STEROID RESPONSIVE MENINGITIS-ARTERITIS: A PROSPECTIVE STUDY OF POTENTIAL DISEASE MARKERS, PREDNISOLONE TREATMENT, AND LONG-TERM OUTCOME IN 20 DOGS (2006 – 2008)

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Abstract

Steroid responsive meningitis-arteritis (SRMA) is a common disease of the dog with no specific ante-mortem diagnostic test and management is complicated by the requirement for prolonged treatment and the potential for relapse. The value of immunosuppressive prednisolone has been established but an optimal regime is not described. The lack of diagnostic and prognostic markers makes management frustrating. This study prospectively evaluates a protocol for the use of a prednisolone monotherapy in the management of SRMA. The utility of conventional and novel protein markers was evaluated by examining their expression at various milestones in the management of SRMA and by comparing this to their expression in other canine inflammatory and non-inflammatory central nervous system (CNS) diseases.

This prospective study recruited twenty dogs with a clinical diagnosis of SRMA presenting to the University of Glasgow Small Animal Hospital between May 2006 and May 2008. These patients were enrolled on a strict prednisolone regimen with serum and cerebrospinal fluid samples taken at key points in the management of this disease.

The protocol was successful at achieving resolution of clinical signs with no relapse in the follow up period in all 20 dogs. Clinical remission was achieved in all cases within 2 weeks of starting the protocol. When relapse occurred during the treatment schedule (4/20), restarting the treatment schedule led to resolution of the disease. The fact that dogs did not relapse post-treatment in this study is supportive of the value of this protocol in managing SRMA.

Overall, the findings detailed in this thesis support the use of an immunosuppressive prednisolone monotherapy in the treatment of canine SRMA. Acute phase proteins are significantly elevated at initial presentation with the remission of clinical signs, induced by immunosuppressive therapy, reducing but not eliminating this increase in serum concentrations. APPs are of particular value in the identification of putative relapse. In contrast, serum and cerebrospinal fluid IgA provides valuable information at diagnosis but a persistent increase throughout the disease limits their value in the monitoring of therapy. This immunoglobulin was found to be also be elevated in the serum and cerebrospinal fluid of other inflammatory and non-inflammatory canine CNS diseases. A robust prognostic marker for SRMA remains elusive.

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Author's Declaration

The work presented in this thesis was performed solely by the author except where the assistance of others has been acknowledged.

Mark L Lowrie, February 2010

Publications and Presentations

Some of the work contained in this thesis has been the subject of the following publications or presentations:

Papers

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Lowrie, M., Eckersall, P.D., Penderis, J., McLaughlin, M., Mellor, D., Anderson, T.J., 2008. The role of acute phase proteins in diagnosis and management of steroid responsive meningitis-arteritis. *Vet Journal* 2009; 182: 125-130.

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Conference proceedings

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2008 Lowrie, M., Eckersall, P.D., Penderis, J., McLaughlin, M., Anderson, T.J., 2008. Acute phase proteins and immunoglobulin A in steroid responsive

meningitis-arteritis. Proceedings of 51st Annual British Small Animal Veterinary Association Congress, Birmingham, UK.

2008 Lowrie, M., Penderis, J., McLaughlin, M., Anderson, T.J., Eckersall, P.D., 2008. The use of acute phase proteins in diagnosis of steroid responsive meningitis-arteritis in comparison to immunoglobulin A. Proceedings of 10th Annual Congress of the European Society of Veterinary Clinical Pathology Congress, Barcelona, Spain.

2009 Lowrie, M., Penderis, J., Anderson, T.J., 2009. Prospective evaluation of a prednisolone monotherapy protocol for management of steroid responsive meningitis-arteritis. Proceedings of 52nd Annual British Small Animal Veterinary Association Congress, Birmingham, UK.

Chapter I

REVIEW OF CANINE STEROID RESPONSIVE MENINGITIS-ARTERITIS

1.1 Inflammatory Central Nervous System Disease in the Dog

Inflammatory diseases of the central nervous system (CNS) are one of the most common causes of neurological dysfunction in the dog (Fluehmann et al., 2006). Inflammatory CNS disease can be grouped into two broad categories; those of a known infectious aetiology and those causing meningoencephalomyelitis of unknown aetiology (Tipold, 1994). The latter group consists of diseases such as granulomatous meningoencephalomyelitis (GME), steroid responsive meningitis-arteritis, eosinophilic meningoencephalomyelitis, and the numerous breed specific meningoencephalitides reported (Bailey and Higgins, 1986; Sorjonen, 1990; Tipold et al., 1993; Tipold and Jaggy, 1994; Flegel et al., 2008; Levine et al., 2008; Higgins et al., 2008; Windsor et al., 2009).

The antemortem diagnosis of canine inflammatory CNS disease remains challenging being based on a combination of clinical criteria, non-specific laboratory investigations and exclusion of other diseases. This has been demonstrated in the largest retrospective study of canine inflammatory CNS disease to date whereby clinical distinction between inflammatory CNS diseases and other non-inflammatory CNS diseases was difficult when utilising ante-mortem aids such as history, signalment, clinical examination and clinical pathology (Tipold, 1995). For example, the presence of multifocal signs was been reported as the diagnostic hallmark of inflammatory CNS diseases (Oliver et al., 1997) yet Tipold (1995) found just one third of the 220 dogs retrospectively reviewed to have multifocal clinical signs.

Analysis of cerebrospinal fluid (CSF) is instrumental in confirming the presence of inflammation and is considered the mainstay of diagnosis (Tipold, 1995). Cerebrospinal fluid analysis surprisingly supersedes magnetic resonance imaging (MRI) in sensitivity (Lamb et al., 2005; Bohn et al., 2006). However, the true sensitivity and specificity of CSF analysis in inflammatory CNS disease has not been determined given the difficulties in obtaining a definitive diagnosis in every case (Tipold, 1995). In addition, unperturbed CSF parameters (i.e. total nucleated cell count and total protein concentration) have been described in a number of histopathologically-confirmed inflammatory CNS diseases (Thomas and Eger, 1989; Demierre et al., 2001; Cherubini et al., 2006; Tipold and Jaggy, 1994; Cizinauskas et al., 2000; Levine et al., 2008). Brain biopsy has been suggested as the gold-standard ante-mortem diagnostic aid in inflammatory CNS disease but limited

tissue access, risks of morbidity and mortality together with its limited availability mean other techniques remain common place (Thomas et al., 1996; Kraft et al., 1997; Troxel et al., 2004; Cherubini et al., 2005; Lamb et al., 2005).

These features of inflammatory disease are reflected in steroid responsive meningitis-arteritis (SRMA) in that it is a relatively common disease (representing 2% of neurological referrals in one study; Flehmann et al., 2006), with no definitive diagnostic test (Tipold and Jaggy, 1994), and infrequently causes disease without CSF perturbations (Tipold and Jaggy, 1994; Cizinauskas et al., 2000).

1.2 Steroid Responsive Meningitis-Arteritis

1.2.1 Background

Steroid responsive meningitis-arteritis (SRMA) in the dog is a well-recognised disease in small animal practice (Tipold and Jaggy, 1994). The disease was first recognised as a polyarteritis in young laboratory Beagles in which a predilection for the meningeal vessels and coronary arteries was observed (Harcourt, 1978; Brooks, 1984; Hayes et al., 1989). Subsequently numerous terms have been used to describe this clinical syndrome: 'Beagle Pain Syndrome' (Hayes et al., 1989), 'Canine Pain Syndrome' (Burns et al., 1991), 'Canine Juvenile polyarteritis Syndrome' (Felsburg et al., 1992), 'Necrotizing Vasculitis' (Brooks, 1984), and more recently, steroid responsive meningitis-arteritis (Tipold and Jaggy, 1994). The term SRMA is now the most universally accepted name for this disease as it describes both the predominant pathology and treatment (Tipold and Jaggy, 1994).

A typical presentation would be a juvenile dog (less than 2 years of age) of medium to large breed (often pure-bred) with lethargy, pyrexia, inappetance and some degree of axial skeletal pain. Axial skeletal pain is most commonly evident as reluctance to move, low head carriage, cervical or thoracolumbar pain on examination and occasionally an arched back (Figure 1:1 and Figure 1:2). Clinical signs can wax and wane over a period of months and typically if left untreated the disease can be self-limiting with many dogs over the age of two developing an age-resistance to the disease (Scott-Moncrieff et al., 1992). A caveat to this is the development of neurological deficits due to progression of the meningitis into a myelitis or encephalitis (Tipold and Jaggy, 1994; Wrzosek et al., 2009). The prognosis in these circumstances is guarded with mortality rates varying from 5% to

100% (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). If a rapid diagnosis is achieved and a rigorous treatment protocol is adhered to then the prognosis for remission is usually excellent (Tipold and Jaggy, 1994; Cizinauskas et al., 2000).

Figure 1:1: A young boxer exhibiting low head carriage and an arched back; signs consistent with severe cervical pain.

Figure 1:2: A young Beagle demonstrating neck and spinal pain manifest by a low head carriage and an arched back; signs consistent with steroid responsive meningitis-arteritis.

1.2.2 Aetiology and Pathogenesis

The aetiology of SRMA remains obscure though an immune-mediated cause has been suggested by many authors. The reasons for this are three-fold: high concentrations of immunoglobulin A (IgA) are present both in the serum and CSF (Tipold and Jaggy, 1994; Tipold et al., 1994), a feature shared with some human vasculitides including Kawasaki disease (Felsburg et al., 1992) and Henoch-Schönlein purpura (Saulsbury, 2007); the remission of clinical signs on immunosuppressive doses of steroids (Meric et al., 1985); and the absence of identifiable infectious organisms further implicates immune-mediated disease (Harcourt, 1978; Meric et al., 1985; Meric et al., 1986; Scott-Moncrieff et al., 1992; Poncelet and Balligand, 1993; Tipold and Jaggy, 1994). T cell activation has been identified in this disease, indicating exposure to an antigen (Tipold, 2000), however, no bacterial or viral agent has been identified and the suspicion is that this may be a self-antigen.

The pathology is well-described with the most consistent features being a vasculitis of the meningeal arteries (Harcourt, 1978), a non-suppurative inflammation within the meninges (Meric et al., 1985), and a sub-clinical coronary arteritis (Harcourt, 1978; Spencer and Greaves, 1987; Hayes et al., 1989; Scott-Moncrieff et al., 1992; Snyder et al., 1995). The vascular pathology is similar to that found in confirmed immune-mediated vasculitis due to immunoglobulin deposition in blood vessel walls (Tipold, 2000). However, immunoglobulin complexes have not been identified in the arterial walls of dogs with SRMA. On this basis it is possible that the observed vasculitis is due to some other unknown cause (Tipold, 2000).

1.2.3 Signalment

Specific breeds appear to be predisposed, including beagles (Harcourt, 1978; Meric et al., 1985), Bernese mountain dogs (Presthus, 1991), and boxers (Poncelet and Balligand., 1993; Behr and Cauzinille, 2006) with no sex predilection reported. Young dogs under two years are most commonly affected although there are reports in dogs as old as seven years (Tipold and Jaggy, 1994) with one study suggesting that resistance to relapse develops around two years of age (Scott-Moncrieff et al., 1992).

1.2.4 Clinical Signs

The most common clinical signs include stiffness of the gait, cervical pain, lethargy and pyrexia. Cervical pain is most commonly manifested by a low head carriage and arched back (Figure 1:1 and Figure 1:2). Other clinical signs that may be present from time to time include thoracolumbar pain, muscle rigidity and/or spasms (myoclonus). Two forms of the disease have been reported; a fulminating acute form characterised by a neutrophilic pleocytosis, and a chronic protracted form represented by a mild mononuclear or mixed cell pleocytosis associated with neurological deficits reflecting extension of the inflammation to contiguous structures (e.g. a myelitis or encephalitis) (Tipold and Jaggy, 1994). However, a recent report does indicate that fatal cerebral extension of meningeal inflammation is a complication in acute disease (Wrzosek et al., 2009). Clinical signs therefore vary from per-acute onset with rapid progression, through to a more chronic and insidious course with episodic signs over a course of time (Tipold and Jaggy, 1994).

1.2.5 Clinical Pathology

There is no single diagnostic test that can be used to establish a diagnosis of SRMA. Therefore laboratory findings can only indicate the presence of an inflammatory disease process and as such are not specific for this condition. However, in the correct clinical circumstances, these laboratory findings are useful for supporting the diagnosis of SRMA.

Haematology may demonstrate evidence of a leucocytosis with left shift (Hayes et al., 1989). Biochemistry may reveal a mild hypoalbuminaemia due to the inflammatory disease process, as albumin is a negative acute phase protein; i.e. its concentration decreases in response to inflammation (Cerón et al., 2005). Hyperglobulinaemia has been reported and is likely to be due to the raised serum IgA concentrations observed in this disease (Tipold and Jaggy, 1994; Tipold et al., 1994).

Cerebrospinal fluid analysis is characterised by an increased white blood cell concentration (reference range; < 5-8 wbc/µl (0.005 - 0.008 x 10^9/l)) when CSF is collected from the cisterna magna) (Di Terlizzi and Platt, 2006; Bathen-Noethen et al., 2008). A predominance of neutrophils in the absence of bacteria is seen in the acute form of the disease (Figure 1:3 A and Figure 1:3 B). However, as the disease progresses, mononuclear cells (macrophages, lymphocytes and monocytes) are found in the CSF and therefore the

chronic form of the disease demonstrates a mixed cell pleocytosis (Figure 1:3 C). In association with this inflammatory response an increase in the CSF total protein concentration is also expected (a normal CSF protein concentration is considered as < 250 mg/l if the CSF was collected from the cisterna magna) (Di Terlizzi and Platt, 2006). Cerebrospinal fluid changes appear sensitive to immunosuppressive steroid administration and will be suppressed if the patient is given this medication before CSF collection (Heller and Aurora, 2008), although further investigation is required to investigate whether any particular cell lines are affected preferentially.

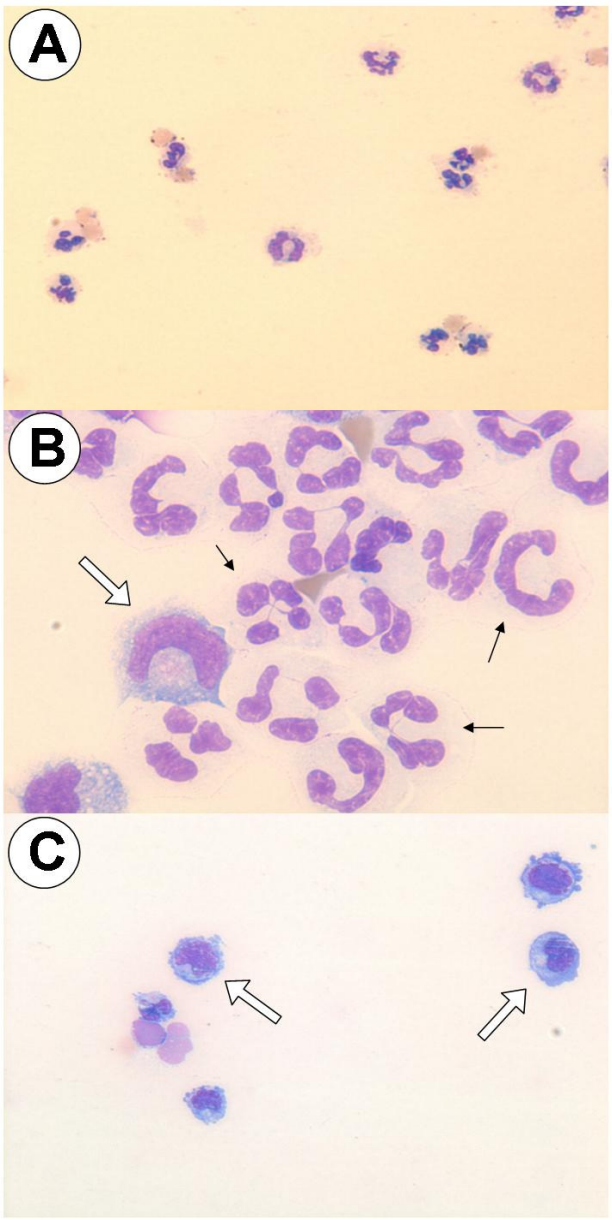

Figure 1:3: Cerebrospinal fluid samples from three dogs (A - C) with steroid responsive meningitis-arteritis (SRMA). A number of mature neutrophils with hypersegmented nuclei can be seen (black arrows). A – The cell count of this sample was 418 cells /µl. A predominance of neutrophils is evident. B – The cell count in this case was 920 cells /µl. There are also a small number of macrophages present (white arrow). C – This sample was taken from a dog with the protracted form of SRMA (i.e. the presence of additional neurological deficits). The predominant cell types present are macrophages (white arrows). Hematoxylin and eosin stain has been used.

1.2.6 Investigation and Diagnosis

The diagnosis of SRMA, once other causes have been ruled-out, is based upon the signalment, history and physical examination in conjunction with an inflammatory CSF analysis, supported by haematological and biochemical values. Important differential diagnoses for cervical pain in a young adult dog include discospondylitis, cervical instability, neoplasia and trauma (Table 1-1).

A neutral lateral cervical radiograph should always be considered in any dog presenting with cervical pain before manipulation of the atlantoaxial joint is performed due to the possibility of instability within this region. Conscious radiographs are preferable if instability is suspected, although radiographs can also be obtained under general anaesthesia provided careful handling is observed, particularly during intubation. Radiography may also allow evaluation of the intervertebral disc spaces to look for evidence of disconspondylitis, i.e. radiopaque irregular proliferative lesions located at the vertebral end plates (sclerosis) with associated lysis.

Once these procedures have been performed to rule-out instability then it is safe to procede with collecting a CSF sample from the cerebromedullary cistern (Figure 1:4). Other notable differential diagnoses for inflammatory CNS disease in dogs under 2 years of age include protozoal diseases. Therefore, in the presence of inflammatory CNS disease consideration should be given to evaluation of CSF for the presence of Neospora DNA using a polymerase chain reaction (PCR) test. Bacterial meningitis is uncommon in dogs with CSF culture yielding a low sensitivity (Meric et al., 1988; Radaelli and Platt, 2002; Tipold, 1995). It is therefore usually sufficient to rule out a bacterial cause by the absence of intracellular or free-living organisms on a smear.

Other potential causes of inflammatory brain disease include granulomatous meningoencephalomyelitis (GME) and many of the breed specific meningoencephalitides. These diseases should be suspected when neurological deficits are present making them difficult to distinguish from the protracted form of SRMA. In these circumstances, advanced imaging studies are recommended to exclude parenchymal CNS involvement.

Immunoglobulin A is reported to be increased in both the serum and CSF of dogs with SRMA (Tipold et al., 1994). As previously mentioned the reference range for IgA in normal dogs is ambiguous and patients with other inflammatory CNS diseases can cause increased IgA concentrations within serum and CSF (Tipold et al., 1994; Tipold et al., 1995). Similarly, IgA has also been found to be normal in cases of SRMA (Behr and Cauzinille, 2006; Wrzosek et al., 2009). Therefore diagnosis of SRMA remains tentative and the lack of a specific disease marker makes management frustrating.

Differential Diagnosis for Cervical Pain in a Young Dog	
Inflammatory	SRMA, Granulomatous meningoencephalomyelitis, Breed specific encephalitis and meningitis, Infectious meningitis/myelitis, Discospondylitis, Osteomyelitis, Empyema, Polyarthritis, Polymyositis
Anomalous	Atlantoaxial Instability, Chiari-like Malformations, Osteochondromatosis, Perineurial (Tarlov) cysts, Scoliosis/Vertebral abnormalities, Syringohydromyelia
Degenerative	Wobbler syndrome
Trauma	Fractures/luxations, Severe spinal cord contusions, Traumatic disc herniation
Neoplastic	Brain Tumours – primary or secondary with raised intracranial pressure Central Nervous System Neoplasia • Intradural/Extramedullary • Extradural • Intramedullary Non-Central Nervous System Tumours

Table 1-1 Adapted from S. R. Platt (2004) Neck and Back Pain. In: *BSAVA Manual of Canine and Feline Neurology*. 3rd edn. Eds S. R. Platt and N. J. Olby. British Small Animal Veterinary Association, p204.

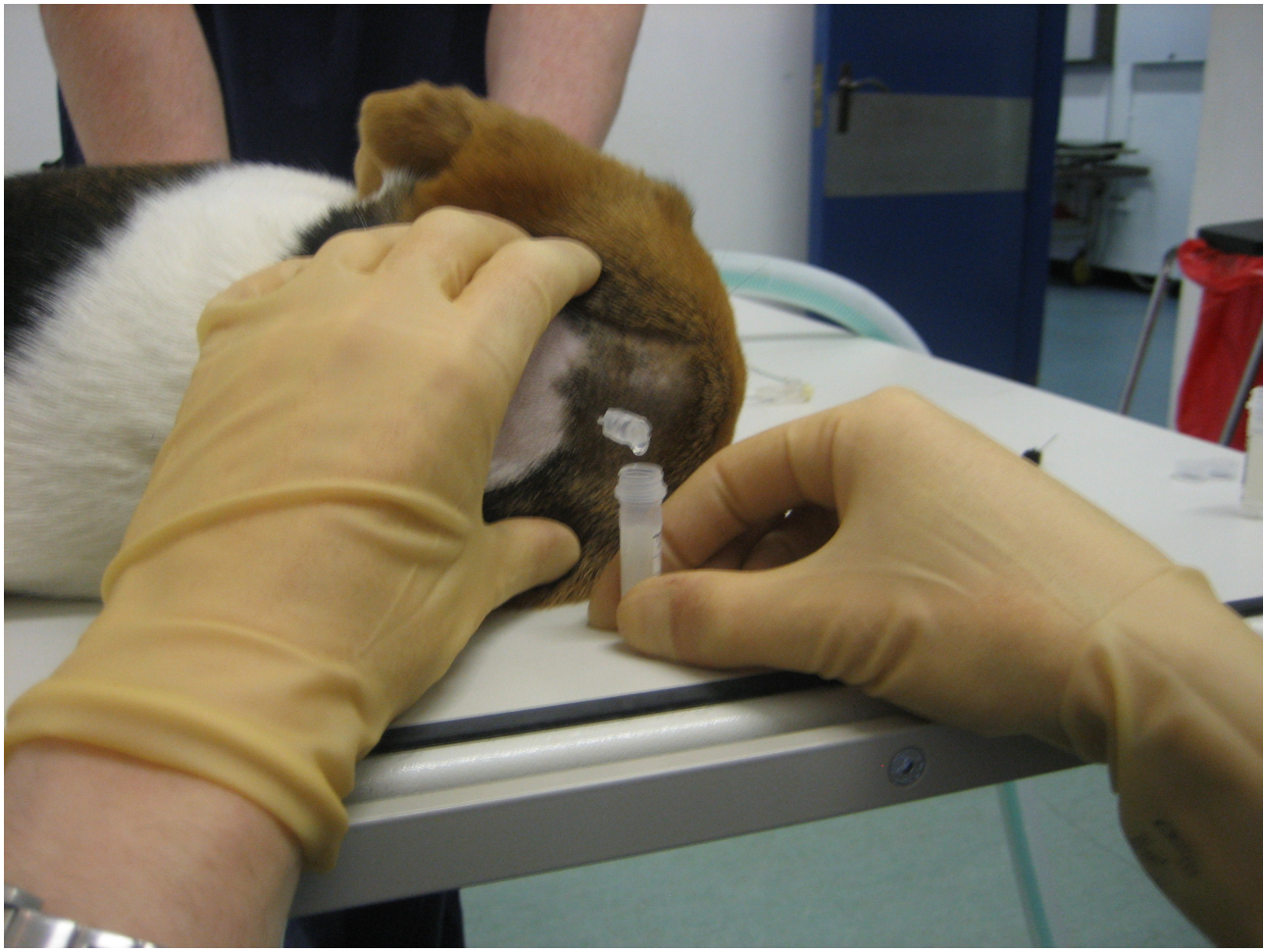

Figure 1:4: Cerebrospinal fluid collection performed at the cerebromedullary cistern.

1.2.7 Treatment

Immunosuppression is the mainstay of treatment. The protocol shown in Figure 1:5 describes a regimen involving an immunosuppressive dose of prednisolone (Prednicare©; Animalcare Limited) that is reduced slowly over several months to the lowest dose necessary to maintain remission of clinical signs. The severity of the clinical signs dictates the starting dose and many cases are deemed severe enough to start at a dose of 2 mg/kg prednisolone twice daily for the first 2 days. Anecdotally it is recommended to counteract the side-effects of this drug by adding in gastroprotectants. All dogs undergoing treatment in our hospital receive sucralfate (Antepsin©; Chugai Pharma UK Ltd) (1 - 2 g/dog orally three times daily), ranitidine (Zantac©; GlaxoSmithKline) (2 mg/kg orally twice daily) or famotidine (Pepcid©; Merck, Sharp & Dohme Limited) (0.5 - 1 mg/kg orally once daily) for the duration of the corticosteroid therapy. Furthermore gastrointestinal integrity should be evaluated throughout the course by client education to observe for the presence of vomiting, inappetance, melaena or haematochezia. Steroid responsive meningitis-arteritis is associated with moderate to severe pain and so analgesia is frequently essential at the start of therapy. Some cases require opioid analgesia due to intractable neck pain for the first 24 hours following diagnosis.

Previous studies advocate the use of prednisolone in the treatment of SRMA (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). The reported regime commences with administration of prednisolone at an initial dose of 4 mg/kg/day. After two days this dose was reduced to 2 mg/kg/day for two weeks. Following this the prednisolone was decreased to 1 mg/kg/day for a further two weeks. At this time a re-check examination was performed in which a CSF sample was obtained. These re-check examinations were performed every 4 weeks until both the neurological examination and CSF sample were found to be normal. At this time the dose of prednisolone was slowly tapered to 0.5 mg/kg every alternate day and this was maintained for at least six months.

Cizinauskas and others (2000) report full remission in 8/10 dogs receiving a prednisolone based treatment regime with an in protocol case relapse rate of 60%; the treatment regimen also included the use of other therapeutic agents. Consequently only 4/10 cases received a prednisolone monotherapy. Of these ten cases one was euthanased for reasons related to the disease. Tipold and Jaggy (1994) describe a managed prednisolone monotherapy protocol, involving adjustment of the prednisolone dose in the light of CSF parameters, achieving a case relapse rate of 25%, resolution rate of 60% and a 5% mortality.

Management of SRMA involves monthly serial CSF analyses and repeat neurological examinations until the combined results are normal (Tipold and Jaggy, 1994). Cizinauskas and others (2000) report that between four and 12 re-check examinations were required for each of the 10 dogs with SRMA to obtain a normal neurological examination with unperturbed CSF. This meant that each patient had at least four cerebellomedullary cistern punctures performed in order to achieve remission representing a high intervention management requiring dedicated owner compliance. Failure to comply with such a regime, as reported by Tipold and Jaggy (1994), can result in a high mortality rate. No reported alternative method exists that allows modification of the treatment regime based on patient response. It would be of value to identify a parameter that would predict the potential for relapse and to establish the success of a strict prednisolone monotherapy that caters for relapsing patients. Ultimately, this would give a benchmark on which to evaluate the requirement and success of alternative medications (monotherapies and multimodal therapies).

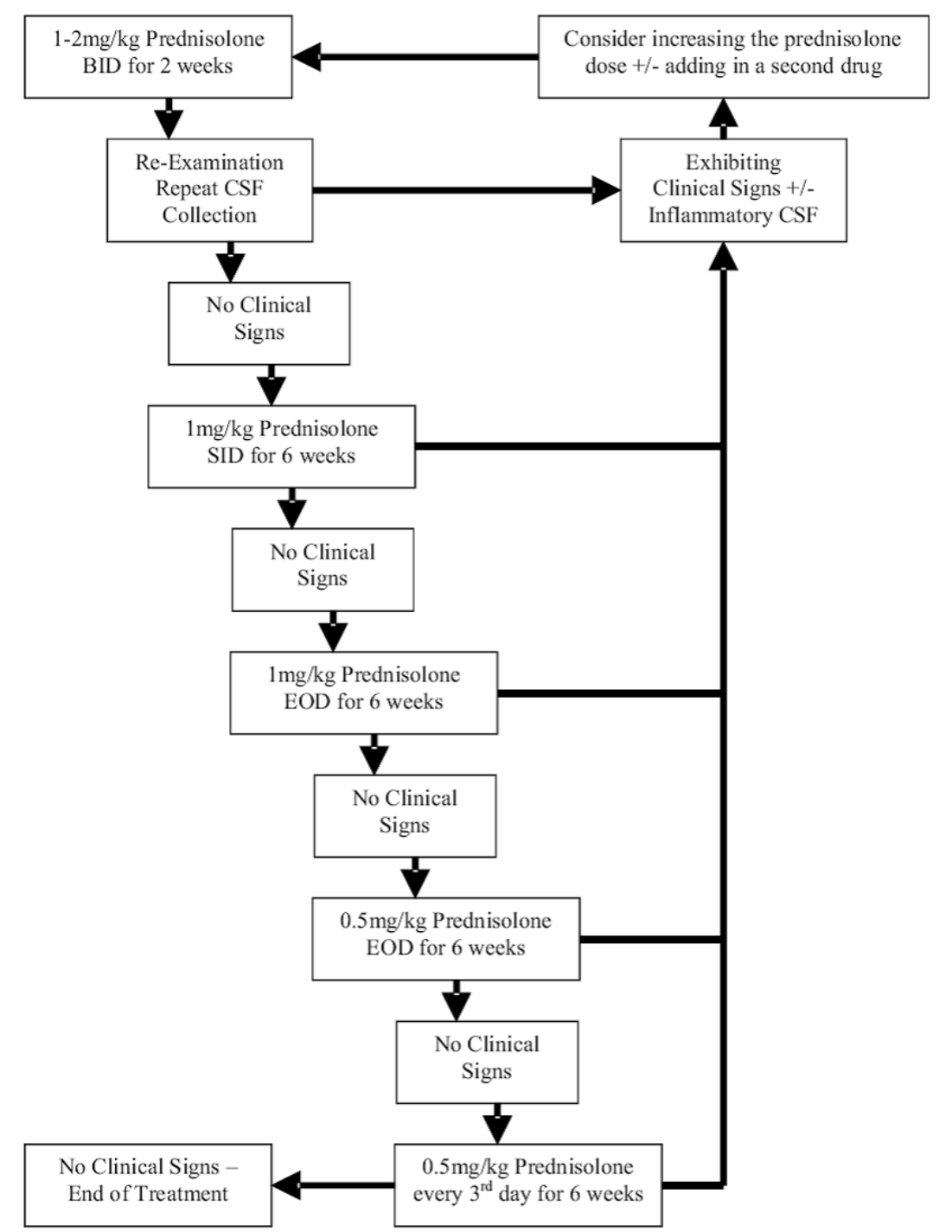

Figure 1:5: A suggested treatment flow chart for the treatment of steroid responsive meningitis-arteritis using guidance from previous studies (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). BID, twice daily; CSF, cerebrospinal fluid; EOD, every other day; SID, once a day.

Treatment with antibiotics may be necessary if the diagnosis is uncertain or if bacterial or protozoal meningitis is suspected. In this situation a good choice of first-line antibiotics would be clindamycin (Antirobe©, Pfizer) (12.5-25 mg/kg orally TWICE DAILY) and trimethoprim/sulphonamide (Trimacare©, Animalcare Limited) (15 mg/kg orally THREE TIMES DAILY) as they are believed to penetrate the CNS better than other available antibiotics (Friedland and McCracken, 1994). If antibiotics are used then treatment should be continued until a negative CSF culture is confirmed.

1.2.8 Investigating potential markers of SRMA

Despite wide recognition of the clinical picture of SRMA the aetiology of this disease remains obscure. Diagnosis relies upon a combination of suggestive clinical indices and exclusion of other diseases. No gold-standard diagnostic modality exists to conclusively confirm a clinical diagnosis of SRMA. Relapse is well recognised in this disease and prolonged therapy appears to reduce, but does not eliminate, its occurrence (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). No technique has been described for identifying when to cease therapy and serial CSF collections combined with an assessment of the patient's clinical signs remain the only indices on which to base modification of the treatment regime (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). The iatrogenic effects of unnecessarily prolonged steroid therapy, though generally reversible, are undesirable (Cizinauskas et al., 2000).

1.2.8.1 Immunoglobulin A

Immunoglobulin A is a non-specific disease marker within the serum and CSF that is not suppressed by clinically effective therapy (Tipold and Jaggy, 1994; Tipold et al., 1994). It has been suggested that IgA CSF and serum levels tend to remain elevated in dogs that have proven difficult to manage (Tipold and Jaggy, 1994; Burgener et al., 1998). However, the relationship between elevated IgA concentrations and relapse has not been reported to be a clinically useful guide for the duration of steroid therapy or prognosis in an individual (Tipold and Jaggy, 1994; Tipold et al., 1994; Cizinauskas et al., 2000).

The reason for the raised systemic and intrathecal IgA concentration is unknown but the phenomenon appears specific to this inflammatory disease in the dog (Tipold and Jaggy, 1994; Tipold et al., 1994). The literature on IgA demonstrates that an increased

concentration may be found in the CSF of SRMA patients, though this feature is shared with other inflammatory CNS diseases giving it a low specificity. Conversely, serum IgA in SRMA is reported to be significantly elevated above the published reference range (10.9 - 100.1 μg/mL; Tipold et al., 1994) in comparison to other CNS inflammatory diseases where concentrations may be normal or only mildly increased (Tipold and Jaggy, 1994; Tipold et al., 1994; Tipold et al., 1995). However, the reported specificity varies, for example, Tipold and Jaggy (1994) report it to be 100% but in a later study Tipold and others (1995) suggest a figure of 83%. A report of normal serum IgA concentrations in four groups of healthy dogs found a wide range of IgA values (from 6.1 – 973 μg/mL) with 1/4 groups (a colony of beagles) exhibiting a mean IgA concentration of 113.9 μg/mL (Griot-Wenk et al., 1999). This suggests that the normal reference range for IgA requires further investigation. However, the combination of raised serum and CSF IgA concentrations have been suggested to be useful in the diagnosis of SRMA (Tipold et al., 1995), particularly the chronic form that can be difficult to distinguish from other canine meningoencephalitides. However, the normal reference range and its sensitivity and specificity in the diagnosis of SRMA needs further investigation.

1.2.8.2 Cytokines

Increased serum levels of interleukins, including interleukin-6 (IL-6) and tumour necrosis factor (TNF-α) have been documented in humans with Kawasaki disease; the disease for which SRMA is a model (Lin et al., 1992). The concentrations of these cytokines were measured to investigate their prognostic value. It was found that higher levels of IL-6, intereukin-8 (IL-8) and TNF were present in those children with Kawasaki disease who subsequently develop coronary aneurysms when compared to Kawasaki disease patients that recover (Lin et al., 1993). As a result of these investigations work has been performed in SRMA to establish the role of such markers in the management of SRMA.

In one study, interleukin-6 and TNF-α were measured in serum from a group of laboratory Beagles with suspected SRMA (Hogenesch et al., 1995). The concentrations of IL-6 were increased in Beagles with clinical disease but were reduced (6/12) or undetectable (6/12) in the same dogs when clinical remission had been achieved following prednisolone administration. Interleukin-6 can cause fever, induce acute-phase proteins (APPs) and cause weight loss (Van Snick, 1990). The significance of the varying concentrations of IL-6 in clinical remission is unknown. However, experimental studies reveal that fever is not seen until a threshold of IL-6 is achieved. This was done by serial monitoring of the core

body temperature and serum IL-6 concentrations in dogs that had been challeneged with an injection of lipopolysaccharide (LeMay et al., 1990). Hogenesch and others (1995) were able to show that the level of this threshold varied amongst individuals and IL-6 concentration had no correlation with the severity of clinical signs. In this same study serum TNF-α was undetectable in both controls and dogs with SRMA. This finding may suggest that TNF-α has no role in the pathogenesis of SRMA. However, systemic TNF-α may not be a true reflection of its presence intrathecally and, unlike IL-6, TNF-α peaks 1-4 hours post-induction and decreases to undetectable concentrations after 5-6 hours suggesting that sampling time is integral to its detection (Hogenesch et al., 1995).

A second study investigated concentrations of IL-8 in the CSF of four patients with SRMA throughout the course of the disease (Burgener et al., 1998). Interleukin-8 remained elevated throughout the disease and correlated with concentrations of IgA in the CSF. Three of the four dogs had persistently high CSF IL-8 and IgA concentrations during therapeutic management of the disease and this was associated with frequent and severe relapse. The fourth dog, however, had elevated concentrations of both of these CSF markers and yet responded to a tapering prednisolone regime as described by Tipold and Jaggy (1994) without experiencing relapse. The conclusions from this study remarked that IL-8 and IgA may both increase in response to a stimulus or that IL-8 may trigger the production of IgA.

1.2.8.3 Acute Phase Proteins

The acute phase response provides markers for diagnosing and prognosticating inflammatory diseases in both humans and dogs (Jergens et al., 2003; Vermeire et al., 2004; Agrawall, 2005; Mischke et al., 2007). Serum APP concentrations have been found to be eight times more sensitive to inflammatory disease than white blood cells (Cerón et al., 2005). In a study investigating milk SAA concentration in cattle with mastitis it was found that maximum values of SAA preceded that of somatic cell count (SCC) and clinical signs during both episodes of mastitis recorded in one of the four cows studied (Figure 1:6). The situation for the remaining three cases was slightly more complex, and although SAA did not reach maximum values before the detection of clinical signs, they increased in concentration either prior to or in conjunction with SCC. A further study established that in the early stages of infection serum c-reactive protein (CRP) increases before an elevated rectal temperature is observed in cattle (Saini et al., 1991). These findings

suggest that APPs may provide valuable information in reaching an early diagnosis of SRMA and allow the clinician to respond quickly to relapse.

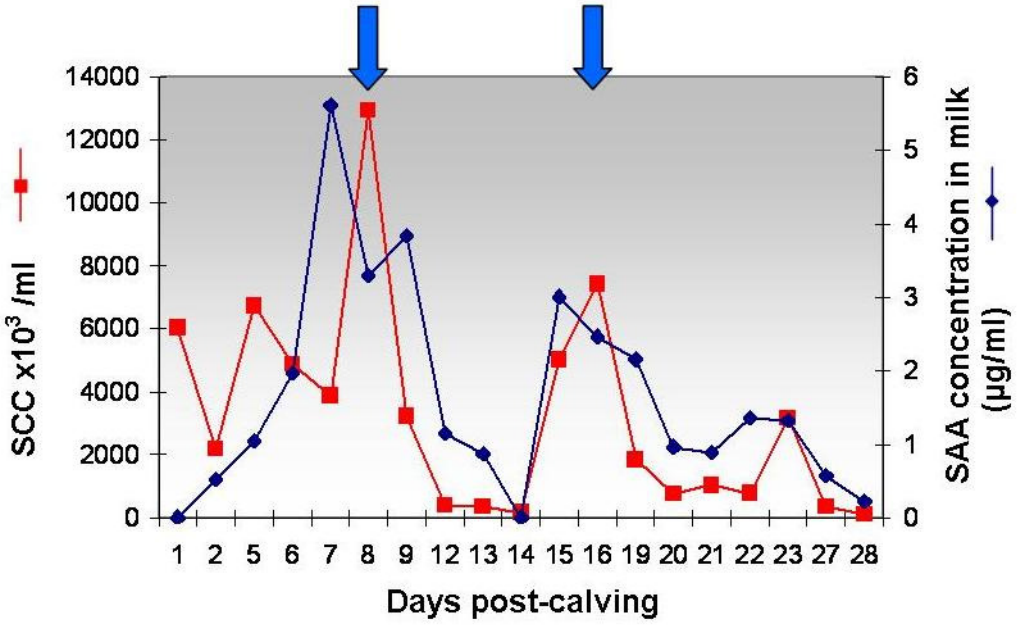

Figure 1:6: Profiles for somatic cell count (SCC) and serum amyloid-A (SAA) in milk in a post-parturient cow. Clinical mastitis (caused by *Streptococcus uberis*) developed on day 8 and again on day 16 (as indicated by the blue arrows) (from Hogarth, 2004).

1.3 Acute Phase Response

1.3.1 Background

The acute phase response (APR) is a non-specific inflammatory response affecting various host organs, that commences within hours of any tissue injury and can last for several days (Cerón et al., 2005). The stimulus resulting in this APR may include bacterial infection, trauma, neoplasia, tissue infarction, and immune-mediated inflammatory disease amongst others (Murata et al., 2004). All of these conditions induce a local inflammatory response and the early release of soluble mediators, which in turn leads to a systemic APR encompassing metabolic and physiological changes, providing the host with an optimal "infection-fighting" environment. The proposed purpose of the APR is to prevent ongoing tissue damage, to isolate and destroy the infective organisms, to remove harmful molecules and debris, and to activate the repair processes that are necessary to return the organ to normal function (Baumann and Gauldie, 1994).

The APR is an adaptive response that helps host survival and aims to restore physiological homeostasis, as deviations from a normal internal environment can impose a serious threat to the health of an individual. The APR may be relatively transient, subsiding over 24 to 48 hours, with the host returning to normal function within a few days, demonstrating the protective and homeostatic nature of this host response. In contrast, chronic disease can result in a more persistent APR (Baumann and Gauldie, 1994). Clinical changes observed in these homeostatic mechanisms during the APR include pyrexia, lethargy, anorexia, increased synthesis of certain endocrine hormones, decreased erythropoiesis, thrombocytopenia, alterations in plasma cation concentrations, inhibition of bone formation, negative nitrogen balance (as a result of proteolysis and decreased protein synthesis in skeletal muscle) with consequent gluconeogenesis, and alterations in lipid metabolism (Cerón et al., 2005).

1.3.2 Acute Phase Proteins

Acute phase proteins (APPs) are part of the host response to challenges such as inflammatory, infectious, traumatic or neoplastic stimuli. Their expression is moderated by the release of pro-inflammatory cytokines either in a positive (positive APPs) or negative (negative APPs) manner, or in an alteration of the protein isoform (Murata et al., 2004; Cerón et al., 2005). The majority of APPs are synthesised in the liver and released into the blood stream. However, extra-hepatic production sites have been identified for C-reactive protein (CRP) (Jabs et al., 2003), serum amyloid-A (SAA) (Liang et al., 1997; Lin et al., 2001; Eckersall et al., 2001), alpha-1-acid glycoprotein (AGP) (Gahmberg et al., 1978; Fournier et al., 2000, Poland et al., 2002), and haptoglobin (Hp) (Dobryszycka, 1997; Ebersole et al., 2000) although the significance and extent of this non-hepatic expression is unknown.

Acute phase proteins can be divided based on their response to stimulation with positive APPs increasing in response to a stimulus and negative APPs decreasing in concentration (Cerón et al., 2005). Positive APPs are further divided into two groups. Major APPs have very low or undetectable levels in the serum of healthy animals, but increase more than 100 fold during an APR (Cerón et al., 2005). Minor or moderate APPs are present in the serum of healthy animals and their concentrations do increase during an APR, but only an increase of up to 10 fold is observed (Gruys et al., 1994; Eckersall, 1995b). In humans, the concentration of SAA and CRP can increase up to 1000-fold during acute disease states (Cerón et al., 2005). Consequently these proteins are of diagnostic interest in human and veterinary medicine.

It is now recognised that there is considerable variation among species in the pattern of the APR (Eckersall, 1995b). An early indication of this inter-species variation was the observion that some APPs originally identified in man did not give a similar response in animals. For example, the response of CRP in cattle is different in that it acts as a moderate APP. In contrast, Hp is a major APP in cattle with levels increasing from being undetectable in normal animals, to over 100-fold on stimulation (Eckersall 1995a). In dogs, Hp acts as a constitutive serum protein and is a moderate APP (Conner et al., 1988; Eckersall et al., 1996). The pattern of the APP response of selected species is summarised in Table 1-2.

Acute Phase Protein	Major	Moderate	Minor	Negative
C-Reactive Protein	Canine, Porcine		Bovine	
Alpha-1-Acid Glycoprotein		Bovine, Ovine, Canine, Feline	Porcine	
Serum Amyloid-A	Bovine, Canine, Feline, Porcine			
Haptoglobin	Bovine, Ovine	Porcine, Canine, Murine, Rat		
Fibrinogen		All species		
Albumin				All species

Table 1-2: Classification of the acute phase protein response in selected species (adapted from Murata et al., 2004; Cerón et al., 2005). Major = 10 to several hundred times increase, moderate = 5 to 10 times increase, minor = 1 to 5 times increase, negative = decrease.

1.3.3 C-reactive Protein

C-reactive protein (CRP) was the first APP to be described. It was identified in humans suffering from infections and named after its ability to precipitate pneumococcal C-polysaccaride (Tillett and Francis, 1930). It has a molecular weight of 100 kD and consists of 5 subunits of 20 kD each. The major difference between human and canine CRP is that the canine form has glycosylation of 2 of the 5 subunits and this may be the reason as to why using antibodies against human CRP to measure canine CRP causes problems (Eckersall and Conner, 1988). The most established biological role for CRP is in complement activation and opsonisation (Volanakis, 1982).

In human medicine, some authors have suggested CRP is a reliable indicator for distinguishing bacterial from viral meningitis. As an example, bacterial meningitis results in a significantly elevated serum concentration of CRP whereas no changes are seen in viral meningitis (Peltola, 1982).

1.3.4 Serum Amyloid A

Serum amyloid A, a 15 kD apolipoprotein is the precursor of the Amyloid A protein, which is the principal component of secondary amyloid plaques, which may be deposited in major organs as an occasional consequence of chronic inflammatory disease (Husebekk et al., 1986). During inflammation SAA associates predominantly with the third fraction of high-density lipoprotein (HDL3) (Benditt et al., 1979), and constitutes as much as 30-50% of HDL3 protein during the APR. Serum amyloid A is synthesised primarily by the liver although a number of extra-hepatic sites have been shown to exist (Lin et al., 2001; Eckersall et al., 2001).

The structure of SAA has yet to be determined (Uhlar and Whitehead, 1999). Few attempts have been made to elucidate the role of SAA; thus the normal physiological function of SAA remains unclear. However, a high degree of conservation of the SAA genes and proteins has been maintained throughout the evolution of mammals suggesting they have an important biological role, perhaps in survival of the species (Hulten et al., 1999) in the face of infection by pathogens. One isoform of SAA (SAA3) is constitutively expressed in a number of diseased tissues, in particular endothelial cells, suggesting SAA plays an important role in the local defence mechanisms against invading micro-organisms

(Meek et al., 1994; Urieli-Shoval et al., 1998). Serum amyloid-A has been shown to work as scavenger of potentially dangerous oxidised cholesterol but also has immunomodulatory activities (He et al., 2006).

1.3.5 Alpha-1-Acid Glycoprotein

Alpha-1-acid glycoprotein, previously known as orosomucoid, belongs to the lipocalin family, a group of extracellular binding proteins specific for hydrophobic molecules, and specifically to the immunocalin subfamily, which includes proteins with immunomodulating properties (Lögdberg and Wester, 2000). Human AGP has a low molecular weight (41–43 kDa), high solubility, and high percentage of carbohydrates (45%) (Hochepied et al., 2003). Its glycosylation pattern is very variable (12–20 glycoforms) depending on the physiological or pathological conditions, such as parturition, inflammation or neoplasia (Biou et al., 1991; Kim and Varki, 1997). AGP synthesis is mainly by hepatocytes, but extrahepatic synthesis has also been reported (Gahmberg et al., 1978; Fournier et al., 2000, Poland et al., 2002).

The function of AGP has not been completely defined although an immunomodulatory and anti-inflammatory role is suggested as it can down-regulate neutrophil responsiveness, stimulate IL-1R antagonist secretion by macrophages, inhibit platelet aggregation and lymphocyte proliferation, and modulate the production of anti-inflammatory cytokines by peripheral blood leucocytes (Hochepied et al., 2003).

Current veterinary literature on AGP is restricted to its diagnostic use in two chronic inflammatory diseases; feline infectious peritonitis (Duthie et al., 1997) and ovine caseous lymphadenitis (Eckersall et al., 2007) reflecting its role as a moderate APP.

1.3.6 Haptoglobin

Haptoglobin is a major plasma glycoprotein synthesised in the liver and is present in most body fluids (serum, urine, saliva, cerebrospinal fluid, amniotic fluid, ascites, etc) of mammals. It binds free haemoglobin (Hb) in the cardiovascular system of mammals. The binding of Hb to Hp is the strongest non-covalent interaction known among the transport proteins in plasma and is not reversible (Yang et al, 1993). After Hp has bound free Hb,

the complex is degraded by hepatic lysosymes leading to a decrease of Hp concentration in plasma following haemolysis. This reduction in Hp concentration can be profound in severe haemolysis (Yang et al., 2003).

As a positive acute phase reactant, its plasma concentration is increased during inflammation, infections of different aetiology, trauma, tissue damage and malignancy (Dobryszycka, 1997). Haptoglobin is classified as a scavenger protein due to its involvement in the clearance of potentially toxic products (Dennis, 2001). Haemoglobin (Hb) released from ruptured red blood cells is toxic unless rapidly cleared from the circulation (Dennis, 2001). In the normal situation, ageing red blood cells are degraded in the bone marrow, liver or spleen however in certain circumstances, such as infection or inflammatory disorders, red cells may burst within blood vessels. The plasma protein Hp is thought to be involved in promoting the clearance of plasma Hb because it strongly binds free Hb and is depleted during haemolysis (Kristiansen et al., 2001). Consequently it is hypothesised that Hp also has a similar role with regards to scavenging other inflammatory byproducts (Dennis, 2001).

1.3.7 Functions of Acute Phase Proteins

Although several functions have been ascribed to the APPs, speculation still surrounds this topic with regard their absolute role(s). In general, the APPs have a variety of roles in inflammation acting as mediators of cytokines as well as scavengers of cell derived products released from damaged tissue or macrophages (Dennis, 2001). In addition, some members of the family may influence the immune response, which often accompanies inflammation and the release of autologous antigens, through direct protection of the host (Thompson et al., 1992). For example, the binding of free Hb by Hp restricts the availability of free iron to invading bacteria (Dobryszycka, 1997). The fact that APPs have been conserved throughout evolution and that they are induced in large amounts in response to harmful stimuli infers that they have a positive input to inflammatory and tissue repair processes (Hulten, et al., 1999).

1.3.8 Initiation and Progression of Acute Phase Response

Following the discovery of CRP in the 1930s, APPs were used as markers of inflammation for many years, however, from the 1980's, research concentrated on the regulation of APP biosynthesis (Kushner, 1982). *In vitro* models confirmed that the circulating mediators capable of hepatocyte stimulation following local tissue injury were small hormonal proteins called cytokines. Cytokines are intercellular signalling polypeptides produced by a number of different cells, but the most important sources are tissue macrophages and blood monocytes at sites of inflammation. Mononuclear cells are most commonly associated with initiating the cascade of events during the APR. Pro-inflammatory cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor–α (TNF-α) are released from activated macrophages and monocytes at the site of inflammatory lesions or infection (Heinrich et al., 1990) and are the major mediators of the APR in the liver. During the hepatic APR, these pro-inflammatory cytokines enhance the expression and secretion of plasma proteins, namely the positive APPs and decrease the expression and secretion of the negative APPs. The early release of these pro-inflammatory cytokines acts to notify the immune system through local and distant activity. At the local reaction site cytokines activate stromal cells e.g. fibroblasts and endothelial cells causing a secondary release of cytokines (Baumann and Gauldie, 1994). This results in the systemic release of cytokines and initiates a signal transduction cascade ultimately leading to the systemic APR. Many cytokines can regulate the production of other cytokines and their receptors meaning combinations of cytokines may be additive, inhibitory, synergistic, or cooperative in their actions (Gabay and Kushner, 1999).

1.3.9 Applications of Acute Phase Proteins in Veterinary Medicine

The possible diagnostic use of APPs in veterinary medicine has been comprehensively reviewed (Eckersall and Conner, 1988; Kent, 1992; Gruys et al., 1994; Eckersall, 1995b; Pedersen et al., 2004; Murata et al., 2004). Serum CRP has been found to increase rapidly in dogs with arthritis (Ohno et al., 2006), lymphoma (Nielsen et al., 2007), inflammatory bowel disease (Jergens et al., 2003), haematological diseases (Tecles et al., 2005), and in many infectious diseases there is growing evidence for the utility of these proteins in a wide range of conditions (Cerón et al., 2005; Paltrinieri, 2007).

As well as markers of infection or inflammation, APPs may also serve as indicators of prognosis and progress of the animal in response to treatment (Jergens et al., 2003; Vermeire et al., 2004; Agrawall, 2005; Mischke et al., 2007). The evidence that serum APP concentrations correlate with the markers of certain diseases (e.g. somatic cell count and mastitis, Hogarth, 2004) and return to control ranges faster than these established markers suggest that APP concentrations may be valuable in the early identification of disease remission and early convalescence. An example of this is in canine multicentric lymphosarcoma where decreased serum CRP and AGP concentrations, compared with pretreatment concentrations, have been shown to be indicative of remission after chemotherapy in dogs with multicentric lymphosarcoma (Hahn et al., 1999; Nielsen et al., 2007). Another example of this is in dogs with leptospirosis where an increased ratio of CRP to haptoglobin was found to be significantly associated with a negative outcome (Mastrorilli et al., 2007).

Another important feature of the APR in different diseases is their variable response depending on the disease in question. For example, chronic conditions such as canine lymphosarcoma (Merlo et al., 2007) are characterised by continued synthesis of these proteins during periods of disease quiescence or remission (Eckersall, 2004). In contrast, dogs with immune-mediated haemolytic anaemia, having survived their acute illness and achieved disease remission, have serum concentrations of CRP, SAA and AGP within the reference range (Zoia and Morris, 2007). Furthermore, the outcome of the dogs that achieved unperturbed APP concentrations was mixed with some patients clincially resolving after prednisolone treatment and others exhibiting relapse later on. The

implication of this observation on the necessity for chronic immunosuppression remains to be investigated (Eckersall, 2004).

1.4 Preparatory Work

Before commencing this study little was known regarding the APR in SRMA. Increasing evidence suggested that a systemic inflammatory response coincided with the acute clinical signs seen in each patient. The occurrence of an APR in canine SRMA is supported by the findings of moderate to marked leucocytosis with left shift (Hayes et al., 1989) and changes in the serum concentrations of several APPs including albumin and CRP (Bathen-Noethen et al., 2008).

Relapse is an unpredictable and inconvenient complication of SRMA management and infrequently causes disease without CSF perturbations (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). Acute phase proteins may be of use in these difficult cases and may also allow identification of the propensity for a patient to relapse.

Serial monitoring of APPs in dogs with SRMA and study of their association with disease severity, response to treatment and prognosis have not been reported. The possibility that there is an alteration in disease markers between convalescence and quiescence in SRMA and that the expression intensity of this alteration varies between individuals suggests the possibility that these markers may predict disease severity.

1.5 Aims of this Thesis

The major aims of the present study are three-fold:

1. To describe acute phase protein markers related to the diagnosis and management of SRMA as a first step towards confirming them as diagnostic and prognostic markers.

2. To investigate in detail the utility of immunoglobulin A as a diagnostic tool.

3. To implement and prospectively evaluate a refined prednisolone treatment protocol in the management of SRMA.

Chapter II

MATERIALS & METHODS

2.1 Collection of clinical material

2.1.1 Case selection

2.1.1.1 Steroid Responsive Meningitis-Arteritis

All cases of clinically confirmed SRMA seen at the the small animal neurology service, University of Glasgow Small Animal Hospital (UGSAH) between May 2006 and May 2008 were prospectively included in this study. Dogs with a history of steroid administration prior to accession were excluded from the study. Diagnosis was based on supportive clinical signs, history, and CSF characteristics (total nucleated cell count > 5 white blood cells/mL, total protein concentration > 25 mg/dL, and differential cell count).

2.1.1.2 Idiopathic Epilepsy (Negative Controls)

During the same time period cases of IE seen at the the small animal neurology service, University of Glasgow Small Animal Hospital (UGSAH) were prospectively included as negative controls in this study (i.e. non-inflammatory CNS disease). Dogs with a history of steroid administration prior to accession were excluded from the study.

2.1.1.3 Meningoencephalitis of Unknown Aetiology (Positive Controls)

All cases of clinically confirmed meningoencephalitis of unknown aetiology (MUA) seen at the the small animal neurology service, University of Glasgow Small Animal Hospital (UGSAH) between May 2006 and May 2008 were prospectively included in this study as positive controls (i.e. inflammatory CNS disease). Dogs with a history of steroid administration prior to accession were excluded from the study.

2.1.1.4 Steroid Responsive Meningitis-Arteritis Relapse Cases

All on-going cases of SRMA that suffered putative relapse of SRMA between May 2006 and May 2008 were also prospectively included. These dogs previously been clinically confirmed to have SRMA prior to the commencement of this study using the same criteria as described above. Diagnosis of putative relapse was achieved in the same manner to presentation although response to treatment was an additional inclusion criterion in these cases.

2.1.2 Investigation of cases

The signalment was recorded and a detailed history was obtained in each case, including the duration of clinical signs prior to investigation. Each dog was subjected to a complete general and neurological examination by a veterinary neurologist as detailed by Oliver and others (2004). The examination was performed according to a standardised prospective protocol and the investigation was done according to an agreed protocol.

2.1.2.1 Minimum Database

Routine haematological and biochemical evaluation was performed in all cases to rule out the possibility of concurrent systemic disease and as a pre-anaesthetic appraisal of general health. Red blood cell counts, haematocrit, haemoglobin concentration, mean corpuscular volume, mean cell haemoglobin, platelet count and total and differential white blood cell counts were determined. Plasma concentrations of urea, creatinine, sodium, potassium, chloride, calcium, phosphate, glucose, cholesterol, bilirubin, alkaline phosphatase, alanine transferase, aspartate transferase, creatinine kinase, total protein, albumin and globulin were analysed. Results of these tests were compared to reference ranges of UGSAH laboratories and were used to ensure that no other concurrent diseases were present. Identification of concurrent disease was an exclusion criterion in this study. Results of immunoglobulin A and acute phase protein assays were not used as inclusion criteria to select cases.

2.1.2.2 Steroid Responsive Meningitis-Arteritis Group

Further diagnostic procedures were performed in SRMA cases in order to rule out other potential causes of the clinical signs. A minimum database for each dog consisted of the following: complete blood count (CBC), serum biochemistry profile, orthogonal cervical radiographs and CSF analysis (collected from the cerebromedullary cistern) of cytology and total protein. The diagnosis was based on signalment, clinical signs, history, and supportive CSF characteristics (increased total nucleated cell count [TNCC] >5 white blood cells/μL and total protein concentration >25 mg/dL including differential count). Diagnostic investigation of cases where the clinical or neurological findings indicated the possibility of a different underlying disease also included polymerase chain reaction for infectious diseases and MRI to rule-out other diseases. Therefore MRI was not performed in those cases with a clinical presentation consistent with SRMA.

2.1.2.3 Idiopathic Epilepsy Group

In dogs with IE a diagnosis was formed based upon age (between 6 months and 6 years), a normal inter-ictal general clinical and neurological examination, a normal minimum database (CBC and serum biochemistry including a bile acid stimulation test), negative serology and/or polymerase chain reaction for infectious diseases (Neosporosis, Toxoplasmosis and canine Distemper virus), normal CSF analysis (total nucleated cell count <5 white blood cells/μL and total protein concentration <25 mg/dL including differential count) and normal magnetic resonance imaging of the brain. Exclusion of both extra-cranial and intra-cranial causes for seizures supported a diagnosis of IE (Smith et al., 2008).

2.1.2.4 Meningoencephalitis of Unknown Aetiology Group

Diagnostic procedures for cases of MUA consisted of physical and neurologic examination, complete blood examinations, magnetic resonance imaging, and examination of CSF. In the absence of histopathological confirmation of MUA, a diagnosis was based upon multifocal CNS involvement on magnetic resonance imaging, inflammatory CSF analysis (total nucleated cell count >5 white blood cells/μL) and response to treatment, i.e. complete remission following cessation of long-term immunosuppressive therapy using a

combination of cytosine arabinoside and prednisolone as described by Zarfoss and others (2006).

2.1.3 Treatment Protocol

All SRMA dogs were treated with a standard protocol commencing with immunosuppressive doses of prednisolone (Prednicare, Animalcare Limited, York, UK) using a schedule adapted from other studies (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). The protocol is based upon the collective experience of Neurologists at UGSAH treating SRMA cases and the timings of re-evaluation were decided based on when clinical remission is usually expected. Figure 2:1 describes this treatment regime. A re-check examination was scheduled 2 weeks into treatment when clinical remission was anticipated. Clinical remission was defined as the absence of clinical signs in addition to an unperturbed CSF analysis. A failure to achieve clinical remission at the re-check examination resulted in following the protocol according to figure 2:1 with a second re-check examination scheduled 2 weeks later. A failure of the protocol was considered to be two consecutive cycles without achieving clinical remission. Once clinical remission had been achieved no further CSF samples were obtained unless relapse was suspected.

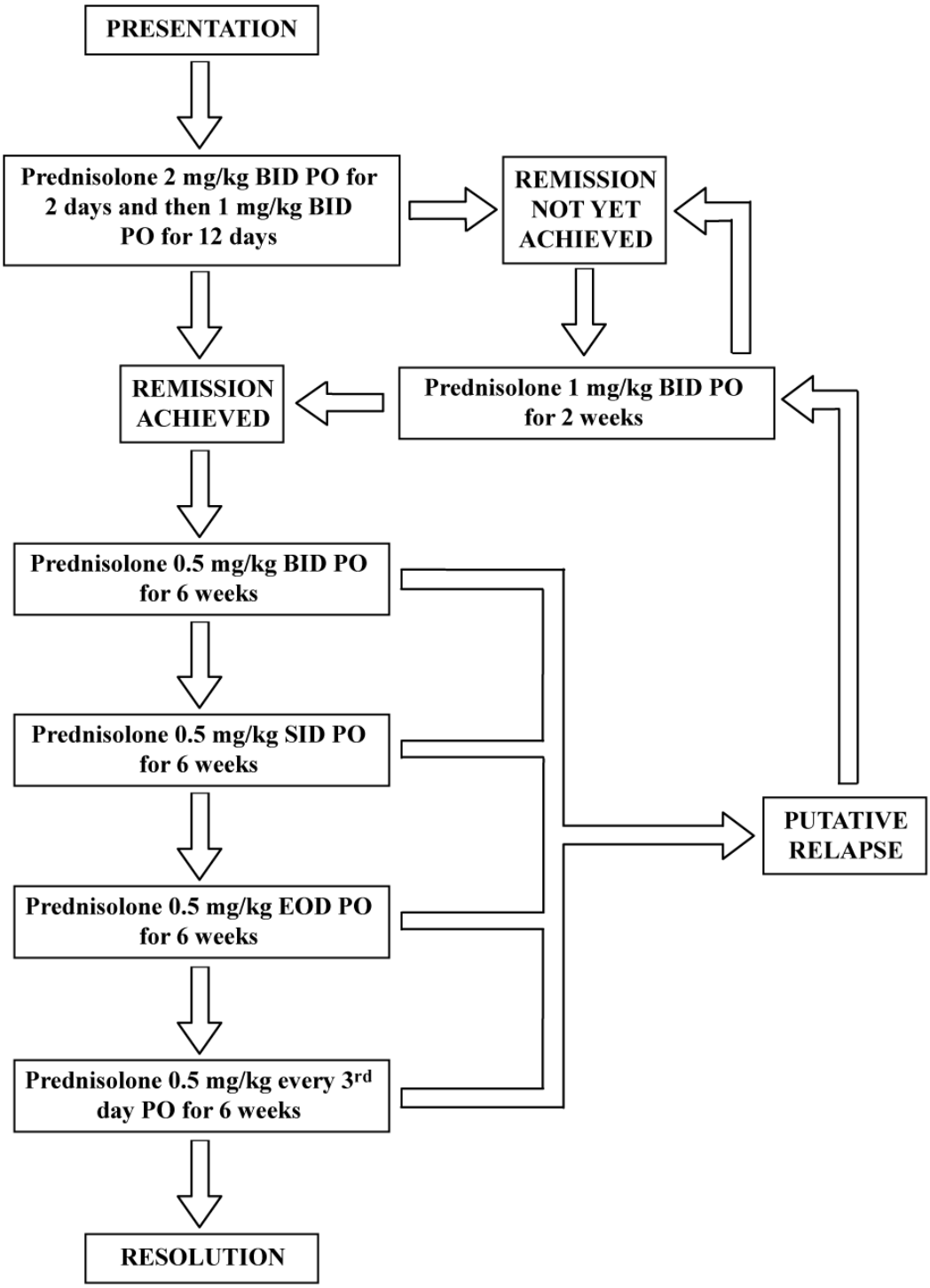

Figure 2:1: Treatment schedule for canine patients with steroid responsive meningitis-arteritis commencing with immunosuppressive doses of prednisolone using a schedule adapted from other studies (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). BID, twice daily; EOD, every other day; PO, per os; SID, once a day.

2.1.4 Timings of Sample Collection

Control Dogs

Serum and CSF was obtained at initial diagnosis in dogs with IE and MUA.

Steroid Responsive Meningitis-Arteritis Dogs

Serum and CSF samples were collected at 3 key stages in disease management: *presentation*, considered as the point of initial diagnosis; *remission*, taken as 2 weeks into treatment when clinical remission (on treatment) was anticipated; and *putative relapse*, defined as the exhibition, during treatment or the six month post-treatment observation period, of clinical signs similar to that demonstrated at presentation. Diagnosis of putative relapse was achieved in the same manner to presentation although response to treatment was an additional inclusion criterion in these cases. Serum samples were also obtained at *resolution*, defined as 4 weeks after cessation of therapy without the recurrence of clinical signs. At presentation, remission and putative relapse a history, general clinical and neurological examination, full CBC and biochemistry, serum and CSF IgA concentration and APP panel was obtained to exclude the possibility of concurrent diseases. The minimum follow-up for each case was six months after cessation of prednisolone based on previous studies (Tipold and Jaggy, 1994; Cizinauskas et al., 2000).

Steroid Responsive Meningitis-Arteritis Relapse Dogs

Serum and CSF samples were collected from dogs with putative relapse during the observation period that had been previously diagnosed with SRMA prior to the commencement of this study. Immunoglobulin A was not assayed in the serum or CSF of these cases.

2.1.4.1 Cerebrospinal Fluid Collection and Analysis

Cerebrospinal fluid samples were obtained from the cerebellomedullary cistern and the samples were inspected for colour, consistency, and turbidity. The cell counts were determined by a manual cell count using a modified Fuchs Rosenthal haemocytometer and total protein concentrations were determined using the sulphosalicylic acid method and compared to reference values given by Di Terlizzi and Platt, 2006 (TNCC >5 white blood

cells/μL and total protein concentration >25 mg/dL). Cytological examination of CSF was performed within 30 minutes and a differential count was recorded. The CSF samples were spun down at 1500 g (3000 rpm) for 10 minutes. The supernatant was pipetted off and resuspended in a drop of serum. A smear was made from this suspension and stained using Diff-Quik stain and the differential count recorded. Samples were stored in an eppendorf tube at -20 ˚C until assayed.

2.1.4.2 Serum Sample Collection and Analysis

Serum samples were obtained by collecting whole blood into a 1.5 ml plain tube and the sample was left to clot over 10-15 minutes. The plain tube was then centrifuged at 1500 g (3000 rpm) for 10 minutes. The serum (supernatant) was then pipetted off and placed in an Eppendorf tube and these samples were frozen at -20 ˚C until assayed within 30 minutes of sampling.

2.1.4.3 Acute Phase Protein Assays

C-reactive protein (CRP), serum amyloid-A (SAA), alpha-1-acid glycoprotein (AGP) and haptoglobin (Hp) were measured in the serum at each stage of the disease process or at first diagnosis in the control dogs. Values for CRP were obtained using an immunoturbidimetric assay (Eckersall et al., 1991). Alpha-1-acid glycoprotein was measured using a radial immunodiffusion assay (J-Path Inc, Tokyo, Japan) and SAA was measured using a commercial canine ELISA kit (Tridelta Development Ltd, Kildare, Ireland) (Martinez-Subiela et al., 2005). Haptoglobin was assayed based on its haemoglobin binding capacity (Eckersall et al., 1999; Martinez-Subiela et al., 2005; Mischke et al., 2007). The reference range in our laboratory for serum APP concentrations based on previous studies was 0.46 - 9.6 mg/L for CRP, 0 - 1 mg/L for SAA, 0.5 - 2.2 mg/L for Hp and 0.02 – 0.5 μg/L for AGP concentration. All APP assays were performed by a commercial laboratory.

2.1.4.4 Immunoglobulin A Assays

Immunoglobulin A concentration was measured in serum and CSF at each stage of the disease process (when available) using a commercial dog IgA ELISA quantitation kit

(Bethyl Laboratories, Montgomery, TX) Ninety-six-well polystyrol microtitre plates (Nunc Maxisorp, Basel, Switzerland) were coated overnight at room temperature in a humid atmosphere with a goat antibody against canine IgA (Bethyl Laboratories, Montgomery, TX.) diluted 1:750 in sodium carbonate bicarbonate buffer (pH 9.6). All of the washing steps were performed using 0.9 % sodium chloride solution containing 0.5 % Tween-20 solution and repeated three times. The coated wells were incubated with CSF and serum in duplicates for 90 minutes at room temperature in a humid atmosphere. Cerebrospinal fluid and serum samples were diluted 1:10, 1:100 and 1:1000 in Tris-NaCl solution (0.002 M Tris, pH 8.0; 0.5 M sodium chloride; 0.5 Tween-20) and 100 µl of these dilutions were used for analysis. After washing, mouse monoclonal antibody anti canine IgA (Serotec, Düsseldorf, Germany), diluted 1:100 in Tris-NaCl solution, was added for another 90 minutes at room temperature in a humid atmosphere. Following further washing, peroxidase-conjugated donkey antimouse IgG (Dianova, Hamburg, Germany; diluted 1:2000 in Tris–NaCl) was incubated for 60 minutes. After washing, the enzyme reaction was triggered with 100 µL of substrate solution (0.4 mg/mL phenylenediamine dihydrochloride; 0.4 mg/mL urea hydrogen peroxide; 0.05 M phosphate-citrate, pH 5.0) and stopped by adding 50 µL of phosphoric acid. Standard curves were established using serial dilutions (1:4) of a canine serum pool starting with a concentration of 4.4 µL/mL. Results were quantified using a computer-assisted ELISA reader (Dynatech, Denkendorf, Germany) at 409 nm using the computer software MicroWin (Microtek, Germany). In the control dogs IgA was assayed at first diagnosis in the serum and CSF. The reference range from previous studies is 10.9 - 100.1 µg/mL in the serum and 0 - 0.2 µg/mL in the CSF (Tipold et al., 1994).

2.1.5 Statistical Analysis

Categorical variables were described as fractions or percentages, and comparisons among them were performed using χ^2 test. Non-parametric data were described using median and range with comparisons between paired and non-paired samples performed using the Wilcoxon–Signed–Rank–test and the Mann–Whitney–U–test respectively. Analyses of correlations between the paired variables were performed by calculating Spearman's rank correlation coefficients. For all comparisons, a *P*-value of < 0.05 was considered to be significant. All statistical analyses were performed using GraphPad Prism version 4 (GraphPad Software Inc, La Jolla, CA).

Chapter III

RESULTS

3.1 Results

3.1.1 Prevalence

Steroid responsive meningitis-arteritis represented 0.6 % of the University of Glasgow Small Animal Hospital admissions and 1.6 % of all neurological consultations during the studied time period.

3.1.2 Signalment and History

3.1.2.1 Steroid Responsive Meningitis-Arteritis Group

A total of 20 SRMA patients were included in this study. Fourteen dogs were male (5/14 neutered) and 6 were female (6/6 neutered) though this was not statistically significant (P = 0.074). The median age at time of presentation was 11 months (range: 5 - 19 months). There were dogs of 12 different breeds and a chi-squared test of the data from the two most represented breeds in this study (Boxers n = 4, and English Springer Spaniels n = 4) demonstrated that there was a significant over-representation when compared to the expected hospital population during the same period (χ^2 = 147.35, df = 1, $P < 0.001$). Two Weimaraners and one of each of the following was recorded; beagle, Bernese Mountain Dog, bichon frise, border collie, cocker spaniel, golden retriever, Jack Russell terrier, Labrador, pointer and saluki.

Presentation – The median duration of clinical signs was 6.5 days (range: 2 - 60 days).

Remission – All dogs had entered clinical remission 2 weeks into treatment on the regime described in figure 2:1.

Relapse – 4/20 dogs suffered putative relapse; all during treatment. 1/4 dogs had 2 separate putative relapse episodes and only the first suspected relapse event was analyzed because there was insufficient data to comment on subsequent relapse events, as there was only one instance where this occurred. All responded to an increase in prednisolone dose to 1 mg/kg TWICE DAILY PO for 14 days according to figure 2:1. Putative relapse occurred at a median of 119 days into treatment (range: 74-164 days) and all putatively relapsing dogs were reassessed within 3 days of the recurrence of clinical signs.

An additional 7 dogs suffered putative relapse during the observation period following a previous diagnosis of SRMA. All 7 patients had been following a prednisolone montherapy, although this regime was not implemented in the same strict prospective manner as the 20 patients included in the prospective treatment regime.

3.1.2.2 Idiopathic Epilepsy Group

A total of 10 dogs with IE were included in this study. Five dogs were male (3/5 neutered) and five were female (3/5 neutered). The median age at time of presentation was 47 months (range: 24 - 70 months). All 10 dogs were of different breeds. No patient had exhibited seizure activity within 72 hours of presentation to the UGSAH.

3.1.2.3 Meningoencephalitis of Unknown Aetiology Group

Fifteen dogs were diagnosed with MUA during the observation period of this study. Seven dogs were male (5/7 neutered) and eight were female (8/8 neutered). The median age at time of presentation was 60 months (range: 5 - 91 months). All 10 dogs were of different breeds. A diagnosis of granulomatous meningoencephalomyelitis (GME) was made at post-mortem in 9/15 cases. The remaining dogs (6/15) responded to immunosuppressive therapy (combined prednisolone and cytosine arabinoside) that has since been discontinued with complete resmission in all 6 patients at the time of writing.

3.1.3 Clinical Signs

3.1.3.1 Steroid Responsive Meningitis-Arteritis Group

Presentation – All 20 cases presented with cervical pain and lethargy. 16/20 dog were inappetant, 12/20 dogs had pyrexia (defined as a rectal temperature greater than 39.5 ^{o}C), 3/20 presented with bilateral conjunctivitis and thoracolumbar pain respectively, 2/20 exhibited proprioceptive deficits in both forelimbs, 1/20 had Horner's syndrome, and one dog had cranial nerve VIII deficits.

Relapse – In 4/4 putatively relapsing episodes cervical pain and lethargy were the only clinical signs identified. Pyrexia was not a feature of these dogs.

The 7 dogs exhibiting putative relapse with a prior diagnosis fo SRMA all demonstrated cervical pain and lethargy. No other clinical signs were present and pyrexia was not observed in these individuals.

3.1.3.2 Meningoencephalitis of Unknown Aetiology Group

Presentation – All 7/15 cases presented with multifocal neurological signs. 8/15 had neurological signs attributable to a single neuroanatomical site (Forebrain 3/8; Cerebellum 3/8; Brainstem 1/8; C1 to C5 1/8). 6/15 were inappetant, 3/15 had pyrexia (defined as a rectal temperature greater than 39.5 ^{o}C), and 1/15 had vomiting.

3.1.4 Blood Profiles

3.1.4.1 Steroid Responsive Meningitis-Arteritis Group

Presentation – A neutrophilic leukocytosis was seen in 17/20 cases at first presentation (median: 23.4 x10^9/L, range: 8.4 - 45.7 x10^9/L, reference range: 6 - 12.0 x10^9/L) with band cells seen in 8/17 patients. Hypoalbuminemia was present in 11/20 presenting cases (median: 2.75 g/dL, range: 2.3 - 3.2 g/dL, reference range: 2.9 - 3.6 g/dL).

Remission – At the time of remission 8/20 dogs exhibited a leukocytosis without a left shift (median: 10.6 x10^9/L, range: 5.1 - 18.0 x10^9/L, reference range: 6 - 12.0 x10^9/L) and these same 8 patients had hypoalbuminemia (median: 2.9 g/dL, range: 2.6 - 3.3 g/dL, reference range: 2.9 - 3.6 g/dL).

Resolution – The leucogram and serum albumin concentrations were within normal limits at the time of resolution.

Relapse – A neutrophilic leucocytosis was seen in 3/4 putatively relapsing dogs (median: 11.0 x10^9/L, range: 8.2 - 26.8 x10^9/L, reference range: 6 - 12.0 x10^9/L) and hypoalbuminemia was present in 2/4 (median: 2.9 g/dL, range: 2.6 - 3.0 g/dL, reference range: 2.9 - 3.6 g/dL).

In the dogs suffering putative relapse following a previous diagnosis of SRMA, 3/7 dogs had a leucocytosis, though only one of these cases demonstrated a left shift (median: 11.3 x10^9/L, range: 8.22 – 26.9 x10^9/L, reference range: 6 - 12.0 x10^9/L).

3.1.4.2 Meningoencephalitis of Unknown Aetiology Group

Presentation – A neutrophilic leukocytosis was seen in 3/15 cases (median: 8.1 $x10^9$/L, range: 3.7 – 26.5 $x10^9$/L, reference range: 6 - 12.0 $x10^9$/L) with no band cells seen. Hypoalbuminemia was present in 3/15 presenting cases (median: 3.3 g/dL, range: 2.4 - 4.1 g/dL, reference range: 2.9 - 3.6 g/dL).

3.1.5 Cerebrospinal Fluid Analysis

All samples had red blood cell counts of less than 5 /μL.

3.1.5.1 Steroid Responsive Meningitis-Arteritis Group

Presentation – Total nucleated cell count was increased in 20/20 cases ranging from 25 to 2500 white blood cells/μL; reference range: < 5 white blood cells/μL (DiTerlizzi and Platt, 2006). Total protein concentration was elevated in 15/20 cases with a range of 17–480 mg/dL (median: 60 mg/dL; reference range: < 25 mg/dL) (DiTerlizzi and Platt, 2006). A predominantly neutrophilic pleocytosis (> 80 %) was identified in 12/20 dogs and a predominantly monocytic pleocytosis (> 80 %) was identified in the remaining 8/20 dogs. Those dogs in which a predominantly neutrophilic pleocytosis was observed had clinical signs for ≤ 7 days (median: 4.5 days; range: 2 - 7 days) and dogs with a predominantly monocytic pleocytosis had clinical signs for > 7 days (median: 14 days; range: 10 - 60 days).

Remission – Cerebrospinal fluid parameters were within normal limits in 20/20 cases.

Relapse – 1/4 putatively relapsing dogs exhibited a pleocytosis (median: 0 /μL; range: 0 - 512 /μL) and 2/4 exhibited an elevated total protein concentration (median: 25 mg/dL; range: 7 - 40 mg/dL).

6/7 putatively relapsing dogs with a previous diagnosis of SRMA exhibited an unperturbed CSF analysis (median: 0 /μL; range: 0 - 96 /μL). Total protein concentration was within the reference range in 6/7 (median: 1.8 mg/dL; range: 5 - 38 mg/dL).

3.1.5.2 Meningoencephalitis of Unknown Aetiology Group

Presentation – Total nucleated cell count was increased in all 15/15 cases ranging from 24 to 1000 white blood cells/μL with a median value of 161 white blood cells/μL; reference range: < 5 white blood cells/μL (DiTerlizzi and Platt, 2006). Total protein concentration was elevated in 15/15 cases with a range of 28–292 mg/dL (median: 68 mg/dL; reference range: < 25 mg/dL) (DiTerlizzi and Platt, 2006). A predominantly neutrophilic pleocytosis (> 80 %) was identified in 3/15 dogs and a mixed cell pleocytosis was identified in 10/15 dogs. 2/15 had a predominantly lymphocytic pleocytosis (> 80 %) There was no correlation between duration of clinical signs and the differential CSF count.

3.1.6 Serum Acute Phase Protein and Immunoglobulin A Response

3.1.6.1 Steroid Responsive Meningitis-Arteritis Group

Presentation – Serum CRP, SAA and Hp concentrations were above the reference range in 20/20 dogs (CRP median: 75.2 mg/L, range: 22.4 - 328.9 mg/L, reference range: 1.0 - 9.6 mg/L; SAA median: 261.5 mg/L, range: 33.5 - 6100.0 mg/L, reference range: 0.0 - 1.0 mg/L; Hp median: 16.4 g/L, range: 4.6 - 23.8 g/L, reference range: 0.5 - 2.2 g/L). Alpha-1-acid glycoprotein was above the reference range in 19/20 cases (median: 0.96 g/L, range: 0.24 - 3.79 g/L, reference range: 0.02 – 0.5 μg/L). Immunoglobulin A was increased in the serum of 19/20 patients at presentation (median: 480 μg/mL; range: 82.0 - 3530 μg/mL) (Figures 3:1, 3:2, 3:3, 3:4 and 3:5).

Remission – Serum CRP remained above the reference range in 16/20 dogs but decreased significantly when compared to presentation (CRP median: 14.6 mg/L, range: 5.0 - 29.2 mg/L, $P < 0.0001$) (Figure 3:1). Serum SAA and AGP significantly decreased on treatment when compared to presentation (SAA median: 0.57 mg/L, range: 0.11 - 57.50 mg/L, $P < 0.0001$; AGP median: 0.32 g/L, range: 0.13 - 1.14 g/L, $P = 0.0005$) (Figures 3:2 and 3:3). Haptoglobin concentration remained similar to presentation (median: 15.1 g/L, range: 4.5-29.0 g/L, $P = 0.9777$) (Figure 3:4). Immunoglobulin A was not significantly different to presentation ($P = 0.2079$), although demonstrated a tendency towards increasing over that of presentation with a median of 477 μg/mL (range: 86-3600 μg/mL) (Figure 3:5).

Resolution – Serum CRP decreased significantly when compared to remission and was within the reference range in 20/20 dogs at resolution (CRP median: 5.0 mg/L, range: 2.1 - 9.4 mg/L, *P* = 0.0001) (Figure 3:1). Serum SAA, AGP and Hp all significantly decreased at disease resolution when compared to remission (SAA median: 0.23 mg/L, range: 0.05 - 1.65 mg/L, *P* = 0.0036; AGP median: 0.15 g/L, range: 0.01 - 0.46 g/L, *P* = 0.0005; Hp median: 2.75 g/L, range: 0.4 - 11.4 g/L, *P* = 0.0001) (Figures 3:2, 3:3 and 3:4). Immunoglobulin A had significantly decreased compared with presentation (*P* = 0.0001) and remission (*P* = 0.0004) but still remained above the reference range in 15/20 dogs (median: 284.2 µg/mL; range: 41 - 2753.7 µg/mL) (Figure 3:5).

Relapse – Serum CRP and SAA was increased in 4/4 suspected relapse cases (CRP median: 83.7 mg/L, range: 56.2 - 402.0 mg/L; SAA median: 262.8 mg/L, range: 11.6 - 480.5 mg/L). Alpha-1-acid glycoprotein was within the reference range in 3/4 (median: 0.65 g/L, range: 0.62 - 0.93 g/L) and Hp was elevated in all 4/4 patients (median: 18.3 g/L, range: 19.0 - 19.8 g/L). Immunoglobulin A was increased in 4/4 cases (median: 463 µg/mL, range: 406-2597 µg/mL) (Table 3-1).

The serum concentrations of CRP and SAA in dogs with suspected relapse with a previous diagnosis of SRMA were at least 7 and up to 10 times greater than the reference range respectively (CRP median: 142 mg/L, range: 33 - 407.0 mg/L; SAA median: 408.5 mg/L, range: 10.6 – 744.0 mg/L). Alpha-1-acid glycoprotein was within the reference range in 4/7 (median: 0.28 g/L, range: 0.01 - 1.38 g/L) and Hp was elevated in all 7/7 patients (median: 17.6 g/L, range: 10 – 31.4 g/L) (Table 3-2).

3.1.6.2 Idiopathic Epilepsy Group

Presentation – Serum CRP and SAA concentrations were within the reference range in 10/10 dogs (CRP median: 7.53 mg/L, range: 5.2 – 9.6 mg/L, reference range: 1.0 - 9.6 mg/L; SAA median: 0.22 mg/L, range: 0 – 0.67 mg/L, reference range: 0.0 - 1.0 mg/L) (figures 3:6 and 3:7). Haptoglobin was above the reference range in 6/10 cases (Hp median: 3.75 g/L, range: 0 – 8.3 g/L, reference range: 0.5 - 2.2 g/L) (figure 3:8). Alpha-1-acid glycoprotein was above the reference range in 3/10 cases (median: 0.47 g/L, range: 0.12 - 0.92 g/L, reference range: 0.02 – 0.5 µg/L) (figure 3:9). Immunoglobulin A was increased in the serum of 10/10 patients at presentation (median: 383 µg/mL; range: 106 - 874 µg/mL) (figure 3:10).

3.1.6.3 Meningoencephalitis of Unknown Aetiology Group

Presentation – Serum CRP was raised in 13/15 cases (CRP median: 26.2 mg/L, range: 7.1 – 56.6 mg/L, reference range: 1.0 - 9.6 mg/L) (figure 3:6) and serum SAA was increased in 9/15 dogs (SAA median: 9.6 mg/L, range: 0.24 – 26.4 mg/L, reference range: 0.0 - 1.0 mg/L) (figure 3:7). 9/15 dogs had Hp concentrations above the reference range (Hp median: 3.0 g/L, range: 0 – 16.2 g/L, reference range: 0.5 - 2.2 g/L) (figure 3:8) and AGP was above the reference range in 8/15 cases (median: 0.47 g/L, range: 0.12 - 0.92 g/L, reference range: 0.02 – 0.5 µg/L) (figure 3:9). Immunoglobulin A was increased in the serum of 13/15 patients at presentation (median: 396 µg/mL; range: 62 - 2680 µg/mL) (figure 3:10).

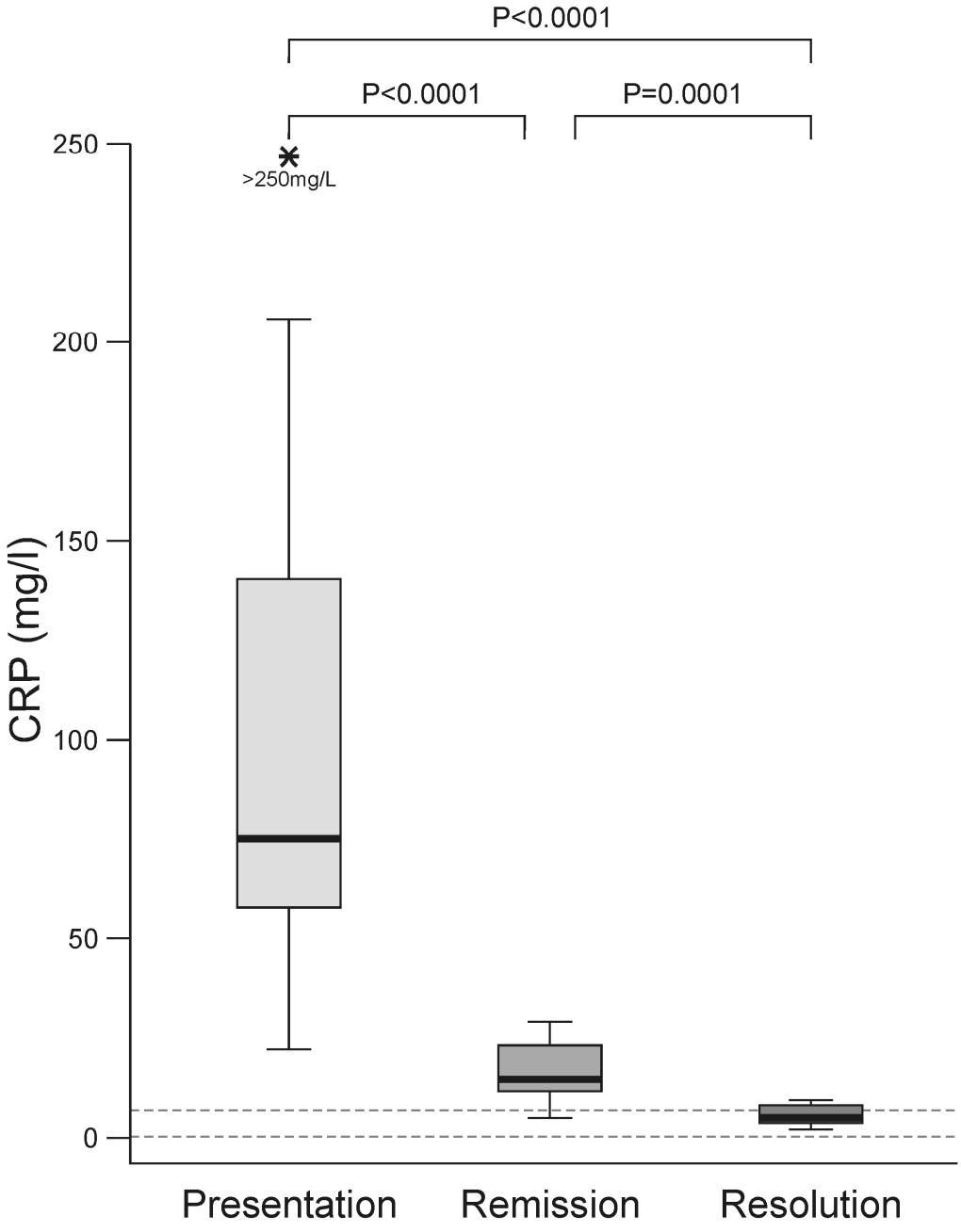

Figure 3:1: Serum C-reactive protein (CRP) acute phase response in 20 canine SRMA patients at presentation, remission (+14 days on prednisolone treatment) and resolution (4 weeks after cessation of therapy). The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

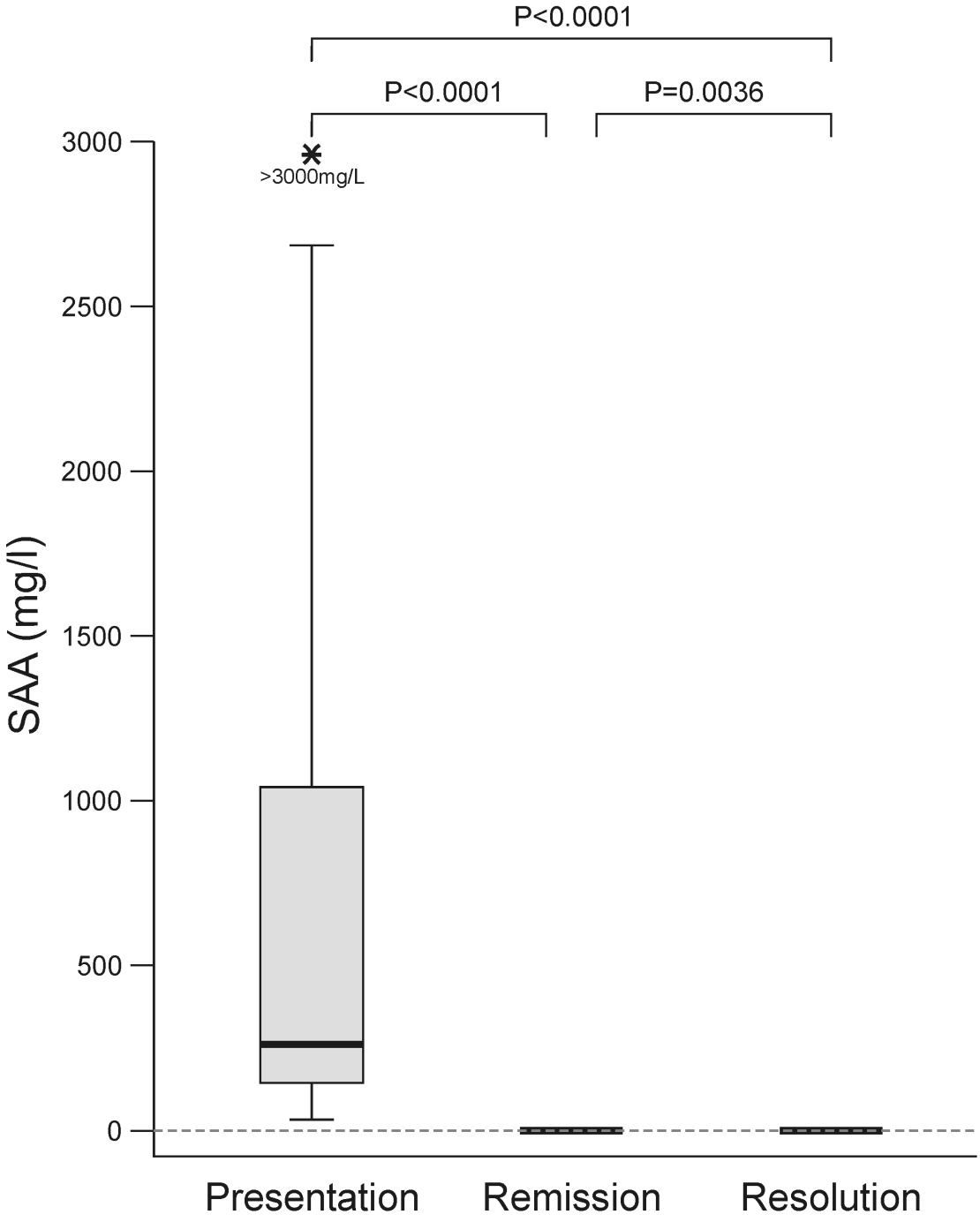

Figure 3:2: Serum amyloid-A (SAA) acute phase response in 20 canine SRMA patients at presentation, remission (+14 days on prednisolone treatment) and resolution (4 weeks after cessation of therapy). The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

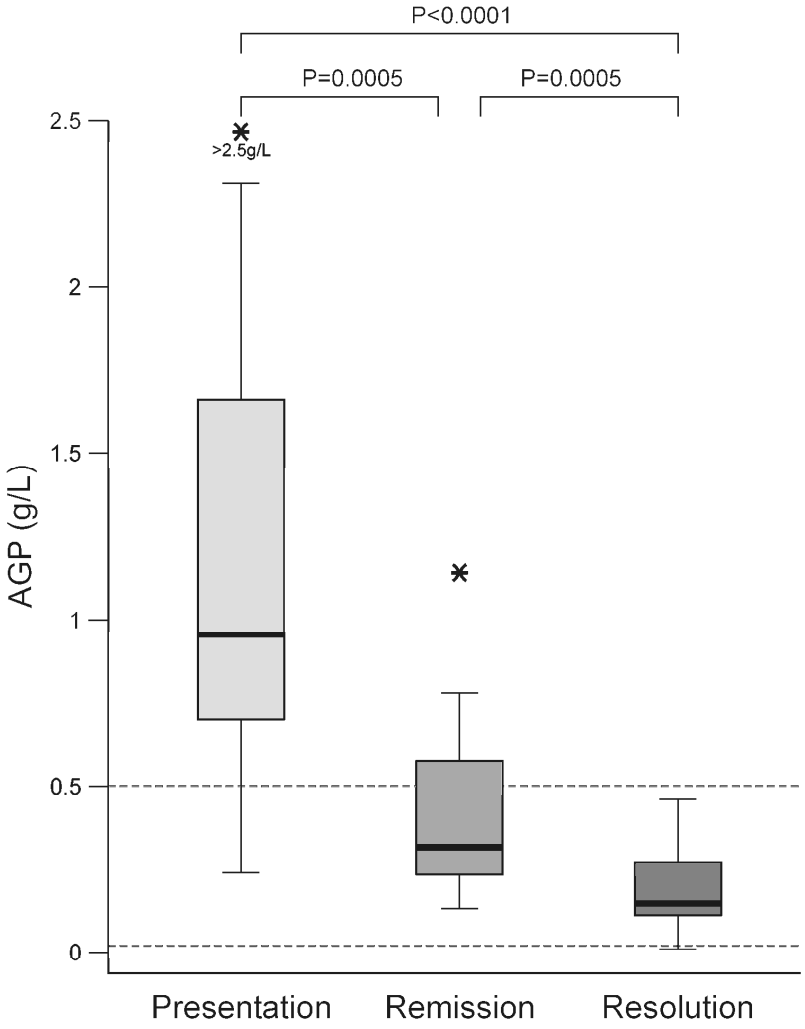

Figure 3:3: Serum alpha-1-acid glycoprotein (AGP) acute phase response in 20 canine SRMA patients at presentation, remission (+14 days on prednisolone treatment) and resolution (4 weeks after cessation of therapy). The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

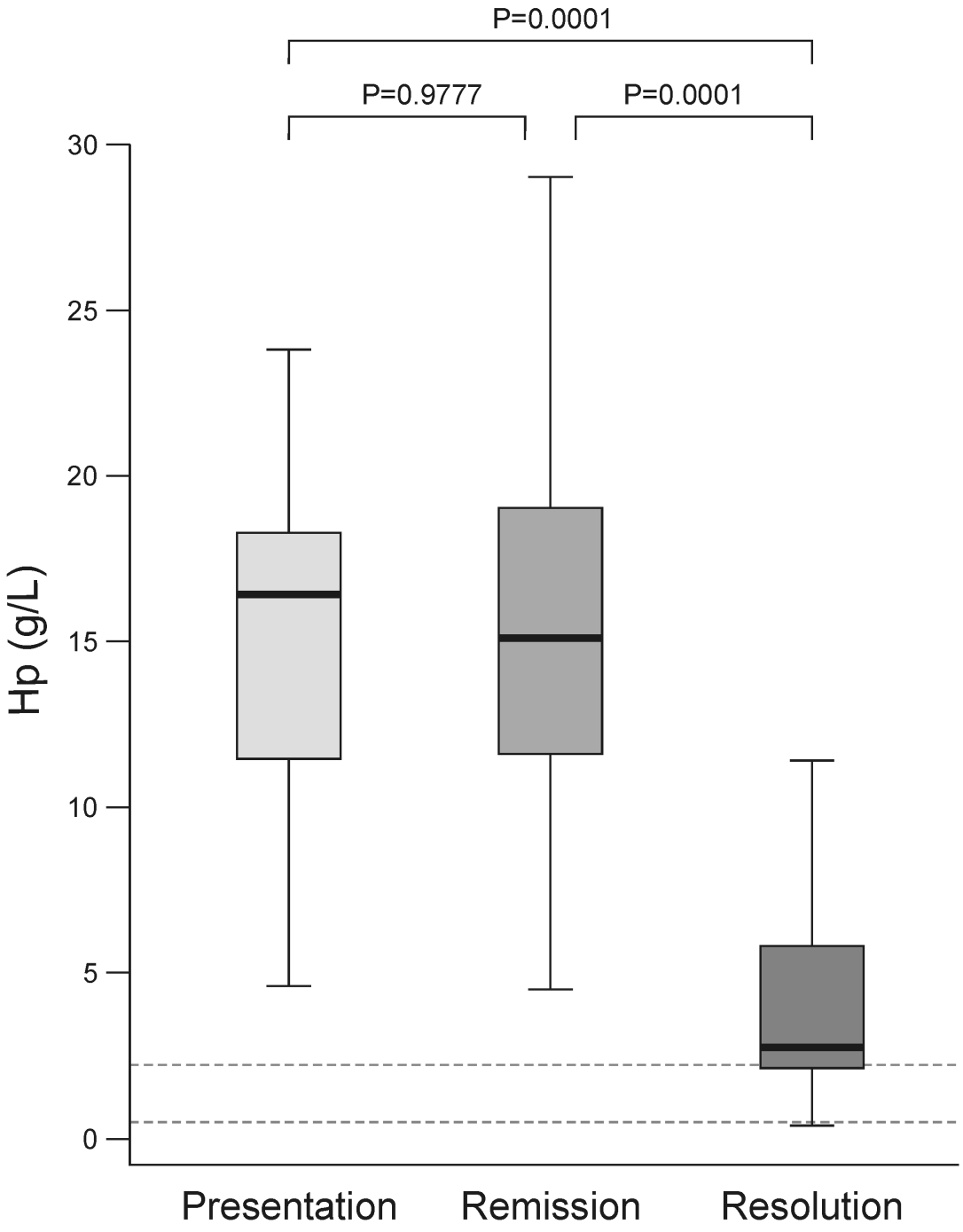

Figure 3:4: Serum haptoglobin (Hp) acute phase response in 20 canine SRMA patients at presentation, remission (+14 days on prednisolone treatment) and resolution (4 weeks after cessation of therapy). The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines.

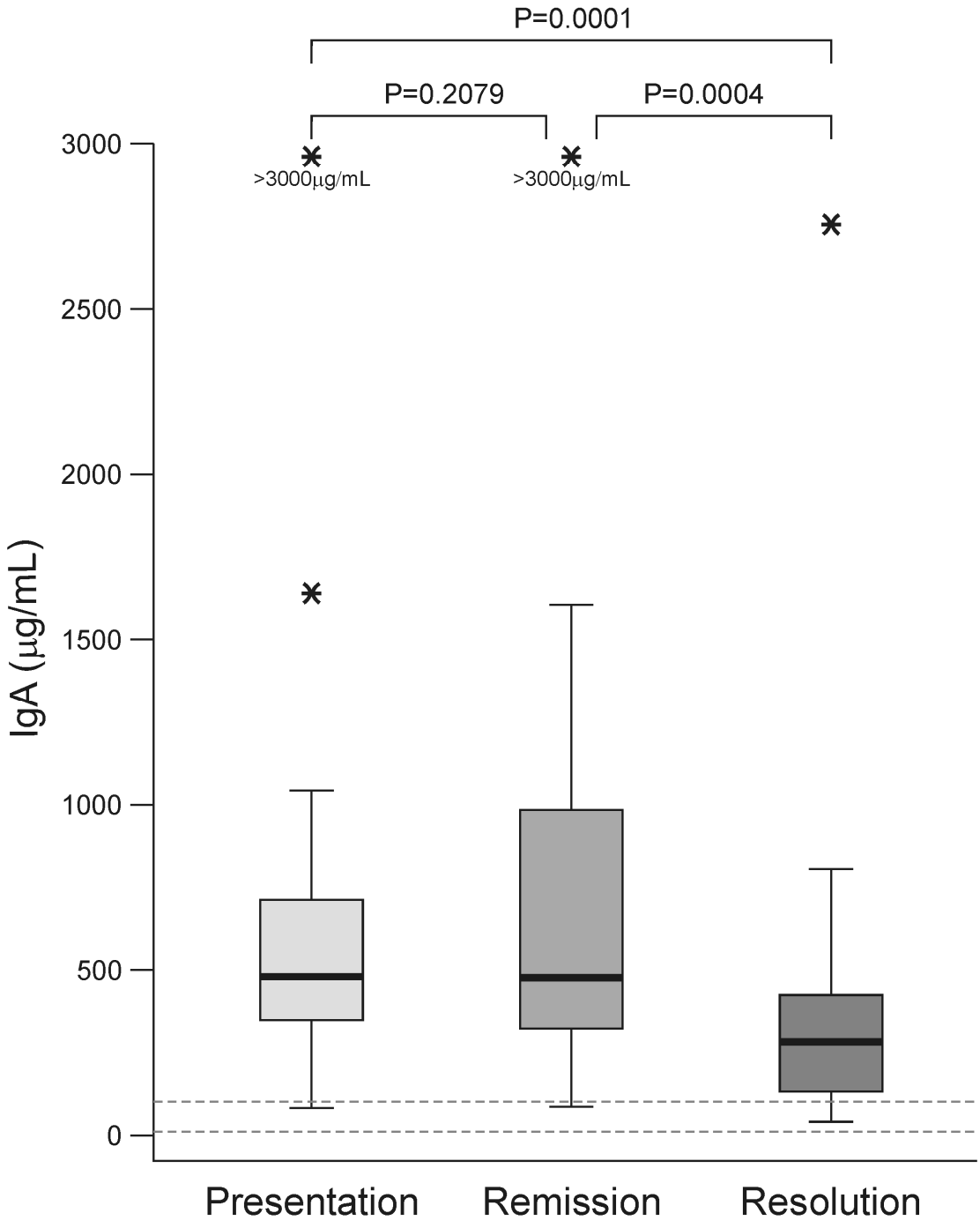

Figure 3:5: Serum immunoglobulin A (IgA) response in 20 canine SRMA patients at presentation, remission (+14 days on prednisolone treatment) and resolution (4 weeks after cessation of therapy). The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

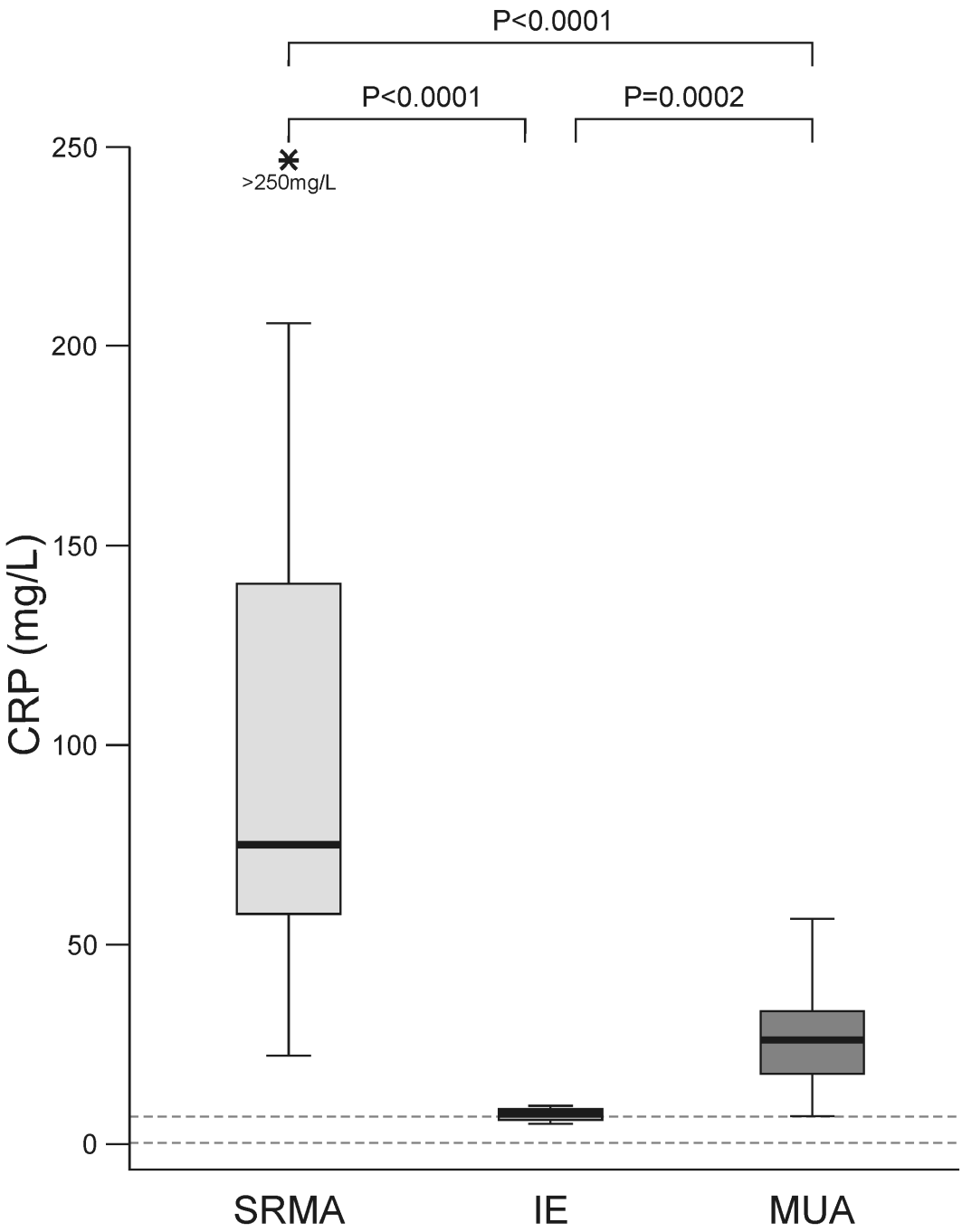

Figure 3:6: A comparison of the serum C-reactive protein (CRP) acute phase response in three groups of dogs suffering from steroid responsive meningitis-arteritis (SRMA), idiopathic epilepsy (IE) and meningoencephalitis of unknown aetiology (MUA) respectively. The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by *, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

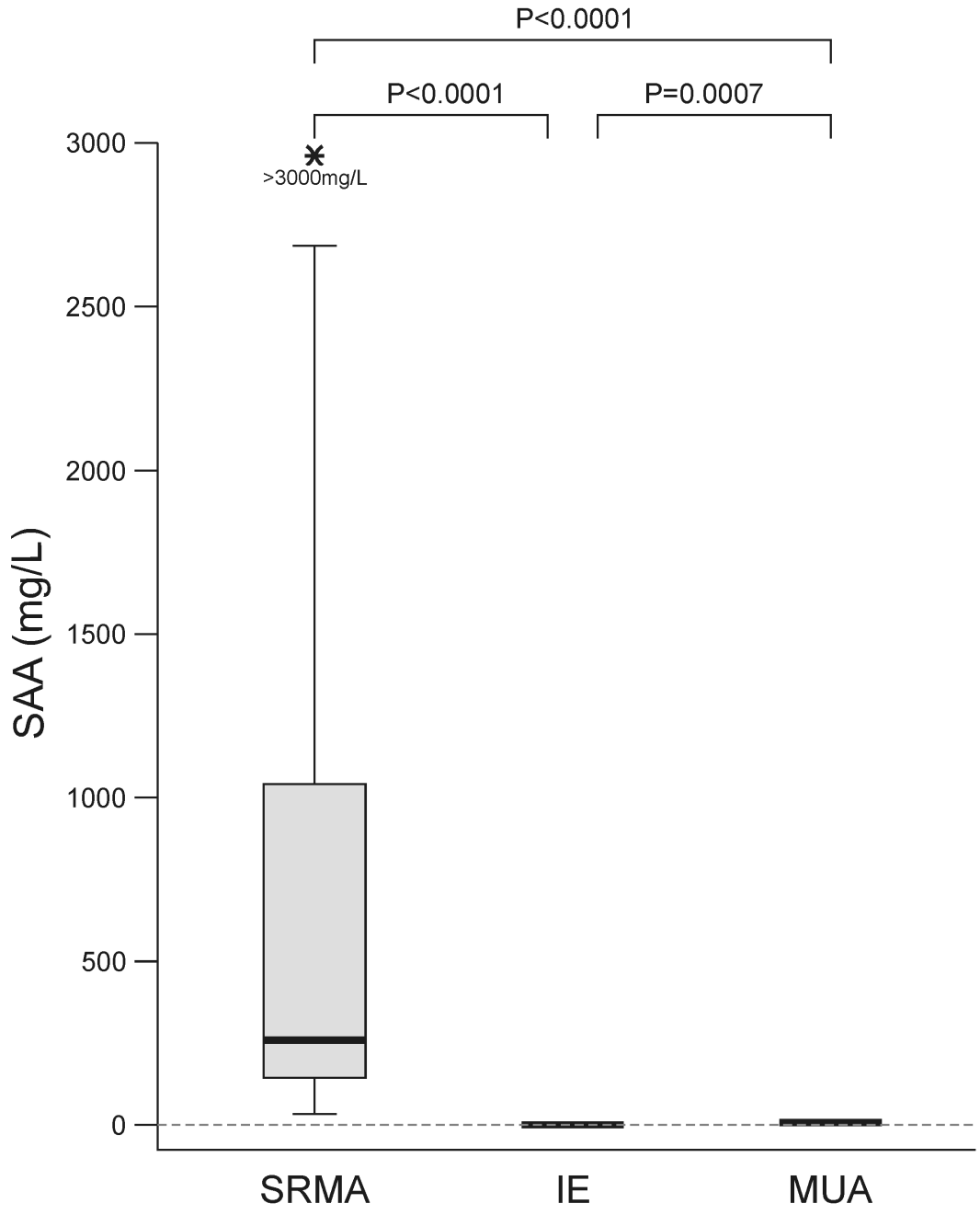

Figure 3:7: A comparison of the serum amyloid-A (SAA) acute phase response in three groups of dogs suffering from steroid responsive meningitis-arteritis (SRMA), idiopathic epilepsy (IE) and meningoencephalitis of unknown aetiology (MUA) respectively. The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

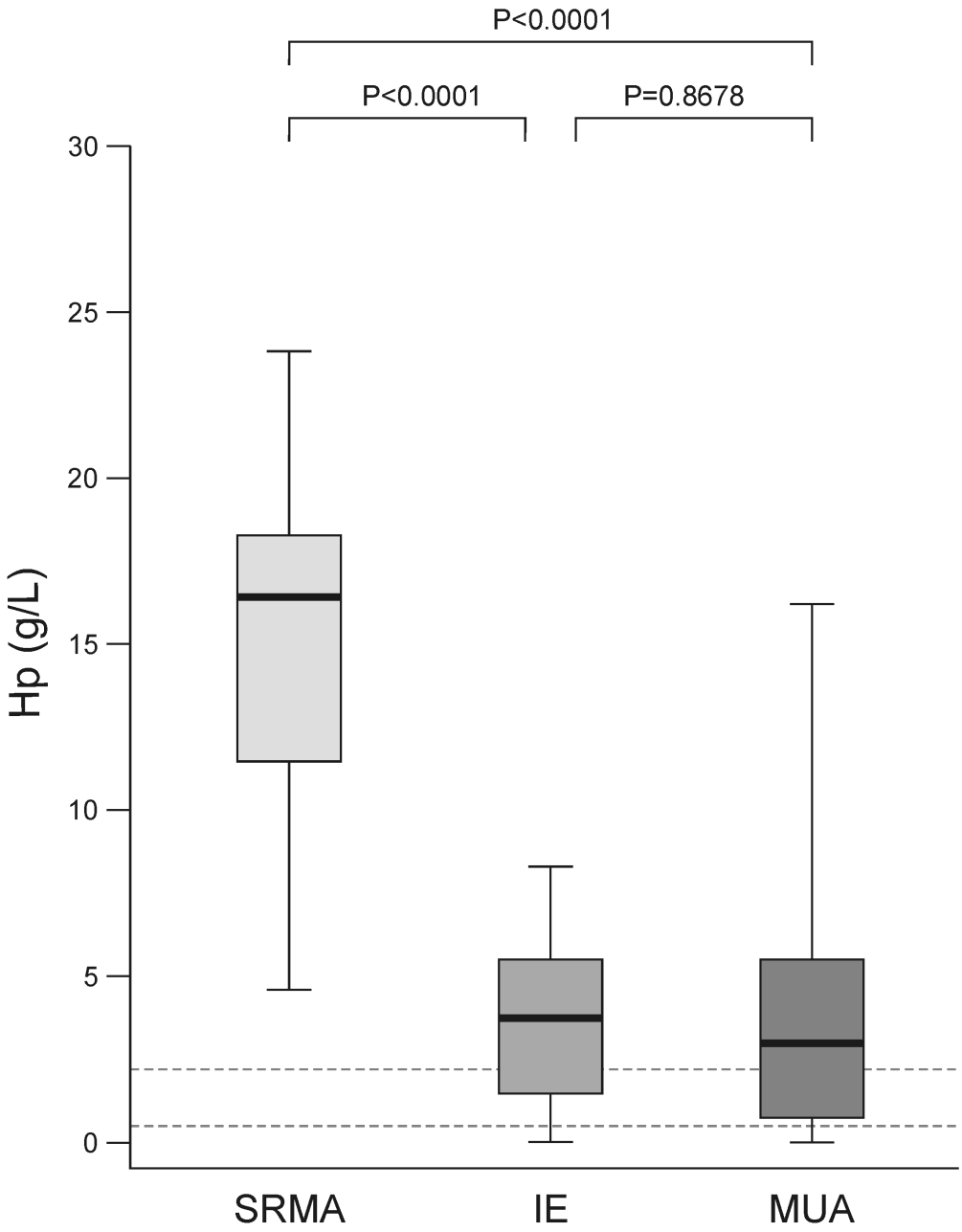

Figure 3:8: A comparison of the serum haptoglobin (Hp) acute phase response in three groups of dogs suffering from steroid responsive meningitis-arteritis (SRMA), idiopathic epilepsy (IE) and meningoencephalitis of unknown aetiology (MUA) respectively. The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, and the highest and lowest values of the laboratory normal reference range are represented by dashed lines.

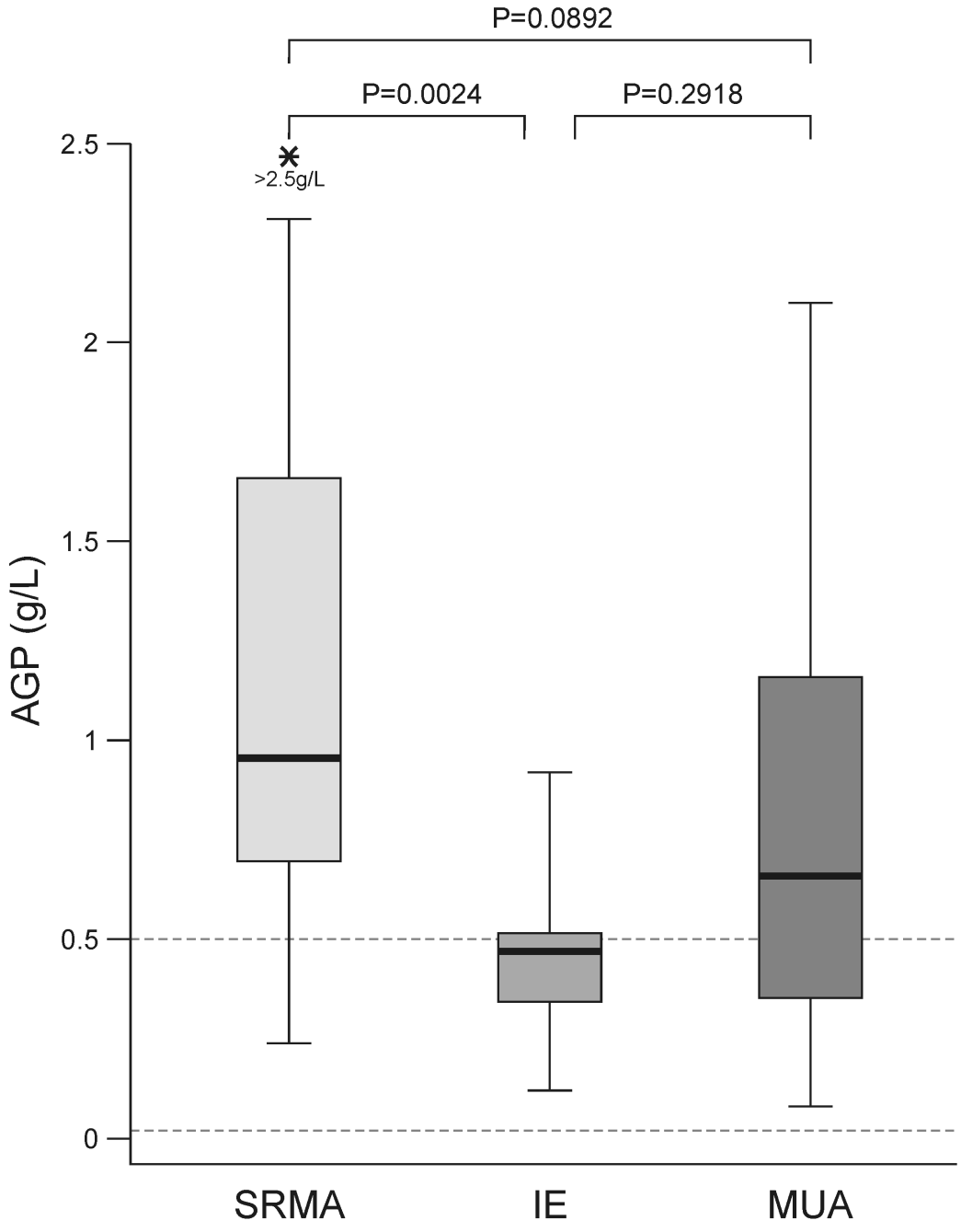

Figure 3:9: A comparison of the serum alpha-1-acid glycoprotein (AGP) acute phase response in three groups of dogs suffering from steroid responsive meningitis-arteritis (SRMA), idiopathic epilepsy (IE) and meningoencephalitis of unknown aetiology (MUA) respectively. The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

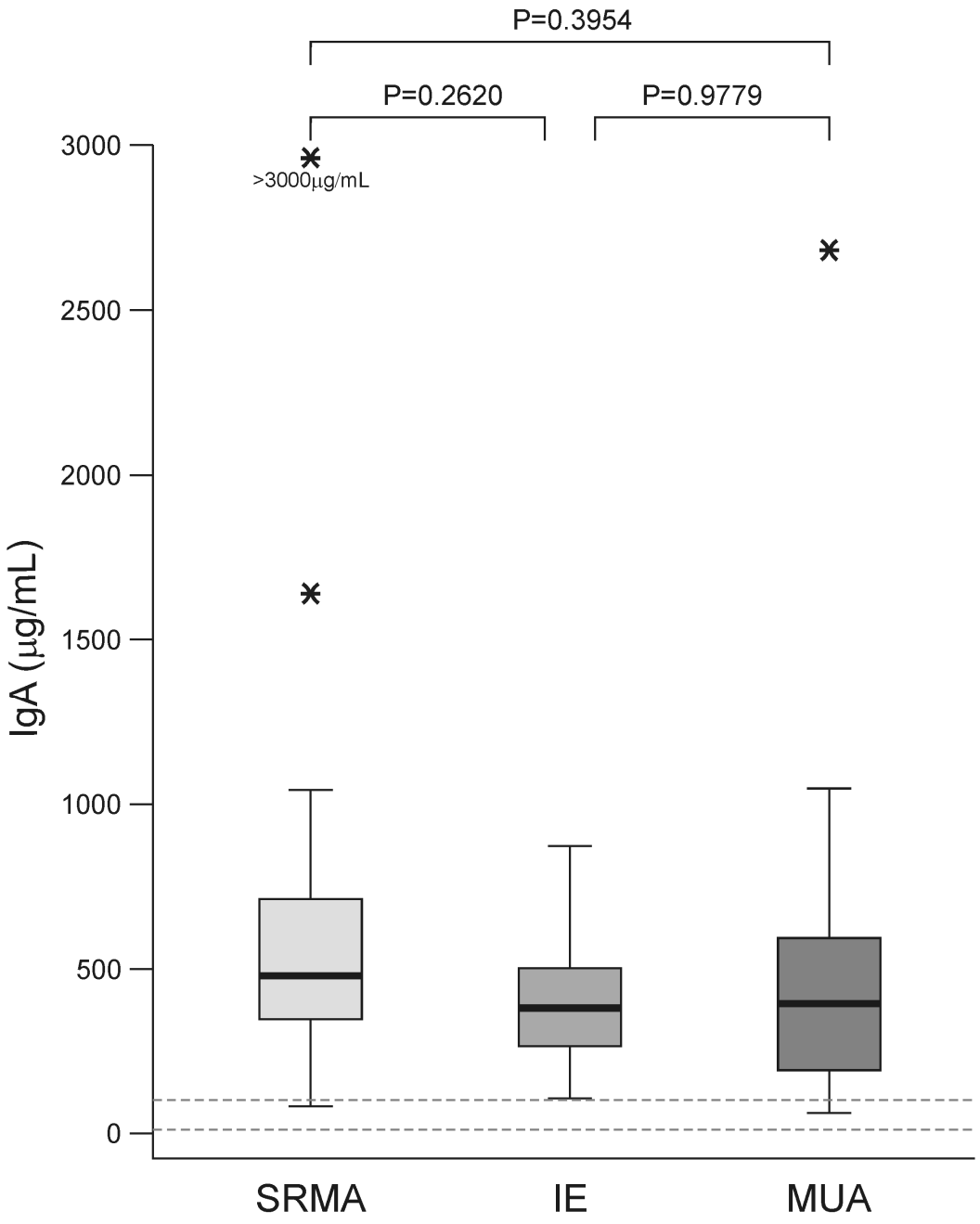

Figure 3:10: A comparison of the serum immunoglobulin A (IgA) response in three groups of dogs suffering from steroid responsive meningitis-arteritis (SRMA), idiopathic epilepsy (IE) and meningoencephalitis of unknown aetiology (MUA) respectively. The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

Relapse Patient	Signalment at Relapse	Dose of prednisolone at relapse	IgA (µg/mL)		CRP (mg/L) Serum	SAA (mg/L) Serum	AGP (g/L) Serum	Hp (g/L) Serum
			Serum	CSF				
1	18 month old FN Labrador	0.5 mg/kg EOD	460	0.81	83	117	0.62	19.2
2	13 month old ME Jack Russell Terrier	0.5 mg/kg SID	406	0.63	84.4	408.5	0.62	19.8
3	13 month old ME Boxer	0.5 mg/kg every 3rd day	2597	4.87	402	11.6	0.72	18.3
4a	10 month old MN Springer Spaniel	0.5 mg/kg EOD	465	0.76	56.2	480.5	0.93	19.0
4b	15 month old MN Springer Spaniel	0.5 mg/kg EOD	357	0.62	69.6	0.4	0.43	21.4

Table 3-1: Serum and cerebrospinal fluid (CSF) concentrations of immunoglobulin A (IgA) and serum concentrations of C-reactive protein (CRP), serum amyloid-A (SAA), alpha-1-glycoprotein (AGP), and haptoglobin (Hp) in putatively relapsing steroid responsive meningitis-arteritis dogs. Regarding patient 4, 'a' denotes the first relapse event and 'b' represents a second relapse event. EOD, every other day; FN, female neutered; ME, male entire; MN, male neutered; SID, once a day.

Signalment	Serum CRP (mg/L)	Serum SAA (mg/L)	Serum AGP (g/L)	Serum Hp (g/L)
1 year old ME Saluki	33	480.5	0.93	10
9 month old FN Labrador	83.3	116.5	0.76	14
1 year old FN Golden Retriever	341	744	0.13	12.5
16 month old MN Boxer	84.4	408.5	1.38	17.6
9 month old ME Jack Russell Terrier	142	56	0.28	31.4
18 month old FN Springer Spaniel	407	10.6	0.22	18.2
9 month old FN Boxer	247.3	475.2	0.01	27.2
13 month old ME Boxer	33	480.5	0.93	10
1 year old ME Weimaraner	83.3	116.5	0.76	14

Table 3-2: Serum concentrations of C-reactive protein (CRP), serum amyloid-A (SAA), alpha-1-glycoprotein (AGP), and haptoglobin (Hp) in putatively relapsing steroid responsive meningitis-arteritis dogs diagnosed prior to the start of this study. FE, female entire; FN, female neutered; ME, male entire; MN, male neutered.

3.1.7 Cerebrospinal Fluid Immunoglobulin A Response

3.1.7.1 Steroid Responsive Meningitis-Arteritis Group

Presentation – The IgA concentration in CSF was elevated above the reference range in 19/20 patients (median: 0.69 µg/mL; range: 0.15 - 4.67 µg/mL; reference range: 0.0 - 0.2 µg/mL) (Figure 3:11).

Remission – The IgA concentration in CSF was increased above the reference range in 20/20 patients (median: 0.62 µg/mL; range: 0.28 - 3.24 µg/mL; reference range: 0.0 - 0.2 µg/mL) (Figure 3:11).

Relapse – The IgA concentration in CSF was increased above the reference range in 4/4 putatively relapsing patients (median: 0.79 µg/mL; range: 0.63 - 4.87 µg/mL) (Table 3-1).

3.1.7.2 Idiopathic Epilepsy Group

Presentation – The IgA concentration in CSF was elevated above the reference range in 8/10 patients (median: 0.53 µg/mL; range: 0.11 – 1.23 µg/mL; reference range: 0.0 - 0.2 µg/mL) (Figure 3:12).

3.1.7.3 Meningoencephalitis of Unknown Aetiology Group

Presentation – The CSF IgA concentration was increased above the reference range in 15/15 patients (median: 0.84 µg/mL; range: 0.22 – 1.92 µg/mL; reference range: 0.0 - 0.2 µg/mL) (Figure 3:12).

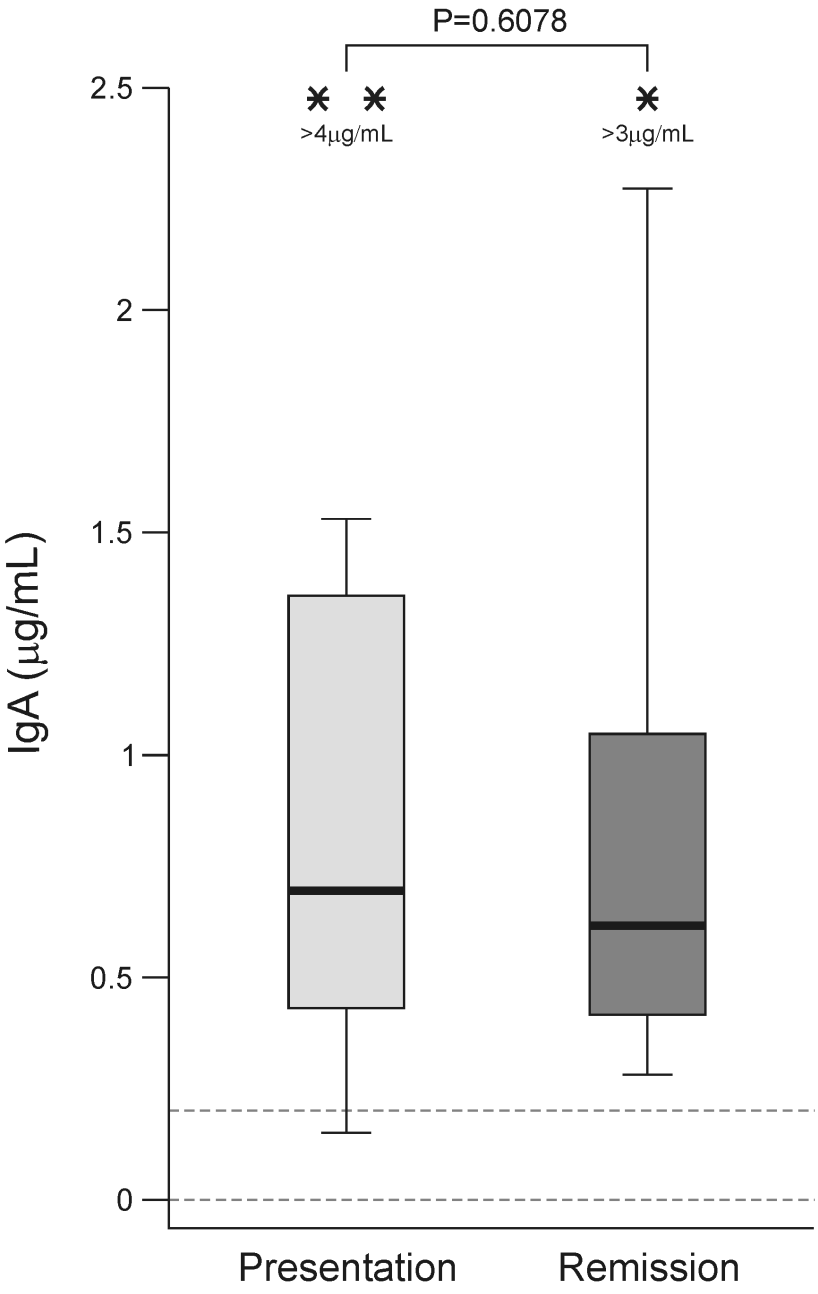

Figure 3:11: Cerebrospinal fluid immunoglobulin A (IgA) response in 20 canine steroid responsive meningitis-arteritis patients at presentation and remission (+14 days into prednisolone treatment). The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values and outliers are represented by *. Extreme outliers are presented as censored data. The highest and lowest values of the laboratory normal reference range is represented by dashed lines (Tipold and Jaggy, 1994).

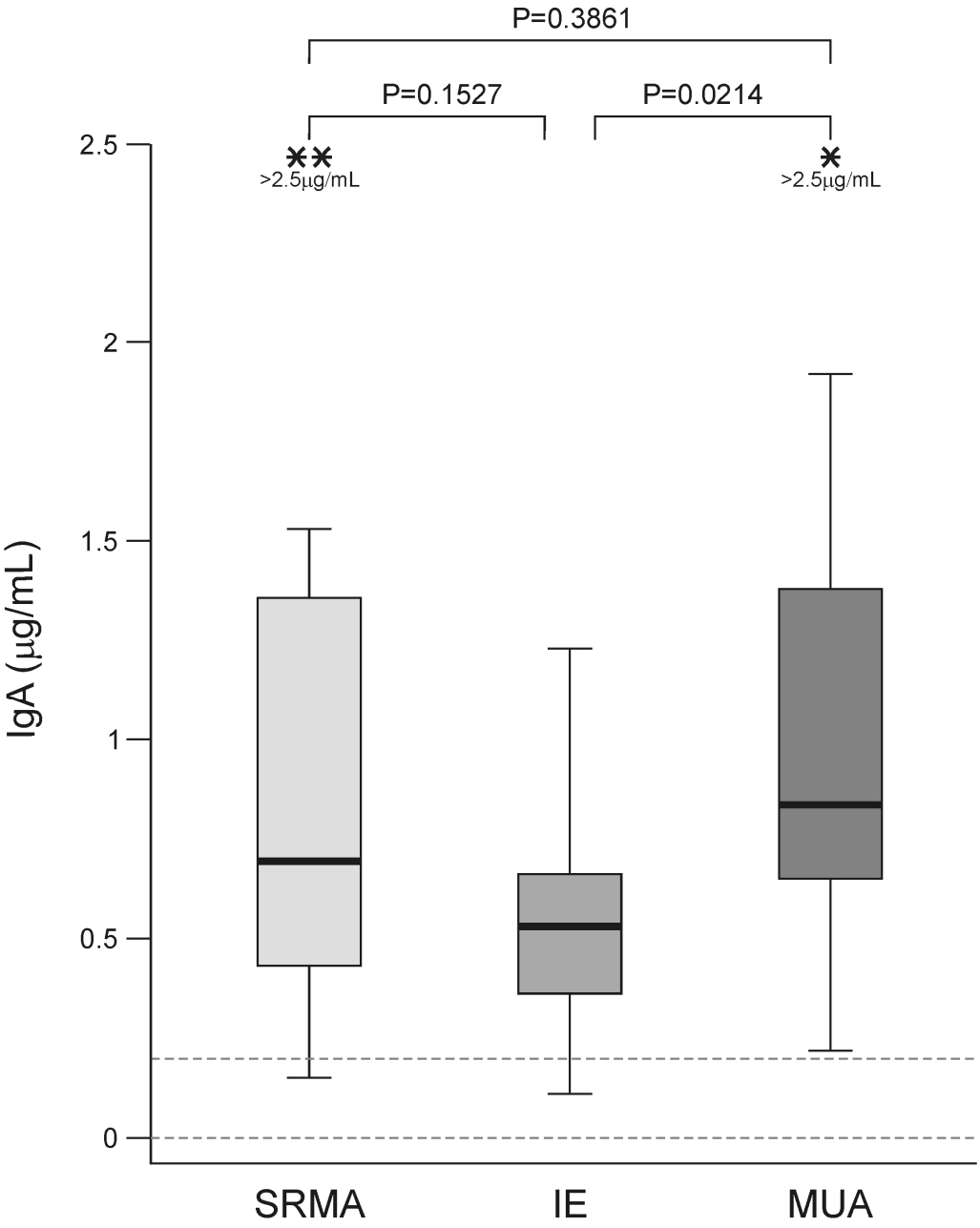

Figure 3:12: A comparison of the cerebrospinal fluid immunoglobulin A (IgA) response in three groups of dogs suffering from steroid responsive meningitis-arteritis (SRMA), idiopathic epilepsy (IE) and meningoencephalitis of unknown aetiology (MUA) respectively. The box represents the 25 – 75th percentile range, the line through represents the median, the range lines correspond to the highest and lowest values, outliers are represented by ⁎, and the highest and lowest values of the laboratory normal reference range is represented by dashed lines. Extreme outliers are presented as censored data.

3.1.8 Statistical Comparisons Between Groups

3.1.8.1 Acute Phase Proteins

Serum CRP was significantly different between all three disease groups at first presentation (SRMA versus IE, $p < 0.0001$; IE versus MUA, $p = 0.0002$; SRMA versus MUA, $p < 0.0001$; figure 3:6). Serum SAA was also significantly different between all three disease groups at first presentation (SRMA versus IE, $p < 0.0001$; IE versus MUA, $p = 0.0007$; SRMA versus MUA $p < 0.0001$; figure 3:7). Serum Hp was significantly different when comparing the SRMA dogs with IE or MUA ($p < 0.0001$; figure 3:8) but was not significantly different when comparing the IE group with dogs diagnosed with MUA ($p = 0.8678$; figure 3:8). Alpha-1-acid glycoprotein was only significantly different when comparing the SRMA group to the IE group at first presentation ($p = 0.0024$; figure 3:9).

3.1.8.2 Immunoglobulin A

Serum and CSF IgA were not significantly different between any of the three disease groups studied at first presentation (Serum: SRMA versus IE, $p = 0.2620$; IE versus MUA, $p = 0.9779$; SRMA versus MUA, $p = 0.3954$; CSF: SRMA versus IE, $p = 0.1527$; IE versus MUA, $p = 0.0214$; SRMA versus MUA, $p = 0.3861$; figure 3:10).

3.1.9 Correlation Analyses

Serum CRP demonstrated a significant positive correlation with the CSF TNCC ($r = 0.902$, $P < 0.001$).

3.1.10 SRMA Treatment Protocol

All 20 dogs responded to the six-month course of prednisolone therapy. Four dogs were suspected to suffer from putative relapse with 1/4 having two separate episodes. All 4/4 suspected relapse dogs remained within the treatment protocol and increasing the prednisolone dose as described resulted in resolution of clinical signs with a six-month long follow-up period off treatment (clinical resolution).

Side-effects of prednisolone therapy were recorded at the first re-check examination. The most commonly reported side-effects at this stage were diarrhoea (n=14), polyuria/polydipsia (n=13), weight gain (10/20) and polyphagia (n=6). All reported side-effects resolved following the reduction as described in the treatment regime (Figure 2:1).

CHAPTER IV

DISCUSSION

4.1 Discussion

Inflammatory diseases of the CNS are one of the most common causes of neurological dysfunction in the dog (Fluehmann et al., 2006). The antemortem diagnosis of canine inflammatory CNS disease remains challenging being based on a combination of clinical criteria, non-specific laboratory investigations and exclusion of other diseases. Cerebrospinal fluid analysis appears superior to other diagnostic modalities in reaching a diagnosis (Tipold, 1995) and surprisingly supersedes magnetic resonance imaging (MRI) in sensitivity (Lamb et al., 2005; Bohn et al., 2006). However, the true sensitivity and specificity of CSF analysis in inflammatory CNS disease has not been determined given the difficulties in obtaining a definitive diagnosis in every case (Lowrie et al., 2008). In addition normal CSF parameters have been described in a number of histopathologically-confirmed inflammatory CNS diseases (Thomas and Eger, 1989; Demierre et al., 2001; Cherubini et al., 2006; Tipold and Jaggy, 1994; Cizinauskas et al., 2000). These features of inflammatory disease are reflected in SRMA in that it is a relatively common disease (representing 2% of neurological referrals in one study; Flehmann et al., 2006), with no definitive diagnostic test (Tipold and Jaggy, 1994), and infrequently causes disease without CSF perturbations (Tipold and Jaggy, 1994; Cizinauskas et al., 2000).

This thesis describes a series of 20 dogs with SRMA with respect to response to therapy, long-term outcome, and expression of potential disease markers at milestones in management. A comparison is made of each of these markers to determine their value at the various stages of this disease and an investigation is performed into their expression in other inflammatory and non-inflammatory CNS diseases. In order for inclusion into this study the SRMA patients had to present with typical historical and clinical signs of SRMA, a CSF pleocytosis and exclusion of other disease. In the unlikely event that the presenting clinical signs were related to a different underlying disease, patients were furthermore excluded if they developed other disease (unrelated to SRMA) within the period of at least six months post-treatment. Findings of this prospective study demonstrate that prednisolone monotherapy is successful at achieving resolution of SRMA.

In this study all dogs presenting with SRMA were under two years old. This finding is not unexpected as it is reported that dogs can develop an age-resistance to the disease at approximately two years of age (Scott-Moncrieff et al., 1992). Previous SRMA studies have reported older patients wih a diagnosis of SRMA. For example, Tipold and Jaggy

(1994) report a dog of seven years old and Cizinauskas and others (2000) report ten dogs with SRMA of which 2/10 were over 24 months of age. Therefore although age would give an indication as to the likelihood of SRMA it cannot be used as an exclusion criterion.

Clinical signs previously reported in SRMA include pyrexia, depression, anorexia, neck pain, stiff gait, conjunctivitis, tonsillitis, hyperaesthesia of the ribs, postural reaction deficits of the limbs, hypermetria, and a decreased menace response (Tipold and Jaggy, 1994; Cizinauskas et al., 2000). These signs can vary according to time and a comparison between studies is difficult, as this would compare individuals at different stages of disease. In this study cervical pain and lethargy was the most common finding and was present in all cases of SRMA reported. Then in decreasing order of representation, other signs included inappetance, pyrexia, bilateral conjunctivitis, thoracolumbar pain, proprioceptive deficits in the forelimbs, Horner's syndrome, and cranial nerve VIII deficits. On this basis, cervical pain and lethargy were suggestive in the diagnosis of SRMA although these signs are non-specific and common to other diseases, including non-inflammatory and non-neurological diseases, making them non-specific.

Two forms of the disease have been reported; a fulminating acute form characterised by a neutrophilic pleocytosis, and a chronic protracted form represented by a mild mononuclear or mixed cell pleocytosis associated with neurological deficits reflecting extension of the inflammation to contiguous structures (e.g. a myelitis or encephalitis) (Tipold and Jaggy, 1994). In a retrospective study investigating inflammatory and infectious diseases of the CNS it was found that although CSF perturbations were non-specific for a particular disease they were 88% sensitive at detecting the presence of intrathecal inflammation or infection (Tipold, 1995). Additionally, SRMA patients frequently had extremely high total nucleated cell counts and this feature was shared with bacterial meningitis (an extremely rare cause of inflammatory CNS disease; Radaelli and Platt, 2002) and the occasional case of GME. Therefore CSF analysis is non-specific and although the magnitude of nucleated cells in the CSF may give an indication of the disease process it is important to use this test in combination with other clinical indices and to exclude other causes of a pleocytosis. The acute form of SRMA, characterised as a neutrophilic pleocytosis (Tipold and Jaggy, 1994), was observed in 12/20 SRMA dogs presented in this study; these cases having clinical signs ≤ 7 days duration. The 8/20 remaining SRMA cases in this study had a monocytic pleocytosis and were reported to have exhibited clinical signs for > 7 days with 4/8 demonstrating additional neurological deficits. However, these 8 cases of mononuclear

pleocytosis do not share all the characteristics of the protracted form of SRMA as described by Tipold and Jaggy (1994).

The chronic form of SRMA can be difficult to distinguish from meningoencephalitis of unknown aetiology and for this reason raised serum and CSF IgA concentrations have been suggested to be a suitable marker for differentiation of these diseases (Tipold and Jaggy, 1994). The literature on IgA demonstrates that an increased concentration may be found in the serum and CSF of SRMA patients though this feature is shared with other inflammatory central nervous system (CNS) diseases giving it a low specificity (Tipold and Jaggy, 1994; Tipold et al., 1994; Tipold et al., 1995) and the extent of the normal range for serum IgA may vary with breed (Griot-Wenk et al., 1999). The sensitivity of this combination, in contrast, is reported to be as high as 100 % (Tipold and Jaggy, 1994) and this study found 19/20 cases had increased serum and CSF IgA at presentation (95 % sensitivity) supporting this figure and giving this immunoglobulin diagnostic value in SRMA. Furthermore serum and CSF IgA remained elevated in 20/20 patients at remission and serum IgA remained elevated in 15/20 dogs at disease resolution despite remaining asymptomatic for the six month post-treatment observation period. Although a humoral immune response is initiated in SRMA the reason for this persistently raised intrathecal and systemic IgA is not known (Schwartz et al., 2008).

Acute phase proteins are serum proteins produced predominantly by the liver in response to bacterial infection, trauma, neoplasia, tissue infarction, and immune-mediated inflammatory disease and as such are non-specific (Cerón et al., 2005). Serum CRP and SAA correlate with the clinical course of SRMA with relatively increased concentrations at diagnosis and a resolution of clinical signs being associated with normal to mildly elevated serum concentrations. Their production is not reported to be affected by corticosteroids with the exception of Hp which is known to increase in concentration with high-dose prednisolone therapy and this supports the results of the present study (Martinez-Subiela et al., 2004, Cerón et al., 2005).

In contrast to a previous study (Bathen-Noethen et al., 2008) serum CRP demonstrated a significant positive correlation with the TNCC of CSF ($r = 0.902$, $P < 0.001$). Also in contrast to the same study, serum CRP remained above the reference range in 16/20 SRMA dogs at the time of remission, despite an unperturbed CSF analysis in 20/20 SRMA cases. However, it should be noted that there is a difference in timing of the first assessment between the two studies (2 weeks vs 4 weeks on therapy) that makes a direct

comparison difficult. The current study suggests that initial immunosuppressive doses of prednisolone suppress but do not eliminate inflammation in dogs affected by SRMA as serum CRP remains elevated but markers of inflammation in the CSF (TNCC and total protein) are resolved. This is in keeping with the concept that CRP is a more sensitive marker of inflammation than more traditional markers e.g. peripheral neutrophilia (Cerón et al., 2005).

Serum amyloid-A was raised at least 30 times above the reference range in all presenting SRMA cases and occasionally was found to be orders of magnitude greater than the normal range. This appears to be a unique observation to this canine inflammatory disease. Given the large-scale production of SAA, hepatic synthesis is the most likely source of this protein. However, extra-hepatic intrathecal or vascular production of SAA cannot be excluded by these results and further studies are required to elucidate the site(s) of synthesis.

Serum Hp was found to remain elevated during treatment of SRMA in keeping with previous studies that found Hp was induced by steroid medication (Cerón et al., 2005; Martinez-Subiela et al., 2004), but also supports its use in investigation prior to steroid administration. In inidividual cases Hp concentrations were found to increase or decrease during therapy, and this probably reflects the conflicting influence of disease resolution versus steroid induction in these patients. Alpha-1-acid glycoprotein concentrations were above the reference range at initial presentation of SRMA in 19/20 dogs. However, there was a tendency towards higher concentrations of this protein in those patients that had shown clinical signs for >7 days. These patients also had a predominance of monocytes (>80%) in the CSF. This observation would agree with findings in other species where AGP has found to be particularly associated with chronic conditions (Horadagoda et al., 1999; Eckersall et al., 2007).

Occasionally SRMA can have an atypical presentation in which traditional CSF parameters (TNCC and total protein concentration) may be unperturbed or exhibit albuminocytological dissociation. Such observations have been reported in the protracted form of the disease (Tipold and Jaggy, 1994; Cizinauskas et al., 2000) and in cases of putative relapse (Cizinauskas et al., 2000; Lowrie et al., 2008). In the present study 3/4 putatively relapsing dogs had a normal TNCC and 2/4 had normal total protein concentrations. A further seven dogs with a previous diagnosis of SRMA demonstrated putative relapse during the observation period of which 6/7 patients had a normal TNCC

and total protein concentration. A diagnosis was made in these cases based on a previous recent diagnosis of SRMA, clinical signs similar to those at initial diagnosis, raised serum and CSF IgA concentrations, increased serum APP concentrations, and response to treatment. A neutrophilic leucocytosis was also seen in 3/4 putatively relapsing dogs enrolled on the treatment protocol and 4/7 suspected relapse patients previously diagnosed with SRMA. Although this may be a manifestation of the effect of steroids, these dogs lacked the hallmarks of a steroid-induced leucogram; notably concomitant lymphopenia, eosinopenia or right shift (Raskin et al., 1999). Indeed in the presence of unperturbed CSF parameters (2/4 and 6/7 putative relapsing cases) and a normal leucogram (4/4 and 4/7 putative relapsing cases), CRP and SAA were found to be elevated in all patients exhibiting clinical signs consistent with SRMA relapse. The literature demonstrates that 1/32 dogs (Tipold and Jaggy 1994) and 1/6 dogs (Cizinauskas et al., 2000) with suspected SRMA relapse did not show an elevated CSF cell count. The elevated APP concentrations in these cases, though unspecific for SRMA, suggested the presence of an inflammatory disease, which in the clinical context was considered to support the diagnosis of relapse. Re-starting or increasing the dose of prednisolone in these patients resulted in remission with a full recovery in all cases. All putative relapse cases had a minimum follow-up of 6 months and did not develop any concurrent diseases.

Cerebrospinal fluid analysis is considered the gold standard ante-mortem diagnostic modality in confirming CNS inflammation (Tipold, 1995). A novel observation in this study was the identification of an apparently inflammatory environment (raised serum APPs) in the presence of an unperturbed CSF analysis at clinical remission of SRMA. In this study it was difficult to prove whether or not raised systemic APPs truly represented an inflammatory CNS environment. However, a recent report demonstrated increased IL-6 concentrations in unperturbed CSF samples taken from putatively relapsing SRMA patients providing preliminary evidence that CNS/meningial inflammation may be present without a pleocytosis (Lowrie et al., 2008). Twelve percent of CSF samples obtained from 188 dogs with inflammatory or infectious CNS disease were within the normal reference ranges for total nucleated cell count and total protein concentration (Tipold, 1995). The presence of an apparently inflammatory CNS environment in the absence of CSF perturbations is recognised in the veterinary literature, in particular it has been associated with histopathologically confirmed cases of GME (Thomas and Eger, 1989; Dermierre et al., 2001; Cherubini et al., 2006), but the reason for unperturbed CSF remains elusive. These circumstances make diagnosis challenging and so the availability of other markers of inflammation are of value in these rare circumstances.

The diagnostic utility of IgA and APPs was also examined in this study by comparing the synthesis of these proteins in inflammatory and non-inflammatory CNS diseases. Serum IgA was found to be increased above the reference range (Tipold et al., 1994) in 13/15 MUA cases, 10/10 IE dogs and 19/20 SRMA cases. Cerebrospinal fluid IgA concentrations were above the published reference range (Tipold et al., 1994) in 15/15 MUA cases, 8/10 IE dogs and 19/20 SRMA cases. Statistical comparison of serum and CSF IgA concentrations did not reveal any significant differences between the three different disease groups. The combination of increased serum and CSF IgA concentrations is reported to have a specificity of 83 to 100 % (Tipold and Jaggy, 1994; Tipold et al., 1995; Maiolini et al., 2009). Although the sensitivity of combined raised serum and CSF IgA concentrations was high for SRMA (95 %) in this study, the specificity was low when using the calculations described by Lamb (2007). The specificity of raised serum and CSF IgA concentrations to correctly diagnose SRMA in our population of dogs with inflammatory CNS disease (SRMA and MUA) was 13 %. This specificity decreased to 8 % when considering the diagnostic utility of IgA in SRMA in the study population as a whole. In contrast to this study, the sensitivity of combined raised serum and CSF IgA concentrations in diagnosing SRMA in a group of 12 Boxers with the disease was 25 % (Behr and Cauzinille, 2006). A recent report of histopathologically confirmed SRMA in a Boxer also reported normal serum and CSF IgA concentrations (Wrzosek et al., 2009). These results suggest that IgA concentrations in the serum and CSF are influenced by multiple extraneous sources. Griot-Wenk and others (1999) have demonstrated that genetic factors may influence serum IgA concentration. The discovery of raised serum and CSF IgA in SRMA was made on dogs from Switzerland (Tipold et al., 1994). The study reporting a 25 % sensitivity of this combination in SRMA was performed on patients from France. The current study was performed on dogs from the United Kingdom. All three geographical areas appear to harbour dogs with a wide range of serum and CSF IgA concentrations, regardless of breed. The lack of a 'true' control population in this study is a limitation and the original reference range was determined on a group of 15 healthy laboratory Beagles (Tipold et al., 1994).

Serum CRP was found to be increased above the reference range in 20/20 dogs with SRMA and 13/15 with MUA. Serum SAA was elevated in 20/20 dogs with SRMA and 9/15 patients with MUA. Serum CRP and SAA were within the reference range in all 10 dogs with IE. Statistical comparison of serum CRP and SAA concentrations revealed significant differences between all three different disease groups. C-reactive protein and

SAA are described as fast APPs responding rapidly to inflammation and its resolution (Cerón et al., 2005). SRMA and MUA both share the clinicopathological features of acute inflammation that presumably drives this APR. However, CRP and SAA were statistically different between these two groups demonstrating a difference in the magnitude of this response between the two conditions. Meningioencephalitis of unknown aetiology is considered a chronic inflammatory CNS disease charcterised by chronic and coexisting acute inflamamtory meningeal and parenchymal lesions (Cordy, 1979; Braund, 1985; Munana et al., 1998; Higgins et al., 2008). In contrast SRMA more frequently presents in its acute form with meningeal pathology alone (Harcourt, 1978; Meric et al;., 1985). Patients with IE did not appear to have raised serum CRP or SAA concentrations.

Haptogolobin and AGP are considered more moderate APPs and as such they are considered responsive to more chronic conditions (Horadagoda et al., 1999; Eckersall et al., 2007). AGP concentrations were above the reference range in a proportion of individuals from all three groups of dogs. Meningoencephalitis of unknown aetiology is a chronic inflammatory CNS disease and so increases in these proteins would not be surprising (Cordy, 1979; Braund, 1985; Munana et al., 1998; Higgins et al., 2008). An APR has been described in humans with epilepsy although this is transient and associated with seizure activity (Peltola et al., 2002). The precise nature of this response is unknown and may relate to neuronal injury (Correale et al., 1998) or the seizure activity itself (Peltola et al., 2002). All patients with IE in this study had been seizure free for > 72 hours and this may explain the observation of a 'moderate' APR in the absence of CRP and SAA elevations. Another explanation is the observation that phenobarbitone induces AGP in the dog (Bai and Abramson, 1983) and all 10 pateints in this study were receiving phenobarbitone at doses sufficient to achieve a therapeutic concentration. A limitation of the current study is the lack of a true negative control group (i.e. healthy dogs) although the published literature provides reference ranges for IgA and serum APPs on which to interpret the results.

The design of the protocol is derived from the collective experience of Neurologists based at UGSAH over several years combined with guidelines from previous studies (Tipold and Jaggy, 1994; Cizinausakas et al., 2000). The idea was to design a protocol that would be simple to follow and easy to understand so that the owner and veterinary surgeon alike, would always know the process involved in obtaining complete resolution. In doing this the protocol was the first to incorporate a regime to manage suspected relapse cases and therefore each patient was treated uniformly with regards to medciation when this

complication arose. This is the first study to cater for relapse patients, offering a rigid management regime to which they can adhere. In the event of this protocol failing (defined as two consecutive cycles without achieving clinical remission) then other medications may be of benefit in treating SRMA, however, this was not necessary in the 20 patients recruited in this study.

The follow-up period of 6 months was based on observations on the pattern of relapse in previous studies (Tipold and Jaggy, 1994; Cizinausakas et al., 2000). With the wealth of immunosuppressive therapy available in veterinary medicine today there is a tendency towards multi-modal therapy in the management of inflammatory CNS disease. In this case series a prednisolone monotherapy protocol for SRMA was successful at achieving full remission in 20/20 affected dogs, with an in protocol case relapse rate of 20% (4/20). In contrast Cizinauskas and others (2000) report full remission in 8/10 dogs receiving a prednisolone based treatment regime with an in protocol case relapse rate of 60%; the treatment regimen included the use of other therapeutic agents. Consequently only 4/10 cases received prednisolone monotherapy. Of these ten cases one was euthanased for reasons related to the disease. Tipold and Jaggy (1994) describe a managed prednisolone monotherapy protocol, involving adjustment of the prednisolone dose in the light of CSF parameters, achieving a case relapse rate of 25 %, resolution rate of 60 % and a 5 % mortality. It should be borne in mind that the two case series referred to and the current study are likely to contain a differing spectrum of the stages of SRMA at the time of presentation, which may influence the response of the cohort to therapy. It is reported that long-term prednisolone to treat SRMA is associated with mild clinical side-effects and this study mirrors these findings, with diarrhoea being the most commonly occurring side-effect (Cizinauskas et al., 2000). Our findings support the use of an immunosuppressive prednisolone monotherapy, using the described protocol, in the treatment of SRMA.

In order to most effectively manage a patient with SRMA it would be of value to identify a parameter that would predict the potential for relapse. It has been observed that dogs that go onto exhibit more than one relapse have a tendency towards higher serum CRP concentrations after 4 weeks on immunosuppressive prednisolone therapy (Bathen-Noethen et al., 2008). In this study assessment of clinical and laboratory parameters did not identify any obvious modality for predicting relapse. However, a direct comparison between these studies is inappropriate as re-check examinations were scheduled at different time points during treatment. However, the present study confirms that relapse has a propensity to occur on treatment and not following cessation of treatment in

agreement with a previous study (Cizinauskas et al., 2000). Only first relapse events were analysed in this study, however inclusion of the single second relapse event did not alter the outcome of the statistical tests for any of the parameters and therefore inclusion or exclusion of these results do not bias our data.

4.2 Summary

The described prednisolone monotherapy resulted in resolution of SRMA in 20/20 cases with a disease free post treatment interval of at least six-months. Remission of clinical signs was associated with an unperturbed CSF analysis in the presence of elevated serum CRP concentrations. Serum AGP and Hp support initial diagnosis alone. In contrast to APPs, serum IgA remains elevated at remission and resolution suggesting different aspects of the disease are reflected by these different markers. Therefore serum and CSF IgA concentrations aid in initial diagnosis but are not a marker of clinical remission or resolution of disease. Immunoglobulin A is elevated in other inflammatory and non-inflammatory CNS diseases of the dog. A robust prognostic indicator at initial presentation for potential relapse remains elusive. This study has suggested that the traditional markers of CNS inflammation, i.e. CSF TNCC and total protein concentration, are not sensitive in all circumstances of inflammatory CNS disease and serum APPs may offer clinical utility in aiding a diagnosis when other diseases have been ruled-out.

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