presentation, which occurred immediately after placement of a microchip, and had been treated with antibiotics.

A number of dogs underwent additional diagnostic tests. These included: *Toxoplasma* and *Neospora* serology (on blood) or PCR (on blood or CSF) in 17 cases; *Rickettsae* PCR (on blood) in 2 cases; *Bartonella* PCR (on blood) in 3 cases; *Anaplasma* PCR (on blood) in 5 cases*; Ehrlichia* PCR (on blood) in 7 cases; *Borrelia* PCR (on blood) in 11 cases; joint taps in 17 cases; faecal culture in 2 cases; CSF culture in 5 cases; and blood culture in 1 case. Five out of 17 cases documented a neutrophilic inflammation on joint taps but no pathological organisms were identified on cytology. All other tests were unremarkable.

Twenty of the 21 SRMA dogs were treated in the immediate period (7 days) prior to referral as follows: 18 received antibiosis; 14 had NSAIDs as a sole anti-inflammatory medication; 1 had steroids as their sole anti-inflammatory medication; and 1 had steroids and NSAIDs.

Discussion

No infectious or neoplastic triggers were definitively identified in any of the 21 SRMA cases subjected to screening diagnostics recommended for IMHA cases. This included screening for bacterial and protozoal diseases. This mirrors previous studies that have failed to identify triggers for SRMA with antemortem diagnostic tests (Scott-Moncrieff *et al.,* 1992) or necropsy and bacteriology, virology and histopathology (Harcourt, 1978). In contrast an infection or neoplasm was identified as the trigger in 35 of 181 cases of IMHA with the same screening diagnostics, although it is unclear if these cases were pre-treated with antibiosis and anti-inflammatory drugs as in some of the SRMA cases presented here (Wienkle *et al.*, 2005).

Screening diagnostics did identify other inflammatory foci in the abdomen of 4 of the 21 SRMA cases. Similarly joint taps identified neutrophilic inflammation in 4 out of 17 SRMA cases. Other inflammatory foci have previously been found in association with SRMA ante- and post-mortem (Scott-Moncrieff *et al.,* 1992; Webb *et al*., 2002) and these results may reflect part of the systemic inflammatory response that is reported in this disease. Abdominal lymphadenitis may reflect signs of a gastrointestinal or other abdominal infectious or neoplastic trigger for SRMA but none was identified in the present cases. Faecal culture was not routinely performed and has not been done so by others in any significant numbers. A faecal isolate of *C. perfringens* was identified in a previous study of SRMA but this was not considered an antigenic trigger for SRMA (Scott-Moncrieff *et al.,* 1992). This theory is supported by evidence that in one study 71% of clinically normal dogs had *C. perfringens* isolated in their faeces (Weese *et al*., 2001).

Despite negative urine culture results, two of the SRMA cases had a mild increase in UP:C, as has been described previously (Scott-Moncrieff *et al.,* 1992). In the absence of active urine sediment or culture indicating infection or gross haematuria this could be secondary to glomerular protein loss (Meryl and Littman, 2011). This is most likely secondary to the systemic inflammatory nature of SRMA

as renal inflammatory foci have been identified previously in SRMA cases (Harcourt, 1978). This assumption is supported by the fact that the elevated UP:C subsequently resolved at recheck appointments (2 or 4 weeks later) after immunosuppressive steroid therapy and cessation of antibiotics.

CBC documented a neutrophilia and monocytosis with or without a lymphocytosis and eosinopenia in the SRMA cases. Neutrophilia, monocytosis and eosinopenia have been described previously in SRMA cases (Scott-Moncrieff *et al.,* 1992; Cizinauskas *et al.,* 2000) but lymphocytosis has not. In IMHA the CBC usually demonstrates a leukocytosis with neutrophilia and left shift without changes to the lymphocyte and eosinophil counts (McManus and Craig, 2001). The lymphocytosis in SRMA may be related to the age of these animals, a physiological response to adrenaline release or could reflect prolonged immune stimulation that has an alternative immune mediated mechanism compared to IMHA (Blackwood, 2005). Eosinopenia in the SRMA cases is most likely secondary to a stress leukogram but could reflect acute infection (Blackwood, 2005). Biochemistry documented hypoalbuminaemia, hyperglobulinaemia, increased ALP activity, hyperphosphataemia, increased total calcium, hypercholesterolaemia, hyperkalaemia, increased urea and increased ALT activity in some of the SRMA cases. Hypoalbuminaemia, hyperglobulinaemia, increased ALP, increased ALT and hypercholesterolaemia have been described previously in SRMA (Scott-Moncrieff *et al.,* 1992; Cizinauskas *et al.* 2000). It is likely that that raised total calcium and hyperphosphataemia is secondary to the young age of the SRMA population whilst the raised potassium may be secondary to drug therapy (i.e. NSAIDs), diet or increased leukocyte counts (Skelly and Mellanby, 2005). The increased urea was presumptively due to dehydration (supported clinically by tacky mucous membranes and a skin tent) in the one case where it was present.

It is possible that the case with the cervical abscess could have had a trigger for its SRMA. The time course between diagnosis of the abscess and SRMA and the fact that no infectious organism was identified on cytology or culture of the abscess make this less likely. However, this case was treated with antimicrobials prior to referral and an infection may have been present initially.

The same issue of treatment with antibiotics prior to performing screening diagnostics may have prevented the identification of a bacterial trigger for SRMA in many of the present cases. In one other study antibiotics were instigated before trying to find a trigger for SRMA post mortem but blood, urine, CSF and faeces were sent for culture, and abdominal and thoracic radiographs were taken before antibacterial therapy began (Scott-Moncrieff *et al.* 1992). Bacteria were identified on culture in some of these samples but felt unlikely to be triggers of SRMA for three reasons: on repeated sampling and culture no bacteria were identified in the same cases where bacteria were initially cultured; dogs responded to immunosuppressive doses of steroids; and no dogs clinically responded to antibiotics that the cultured bacteria were sensitive to. Harcourt (1978) stated that antibiotics did not prevent the recurrence of disease in the Beagle colony studied, but it is unclear whether dogs had been treated with these drugs at the time of the investigations to try and establish a trigger. Given these findings it would seem unlikely that a bacterial trigger was evident in these cases but it cannot be excluded from the current study.

The main limitations of this paper are the previous treatment of most cases with antibiotics preventing identification of infectious agents and the small numbers described. The low numbers make statistical analyses difficult and therefore conclusions are not definitively proven. Case numbers could have been increased by including cases over the age of 18 months (5 extra cases), those with other neurological signs (3 extra cases) (i.e. paresis, ataxia, menace deficits, anisocoria, strabismus) and those with a mixed or mononuclear pleocytosis (7 extra cases) as these have previously been described with SRMA (Tipold and Jaggy, 1994). However, including these cases could have resulted in the inclusion of other inflammatory meningioencephalitides (Lowrie, M., *et al*. 2009). Measuring IgA in serum and CSF has been recommended to differentiate SRMA from other inflammatory meningioencephalitides (Tipold, 1995) but this was not performed on the dogs included in this retrospective study. Additionally increased case numbers in this study would have been possible by reducing or changing the number of screening tests performed. If all cases that had some of the screening tests noted in the inclusion criteria, a further 21 cases could have been reported. Of these further 21 cases that had some of the screening tests performed (including some of the additional diagnostics for bacterial and protozoal diseases) no triggers for SRMA were identified. A case control study would have allowed statistical comparison between groups and allowed identification of the

number of inflammatory foci that may be present in dogs presenting with other conditions but similar clinical signs. Additionally the relationship between vaccination or drug history and SRMA was not examined for these cases despite the link to cases of IMHA. Unfortunately the previous exposure to drugs was incomplete in the clinical records.

It is concluded that SRMA cases, with a neutrophilic pleocytosis on CSF analysis, that fall into a specific clinical picture (i.e. <18 months of age, with pyrexia, neck pain, depression and no other neurological signs) are unlikely to have abnormal findings when screened for infectious or neoplastic diseases. This may aid clinicians to define the most cost effective approach in such SRMA cases by avoiding further screening diagnostic tests (i.e. cystocentesis urine analysis (including culture), complete abdominal ultrasound and orthogonal inflated thoracic radiographs) when not indicated by other abnormalities on history or clinical examination.

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