


NEW INSIGHTS INTO THE PATHOGENESIS OF SYRINGOMYELIA SECONDARY TO CHIARI-LIKE MALFORMATION IN DOGS

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New insights into the pathogenesis of syringomyelia secondary to Chiari-like malformation in dogs

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
bFFE	Balanced fast field echo
CCF	Caudal cranial fossa
CCFP	Caudal cranial fossa parenchyma
CI	Confidence interval
CKCS	Cavalier King Charles Spaniels
CM	Chiari-like malformation
CM-I	Chiari malformation type I
CSF	Cerebrospinal fluid
ECG	Electrocardiogram
EEG	Electroencephalogram
FLAIR	Fluid attenuated inversion recovery
GB	Griffon Bruxellois
GRPR	Gastrin-releasing peptide receptors
IQR	Inter-quartile range
LD	Labradors
MRI	Magnetic resonance imaging
NeP	Neuropathic pain
NSAIDS	Non-steroidal anti-inflammatory drugs
PVS	Peri-vascular spaces
QOL	Quality of life
RVC	Royal Veterinary College
SAS	Sub-arachnoid space
SB	Small breed dogs
SD	Standard deviation
SEM	Standard error of the mean
SSFP	Steady-state free precession imaging
TE	Echo time
TR	Repetition time
TSE	Turbo-spin echo

UK	United Kingdom
V	Ventricular system
VAS	Visual analogue scale

SECTION I

GENERAL INTRODUCTION

THEORIES FOR THE PATHOGENESIS, DIAGNOSIS, AND TREATMENT OF SYRINGOMYELIA SECONDARY TO CHIARI-LIKE MALFORMATION IN DOGS

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Summary

The term syringomyelia (SM) refers to non-cerebrospinal fluid (CSF) filled cavities within the spinal cord parenchyma. These cavities are thought to develop due to alterations in CSF flow, secondary to multiple chronic diseases of the central nervous system (CNS). SM in dogs is most frequently associated with 'Chiari-like malformations' (CM) of the craniocervical junction; however, the pathogenesis of this debilitating disease complex has not yet been fully elucidated. Clinical signs of CM/SM range from cervical hyperaesthesia to tetraparesis. Magnetic resonance imaging (MRI) is typically employed to diagnose the disease. Treatment of CM/SM can be medical or surgical but objective data concerning treatment outcomes and prognosis is currently lacking.

Introduction

SM is an enigmatic disease in dogs characterised by fluid-cavitation of multiple spinal cord segments. [1,2] SM can be classified as either 'communicating' or 'non-communicating'.[3] The term 'communicating SM' was introduced by Williams[4], who suggested that the syrinx cavities contained a fluid similar to CSF. Subsequently, Gardner[5] postulated that this fluid arrived in the syrinx directly from communication with a distended central canal under high pressure. Experimental reproduction of human Chiari-type I malformation (CMI) in dogs, by the injection of kaolin into the cisterna magna, produces syringes with a patent connection to the central canal and hydrocephalic ventricles.[6] Cavitation and damage to the spinal cord, typically the dorsal horn, results in abnormal processing of sensory information that manifests clinically as pain and dysaesthetic behaviour.[7,8]

SM occurs in dogs secondary to congenital and acquired neurologic disorders such as craniocervical malformations[1], subarachnoid diverticulae[9], and intra-cranial neoplasms.[10,11] These diseases share reduced cross-sectional area of the subarachnoid space (SAS) and altered CSF hydrodynamics as common features. Alteration of CSF flow is suspected to drive the accumulation of fluid in the spinal cord causing central canal dilation and eventually parenchymal cavities.[12,13] In both humans and dogs, SM is more often associated with reduced CSF flow at the foramen magnum than at other levels in the neuraxis.[14-17]

Chiari type 1 and Chiari-like malformation

Human Chiari type I malformations (CMI) are associated with reduced posterior fossa volume[15] and caudal descent of the cerebellar tonsils.[18] The prevalence of SM secondary to CMI is high[17] as altered CSF hydrodynamics occur at the level of the foramen magnum and the cranial cervical spinal cord.[19,20] Similarly, SM is a common sequel of similar craniocervical malformation in dogs, but dogs lack cerebellar tonsils.[1,16] The term *Chiari-like* malformation in Cavalier King Charles Spaniels (CKCS), as defined by an international working group[21], encompasses a mismatch between caudal cranial fossa (CCF) volume and brain parenchyma within, leading to a caudal herniation of part of the cerebellum and brainstem into or through the foramen magnum (Figure 1). This terminology is advantageous

in the CKCS where all these features are typically present and are somewhat analogous to CMI.

Another advantage of studying the disease in CKCS is the high prevalence of CM[22,23]; in one study 92% of CKCS had at least one morphologic abnormality consistent with CM on MRI, such as occipital hypoplasia, cerebellar herniation into the foramen magnum and medullary kinking .[24] Diagnosis of CM requires MRI, with indentation and herniation of the cerebellar vermis most commonly cited as key diagnostic features (Figure 1).[25,26]

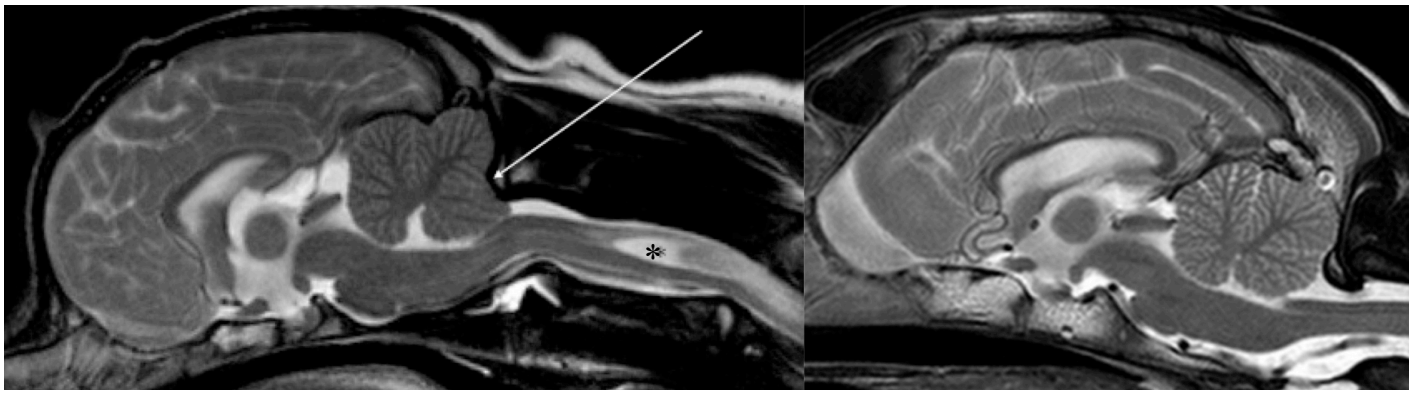

Figure 1: Sagittal T2-weighted MRI of a dog's brain with a Chiari-like malformation (left); there is indentation (arrowed) and herniation of the cerebellum through the foramen magnum and syringomyelia (asterisk). An MRI from a Labrador (right) is included for comparison.

Syringomyelia secondary to Chiari-like malformation

Unlike human medicine, it is contentious in the veterinary literature as to whether CM alone causes clinical signs[24,25]. Conversely, SM alone has been associated with neurologic signs and neuropathic pain in CKCS[7,24], with large and asymmetrical syringes being the strongest predictors of pain.[7] SM secondary to CM is a complex oligogenic trait in CKCS with a moderately high estimate of hereditability.[27] Preventative breeding schemes have existed for several years with some recent success in reducing the disease prevalence.[28] It is from these breeding schemes that estimates of the prevalence of asymptomatic SM have been made; 25% of CKCS aged 12 months have SM, increasing to 70% in CKCS aged 72 months or more.[29] It is difficult to predict which dogs will develop clinical signs following incidental diagnosis,

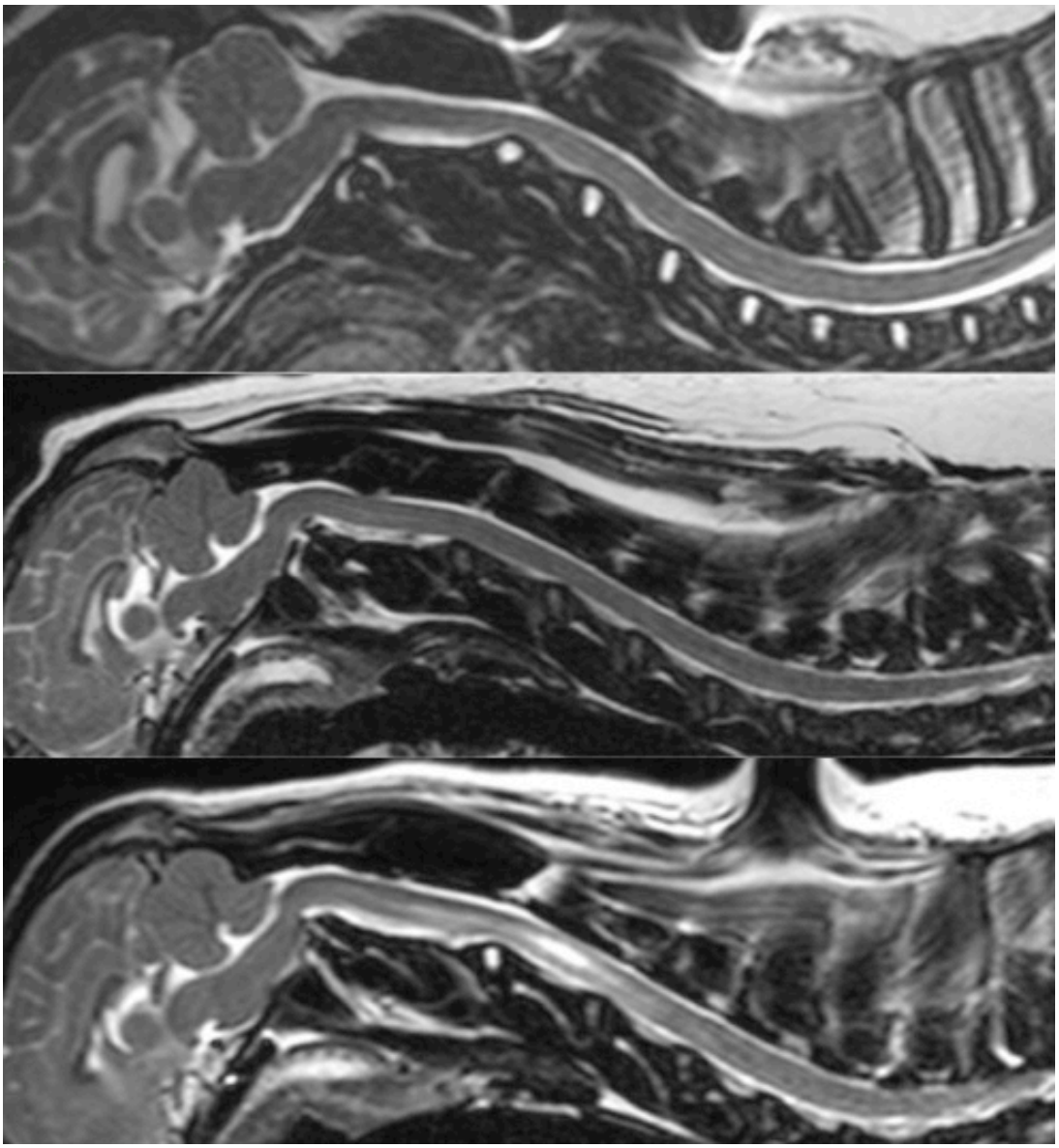

Figure 2: Sagittal T2-weighted MRI scans of the brain and cervical spine in a flexed position from (top to bottom) a brachycephalic (Pug) dog with a normal cervicomedullary junction, a Cavalier King Charles Spaniel with Chiari-like malformation (indentation of the cerebellum by the occipital bone and herniation of the cerebellar vermis into the foramen magnum) and lastly a Cavalier King Charles Spaniel with Chiari-like malformation that has developed syringomyelia in the cervical spinal cord.

which is a source of concern and frustration for dog owners, breeders and veterinarians. CM/SM is not infrequently observed in an increasing number of other small or 'toy' dog breeds, including the French Bulldog, Griffon Bruxellois (GB), Chihuahua, Pomeranian, Maltese terrier, Pug and Yorkshire terrier[30] (Figure 2). CM is an important risk factor for the

development of SM in GB, however SM does occur in this breed without apparent cerebellar herniation.[31] Moreover, other morphologic abnormalities affecting the volume of the subarachnoid space in the craniocervical region such as atlantooccipital overlapping may contribute to the disease and should possibly be included as defining features of CM.[32] The pathogenesis of SM secondary to CM (in its broadest sense) in dogs is therefore likely complex.

Current theories for the pathogenesis of SM secondary to CM in dogs

Morphometric analysis of CM/SM

In canines, breed selection pressure has resulted in a vast variation of skull shape and size. In particular, canine brachycephaly is related to paedomorphosis[33], as in certain dog breeds it is considered desirable (for various functional and aesthetic reasons) to retain the shorter skull confirmation that is common to all juvenile dogs. CKCS are classified as toy-breed brachycephalic dogs (Figure 3). Brachycephalic dogs have a rounded, relatively broad head with shortened facial bones.[34]

Figure 3: The Cavalier King Charles Spaniel. The UK Kennel Club classifies this breed into the 'toy dog' group.

Brachycephalism has lead to the alteration of other cranial features, not just limited to the facial bones; for example the virtual abolition of frontal sinuses[35] and morphologic differences in the neurocranium, specifically a large cephalic index (Figure 4).[36] Further,

brachycephalism has had effects on cerebral tissue contained within the calvarium such as a more ventral pitch of the primary longitudinal brain axis and ventral shift in the position of the olfactory lobe.[36]

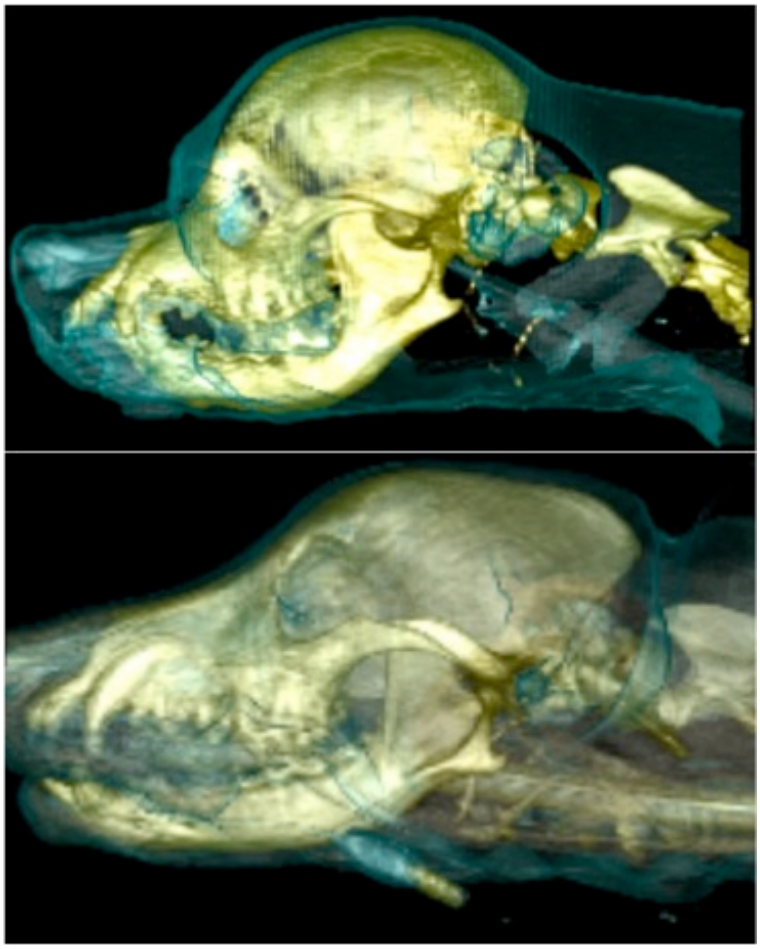

Figure 4: Three-dimensional reconstructions of computed tomography scans from a CKCS (top) and a Labrador (bottom). The skull of the CKCS is short and wide, with decreased facial and cranial length.

At a basic level, CM is suspected to be a disease of abnormal geometric craniocerebral morphology that may also be associated with brachycephalism and miniaturisation. The traditional view of human CMI is that occipital bone hypoplasia facilitates caudal descent of the cerebellum.[15,18,37,38] Occipital bone hypoplasia has been proposed as a cause of CM in dogs.[1,39] However, abnormalities of the occipital bones in isolation are not consistent with the observation that dogs with CM display shortening of the entire basicranium.[31]

Caudal cranial fossa morphology

The *fossa cranii caudalis* is present on the ventral surface of the internal aspect of the skull and is formed by the dorsal aspect of the basioccipital bone.[34] This concavity houses the pons,

medulla oblongata and associated vessels; it is caudal to the rostral and middle cranial fossae. In this thesis, the CCF is defined as the intracranial compartment that contains the cerebellum as well as the pons and medulla oblongata; a potential cavity that is analogous to the posterior fossa of humans. Similarly, the rostral and middle cranial fossae are grouped and defined as the intracranial compartment containing all supratentorial structures. Internally the CCF is delineated dorsally and rostrally by the tentorium cerebelli (which is not predominantly osseous in the dog) and ventrally it extends from the petrosal crests and dorsum sellae to the foramen magnum, as defined by the *fossa cranii caudalis*[34]. We define the CCF externally by the occipital bone, which forms from four centres: the squamous part (*squama occipitalis*), the paired lateral parts (*partes laterals*) and the basilar part (*pars basilaris*). These parts are referred to hereafter as the supraoccipital, paired exoccipitals and basioccipital bones (Figure 5).[34,40] The foramen magnum is the cavity through which the medulla and associated vessels are continuous with the spinal cord and associated vessels.

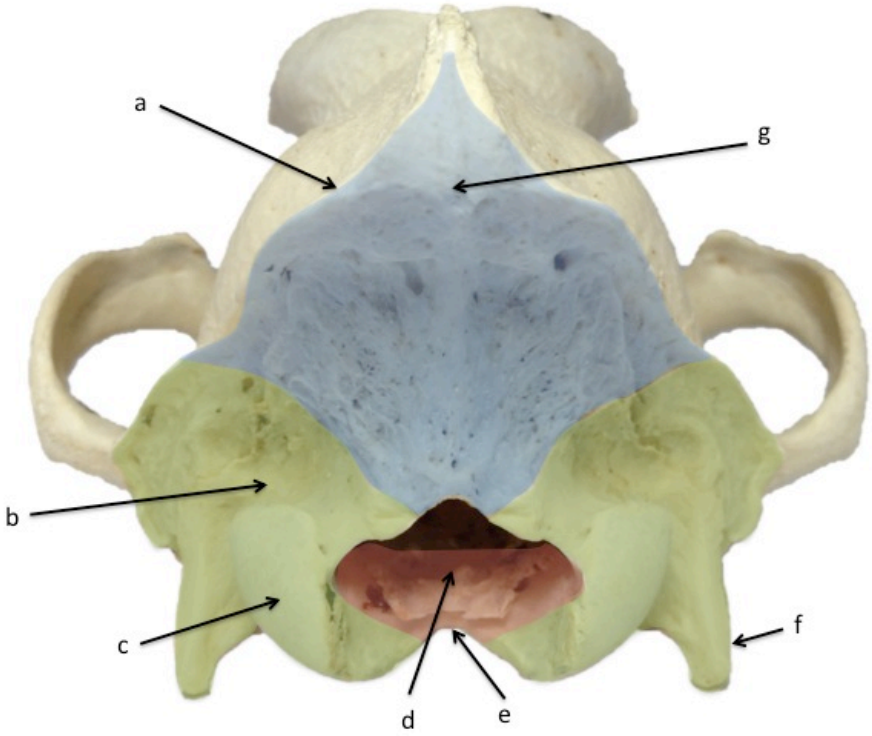

Figure 5: Caudal aspect of the occipital bone. There are four centres of occification: the squama occipitalis (supraoccipital bone; blue), the partes laterales (paired exoccipitals, green) and the pars basilaris (basioccipital bone, red). Key: a = nuchal crest, b = dorsal condyloid fossa, c = occipital condyle, d = foramen magnum, e = intercondyloid notch, f = paracondylar process, g = external occipital protruberance.

The volume of the posterior fossa plays a role in CMI[41], which is significantly smaller in children with both CMI and SM.[42] It is conceivable that more severe morphologic abnormalities affecting the CCF may be associated with a more severe disease phenotype in dogs, in particular CCF volume and the presence of clinically significant SM. As in CMI, CKCS have been found to have a shallower caudal cranial fossa with abnormalities of the supraoccipital and basioccipital bones when compared to mesaticephalic breeds[23]. However, independent studies have so far not established a role for CCF volume in the development of SM.[22,24] A three-dimensional volumetric technique has been employed in dogs to compare cranial and cerebral volumes in CKCS to those of other small breed dogs and Labrador retrievers.[26] Interestingly, CKCS appeared to have a similar sized CCF to small breed dogs, but had a parenchymal volume within the CCF that was similar to Labradors.[26] The authors suggested therefore that parenchymal overcrowding of the CCF might exist. It is not currently known if this is the case, nor if such overcrowding is related to the presence and severity of SM.

Vascular morphology

The rate of drainage of CSF is related to venous blood pressure, as arachnoid villi act as a one-way valve such that CSF will only drain when CSF pressure exceeds venous pressure.[43] Consequently, researchers have investigated reduced venous outflow as a mechanism for raised CSF pressure, particularly in relation to the aforementioned osseous abnormalities of CM/CMI. Reduced longitudinal extension of the braincase may have other consequences; in humans with achondroplasia, earlier closure of cranial base synchondroses occurs[44], which is associated with clinical signs that are attributable to raised intracranial pressure.[45,46]

Venous hypertension is believed to occur due to congestion of outflow[47], specifically the venous sinuses at the level of stenotic cranial foramina.[48-50] The jugular foramen encloses the sigmoid sinus that receives blood from the petrosal and transverse sinuses. In man, the jugular foramen is the major site of cerebral drainage.[51,52] Children with craniosynostosis and raised intracranial pressure had significantly narrower jugular foramina.[49] It is conceivable that dogs with SM may have stenosis of this and other cranial foraminae with secondary venous hypertension. In dogs, the large temporal sinus (*sinus temporalis*) becomes the emissary vein at the retroarticular foramen (*v. emmisaria foraminis retroarticularis*), which is the major site of drainage of cerebral blood into the maxillary vein. Stenosis of cranial foraminae has

previously been proposed as the mechanism by which some dogs suffer SM in the absence of cerebellar herniation.[31]

Spinal morphology

The formation and propagation of syringes may occur due to distending centrifugal forces exerted on the spinal cord.[20,53] Variation in subarachnoid space width is suspected to cause these forces due to increased CSF velocity and the *Venturi* effect.[40] It was hypothesised by Rusbridge[40] that vertebral canal narrowing caudal to C1, in the C1-3 region, might result in SM. However, Carruthers[54] found no association between vertebral canal narrowing or angulation at C2/3 and the development of SM. A limitation of this study is that dogs with SM were significantly older than the control group, which would be expected in a progressive disease. Other stenoses of the C1 region have been reported in conjunction with SM, including dorsal impingement by soft tissue and atlantoaxial instability.[24] Conversely, Stalin[55] noted that although atlantoaxial morphology in CKCS differs from other breeds, it does not correlate with the appearance of SM in individual CKCS. Jull and others[56] took linear vertebral measurements from mid-sagittal MRI scans and found that CKCS with SM had an increased spinal cord to spinal canal ratio compared to CKCS without SM in the C2-5 region. It is not clear if this increase in relative spinal cord width is the cause, due to the Venturi effect, or the effect of SM. The authors of this study hypothesised that increased cord width could be associated with increased pressure within the spinal cord.

Dynamic analysis of CM/SM

Recent advances in MRI-based diagnostic tools for the investigation of CMI have been made. Some of these techniques have recently been described for the evaluation of CM/SM in dogs.

CSF hydrodynamics

Phase contrast 'cine' MRI (cMRI) combines the flow-dependent contrast of phase contrast MRI previously discussed with the ability of cardiac gating (MRI sequence acquisition triggered by an electrocardiogram recorded during the examination) to produce images throughout the cardiac cycle. Phase contrast cMRI is recommended to quantitatively assess CSF flow velocity in patients with CMI[57], and clinical signs appear to correlate with the

severity of the abnormality.[58] This technique has been used to visualise CSF flow in CKCS with CM.[59,60] In an experimental study, turbulent CSF flow at the foramen magnum was associated with SM severity and CSF velocity dorsal to C2/3 was inversely related to the presence of SM.[60] Whilst the clinical relevance of this finding remains uncertain, the study highlights that the common path leading to SM is abnormal CSF hydrodynamics as in CMI/SM patients.[12,13] Unfortunately, the usefulness of this technique in dogs may be limited given the approximately 10-fold lower peak CSF flow velocity versus human patients, despite positioning dogs with a flexed neck to mimic standing physiology.[60,61]

Cerebellar pulsation

As the cerebral vessels engorge in systole the hindbrain moves towards the foramen magnum, a phenomenon previously coined the *piston action* of the brain.[62] Most of the theories for the formation of syrinx cavities (reviewed by Rusbridge and others[40]) share the belief that cerebellar descent in systole creates a high-pressure CSF pulse wave.[20,63] As this pulse wave establishes a difference in pressure between the subarachnoid space and the spinal cord, the cord is subjected to a distending centrifugal force distal to blockages, favouring the formation of a syrinx[20]. Lu and others[25] did not find an association between the degree of cerebellar herniation and SM in CKCS with CM, however the position of the cerebellar vermis may change with patient positioning for MRI.[61]

CMI patients have greater movement of the cerebellar tonsils during the cardiac cycle when measured using cardiac-gated phase contrast cMRI[64], however the low spatial resolution of this technique would most likely render it useless for studying CM in dogs. The relationship between cerebellar pulsation and SM has not been investigated in dogs, newer techniques with improved spatial resolution, such as steady-state free precession imaging[65], could facilitate this.

Histological analysis of CM/SM

Spinal cord histology

Hu and others[66] described the histopathologic findings of SM in symptomatic and asymptomatic CKCS. In addition to cavitations, the most notable findings were evidence of

spongy degeneration of the neuropil, plus neuronal necrosis and Wallerian degeneration in the white matter; gliosis in the regions immediately adjacent to the central canal or syrinx; a suggestion of increased vascularity; and in some animals, a proliferative fibrous response that lined the inner aspect of the central canal and syrinx. In support of the radiologic findings of Rusbridge[7], CKCS with asymmetrical syrinx cavities were more likely to suffer clinical signs.[66]

Diagnosis of CM/SM in dogs

Clinical signs

As the expanding syrinx damages the dorsal horn of the spinal cord, clinical signs tend to be associated with a change of the somatosensory processing of information resulting in the perception of neuropathic pain.[67] Neuropathic pain is different to physiological and inflammatory pain, in that it never benefits an animal and can be considered a disease in its own right. Approximately 35% of dogs with SM are reported to suffer pain.[7] The presence and severity of neuropathic pain is associated with syrinx dimensions, with syrinx width and asymmetry being the best predictors of pain.[7]

There are several proposed mechanisms for the development of neuropathic pain, related to anatomical and neurochemical changes in the dorsal horn of the spinal cord. These mechanisms have been reviewed elsewhere.[67]

Neuropathic pain can manifest as vocalisation, reluctance to exercise and fearful behavioural changes that tend to be made worse with anything that raises CSF pressure, such as exercise, coughing and defecation. Owners often note their pets to be painful when they are picked up. Excessive grooming or "phantom scratching"/"air guitar playing" behaviour is often described and probably represents dysaesthesia, i.e. spontaneous abnormal sensations (Figure 6).[1] Affected dogs occasionally have a trigger spot in the cervical region that when touched elicits this behaviour, which may represent a form of allodynia; i.e. pain in response to a normal stimulus.[1]

Other clinical signs relate to abnormal postural information, for example scoliosis, ataxia, muscle wastage and thoracic limb paresis. As the syrinx may be asymmetrical in shape, postural deficits are often lateralized.[1,7,66]

Figure 6: A CKCS with CM/SM with 'phantom' pruritus; scratching without contacting the skin, often associated with gentle touch applied to a focal spot in the cervical region.

On clinical examination cervical spinal pain is the most common finding, but pain can often be elicited along the entire spine, as the distribution of syringes tends to be multi-focal along its length.[2] This may raise the suspicion of SM versus more focal causes of spinal pain. Postural reactions may be delayed in affected dogs, particularly hopping tests.

Important differential diagnoses include dermatological diseases, intervertebral disc disease, discospondylitis, spinal neoplasia, subarachnoid diverticulae, atlantoaxial subluxation and inflammatory central nervous system diseases, whether sterile, such as granulomatous meningoencephalomyelitis, or infectious in the case of distemper, neospora and toxoplasma myelitis. Occasionally one or more diseases occur concomitantly and may result in acute decompensation (for example, mild SM and fibrous intervertebral disc protrusion), which has implications for management of the condition. CKCS are also overrepresented for primary secretory otitis media (PSOM), a middle ear condition analogous to glue ear in children that

can cause head shyness, bulla pain, vestibular signs and potentially scratching behaviour that can confuse diagnosis.[68] The pathogenesis of PSOM in CKCS may be related to brachycephalic conformation and stenosis of the eustachian tube; one retrospective study found CKCS with bilateral middle ear effusions to have a significantly greater thickness of the soft palate and a reduced cross-sectional area of the nasopharynx compared with CKCS without middle ear effusions.[69]

Diagnostic tests

Plain spinal radiographs may be useful in assessing for the presence of atlantoaxial subluxation and some causes of diffuse spinal pain that produce lytic lesions such as vertebral neoplasms (osteosarcomas, multiple myeloma) or infections (osteomyelitis, discospondylitis). Analysis of CSF typically reveals a mild mononuclear pleocytosis and elevated protein concentration in patients with CM/SM compared with CM alone.[70] Lumbar myelography can only detect SM when contrast is inadvertently injected into the central canal. Computed tomography (CT) may detect large syringes but MRI is traditionally considered a better modality for these cases.

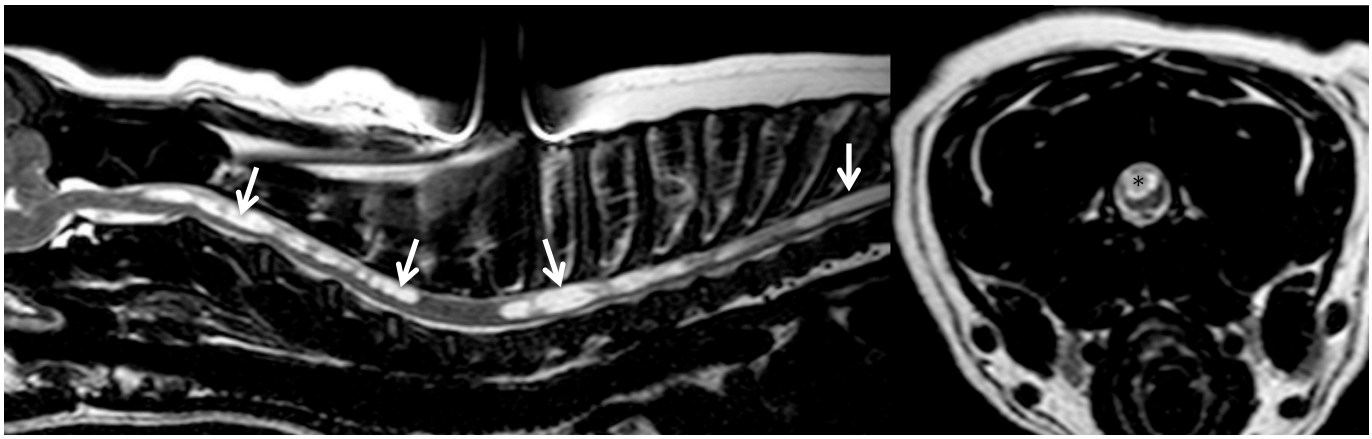

Figure 7: Sagittal T2-weighted MRI of the cervicothoracic spine of a CKCS (left) displaying the diffuse nature of syringomyelia (arrows) secondary to Chiari-like malformation. A transverse T2-weighted MRI section (right) through a syrinx shows its large dorsal distribution and asymmetry (asterisk).

Despite its multifocal nature, SM is most often found with MRI of the cervical spinal cord, but images should include the whole spine if clinically indicated (Figure 7).[2] Imaging features reveal indentation of the cerebellum by the occipital bone and herniation of the cerebellar

vermis with fluid filled dilatations of the spinal cord that are hyperintense on T2-weighted images and hypointense on T1-weighted images to spinal cord tissue.[25] There is often surrounding oedema evident as poorly delineated T2-weighted hyperintensity. MRI often reveals other related problems such as enlarged ventricles (ventriculomegaly) and intra-cranial (quadrigeminal) fluid accumulations ("cysts").

Treatment of CM/SM in dogs

Little is known about the natural progression of CM/SM in dogs. Typically SM is described as a progressive condition, as evidenced by the increasing disease prevalence with age.[29] Medical and surgical treatment options exist for dogs with CM/SM. Many of the suggested medical treatments are not licensed and the full pharmacokinetic profiles of some drugs are not established in dogs. In addition, there is no scientific evidence to prove the efficacy of these drugs in the management of neuropathic pain associated with CM/SM in dogs.[40,67,71] The long-term outcome of medical management requires investigation. Medical treatment is generally aimed at one of three targets:

Reducing inflammation

Spinal cord injury as a result of CSF flow disturbances has an inflammatory component that may be mediated by cytokines or neurotransmitters such as substance P.[66] There is a rationale therefore for treating patients with CM/SM with short courses of non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids.[40] As the cyclooxygenase-2 (COX-2) enzyme contributes to the development in neuropathic pain, COX-2 specific NSAIDs such as firocoxib may be of additional benefit.[72]

Corticosteroids such as prednisolone can almost be considered in their own class, as they have several modes of action that include reducing inflammation medicated via the arachidonic acid pathway[73], reducing neurotransmitter transcription[74] and CSF production. However their side effects often outweigh the perceived benefits to owners. Corticosteroids should be considered in conjunction with other oral medications described below if the clinical signs prove difficult to control and surgical treatment is not possible.

Reducing CSF production

Proton pump inhibitors (omeprazole), diuretics (furosemide), carbonic anhydrase inhibitors (acetazolamide) and corticosteroids reduce CSF production and lower intra-cranial pressure that may aid in control of SM related pain.[40] However, their long-term use is often associated with side effects and metabolic derangements, therefore they are not recommended as first line treatments any more due to the relative success of other treatments such as NSAIDs.

Neuropathic pain

Neuropathic pain does not tend to respond to traditional opioid analgesics.[75] In the acute setting however methadone may be useful due to its antagonism of the N-methyl-D-aspartate (NMDA) receptor that is often responsible for CNS sensitization.[67] Other than NSAIDs and corticosteroids, some anti-convulsant treatments have benefits in reducing neuropathic pain syndromes such as allodynia[76], typically by reducing the release of neurotransmitters such as glutamate and substance P in the damaged dorsal horn of the spinal cord[77]. The best example is gabapentin, an analogue of gamma aminobutyric acid (GABA), which acts via modulation of the alpha 2-delta subunit of voltage-gated calcium channels.[78] Gabapentin has been found to be more useful for the treatment of pain than epilepsy in people.[79] Side effects are rare in dogs but typically include sedation and ataxia. Other side effects reported in people include weight gain and swollen extremities, however as yet this has not been displayed in canine patients. Pregabalin is an alternative to gabapentin for non-responders and may even be more efficacious, however the cost of this drug is often prohibitive for its use in small animal practice. As with most chronic drug therapies, the lowest daily dose required to control the symptoms is ideal.

Surgical treatment

Surgical management (craniocervical decompression) is frequently performed in people with Chiari-I malformation, with and without SM, to alleviate clinical signs.[80] In theory decompressing the foramen magnum by suboccipital craniectomy should restore normal CSF flow and resolve SM. In addition, patients with hydrocephalus can have CSF shunts placed to facilitate physiological CSF drainage levels, normally from the lateral ventricles to the peritoneal cavity. Several surgical techniques for CM/SM have been described in dogs; the

most commonly employed technique involves suboccipital craniectomy, dorsal laminectomy of the first cervical vertebra and placement of a synthetic cranioplasty over the defect to minimise adhesions.[30,81] Following surgery, 80% of dogs improved, but there was no resolution of the syringes, and nearly all dogs continued to exhibit clinical signs suggestive of neuropathic pain postoperatively. [30,81-83] Additionally, 25–47% of the operated dogs showed recurrence or deterioration of the clinical signs within 0.2–3 years after surgery.[30,81-83] This may be related to important differences in the pathogenesis between humans and dogs that do not result in SM resolution after surgery and are as yet undetermined.

Conclusions

SM is a relatively common source of neuropathic pain and other neurological deficits in small breed dogs, particularly CKCS. The pathogenesis of SM secondary to CM is suspected to be associated with abnormal craniocerebral morphology, cerebellar pulsation and CSF hydrodynamics. Morphometric studies are required to investigate the relationship between cranial and cerebral volumes and the presence and severity of syringes. New advanced magnetic resonance imaging techniques are required to evaluate the role of cerebellar pulsation in the formation of syringes. There is insufficient knowledge concerning the natural progression of SM secondary to CM in dogs, radiologically and clinically. It is currently unknown whether the medical treatment of canine CM/SM is successful and further study is required.

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SECTION II

SCIENTIFIC AIMS

SCIENTIFIC AIMS

Canine syringomyelia (SM) is a debilitating spinal cord disease affecting multiple small dog breeds. SM is most often diagnosed in association with 'Chiari-like' malformation (CM) of the craniocervical junction. The disease complex has a particularly high prevalence in Cavalier King Charles Spaniels (CKCS). At this time the pathogenesis of SM secondary to CM is poorly understood. Whilst multiple surgical procedures for the treatment of CM/SM have been proposed, there is limited knowledge concerning the natural progression and medical treatment of canine CM/SM.

Therefore, the general aim of our study was to gain more information about the pathogenesis, natural progression and treatment of canine CM/SM using CKCS as a disease model. The specific aims of this study were:

1. To examine the relationship between cranial and cerebral morphology to syringomyelia lesions
2. To evaluate the relationship of CM/SM to clinical signs in dogs
3. To evaluate the long-term outcome on medical management of CM/SM in dogs
4. To determine the natural progression of CM/SM in dogs with time
5. To evaluate whether cerebellar pulsation occurs in dogs with CM and whether cerebellar pulsation is related to SM presence and severity

SECTION III

MATERIALS AND METHODS

MATERIALS AND METHODS

In this thesis, Cavalier King Charles Spaniels (CKCS) were used as the main study population. This is because Chiari-like malformations (CM) are ubiquitous in the breed and syringomyelia (SM) is highly prevalent.

48 CKCS were prospectively and 75 CKCS were retrospectively studied. For all prospectively included dogs, written owner consent was obtained prior to study enrollment.

The information regarding the 75 **retrospectively** included CKCS was retrieved from the clinical databases of three institutions: the Queen Mother Hospital for Animals of the Royal Veterinary College (RVC), University of London, UK; Stone Lion veterinary Hospital, London, UK; and the Animal Health Trust, Newmarket, UK.

Other than CKCS, retrospective studies compared CKCS with SM to one or several of the following control groups:

1. ***CKCS without SM***

   As previously mentioned this breed was chosen due to the high prevalence of SM and the ubiquitous nature of CM. However, these dogs cannot be considered clinically 'normal' given that CM alone may be associated with clinical signs.

2. ***Clinically normal small breed dogs with brachycephalic conformation***

   These breeds were selected as they have a similar physical conformation to CKCS and CM is reportedly associated with brachycephalism. In addition, dogs of these breeds have been sporadically reported to develop SM secondary to CM.

3. ***Clinically normal Labradors***

   Labradors were selected as controls as they are a breed with mesaticephalic conformation and have never been reported to develop SM secondary to CM.

Information obtained retrospectively included signalment, clinical history, bodyweight, imaging studies and final diagnosis.

Retrospectively recruited dogs were primarily used for morphometric studies. Case numbers and signalement features varied between these studies due to the availability of imaging studies. Imaging studies must have included sagittal and transverse T2-weighted turbo spin echo magnetic resonance imaging (MRI) sequences from the cribiform plate to the third cervical spinal cord segment. CKCS were selected for inclusion if they had radiological findings consistent with CM (indentation of the cerebellum and herniation of the cerebellar vermis into the foramen magnum). In CKCS, the presence of CM was confirmed by a European neurology or diagnostic imaging specialist. Dogs with concurrent diagnoses of space occupying lesions or lesions associated with raised intracranial pressure were not included.

Morphometric studies utilised three-dimensional volumes generated using manual image segmentation techniques, for comparison between groups. These dogs participated in the studies described in section IV, chapters 1.1; 1.2; 1.3; 2.1; 3.1 and 4.1.

A **prospective** cohort study was performed following 48 CKCS. CKCS between the ages of one and 13 years (median 46 months), and bodyweight of 4–13 kg (median 9.5 kg), were recruited from the general population in the UK by advertising through the veterinary press and national CKCS health societies, into a two-week trial of a novel neuropathic pain medication, which was performed under the Animals (Scientific Procedures) Act 1986, and was approved by the institution's ethics committee. After the end of the drug trial, dogs were followed prospectively for a mean period of 39 (±14.3 SD, 4–107) months use in chapter 2.2 of section IV.

SECTION IV

RESULTS

Chapter 1

RELATIONSHIP OF ABNORMAL CRANIOCEREBRAL MORPHOLOGY ASSOCIATED WITH CHIARI-LIKE MALFORMATION TO SYRINGOMYELIA IN DOGS

Chapter 1.1

RELATIONSHIP OF BRAIN PARENCHYMA IN THE CAUDAL CRANIAL FOSSA AND VENTRICLE SIZE TO SYRINGOMYELIA IN CAVALIER KING CHARLES SPANIELS

RELATIONSHIP OF BRAIN PARENCHYMA IN THE CAUDAL CRANIAL FOSSA AND VENTRICLE SIZE TO SYRINGOMYELIA IN CAVALIER KING CHARLES SPANIELS

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Summary

The objective of this study was to assess if the volume of the caudal cranial fossa (CCF), parenchyma within the caudal cranial fossa (CCFP), or ventricles (V) are associated with the presence of syringomyelia (SM) in Cavalier King Charles Spaniels (CKCS) with Chiari-like malformation (CM). In addition, we evaluated whether volumes are associated with transverse syrinx width.

Magnetic resonance images of 59 CKCS with CM were retrospectively reviewed and grouped with or without SM. 3D-images were created and volumes of the fossae, brain parenchyma and ventricular system were calculated from which percentages of CCF, CCFP and V were created. If present, syrinx size was measured from its maximal transverse width. The percentages were statistically compared between groups, and correlation between percentages and syrinx dimensions was made.

CKCS with SM had significantly higher CCFP ($p=0.0001$) and V ($p=0.0003$) to those without but no significant difference in CCF ($p=0.925$). There was a positive correlation between CCFP and syrinx width (Pearson $r=0.437$) and ventricle size to syrinx width (Spearman $r=0.466$).

A more marked overcrowding of the CCF is associated with SM, which may explain the high incidence of SM in CKCS with CM. The association between ventricle and syrinx dimensions supports the theory that SM development is the result of altered cerebrospinal fluid dynamics.

Introduction

Chiari like-malformation (CM) and syringomyelia (SM) is a painful and debilitating disease complex described in dogs, commonly in the Cavalier King Charles Spaniel (CKCS), that is characterised by foramen magnum cerebellar herniation[1,2] and the development of fluid filled cavities (syringes) within the spinal cord.[3] Maximum syrinx width is a strong predictor of pain and spinal cord dorsal horn damage may result in neuropathic pain.[4] The pathophysiology of syrinx formation remains unclear; aberrations of cerebrospinal fluid dynamics caudal to obstructions of the subarachnoid space have been implicated in their development.[5]

CMI in humans are thought to be caused by occipital bone hypoplasia, which results in a shallow and overcrowded posterior fossa.[6-8] Several morphometric analyses of CKCS skulls have been made, particularly the caudal cranial fossa, which contains the cerebellum, pons and medulla, extending from the petrosal crests and dorsum sellae to the foramen magnum.[9] CKCS appear to have a shallower caudal cranial fossa and have abnormalities of the occipital bone when compared to mesaticephalic dogs.[10] However, Cerda-Gonzalez and others[11] showed that in CKCS with CM, caudal fossa size was not associated with SM. Those showing clinical signs did have a smaller caudal fossa volume compared to neurologically normal CKCS. Additionally, Schmidt and others[12] reported no difference in the caudal fossa volume of CKCS compared to brachycephalic breeds. This supports a recent study suggesting that a smaller caudal fossa may not predispose to SM on its own, as small breeds such as Jack Russell Terriers were found to have proportionately a similar size caudal cranial fossa as CKCS for their weight but did not show any signs of CM-SM.[13]

Cross and others[13] showed that CKCS have proportionately the same volume of parenchyma within the caudal cranial fossa as Labradors (a mesaticephalic breed), suggesting a mis-match in volumes and over-crowding of the caudal cranial fossa. As yet no evidence exists to suggest that the volume of parenchyma within the caudal fossa is related to the presence of SM.

Clinical manifestations of CM may be related to cerebrospinal fluid disturbances including hydrocephalus.[7] A unifying hypothesis for hydrocephalus, CM and SM has been suggested.[14] Ventriculomegaly has been reported in some dogs with CM[1] and one study documented that

94.4% of dogs with SM or central canal dilation had moderate or severe ventriculomegaly.[15] More severe SM occurs in dogs less than two years of age and this is often associated with hydrocephalus.[16]

Here we present a retrospective volumetric analysis of MR images to assess if over-crowding of the caudal cranial fossa with hindbrain parenchyma, not the relative size of the caudal cranial fossa alone, is associated with the presence of SM in CKCS affected by CM. Additionally we investigated whether the volume of the ventricular system relative to total cerebral volume is associated with the presence of SM.

Materials and Methods

Electronic patient records[i] from May 2004 to August 2008 stored at the Royal Veterinary College (RVC) were retrospectively reviewed to identify CKCS diagnosed with CM on MRI scans[ii] (3mm slice thickness) by board-certified radiologists. CM was defined as evidence of cerebellar herniation or indentation by the supraoccipital bone, irrespective of the presence of SM.[2] T2-weighted transverse and mid-sagittal MRI scans were reviewed using Image Viewer software[iii] to assess for suitability for inclusion. Transverse scans must have included the cribiform plate cranially and the 1st cervical spinal cord segment caudally. Dogs with parenchymal space-occupying lesions or other conditions thought likely to raise intra-cranial pressure were excluded. Mid-sagittal scans had to include the first three vertebrae of the cervical spine. The sex and age of the dog at the time of scanning were also recorded.

Each dog was assigned a study number so that volume measurements on transverse series could be made blinded to the presence of SM. Suitable series were exported to Mimics®[iv] and measured as described in detail in Cross and others (Figure 8)[13]. Image threshold was set to zero and contrast altered to include all brain parenchyma. Masks were created in two dimensions from individual slices using a pen tablet[v] for accuracy of free hand measurements.

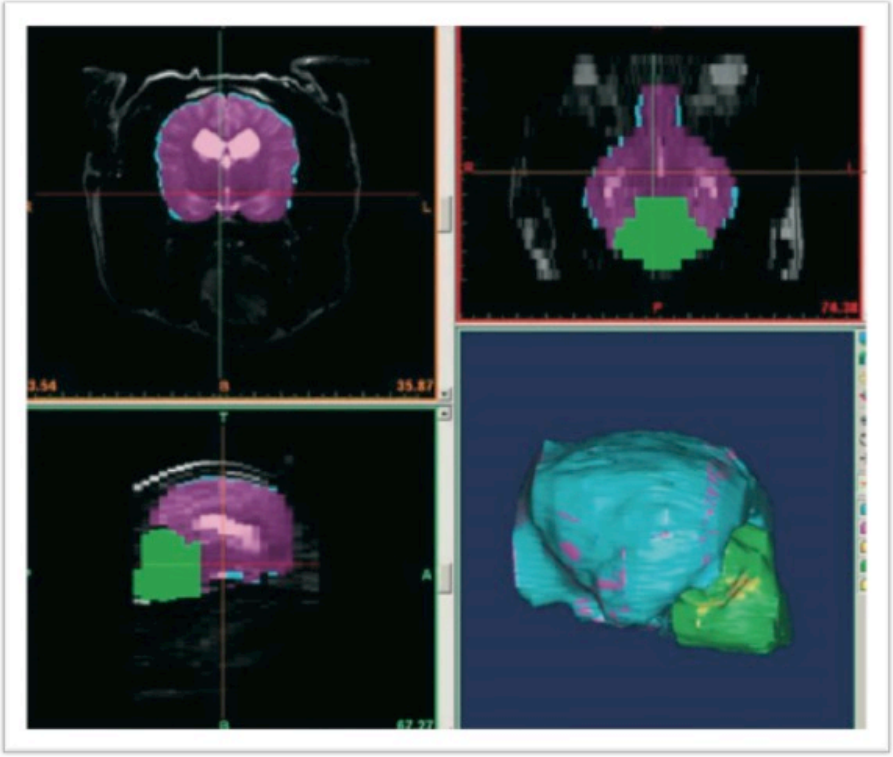

Figure 8. Screenshot from mimics®. Segmentation masks were drawn by hand for various craniocerebral volumes. Key: Blue = rostral and middle cranial fossa; Purple = parenchyma within the rosral and cranial fossae; Green = caudal cranial fossa

All masks were drawn by the same observer (CD) standardising the error.

- rostral and middle cranial fossa (a) : defined as the volume of the internal compartment of the neurocranium, delineated by the internal surface of the sphenoid bones, cribiform plate, frontal, parietal and temporal bones, rostral to the tentorium cerebelli and the dorsum sellae, such that is contains the prosencephalon and mesencephalon.
- parenchyma within the rostral and middle cranial fossae (b) : neural parenchyma contained within the rostral and middle fossa as defined above, excluding the cerebral sulci and ventricles.
- caudal cranial fossa (c) : defined as the volume of the internal compartment of the neurocranium delineated by the internal surface of the occipital bone, caudal to the dorsum sellae and tentorium cerebelli and rostral to the foramen magnum, such that it contains the cerebellum, pons and medulla.
- parenchyma within the caudal cranial fossa (d) : neural parenchyma contained within the caudal cranial fossa as described above, excluding the 4th ventricle.
- ventricle system (e) : the ventricular system from the olfactory recess to the central canal at the level of the foramen magnum.

Volumes of the above were calculated from three-dimensional models reconstructed from individual masks in Mimics®[iv]. The caudal cranial fossa was defined as the tentorium cerebelli cranially and the foramen magnum caudally. Percentages of caudal cranial fossa (CCF), parenchyma within the caudal cranial fossa (CCFP), and ventricles (V) were calculated from these volumes (table 1).

The presence of SM was assessed by a second observer (HV). A syrinx was defined as a fluid-containing cavity within the spinal cord parenchyma with a transverse diameter of greater than or equal to 2mm. If SM was present, the size of the cervical syrinx was measured as the maximal width from transverse T2 weighted images[iii] as in Rusbridge and others[4].

Table 1 Explanation of the calculation of CCF, CCFP and V, where a= rostral and middle fossae, b= parenchyma within the rostral and middle fossae, c= caudal cranial fossa, d= parenchyma within the caudal cranial fossa, e= ventricles

CCF: the percentage of the total neurocranium cavity composed of caudal cranial fossa	$\frac{c}{a+c} \times 100$
CCFP: the percentage of the caudal cranial fossa filled with hindbrain parenchyma	$\frac{d}{c} \times 100$
V: the percentage of total brain parenchyma composed of the ventricular system	$\frac{e}{b+d} \times 100$

Dogs were subsequently split into two groups; with SM and without SM. Statistical analysis was performed with a commercial software package[vi]. Data sets (CCF, CCFP and V) were assessed for normality of distribution with the Shapiro-Wilk W test. Means and standard deviations were calculated for normally distributed continuous data, and medians and ranges were determined for non-normally distributed data. Depending on whether data sets were normally distributed or not, Independent sample t-test or Mann-Whitney U- test were used for statistical evaluation respectively. A separate analysis of CCFP without the 4th ventricle was also made.

In the SM group, the association between over-crowding of the caudal cranial fossa (CCFP) and syrinx size was evaluated by obtaining a Pearsons's rank correlation coefficient. Similarly, the association between ventricle size, V, and syrinx size was evaluated by obtaining a correlation coefficient by Spearman's rho. All tests were used two-sided and $p < 0.05$ was considered significant.

Results

Fifty-nine CKCSs were included in the study; SM was present in 42/59 (71%) dogs. 31/59 dogs (53%) were female. The data collected for dog's ages was normally distributed. There was no significant age difference (p=0.923) between dogs with (60.3 ± 33.8 months; range 6.8-128.9 months) or without SM (59.3 ± 37.1 months; range 3.9-122.8 months).

An example of the three-dimensional reconstructions of the ventricular system can be seen in figure 9. The data sets for CCF, CCFP were normally distributed in both groups. The data set for V was not normally distributed in the SM group. Two outliers with extreme ventriculomegaly were removed from the analysis to prevent statistical bias.

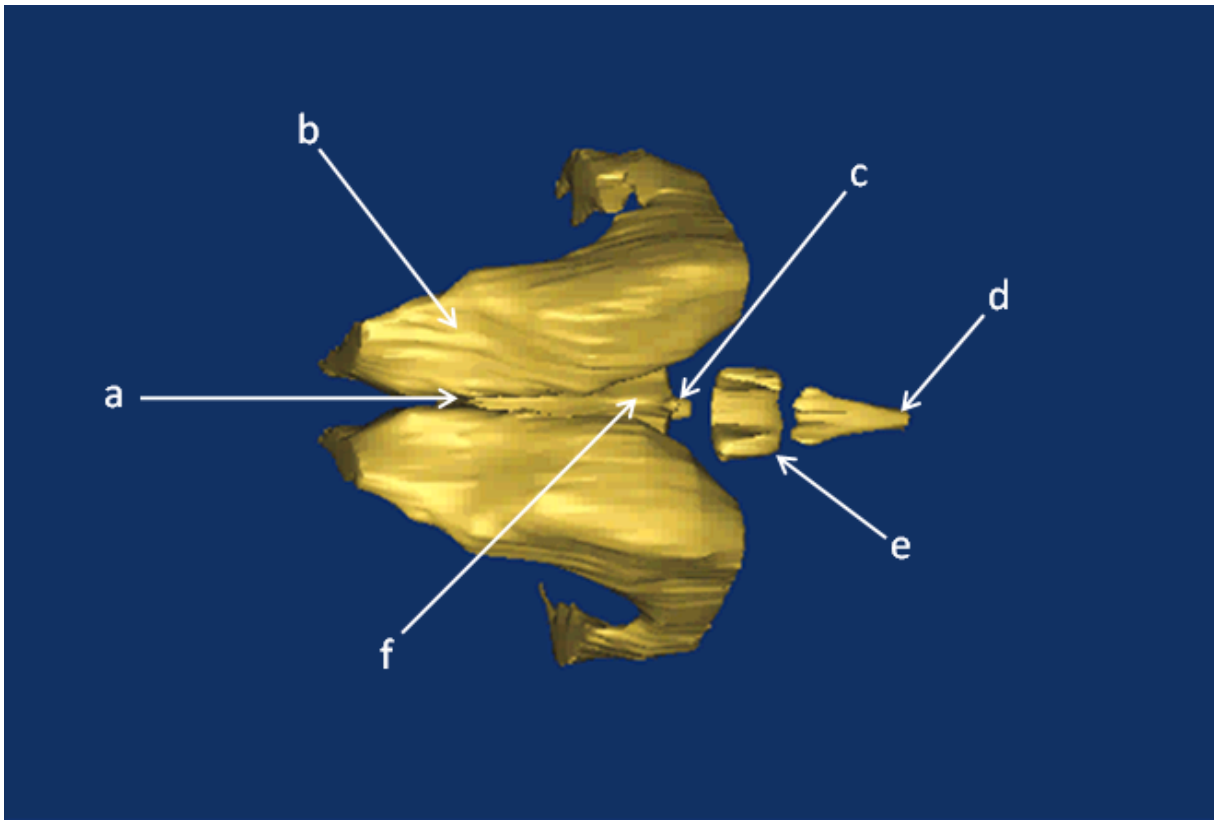

Figure 9. 3D reconstruction of ventricular system from transverse MR series; a = interventricular foramen, b = lateral ventricle, c = mesencephalic aqueduct, d = central canal, e = fourth ventricle, f = third ventricle.

CCF did not differ significantly (p=0.925) between dogs without (14.8 ± 0.3 %) or with (14.8 ±0.2%) SM. However, there was a significant difference (p=0.0001) in CCFP between those without (87.4 ± 0.5%) or with (89.9 ± 0.2%) SM (figure 10). When this analysis was performed with the 4th ventricles removed from the parenchyma volume, it was still significant (p=0.011) between those without (86.1 ± 0.8%) or with (88.2 ± 0.4%) SM. There was also a significant difference (p=0.0003) in V between those without (2.31, 0.37-6.44 %) and with (5.813, 0.93-17.3 %) SM (figure 11).

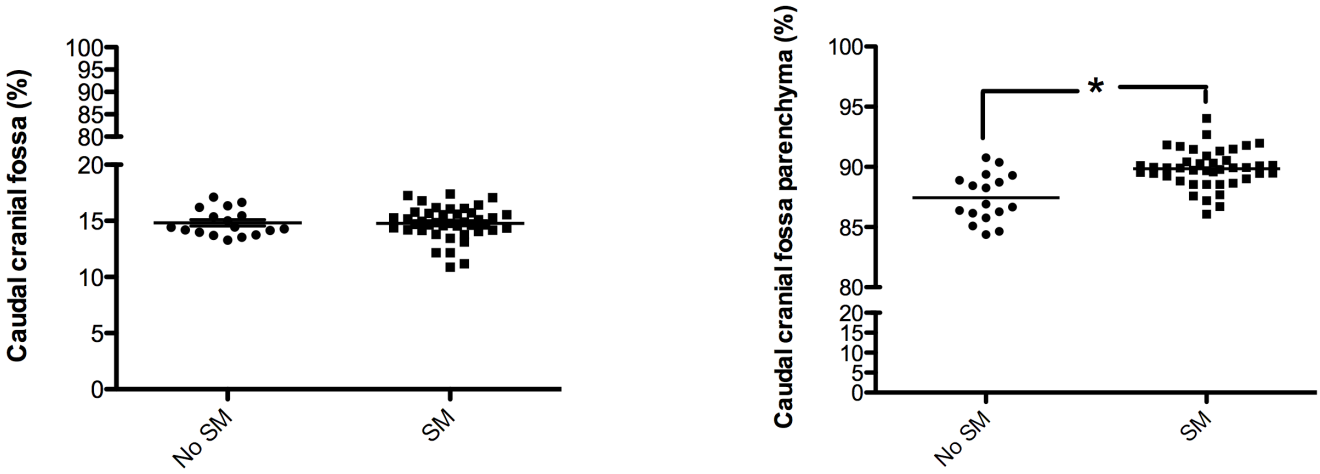

Figure 10. Scatter plots showing distribution of CCF and CCFP between CKCS with syringomyelia (SM) or without (No SM) (bar represents mean; asterisk, p<0.05)

In the SM group a significant positive association of CCFP with maximum transverse syrinx width was found (Pearson r = 0.437). When a syrinx was present the ventricle dimensions, V, were found to have a positive correlation with maximum transverse syrinx width (Spearman r = 0.466).

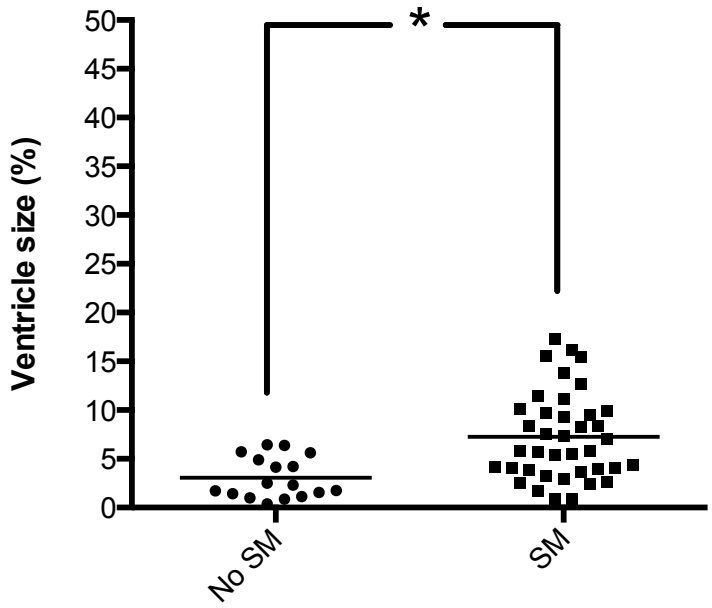

Figure 11. Scatter plot-showing distribution of ventricle size, V (%), between CKCS with syringoymyelia (SM) or without (No SM) (bar represents median; asterisk, $p<0.05$)

Discussion

This study is consistent with previous findings that implied caudal cranial fossa dimensions are not associated with SM in CKCS.[11,12] CKCS have a similar caudal cranial fossa volume to other small breed dogs but have proportionately the same volume of parenchyma within the caudal cranial fossa as Labradors.[13] This study suggested that CM might be the result of paraxial mesoderm insufficiency during embryogenesis as suggested in the human literature.[6,17] These theories suggest that there is failure of communication between the paraxial mesoderm and the cranial somites with the closing neural tube, resulting in loss of coordination between skull and brain growth (unsegmented paraxial mesoderm forms the supraoccipital bone, segmented somitic mesoderm forms the exoccipital and basioccipital bones). Mesodermal growth supports and helps to tether the neural tube to facilitate the closure process. Genetic and environmental influences have a variable effect on mesodermal growth, influencing caudal cranial fossa size. Restricted growth of the posterior fossa in humans is suggested to be associated with other congenital anomalies such as spina bifida and anencephaly.[14] It could also be theorised that overgrowth of the cerebellum causes the mis-match as CKCS have proportionately more hindbrain parenchyma than other small breed dogs.[13] Early growth plate closure may result in CM, as despite the dynamic nature of osseous tissue it would be unable to accommodate the developing brain. Further study is required to better understand the development of CM. This study now provides evidence that more marked brain and skull volume mis-matches result in syringomyelia, as a higher parenchyma percentage (CCFP) is associated with the presence of a cervical syrinx. This could explain the high incidence of SM secondary to CM in CKCS reported by Rusbridge and Knowler.[3]

Syrinx formation has been attributed to disturbances in CSF dynamics caused by obstruction of the foramen magnum by the herniated cerebellum. CSF displaced by each systolic cycle through the compressed subarachnoid space results in a high velocity jet[18], creating a hydrostatic pressure differential between the spinal cord and subarachnoid space favouring fluid accumulation and, eventually, SM.[5] Although statistically significant, the difference in means for CCFP between the two groups appears small. It is therefore hypothesised that only a small difference in parenchymal volume is necessary to influence the development of a cervical syrinx. Furthermore, as the total volume of parenchyma and ventricular CSF within the caudal cranial fossa correlates with cervical syrinx dimensions, it can be hypothesised that

more marked over-crowding of the caudal fossa results in greater compression of the subarachnoid space and subsequent syrinx dilatation.

It has previously been reported that some dogs with CM/SM also have ventriculomegaly[1,15], but there is currently no volumetric evidence for this. The clinical relevance of ventriculomegaly is unclear as there is no correlation between neurological signs and ventricular dilatation[2], but it has been suggested that some CKCS with ventriculomegaly secondary to caudal cranial fossa crowding may be presented with seizures.[5] Compression of the subarachnoid space at the foramen magnum results in turbulent CSF flow, which can result in SM[19] and ventricular dilatation.[16,20] Venous narrowing at the jugular foramina associated with a small skull base can lead to elevated venous pressure.[21] The impairment of CSF absorption reported secondary to raised venous pressures can result in communicating hydrocephalus.[22] It has been suggested that this could be a contributory mechanism to the development of SM in Griffon Bruxellois that had no evidence of CM.[15] This study shows that there is a significant difference in the size of the ventricular system between dogs with and without syringomyelia. Additionally, ventricle dimensions are positively correlated with syrinx width, supporting the theory that the clinical manifestations of CM are related to CSF disturbances.[7] It may be that caudal cranial fossa over-crowding does not just affect CSF flow, but also reduced absorption due to a raised intra-cranial pressure. Further studies are required to assess if SM is a primary disease of reduced CSF absorption.

It has previously been suggested that SM is a disease that can have a late onset. Results from centres offering low cost screening for SM report that it is not unusual for syringes to be detected in middle aged dogs that were free of SM as young adult dogs (unpublished data). In this study there was a wide range of ages within the two groups, but no significant difference between them. This is important as the study includes dogs from each of the three clinical presentations suggested by Rusbridge[23] and it would appear syrinx formation secondary to CM can occur at any age. Further studies comparing old unaffected dogs to young affected dogs would be useful to assess if individuals with early onset SM have a greater parenchymal volume or ventricle size, such that these measurements would be useful as a screening test.

CKCS with other diseases that may affect intracranial pressure (including space occupying lesions) were excluded as this could potentially contribute to cerebellar herniation, affect parenchymal volume measurements and ventricle dimensions. MR images must have

included the entire cerebrum as percentages were calculated relative to the size of the dog so this did not influence outcome. T2 weighted scans were used to highlight CSF and provide a contrast between the brain parenchyma and bone of the skull. Mimics®[iv] facilitated three dimensional reconstructions of cerebral volumes. These volumes are more accurate and reproducible than two-dimensional methods from mid-sagittal scans as used in previous studies[11,24] and therefore 3D morphometric measurements have been used in human studies.[6,25] Thresholds could not be used in Mimcs®[4] due to the similarity in appearance of brain parenchyma and surrounding soft tissues. In this study the measurer of volumes was blinded from the presence of SM as not to introduce bias. Syrinx dimensions were not measured in a similar volumetric fashion, as scans did not normally include the entire cervical syrinx in a transverse plain. Additionally, recent evidence suggests that estimation of only a cervical syrinx underestimates the severity of the condition given that SM frequently affects multiple parts of the spinal cord.[26] Measuring the maximal transverse width of a syrinx is a published estimation of severity that is correlated with clinical signs.[4]

Conclusions

In this study, we have shown that a mild increase of overcrowding of the caudal cranial fossa is associated with SM. Furthermore there is an association between ventricle and syrinx dimensions which supports the theory that SM development is the result of altered cerebrospinal fluid dynamics. The mild but significant difference of CCFP in CKCS with or without SM reflects the clinical difficulty in identifying those dogs that will develop SM. This study does not correlate the presence of SM with clinical signs or disease progression, which would be important for guiding the choice of therapeutic intervention. It therefore remains appropriate to continue to make clinical decisions on the basis of severity of clinical signs. Further studies evaluating these measurements as prognostic indicators are therefore warranted.

Footnotes

[i] Rx Works 3.8.1, RxWorks Inc., 206

[ii] Phillips NT Intera 1.5t MRI scanner slice thickness 6 mm

[iii] Image Viewer 3.7.03, Visbion, 2006iv

[iv] Mimics ® 10.11, Materialse n.v., 2007

[v] XP-Pen ® v.4.04, P-actove Co. Ltd., 2003

[vi] SPSS for Windows ® v 17.0, SPSS inc., 2008

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Chapter 1.2

MORPHOMETRIC ASSESSMENT OF CRANIAL VOLUMES IN AGE-CONTROLLED CAVALIER KING CHARLES SPANIELS WITH AND WITHOUT SYRINGOMYELIA

MORPHOMETRIC ASSESSMENT OF CRANIAL VOLUMES IN AGE-CONTROLLED CAVALIER KING CHARLES SPANIELS WITH AND WITHOUT SYRINGOMYELIA

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Adapted from: Driver CJ, Rusbridge C, McGonnell IM, Volk HA. Morphometric Assessment of Cranial Volumes In Age-Controlled Cavalier King Charles Spaniels With And Without Syringomyelia. Veterinary Record 2010 167;978-979.

Summary

In this retrospective study we used established volumetric measurements of the ventricular system and cranial fossae to assess if there is a significant difference between young CKCS with CM/SM and old CKCS with CM that are considered unlikely to develop SM.

Electronic patient records were reviewed for CKCS <2years with CM/SM and >5years with only CM for which transverse T2 weighted MR images of the whole brain were available. CKCS with diseases that might affect volumetric measurements were excluded. Images were exported to medical imaging software (Mimics v12.0, Materialise n.v, 2007), volumetric analysis was performed based on three-dimensional reconstruction with masks from individual slices.

There was a significant difference in ventricular dimensions between affected young and unaffected old CKCS. Similarly the volume of parenchyma in the caudal cranial fossa was significantly different between the groups Furthermore affected young dogs had a significantly smaller caudal cranial fossa percentage than unaffected old dogs.

This work indicates that severe SM affecting young dogs is associated with more marked volume mis-matches and ventriculomegaly as these changes are not present in older, unaffected dogs. As well as providing evidence for the pathogenesis of this disease, this information could potentially be used to provide better screening of older dogs.

Introduction

Morphological abnormalities of the caudal cranial fossa and craniocervical region, termed Chiari-like malformation (CM), are almost ubiquitous in Cavalier King Charles Spaniels (CKCS).[1] Syringomyelia (SM) is a painful condition that is commonly associated with CM in this breed.[2] CKCS with CM/SM have more brain parenchyma within the caudal cranial fossa and also display ventriculomegaly compared to CKCS with CM alone.[3] One study suggested that CKCS with CM/SM have a smaller relative caudal fossa area, measured on sagittal T2 weighted midline brain images compared to control dogs such as Labradors.[4] However several other studies have failed to find this association between CKCS with and without SM in three dimensions.[1,3,5] This may be due to failure to identify the control population correctly, as some of the younger CKCS might develop SM later in life. Crucially, a volumetric study using age controlled groups to compare two extremes of disease status to identify associations between bony malformation and SM has not been undertaken.

This study describes a retrospective, volumetric comparison of three variables (caudal cranial fossae, parenchyma within the caudal cranial fossae, and ventricular dimensions) between CKCS with SM when less than 2 years of age, versus CKCS older than 5 years without SM.

Materials and Methods

Electronic patient records at the Royal Veterinary College (RVC) and Stone Lion Veterinary Hospital (SLVH) were retrospectively reviewed to identify CKCS with CM for which T2-weighted transverse and mid-sagittal MRI scans of the brain were available. Transverse scans must have included the cribiform plate cranially and the 3rd cervical spinal cord segment caudally. Dogs were grouped into those diagnosed with CM/SM when less than two years of age and those with only CM aged five years or older when scanned. Patients with parenchymal space-occupying lesions or other conditions thought likely to raise intra-cranial pressure were excluded. A syrinx was defined as a fluid-containing cavity within the spinal cord parenchyma with a transverse diameter of greater than or equal to 2mm.

Each dog was assigned a study number so that measurements could be made blinded to patient age and the presence of SM. Suitable series were exported to commercially available medical image software[i] and measured as previously described.[6] A series of masks were created in two dimensions from individual slices using a pen tablet[ii] by the same observer (CD) (a-e, table 2). Volumes of the above were calculated from three-dimensional models reconstructed from individual masks in Mimics®[i]. The caudal cranial fossa was defined as the tentorium cerebelli cranially and the foramen magnum caudally. Percentages of caudal cranial fossa (CCF), parenchyma within the caudal cranial fossa (CCFP), and ventricles (V) were calculated from these volumes (table 2).

Statistical analysis was performed with a commercial software package[iii]. Data sets (CCF, CCFP and V) were assessed for normality of distribution with the Shapiro-Wilk W test. Means and standard deviations were calculated for normally distributed continuous data, and medians and ranges were determined for non-normally distributed data. Independent sample t-test or Mann-Whitney U- test were used for statistical evaluation. Two-tailed tests were used in all cases, with differences associated with $P < 0.05$ considered significant.

Table 2: Explanation of the calculation of CCF, CCFP and V, where a= rostral and middle fossae, b= parenchyma within the rostral and middle fossae, c= caudal cranial fossa, d= parenchyma within the caudal cranial fossa, e= ventricles

CCF: the percentage of the total neurocranium cavity composed of caudal cranial fossa	$\frac{c}{a+c} \times 100$
CCFP: the percentage of the caudal cranial fossa filled with hindbrain parenchyma	$\frac{d}{c} \times 100$
V: the percentage of total brain parenchyma composed of the ventricular system	$\frac{e}{b+d} \times 100$

Results

Twenty-one CKCS less than 2 years were included, all dogs presented with clinical signs related to SM. Fourteen CKCS greater than 5 years were included, which presented for various reasons including idiopathic epilepsy (n=4), intervertebral disc disease (n=3), secretory otitis media (n=2), screening programme (n=3) and facial nerve paralysis (n=2). Affected young CKCS with SM had a smaller CCF, 14.50 ±0.37% than unaffected old dogs, 15.71 ±0.37% (p=0.03, t-test). Similarly CCFP was significantly different between the groups, 90.40±0.33% and 88.19±0.43% (p=0.0007, t-test). There was also a significant difference in ventricular dimensions between affected young, 10.24% (2.46-33.90%) and unaffected old 4.61% (0.88-13.4%) CKCS (p=0.0004, Mann-Whitney U test).

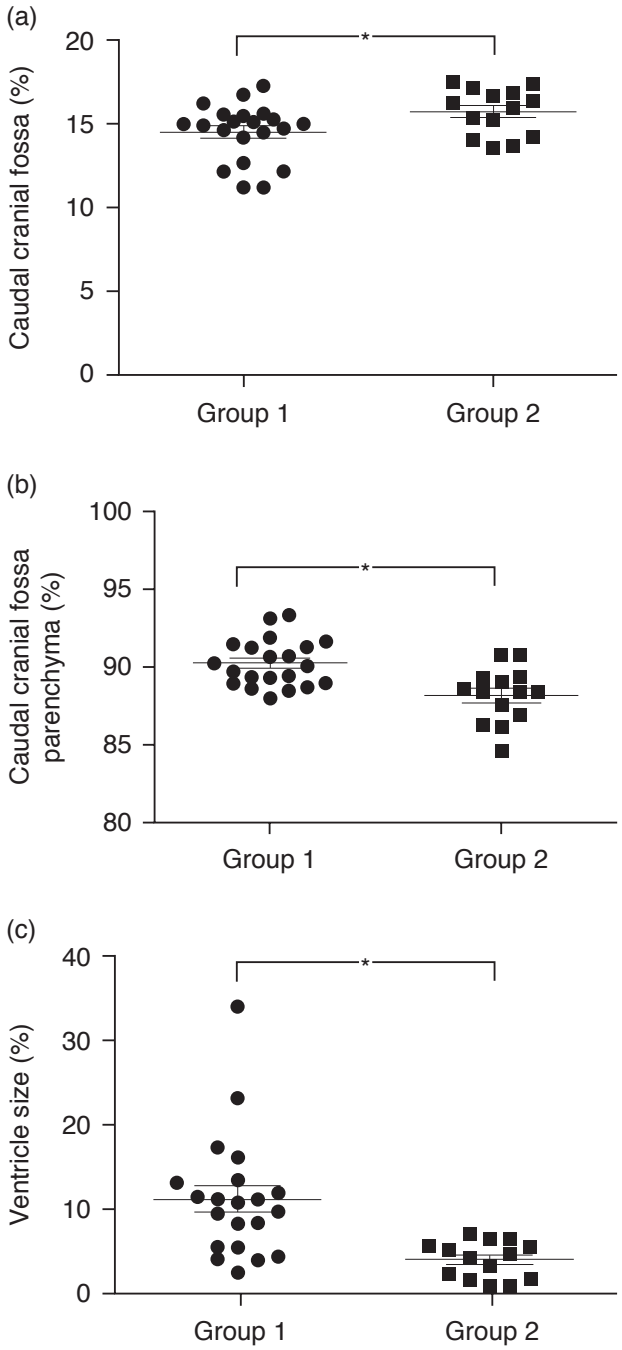

Figure 12. Scatter plots showing distribution of CCF (a), CCFP (b) and V (c) between CKCS with syringomyelia when less than 2 years of age (group 1) or without when greater than 5 years (group 2) (bars represent mean ± SD; asterisk, $p<0.05$).

Discussion

These results are consistent with previous findings that ventriculomegaly and a small, but significant increase in caudal fossa parenchyma is associated with SM.[3] In comparison to previous studies, our results suggest that the CCF volume is also significantly smaller in dogs with SM. This may be due to the fact that these previous studies were not selected by age and failed to distinguish individuals that may develop SM later in life from 'true' normal dogs that are unlikely to ever develop it. The development of SM may be related to a cumulative effect of these factors, however as there is little difference in the groups numerically it is unknown how this would affect CKCS clinically and biologically. Further cohort and longitudinal studies are required to assess if measuring the ventricular dimensions would be useful in discriminating CKCS which will develop SM from those which will not.

Footnotes

[i] Mimics ® 10.11, Materialse n.v., 2007

[ii] XP-Pen ® v.4.04, P-actove Co. Ltd., 2003

[iii] SPSS for Windows ® v 17.0, SPSS inc., 2008

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Chapter 1.3

INCREASE IN CEREBELLAR VOLUME IN CAVALIER KING CHARLES SPANIELS WITH CHIARI-LIKE MALFORMATION AND ITS ROLE IN THE DEVELOPMENT OF SYRINGOMYELIA

INCREASE IN CEREBELLAR VOLUME IN CAVALIER KING CHARLES SPANIELS WITH CHIARI-LIKE MALFORMATION AND ITS ROLE IN THE DEVELOPMENT OF SYRINGOMYELIA

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Adapted from: Shaw TA, McGonnell IM, Driver CJ, Rusbridge C, Volk HA. Increase in cerebellar volume in Cavalier King Charles Spaniels with Chiari-like malformation and its role in the development of syringomyelia. Plos One 7, e33660. doi:10.1371/journal.pone.0033660

Summary

The objectives of this study were to i) compare cerebellar volume in CKCS (a "high risk' group which frequently develops CM/SM), small breed dogs (medium risk – occasionally develop CM/SM), and Labradors (low risk – CM/SM not reported); ii) evaluate a possible association between increased cerebellar volume and CM/SM in CKCS; iii) investigate the relationship between increased cerebellar volume and crowding of the cerebellum in the caudal part of the CCF (i.e. the region of the foramen magnum).

Volumes of three-dimensional, magnetic resonance imaging (MRI) derived models of the CCF and cerebellum were obtained from 75 CKCS, 44 small breed dogs, and 31 Labradors. As SM is thought to be a late onset disease process, two subgroups were formed for comparison: 18 CKCS younger than 2 years with SM (CM/SM group) and 13 CKCS older than 5 years without SM (CM group). Relative cerebellar volume was defined as the volume of the cerebellum divided by the total volume of brain parenchyma.

Our results show that the CKCS has a relatively larger cerebellum than small breed dogs and Labradors and provide evidence that increased relative cerebellar volume in CKCS is associated with crowding of the cerebellum in the caudal part of the CCF. In CKCS there is an association between increased relative cerebellar volume and SM. These findings have implications for the understanding of the pathological mechanisms of CM/SM, and support the hypothesis that it is a multifactorial disease process governed by increased relative cerebellar volume and failure of the CCF to reach a commensurate size.

Introduction

Chiari-like Malformation (CM) and syringomyelia (SM) is a debilitating and painful disease complex in the Cavalier King Charles Spaniel dog breed (CKCS) which is regarded as a complex oligogenic trait of moderately high heritability[1-4] CM is characterised by foramen magnum cerebellar herniation[5,6] and occurs in approximately 95% of the CKCS population.[7] More than half of CKCS over 4 years of age have SM, fluid filled cavities within the spinal cord (syringes).[8] A causal relationship between CM and SM has been hypothesised, the pathophysiology of which is presumed to be mediated by abnormal cerebrospinal fluid flow dynamics.[9,10] CM/SM in the CKCS is commonly associated with pain, especially in the cervical region, and with various neurological dysfunctions such as scoliosis, limb paresis and ataxia.[5,11] Affected dogs might be hypersensitive to touch and often scratch an area on the shoulder, ear, neck or sternum, commonly only on one side of the body and without making skin contact ('phantom scratching').[11] Some dogs perform facial or head rubbing or spontaneous vocalisations.

In humans, 'Classical' Chiari type I malformation is believed to result from hypoplasia of the basioccipital bone and a consequent reduction in the volume of the posterior cranial fossa (which extends from the petrosal crests and dorsum sellae to the foramen magnum), leading to overcrowding by hindbrain parenchyma (cerebellum, pons and medulla oblongata) and herniation of the cerebellum through the foramen magnum.[12-14] Several morphometric analyses have been conducted on the CKCS caudal cranial fossa (CCF), which is homologous to the posterior cranial fossa in humans.[15] As with Classical Chiari type I malformation in humans, CKCS appear to have a shallower caudal cranial fossa and have abnormalities of the supraoccipital and basioccipital bones when compared to mesaticephalic breeds (breeds with a skull of intermediate length and width).[16] However, although CCF overcrowding has been demonstrated in CKCS with CM/SM, independent studies have reported no difference in relative CCF volume in CKCS compared to brachycephalic breeds or other small breed dogs[17,18], or a link between CCF volume and the development of SM.[19-21] In contrast, a recent study has showed that there is evidence of increased volume of brain parenchyma within the CCF in CKCS with syringomyelia compared to CKCS without syringomyelia.[19] A separate study of volumetric breed comparisons showed that the relative volume of hindbrain parenchyma in CKCS is greater than in other small breed dogs (it is approximately equal to the Labrador - a mesaticephalic breed).[18] These are interesting findings as the volume of

parenchyma in the CCF maintains a consistent ratio to the volume of other brain regions in normal dogs.[22]

These previous studies did not identify which of the sub-divisions of the hindbrain, if any, were enlarged in CKCS with syringomyelia. It could be hypothesised that relative cerebellar volume is larger in CKCS than in other breed groups. Further, increased relative cerebellar volume in CKCS could be associated with syringomyelia.

In this study we present a retrospective volumetric study of magnetic resonance (MR) images from small breed dogs, Labradors and CKCS with CM to compare the relative volume of the cerebellum and the remaining hindbrain (i.e. brainstem) in these groups. We chose to compare these breed groups as they represent "high risk" (CKCS have a high rate of CM/SM), "medium risk" (small breed dogs may occasionally develop CM/SM[2,3,23]) and "low risk" (CM/SM has not been recorded in the Labrador). Additionally, CKCS with CM and SM were compared to CKCS with CM but not SM.

Chiari-like malformation in CKCS may be due to a mis-match between the volume of the cerebellum and the CCF, leading to cerebellar crowding in the caudal CCF and consequent herniation of cerebellar tonsils through the foramen magnum. We further hypothesise that increased relative cerebellar volume in CKCS is correlated with increased crowding of the cerebellum in the caudal part of the CCF. We studied this by dividing the CCF into rostral and caudal sections to investigate the crowding of the cerebellum within each part.

Materials and Methods

Subjects

Dogs were retrospectively selected from Electronic patient records[i] from May 2004 to August 2010 (stored at the Royal Veterinary College and Stone Lion Veterinary Hospital) and assessed for suitability by reviewing T2-weighted transverse and mid-sagittal MR imaging scans using Image Viewer software[ii] according to the following criteria:

- Transverse scans had to have included the cribiform plate rostrally and the first cervical spinal cord segment caudally.
- Breeds were selected according to the same criteria as a previous volumetric study on these groups[18]: CKCS, Labrador or a small breed from the toy or utility groups as defined by the Kennel Club; all skeletally mature (over 8 months of age in CKCS and small breed dogs (SB), over 12 months of age in Labradors).
- Dogs with parenchymal space-occupying lesions or other conditions thought likely to raise intracranial pressure were excluded.
- Midsagittal scans had to have included the first three vertebrae of the cervical spine.
- CM was defined as evidence of caudal cerebellar herniation into the foramen magnum or indentation by the supraoccipital bone, irrespective of the presence of SM.[6]
- A syrinx was defined as a fluid-containing cavity within the spinal cord parenchyma with a transverse diameter of greater than or equal to 2 mm.[24]

Subjects were divided into the following groups: Small breed dogs (SB), Labradors (LD) and CKCS. CCF, rostral and middle fossa, and brain parenchyma volumes of all SB and LD individuals and 42 CKCS were used in a previous study.[18] CCF, rostral and middle fossae and brain parenchyma volumes of 49 CKCS individuals were used in another study.[19] SM is thought to be a late onset disease, and previous studies which did not select CKCS according to age may have failed to distinguish dogs that develop SM later in life from 'true' normal dogs that are unlikely to ever develop the condition. CKCS were therefore further subdivided into two groups based on age and presence or absence of SM: the CM/SM group which comprised eighteen individuals under the age of 2 years with SM, all of which presented with clinical signs related to syringomyelia, and thirteen individuals over the age of 5 years with

CM but without SM (the CM group), which presented for various reasons including idiopathic epilepsy (n=4), intervertebral disc disease (n=3), otitis media with effusion (n=2), MR imaging screening programme for breeding (n=3) and facial nerve paralysis (n=2). While the relationship between age and brain atrophy in humans is well documented and could potentially bias a comparison between different age groups, there is no evidence that this is the case in dogs (a recent study has shown that Labradors in the age groups 1-5, 5-10 and 10+ have similar cerebellar volumes)[22] or even chimpanzees.[25] We nevertheless conducted an analysis of relative cerebellar volume and age in SM-negative CKCS to see if this was a potential source of bias.

Approval from the ethics committee of the Royal Veterinary College was not sought as it is the policy of the ethics committee not to subject retrospective studies of images stored in the archive to ethical review.

MR image analysis

The MR imaging report of a board-certified radiologist was consulted for each patient. Suitable series of transverse T2-weighted MR images[iii] (3 mm slice thickness, 0 mm interslice gap) were exported to a 3-D modelling software program[iv] and measured as described in detail in previous volumetric studies.[18,19] Study numbers were assigned to dogs so that observers were blinded to breed, age and the presence of SM. Image threshold was set to zero and contrast altered to include all brain parenchyma. Masks were created in two dimensions from individual slices using a pen tablet[v] for accuracy of free-hand measurements and the following volumes recorded (see Figure 13):

- Parenchyma within the rostral and middle fossae (a)
- Parenchyma within the CCF was measured as:
  - Cerebellum (b)
  - Brainstem (c)
  - CCF (d)

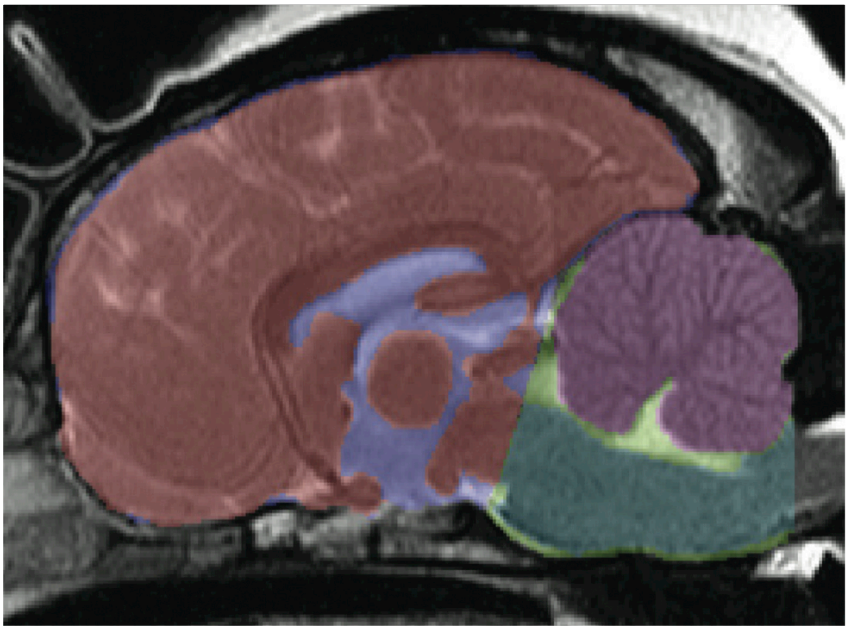


Figure 13. Masks recorded from MR images. Masks were recorded for the following volumes (mid-sagittal view): Parenchyma within the rostral and middle fossae (red), cerebellum (purple), brainstem (dark green), CCF (light green). A mask for the rostral and middle fossae was not recorded but is shown here for completeness (blue).

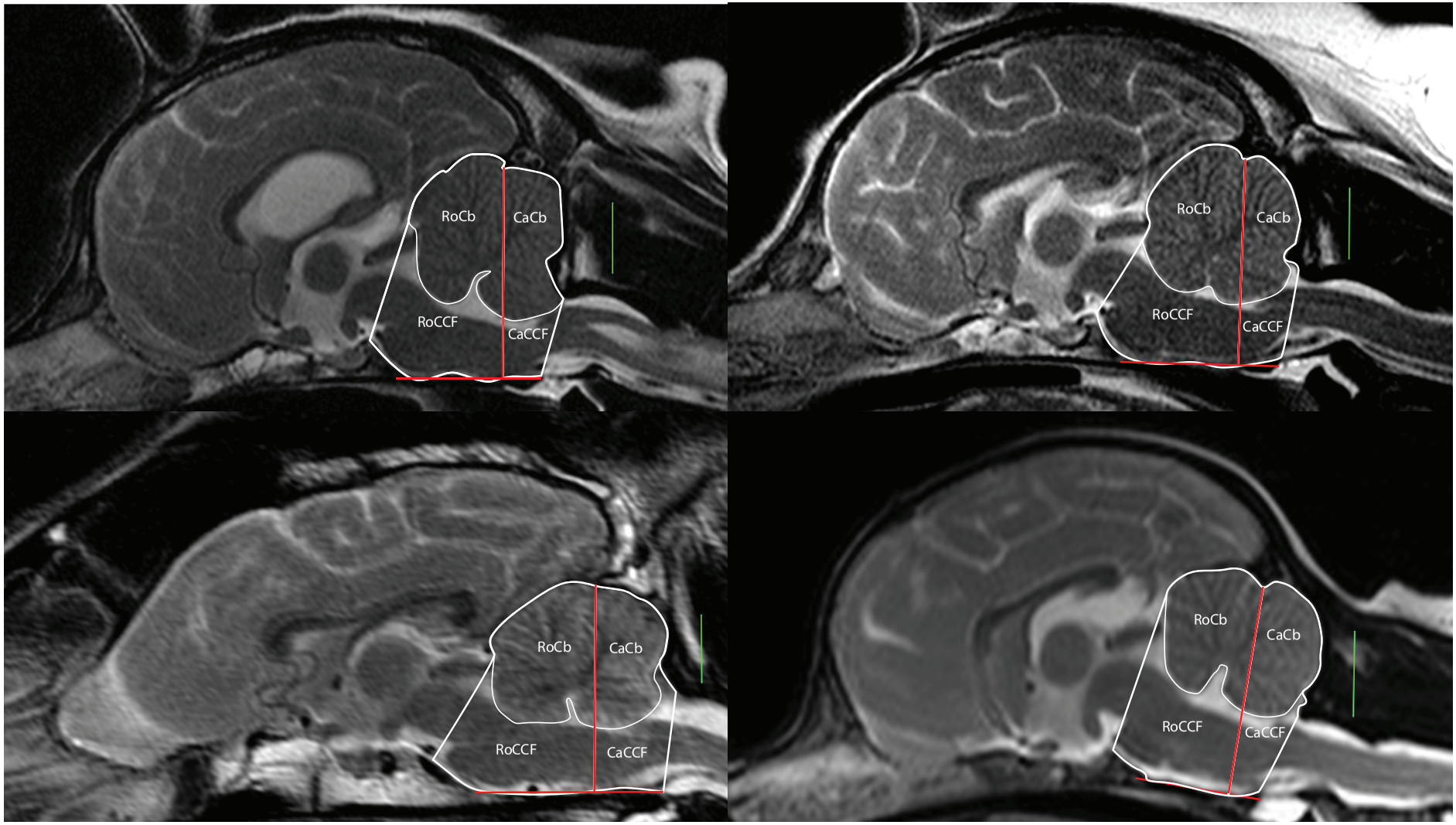
Three-dimensional STL models of the CCF and cerebellum obtained from the masks described above were incorporated into Mimics projects containing sagittal scans of the subjects and aligned with the images. Subjects with missing or incomplete sagittal series were discarded from this part of the study. The CCF was divided into a caudal part and a rostral part by a plane orthogonal to the sagittal images, intersecting the base of the internal occipital protuberance and orientated perpendicular to the basioccipital bone (see Figure 14).

Volumes were recorded for the following:

- cerebellum within the caudal part of the CCF (e)
- cerebellum within the rostral part of the CCF (f)
- caudal part of the CCF (g)
- rostral part of the CCF (h)

See Table 3 for a summary of ratios calculated from masks. Owing to size and conformational differences between dogs, raw volume was considered to be an inappropriate measurement and cerebellar volume was therefore evaluated by using nearby structures, against which it was standardised. We expressed relative cerebellar volume as two

Figure 14. Partitioning of the CCF and cerebellum (mid-sagittal view). CKCS - CM/SM group (top left), CKCS - CM group (top right), Labrador (bottom left), Small breed dog – Chihuahua (bottom right)



percentages: as a percentage of the volume of the CCF (Cerebellar Brain Percentage) and as a percentage of the volume of the entire brain (Cerebellar CCF Percentage). These parameters are accepted standards in canine brain volume measurement as they have been used in a study of cerebellar atrophy[22] and CCF parenchyma volume.[18,19] We used the same percentages to evaluate the relative volume of the brainstem (Brainstem Brain Percentage and Brainstem CCF Percentage). Cerebellar crowding in the rostral and caudal parts of the CCF was expressed as the percentage of that part of the CCF that was occupied by cerebellum (Caudal Cerebellar CCF Percentage and Rostral Cerebellar CCF Percentage).

Table 3. Explanation of the calculation of values.

Cerebellar Brain Percentage	$\frac{b}{a+b+c}\times 100$
Cerebellar CCF Percentage	$\frac{b}{d}\times 100$
Brainstem Brain Percentage	$\frac{c}{a+b+c}\times 100$
Brainstem CCF Percentage	$\frac{c}{d}\times 100$
Caudal Cerebellar CCF Percentage	$\frac{e}{g}\times 100$
Rostral Cerebellar CCF Percentage	$\frac{f}{h}\times 100$

Abbreviations: CCF = caudal cranial fossa. Symbols in equations: a = parenchyma within the rostral and middle fossae, b = cerebellum, c = brainstem, d = CCF, e = cerebellum within the caudal part of the CCF, f = cerebellum within the rostral part of the CCF, g = caudal part of the CCF, h = rostral part of the CCF.

Statistical analysis

A commercial statistical software package[vi] was used for data analysis. Data was analysed with a one-way ANOVA followed by the Bonferroni multiple comparison test (comparison of the different breed groups) or with an unpaired t-test (comparison of CM and CM/SM

groups). Correlations were tested with the Pearson r correlation test. Linear regression was used to model the relationship between variables and ANCOVA was used to determine if the slopes of fitted linear regression models differed significantly. Data are presented as mean ± SEM and $p<0.05$ was considered significant.

Results

Animals

Forty-four small breed dogs (SB), 75 CKCSs and 31 Labradors (LD) were compared. The CM/SM and CM groups consisted of 18 and 13 individuals respectively. A list of dog breeds in the SB group can be found in Table 4.

Table 2. Number of dogs of each breed included in the Small Breed Dog (SB) group.

Breed	Number of Dogs
Bichon Frise	3
Boston Terrier	2
Bulldog	7
Chihuahua	4
French Bulldog	2
Lhasa Apso	1
Maltese Terrier	2
Minature Poodle	2
Pug	4
Shi Tzu	4
Tibetan Spaniel	1
Toy Poodle	1
Yorkshire Terrier	11

Morphology

CKCS had a larger Cerebellar CCF Percentage (CKCS 51.9±0.3% vs. SB 48.1±0.7% [$p<0.0001$] and LD 41.6±0.8% [$p<0.0001$]) and Cerebellar Brain Percentage (CKCS 9.00±0.1% vs. SB 7.63±0.2% [$p<0.0001$] and LD 7.60±0.2% [$p<0.0001$]) compared to the other groups (Figure 15). The CM/SM group had a significantly larger Cerebellar CCF

Percentage (54.0±0.7% vs. 50.5±0.75% [p=0.0034]) and a significantly larger Cerebellar Brain Percentage (9.56±0.2% vs. 8.75±0.2% [p=0.0232]) than the CM group (Figure 15). No significant differences were detected between CKCS and other breed groups in Brainstem CCF Percentage (CKCS 37.8±0.3% vs. SB 37.3±0.8% [p=1.000] and LD 35.8±0.6% [p=0.062]), however Brainstem Brain Percentage was significantly larger in CKCS than small breed dogs (CKCS 6.55±0.1% vs. SB 5.94±0.2% [p=0.003] and LD 6.56±0.2% [p=1.000]). No significant differences were detected between the CM/SM and CM groups in Brainstem CCF Percentage (CM/SM 36.5±0.8% vs. CM 38.5±0.7% [p=0.0917]) or Brainstem Brain Percentage (CM/SM 6.47±0.2% vs. CM 6.68±0.2% [p=0.4239]).

Caudal Cerebellar CCF Percentage was significantly larger in CKCS than the other groups (50.9±1.3% vs. SB 46.1±1.5% [p=0.033] and LD 31.9±1.2% [p<0.0001]; Figure 16), and also significantly larger in the CM/SM group than the CM group (54.8±2.3% vs. 47.3±2.6% [p=0.0386]; Figure 17). Rostral Cerebellar CCF Percentage was significantly larger in CKCS (55.3±0.7%) than Labradors (49.2±2.0% [p=0.004]) but was not significantly larger than small breed dogs (52.5±1.3% [p=0.282]; Figure 16), and significantly larger in the CM/SM group than the CM group (57.1±0.9% vs. 52.5±1.6% [p=0.0146]; Figure 17).

The relationship between cerebellar overcrowding and relative cerebellar volume was tested with a Pearson r correlation of Caudal Cerebellar CCF Percentage and Cerebellar Brain Percentage: The CKCS group demonstrated a positive correlation (r=0.5204 [p=0.0003]), whilst the SB (r=0.1277 [p=0.4449]) and LD (r=0.3016 [p=0.1342]) group did not. A correlation of Rostral Cerebellar CCF Percentage and Cerebellar Brain Percentage revealed a positive relationship in the CKCS group (r=0.4886 [p= 0.0008]), but not in the small breed dog (r=0.2363 [p= 0.1532]) or Labrador (r=0.2562 [p=0.2065]) groups. Linear regression was carried out to compare the trend of the rostral CCF and caudal CCF of the CKCS group. An ANCOVA revealed that the fitted linear regression lines have a significantly different slope (rostral CCF 1.872±0.5159, caudal CCF 4.532±1.148 [p=0.03668], see Figure 18), indicating that in CKCS, crowding of cerebellum in the caudal part of the CCF is more sensitive to changes in the relative volume of the cerebellum than in the rostral part of the CCF. Pearson r correlation between Age vs. Cerebellar CCF Percentage (r=-0.2889, p=0.1084) and Age. vs. Cerebellar Brain Percentage (r=0.2801, p=0.1158) in SM-negative CKCS revealed no statistically significant relationships between relative cerebellar volume and age.

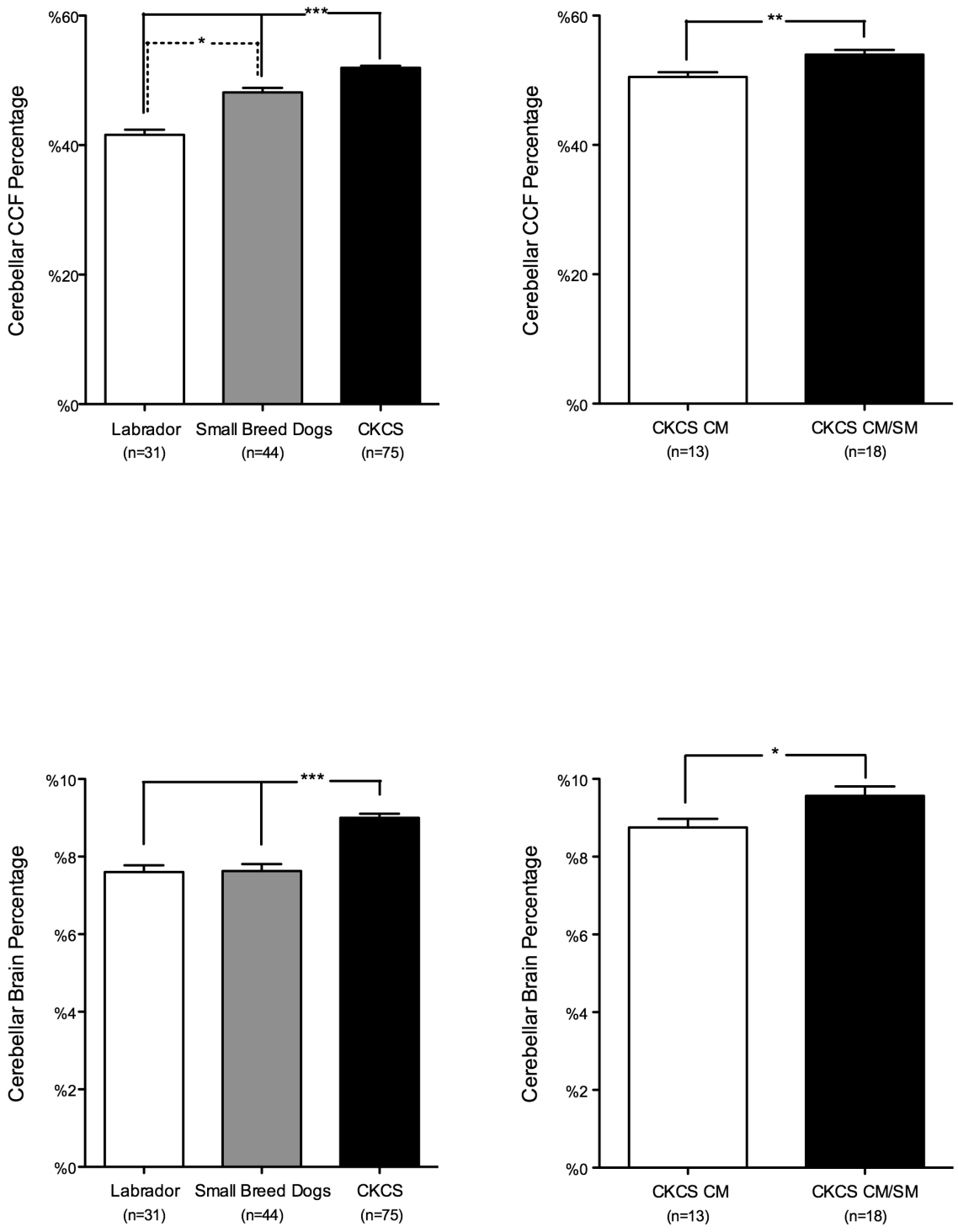


Figure 15. Relative cerebellar volume.

The relative volume of the cerebellum is expressed as a percentage of the caudal cranial fossa volume (Cerebellar CCF Percentage) and a percentage of the total brain volume (Cerebellar Brain Percentage). Cavalier King Charles Spaniels (CKCS) have a relatively larger cerebellum than small breed dogs or Labradors. CKCS under 2 years of age with Chiari-like malformation (CM) and syringomyelia (SM) (CKCS CM/SM) have a relatively larger cerebellum than CKCS over 5 years of age with CM but without SM (CKCS CM). *p<0.05, **p<0.01, ***p<0.001

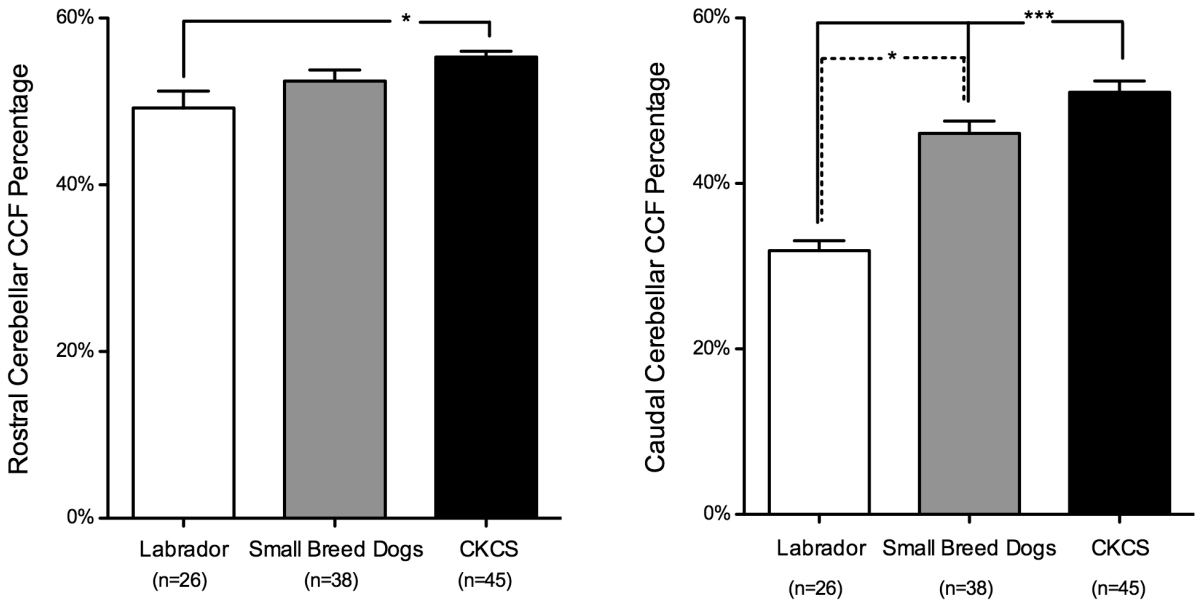


Figure 16. Cerebellar crowding within different parts of the caudal cranial fossa comparing Labradors, small breed dogs and Cavalier King Charles Spaniels. Crowding of cerebellum is defined as the percentage of the volume of each part of the caudal cranial fossa (CCF) which is occupied by cerebellar parenchyma (Rostral Cerebellar CCF Percentage and Caudal Cerebellar CCF Percentage). Cavalier King Charles Spaniels (CKCS) have a more crowded rostral CCF than Labradors and more crowded caudal CCF than small breed dogs or Labradors. *p<0.05, ***p<0.001

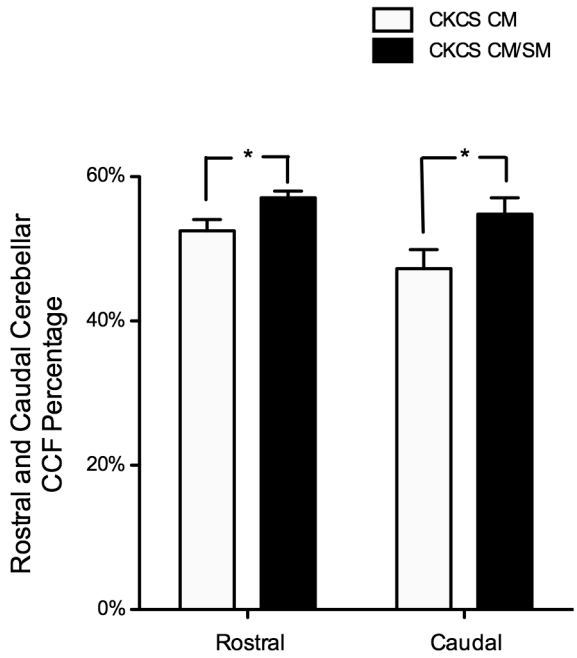


Figure 17. Cerebellar crowding within different parts of the caudal cranial fossa comparing different groups of Cavalier King Charles Spaniels.
Crowding of cerebellum is defined as the percentage of the volume of each part of the caudal cranial fossa (CCF) which is occupied by cerebellar parenchyma (Rostral Cerebellar CCF Percentage and Caudal Cerebellar CCF Percentage). CKCS under 2 years of age with Chiari-like malformation (CM) and syringomyelia (SM) (CKCS CM/SM) have a more crowded Rostral CCF and Caudal CCF than CKCS over 5 years of age with CM but without SM (CKCS CM). *p<0.05

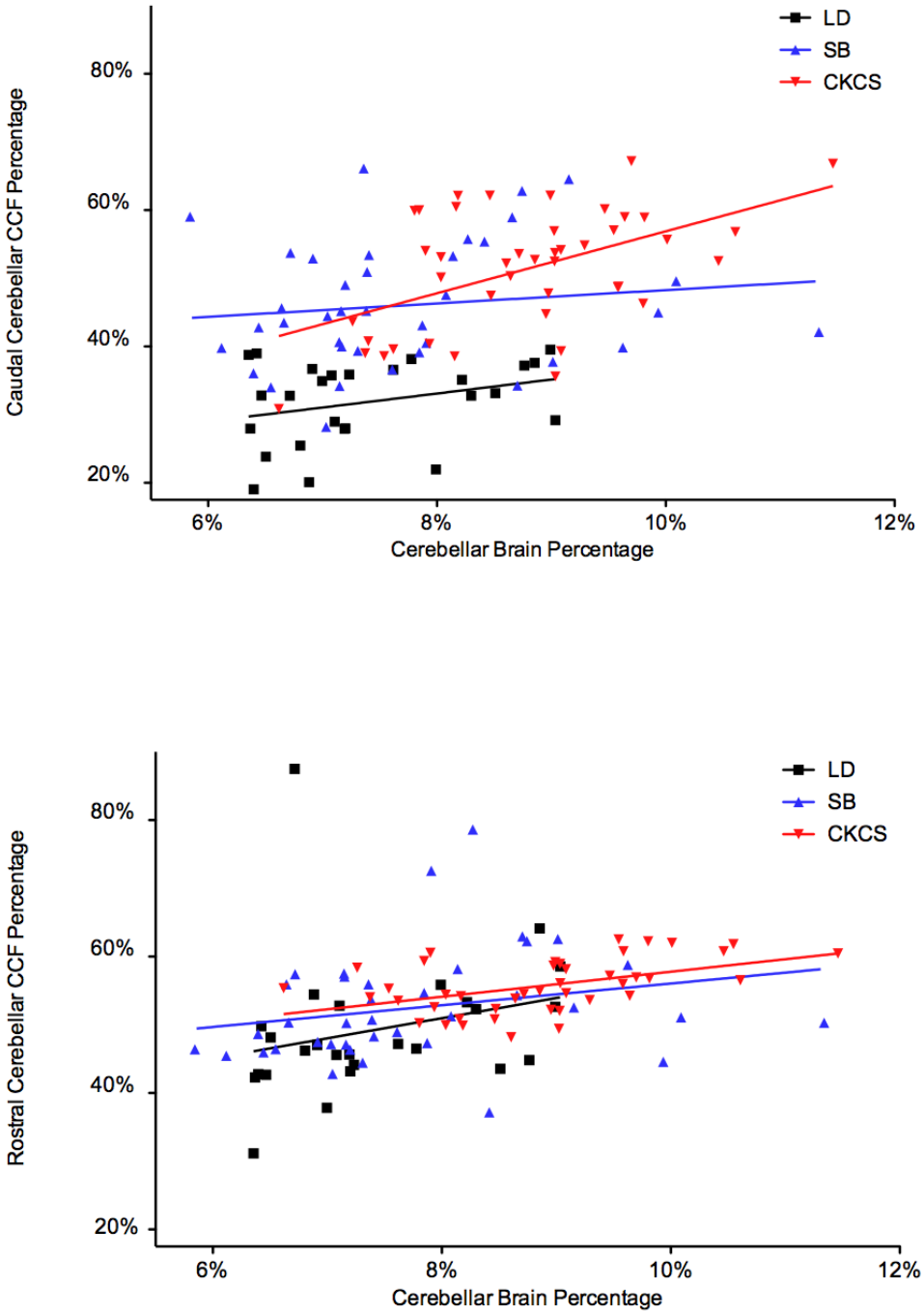


Figure 18. Relationship between Cerebellar Crowding and Cerebellar Volume. Crowding of cerebellum is defined as the percentage of the volume of each part of the caudal cranial fossa (CCF) which is occupied by cerebellar parenchyma (Rostral Cerebellar CCF Percentage and Caudal Cerebellar CCF Percentage). The volume of the cerebellum is expressed as a percentage of the total brain volume (Cerebellar Brain Percentage). Fitted linear regression lines are also displayed. Cavalier King Charles Spaniels (CKCS) showed a relationship between cerebellar crowding and volume in both the rostral CCF (p=0.0008) and caudal CCF (p=0.0003) and Labradors (LD) and small breed dogs (SB) did not. In CKCS the slope of the fitted model in the caudal CCF was significantly steeper than in the rostral CCF (p=0.03668), indicating that crowding in the caudal CCF is more sensitive to changes in cerebellar volume.

Discussion

Our results show that the cerebellum is proportionately larger in CKCS when compared to the rest of the brain of Labradors and small breed dogs and also larger in young CKCS with CM and SM than in older CKCS with CM alone. Furthermore, the degree of cerebellar crowding in the caudal CCF is correlated with increased volume of the cerebellum in CKCS and this is not seen in small breed dogs or Labradors. These findings have implications for the understanding of the pathological mechanisms of CM/SM, and support the hypothesis that it is a multifactorial disease process governed by increased cerebellar volume and failure of the CCF to reach a commensurate size.

SM develops due to abnormal CSF flow dynamics at the cervicomedullary junction.

The presumed pathological mechanism underlying the association between CM and SM is that the development of SM is mediated by abnormal CSF flow dynamics at the cervicomedullary junction.[9,10] This has been attributed to high-velocity jets of CSF caused by obstruction of the foramen magnum by the herniated portion of the cerebellum and overcrowded brain parenchyma in the caudal occipital region[5] which is hypothesised to set up a hydrostatic pressure differential between the spinal cord and subarachnoid space and results in the accumulation of perivascular fluid which eventually forms a syrinx.[9] The degree of crowding may determine the degree of foramen magnum obstruction, and in turn the tendency for syrinxes to form. Relative cerebellar volume is potentially a key factor in determining the degree of obstruction and interference in normal CSF flow through the foramen magnum, which disposes dogs to the subsequent development of SM.

Relative cerebellar volume is larger in CKCS than in the other breed groups

CM in CKCS is the manifestation of cerebellar herniation through the foramen magnum, and previous studies have shown that this is associated with increased crowding of brain parenchyma within the CCF.[18,19] In our study we tested the hypothesis that this crowding was due to increased relative cerebellar volume. Our results show that in CKCS the Cerebellum is more crowded in the caudal CCF than in small breed dogs and Labradors, supporting the theory that CM is due to descent and herniation of the cerebellum through the foramen magnum. CKCS had a relatively larger Cerebellar volume (and a similar brainstem volume)

when compared to small breed dogs and Labradors, supporting hypothesis i) (that CKCS have increased cerebellar volume compared to other breeds of dog).

Uniquely in CKCS, cerebellar volume is correlated with crowding in the caudal CCF

Our results support hypothesis iii) which states that in CKCS an increase in relative cerebellar volume is correlated with an increase in cerebellar crowding in the caudal CCF. It should be noted that small breed dogs and Labradors do not show the same relationship. We infer from this result that during cranial development in Labradors and small breed dogs, a compensatory mechanism maintains the relationship between cerebellar volume and CCF dimensions, and this mechanism is defective in CKCS. We also found in CKCS that cerebellar crowding in the caudal CCF is more sensitive to changes in relative cerebellar volume than cerebellar crowding in the rostral CCF, which is consistent with the theory that increased cerebellar volume results in the cerebellum shifting caudally and causes obliteration of dead space in the caudal CCF. This also causes herniation of the cerebellum through the foramen magnum (i.e. CM). This finding is consistent with previous research, which has shown that CKCS possess a CCF parenchyma proportionately similar in volume to that of a Labrador and a CCF volume similar to small breed dogs, suggesting that CCF growth in CKCS is not keeping pace with the growth of brain parenchyma.[18] This is an interesting finding as it suggests that CM/SM may be a multifactorial disease dependent on the cumulative effects of a small CCF and large cerebellum.

Increased relative cerebellar volume is associated with Syringomyelia in CKCS

In this study we found that CKCS under the age of 2 with SM (the CM/SM group) have an increased relative cerebellar volume when compared to CKCS over the age of 5 without SM. Unlike humans, we found that CKCS do not appear to have age-related atrophy as there was no correlation between relative cerebellar volume and age. This supports hypothesis ii), that increased relative cerebellar volume in CKCS is associated with syringomyelia. Previous volumetric studies in CKCS have shown that there is an association between SM and CCF parenchyma volume[19,26], but this is the first time that relative cerebellar volume has been linked to SM. The cerebellum to brain volume ratio is consistent between normal dogs and has been shown to decrease with cerebellar degenerative disorders[22], but it has never been shown to be increased in size in a canine neurological disorder. To the authors' knowledge,

no studies have examined the role of relative cerebellar volume in human Chiari malformation I and associated SM.

Conditions leading to increased brain volume in humans

Generalised overgrowth of brain parenchyma (megalencephaly) is recognised in over 100 human syndromes[27] and in some of these conditions posterior cranial fossa overcrowding and herniation of the cerebellum have been reported. These include Macrocephaly–Capillary Malformation, also known as macrocephaly-cutis marmorata telangiectatica congenital[28,29], the closely related megalencephaly polymicrogyria-polydactyly hydrocephalus (MPPH) syndrome[30], Rasopathies[31], Alexander's disease[32] and Lhermitte-Duclos disease[33]. Megalencephaly is suggestive of disorders of generalized neuro- and gliogenesis. Some of these conditions are also manifested in different parts of the body, such as Macrocephaly–Capillary Malformation, a poorly understood syndrome which is characterized by malformed capillaries and prenatal somatic overgrowth with numerous asymmetries.[28,29] Other common causes of megalencephaly include Rasopathies, a group of diseases caused by genetic mutations which lead to an upregulation of the mitogen-activated protein kinase (Ras/MAPK) pathway.[31] Affected individuals have multiple defects including progressive brain overgrowth which is caused by proliferation of cortical progenitor cells and premature gliogenesis. A number of Rasopathies which can result in cerebellar herniation include Costello's syndrome[34], Neurofibromatosis type 1[35], Noonan's syndrome[36] and cardio-facio-cutaneous syndrome (CFC).[37]

Conditions leading to increased cerebellar volume in humans

Generalised megalencephaly in the CKCS is not supported by our findings, which indicate that there is enlargement of the cerebellum relative to overall brain volume. However, there are only a few rare syndromes of cerebellar overgrowth in humans that do not involve generalised brain overgrowth. Diffuse cerebellar enlargement (macrocerebellum) is a poorly defined syndrome proposed to be related to the response of the cerebellum to an over-expression of growth factors to augment slow cerebral growth.[38] Primary defects of the cerebellum include Lhermitte-Duclos disease[33], in which a slow-growing hamartoma causes diffuse hypertrophy of the cerebellar stratum granulosum. Chiari Malformation II in humans, although associated with reduced posterior cranial fossa volume, is thought to be

neuroectodermal in origin and involves enlargement of the anterior lobes of the cerebellum.[13,39] The possibility that the pathogenesis of CM in CKCS is due to a neuroectodermal defect should be investigated. The authors are currently investigating the possibility of neurological deficits in CKCS which are referable to cerebellar dysfunction (including a specific "puppy-like" ataxia). Scope for further research would involve histological and embryological studies of the forebrain and cerebellum to evaluate possible developmental disorders.

Bony development of the CCF

It has been proposed that the volume mismatch between the CCF and brain parenchyma in CKCS can be explained by impaired occipital bone development and the consequent reduction in CCF volume.[5,16,21] A recent volumetric study of CKCS has found that individuals in the CM/SM group have a minimally smaller CCF volume than individuals in the CM group.[26] In this study, we find that in CKCS, unlike small breed dogs or Labradors, there is a positive correlation between the volume of the cerebellum and degree of crowding in the caudal CCF, which suggests that CM may be due to CCF development not keeping pace with growth of the cerebellum. This supports the idea that CM/SM in CKCS may in fact be multifactorial and an abnormal development process affecting the CCF may be acting as a disease modifier. Although the occipital bone comprises a single bony plate in an adult individual, its development is complex and mosaic as it develops from the basioccipital, exoccipital, and supraoccipital bones which are derived from distinct acrochordal and parachordal, occipital arch, and supraoccipital cartilages, respectively.[40] Impaired CCF development may be caused by a failure of communication between one or more of these progenitors and the developing neural tube (specifically, rhombomere 1, which gives rise to the cerebellum).[41,42] Alternatively, it could simply be explained by premature closure of growth plates between the bones of the CCF, as has been reported in humans.[14] It has been found that pre-natal posterior cranial fossa development in humans is independent of cerebellar volume but closely parallels the development of the supratentorial bony compartment.[43] If this is also true in dogs it may have implications for the development of CM, as the CCF may have a restricted capacity to adapt to the volume of an enlarged cerebellum through expansion of the sutures between the occipital bones and their neighbours.

Occipital bone resorption

Studies of human skulls have found that the occipital bones adapt to the shape of the growing cerebellum. In one study, the occipital bones showed a resorbtive pattern of bone around the cerebellar hemispheres in adults and in children[44], suggesting that bone remodelling continues long after skull sutures have fused. It has also been noted on post-mortem examination of CKCS and other small breed dogs that the supraoccipital bone overlying the cerebellar vermis is remarkably thin and sometimes eroded so that the foramen magnum is enlarged dorsally[23], which could indicate that there has been substantial bone resorbtion. Work is needed to elucidate the mechanisms of occipital growth in dogs to determine the extent to which a osteo-resorbtive process can mitigate an enlarged cerebellum in CKCS and in other breeds.

Scope for further research

Histopathological studies of occipital bone development are needed in order to compare the CKCS, to other breeds of dog. The possibility of a cerebellar growth disorder also deserves scrutiny. In order to assess the clinical significance of relative cerebellar volume and CCF volume as prognostic indicators, further cohort and longitudinal studies are needed. In human medicine, investigation of the role of increased relative cerebellar volume in the development of Chiari malformation I and associated SM may be warranted.

Conclusions

Our findings show that the CKCS has a relatively larger cerebellum than small breed dogs and Labradors and there is an association between increased relative cerebellar volume and SM in CKCS. In contrast to small breed dogs and Labradors, CKCS exhibit correlation between increased cerebellar volume and cerebellar crowding within the caudal CCF, suggesting that CCF growth in CKCS is not keeping pace with the growth of the cerebellum.

Footnotes:

[i]Rx Works 4.2.1560, RxWorks Inc., 2010

[ii]Image Viewer 4.0.18, Visbion, 2009

[iii]Philips NT Intera 1.5T MRI

[iv]Mimics® 13.10, Materialise n.v., 2009

[v]XP-Pen® v. 4.04, P-Active Co. Ltd., 2003

[vi]Prism® for Windows Version 5.00, Graphpad Software Inc, 2007

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Chapter 2

RELATIONSHIP OF ABNORMAL CRANIOCEREBRAL MORPHOLOGY ASSOCIATED WITH CHIARI-LIKE MALFORMATION TO CLINICAL SIGNS IN DOGS

Chapter 2.1

THE ASSOCIATION BETWEEN CHIARI-LIKE MALFORMATION, VENTRICULOMEGALY AND SEIZURES IN CAVALIER KING CHARLES SPANIELS

THE ASSOCIATION BETWEEN CHIARI-LIKE MALFORMATION, VENTRICULOMEGALY AND SEIZURES IN CAVALIER KING CHARLES SPANIELS

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Summary

Cavalier King Charles Spaniels (CKCS) with Chiari-like malformation (CM) suffering seizures are frequently diagnosed with idiopathic epilepsy. There may be an association between ventriculomegaly (V) or caudal fossa overcrowding (CCFP) and seizures. This retrospective case-controlled study uses MRI to investigate the association between these morphologic abnormalities and seizures. Seizure semiology and, where possible, electroencephalographic (EEG) abnormalities are described. Eighty-five CKCS with CM were recruited, 27 with seizures. There was no association between V or CCFP and seizures (P = 0.10 and 0.71 respectively). Another cause of recurrent seizures in CKCS is suspected, such as familial epilepsy as previously reported.

Introduction

Chiari-I malformations (CM) in children are congenital anomalies associated with hypoplasia of the bones of the posterior fossa[1]. They result in compression of neural tissue and most commonly pain; seizures are also seen frequently in human patients with posterior fossa abnormalities and it has been suggested to have a role in epileptogenesis.[2] There is currently no evidence in the human literature to suggest volume overcrowding is associated with seizures. The analogous canine condition, CM, is prevalent in CKCS and is often associated with syringomyelia and neuropathic pain.[3] In a previous report of CM in CKCS, 32% of the study population had seizures.[4] There is a heritable basis for idiopathic epilepsy in CKCS, however this appears to be a separate genetic subset from dogs with occipital bone hypoplasia.[5]

In CKCS with CM, ventriculomegaly is associated with overcrowding of the caudal fossa and syrinx dimensions[6], possibly as a result of altered CSF flow dynamics. A unifying theory associating Chiari-I malformation, hydrocephalus and syringomyelia with reduced venous drainage from the central nervous system has been proposed in humans.[7] Hydrocephalus has previously been associated with seizures in dogs.[8] Rusbridge and others[9] postulated that progressive ventriculomegaly in CKCS with CM may be associated with seizures. Conversely, a separate study found the presence of neurologic signs correlated poorly with morphologic abnormalities including the degree of cerebellar herniation, the relative size of the cerebellum, the size of the lateral ventricles and the proportion of the foramen magnum occupied by the spinal cord.[4] Therefore, it is unclear whether the presence and severity of CM and ventriculomegaly should be considered incidental in CKCS with CM that also present with seizures.

The primary aim of this study is to use the analysis of cranial volumes on MRI to determine whether there is an association between morphologic abnormalities in CKCS with CM and seizures. The secondary aim is to describe the seizure semiology and electroencephalographic (EEG) findings obtained from the affected CKCS.

Materials and Methods

Electronic patient records at the Royal Veterinary College were searched between November 2005 and 2010 for CKCS with full cranial MRI consistent with CM (cerebellar indentation and herniation into the foramen magnum) for which T2-weighted transverse and mid-sagittal MRI scans of the brain were available. Dogs were excluded if there were abnormalities on routine complete blood count, serum biochemical profile, bile acid stimulation test, cerebrospinal fluid analysis or if there were MRI lesions other than CM that may be associated with seizures. Recorded data included age when scanned, presenting clinical complaint and when present, seizure semiology. Dogs were grouped by the presence or absence of seizures at presentation.

Seizures were classified on the basis of owner description as used by Berendt and Gram[10]. When possible, inter-ictal EEG was performed under light sedation with 0.005 mg/kg medetomidine hydrochloride[i] using a digital EEG unit[ii]. A total of 16 platinum needle electrodes, including a ground, were used to record the EEG. An electrocardiogram (ECG) was obtained by using a base-apex electrode configuration.

Each dog was assigned a study number so that an observer (CD) blinded to the dog's age and the presence of seizures could make measurements. Suitable MRI series were exported to commercially available medical image software[iii] and accurately measured in three dimensions as previously described.[6] The percentage volumes of parenchyma within the caudal cranial fossa (CCFP) and ventricle size relative to total brain volume (V) were recorded.

Statistical analysis was performed with a commercial software package[iv]. The Shapiro-Wilk W test was used to assess for normality in datasets (CCFP, V, Age). Means and standard deviations were calculated for normally distributed continuous data, and medians and ranges were determined for non-parametric data. Independent sample t tests or Mann-Whitney U tests were used for statistical evaluation. Two-tailed tests were used in all cases with $P < 0.05$ considered significant.

Results

Eighty-five CKCS were included. They presented for the investigation of seizures (n = 27), peripheral vestibular disease (n = 3) and neuropathic pain (n = 55). There were therefore 27 dogs in group 1 (seizures) and 58 dogs in group 2 (control). There was no statistically significant difference between group 1 and 2 for CCFP (89.83 ± 2.37% vs 89.66 ± 1.80%; P = 0.71), V (4.63%, 1.00 - 14.7% vs 5.813%, 0.88 - 33.9%; P = 0.10) or age (4.48 ± 2.92 years vs 4.48 ± 2.73 years; P = 0.99). Seizure classification is summarised in Table 5. Seizures were classified as having partial onset in 17 individuals (61% of the study population, 95% CI 42.41 - 76.43%). EEG was performed in 4 dogs with seizures and was found to be abnormal in three cases, findings are summarised in Table 6.

Table 5 Seizure Classification by onset, type and subtype (Berendt and Gram 1999).

Onset	**Number (%)**
Partial onset	17 (61)
Generalised onset	11 (39)

Type	**Number (%)**	**Subtype**	**Number (%)**
Partial	10 (36)	Simple partial Complex partial	5 (50) 5 (50)
Partial with secondary generalisation	7 (25)	Generalised Tonic-clonic Generalised Tonic Generalised Atonic*	13 (46) 3 (11) 2 (7)
Generalised	11 (39)		

* Patients with atonic seizures had normal cardiac evaluation including 24-hour electrocardiographic holter monitor

Table 6 Results of inter-ictal electroencephalography studies in three CKCS.

	Signalment	Seizure Semiology	EEG report
Case 1	1.03y FN	Complex partial seizures; repetitive fly catching	Generalised spike-wave activity, more severe in the right fronto-temporal regions*. There was further spike activity with underlying delta activity on waking (bifrontal, worse on the right).
Case 2	0.92y MN	Generalised tonic seizures	Generalised inter-ictal spike waves, mostly observed from the fronto-temporal regions.
Case 3	6.04y MN	Complex partial seizures	Transient sharp waves during sleep in the rostral cortical regions particularly in the frontocentral areas. After reversal of sedation, further rhythmic sharp wave activity, which was often associated with witnessed seizures.
Case 4	2.04y F	Generalised tonic-clonic seizures	No abnormalities detected

*The generalised spike wave activity recorded may reflect the poor spatial resolution of EEG in detecting an epileptic seizure focus in non-superficial cerebral tissue

Discussion

These findings suggest that there is no significant difference in ventricle size or caudal fossa overcrowding between CKCS with seizures and CM or CKCS with CM with other clinical signs but not seizures. This supports previous evidence that morphologic abnormalities cannot be associated with individual clinical signs.[4] There may, therefore, be another source of epileptogenesis in this subset of dogs. In human patients with posterior fossa malformations, the two risk factors identified for epilepsy are familial antecedents for epilepsy and the involvement of the cerebellum in the malformation.[2] As there is a hereditable basis for idiopathic epilepsy in CKCS[5], the former may be considered in these dogs. Another suspected cause of seizures and focal paroxysmal abnormalities in humans with Chiari-I malformation is cerebral microdysgenesis[11], which remains to be investigated in dogs.

The EEG abnormalities described in this report are similar to those found in a small series of humans with Chiari-I malformation, where paroxysmal abnormalities were mainly located over the frontal and temporal regions rather than the cerebellum[11] suggesting they are not correlated with CM. However, there is a role for the cerebellum in epileptogenesis or seizure inhibition that has been demonstrated in animals.[12] Lesions of the cerebellum such as ganglioglioma can cause seizures.[13] Rhythmic output from the cerebellum may contribute to the maintenance of generalised petit mal seizures[14]. Conversely, several subcortical structures including the cerebellum influence the susceptibility of the forebrain to seizures by modulating the threshold for seizure initiation.[15] As EEG electrodes are not routinely placed over the cerebellum in our clinic, we would not be able to exclude the possibility of paroxysmal electrical activity originating in the cerebellum of CKCS with CM. There could therefore be a subset of CKCS for which epileptogenesis occurs in the cerebellum as previously postulated in a small group of human patients.[11]

61% of dogs in this study had seizures that were classified as having partial onset, which is similar to previous studies regarding the general canine population.[10] This is the first report of seizure semiology of dogs with CM and epilepsy. Similarly, small case series in humans with Chiari-I malformation and epilepsy have revealed a predominance of partial seizures, typically with secondary generalisation.[11,16] Two CKCS suffered from unusual acute onset and brief generalised atonic seizures. These may be similar to ‘drop’ seizures associated with

Chiari-I in children that are believed to originate in the cerebellum.[17] These are paroxysmal attacks of collapse with or without loss of consciousness, abnormal extensor posturing and varying degree of respiratory compromise that are commonly associated with structural lesions of the cerebellum. This syndrome is often misdiagnosed as epilepsy in children with Chiari-I malformation.[16] Further study would be required to evaluate this association in dogs.

There are limitations to this retrospective study. A control group of CKCS without CM could not be recruited as the disease is almost ubiquitous in this breed. In one study by Cerda-Gonzalez and others[18], 59/64 (92%) of CKCS had one craniocervical morphologic abnormality associated with CM detected on MRI. We therefore investigated the relationship of individual morphometric abnormalities associated with CM and the presence of recurrent seizures. However, volumes of the ventricular system and parenchyma within the caudal fossa, which may be associated with syringomyelia[6], were not associated with recurrent seizures in this study.

Footnotes:

[i] Animalcare, UK

[ii] Trackit, Lifelines Ltd, UK

[iii] Mimics 10.11; Materialise

[iv] SPSS for Windows v17.0; SPSS

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Chapter 2.2

LONG-TERM OUTCOME OF CAVALIER KING CHARLES SPANIELS WITH CLINICAL SIGNS ASSOCIATED WITH CHIARI-LIKE MALFORMATION AND SYRINGOMYELIA

LONG-TERM OUTCOME OF CAVALIER KING CHARLES SPANIELS WITH CLINICAL SIGNS ASSOCIATED WITH CHIARI-LIKE MALFORMATION AND SYRINGOMYELIA

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Summary

This purpose of this prospective cohort study was to follow 48 Cavalier King Charles Spaniels (CKCS) with Chiari-like malformation (CM) and/or syringomyelia (SM) and clinical signs suggestive of neuropathic pain (NeP) for a period of 39 (±14.3) months from diagnosis. At the end of the study, 36 dogs were still alive; five dogs died of an unrelated or unknown cause, and seven were euthanased due to severe clinical signs suggestive of NeP. During the follow-up period, the clinical signs of scratching, facial rubbing behaviour, vocalisation and exercise ability were evaluated. Nine out of 48 dogs stopped scratching ($P<0.001$), but there was no statistically significant change in the number of dogs exhibiting exercise intolerance, vocalisation or facial rubbing behaviour. The overall severity of clinical signs based on a visual analogue scale (VAS) (0 mm: no clinical signs 100 mm: severe clinical signs) increased (from median 75 mm (interquartile ranges (IQR) 68–84) to 84 mm (IQR 71.5–91), $P<0.001$). A quarter of the dogs were static or improved. In general, the majority of the owners felt that the quality of life of their dogs was acceptable. Medical treatments received were gabapentin or pregabalin and/or intermittently, carprofen. The owner's perception of their animal's progress, and progress based on VAS, had strong positive correlation (Spearman's rank correlation (sr) 0.74, $P<0.001$). Overall, this study suggests that clinical signs suggestive of NeP progress in three-quarters of CKCSs with CM and/or SM.

Introduction

Chiari-like malformation (CM) and syringomyelia (SM) is an enfeebling disease complex most prevalent in Cavalier King Charles spaniels (CKCS).[1] CM refers to the apparent mismatch in volume between the caudal brain structures and the caudal skull[2-4], and is associated with herniation of the cerebellum through the foramen magnum.[1,5] SM refers to accumulation of fluid within the parenchyma of the spinal cord, and is thought to result from CM and resultant changes in the dynamics of cerebrospinal fluid (CSF) flow through the foramen magnum and the cranial part of the cervical spinal cord.[6-11]

The estimated prevalence of CM in this breed varies from 92%[9] to 100%.[2,12] SM affects almost half of asymptomatic (as perceived by their owners) young CKCS, which increases up to 70 per cent at the age of six years.[12,13] The prevalence of CKCS with SM that suffer clinical signs suggestive of NeP is unknown.

Clinical signs typically associated with CM and/or SM include cervical scoliosis, thoracic and pelvic limb ataxia, thoracic limb paresis and signs suggestive of neuropathic pain (NeP).[10] NeP most often manifests as allodynia (pain arising from a non-noxious stimulus, i.e. gentle palpation) or dysaesthesia (spontaneous or evoked unpleasant sensation which manifests as phantom scratching, facial/ear rubbing).[14] One frequent sign of SM attributed to dysaesthesia and/or allodynia is phantom scratching. Phantom scratching is characterised by a pelvic limb scratching action to the shoulder and neck area, often without making skin contact, and typically on one side only. Unlike scratching associated with skin disease, dogs will often scratch whilst walking.[14] It is not yet fully understood how CM/SM causes NeP. However, histopathological studies of SM in CKCS have found that dogs which had expressed signs of NeP suffered an asymmetrical syrinx with profound alteration of the structure of the dorsal horn laminae, and had reduced expression of the pain-related neuropeptides substance P, and calcitonin gene-related peptide.[15] Glial and fibrous proliferations were also associated with expression of clinical signs.[16] Chiari-I malformation/SM causes clinical signs of NeP in up to 80% of human beings with this disorder, and up to 35% of affected dogs.[17,18] NeP has an important impact on the affected person's quality of life (QOL) and neurobehaviour[19], and a recent study in dogs[20] confirmed an association between the degree of NeP and fear/anxiety-related behavioural changes.

Medical and surgical treatment options exist for dogs with CM/SM. Medical management includes the use of NSAIDs, drugs that reduce CSF production (omeprazole, cimetidine), corticosteroids and antiepileptic drugs that have analgesic properties (gabapentin, pregabalin). However, there is no scientific evidence to prove the efficacy of these drugs in the management of NeP associated with CM/SM in dogs.[10,14,21] Surgical management (craniocervical decompression) is frequently performed in people with Chiari-I malformation, with and without SM, to alleviate clinical signs.[22] Following surgery, 80% of dogs improved, but there was no resolution of the syringes, and nearly all dogs continued to exhibit clinical signs suggestive of NeP postoperatively.[23-26] Additionally, 25–47% of the operated dogs showed recurrence or deterioration of the clinical signs within 0.2–3 years after surgery.[24-26]

To the authors' knowledge there are currently no data regarding the long-term outcome of non-surgically managed dogs with clinical signs suggestive of NeP secondary to CM/SM. This prospective cohort study follows 48 CKCS dogs with clinical signs suggestive of NeP due to CM and/ or SM for a period of 39 (±14.3) months.

Materials and Methods

Study design

A prospective cohort study was performed following 48 CKCS dogs with CM and/ or SM disease complex for a mean period of 39 (±14.3) months from treatment termination.

Animals

CKCSs between the ages of one and 13 years (median 46 months), and bodyweight of 4–13 kg (median 9.5 kg), were recruited from the general population in the UK by advertising through the veterinary press and national CKCS health societies, into a two-week trial of a novel neuropathic pain medication, which was performed under the Animals (Scientific Procedures) Act 1986, and was approved by the institution's ethics committee. After the end of the drug trial, dogs were followed prospectively for a mean period of 39 (±14.3 SD, 4–107) months for this study.

Dogs with other medical or neurological conditions that could have influenced the preceding pharmacological study were excluded, such as brain or spinal cord diseases, other than CM/SM; CKCS which had undergone occipital or craniocervical decompression; those with evidence of inflammation in the external ear canal (erythema, discharge, lichenification); with a history and clinical signs of skin disease; seizures at the time of diagnosis; or with a systolic heart murmur of greater than grade II/VI. Thus, owners were questioned about general health status, exercise intolerance and clinical signs suggestive of pruritus and pain.

Recruitment and inclusion criteria were: CKCS with clinical signs suggestive of NeP (such as spinal hyperaesthesia on palpation, facial rubbing, vocalisation and/or phantom scratching)[11,14] which scored 50 or more on the visual analogue scale (VAS), underwent MRI of the brain and spinal cord[4,27], and were subsequently diagnosed with CM and/or SM by a board-certified neurologist. CM was defined as evidence of cerebellar herniation or indentation by the supraoccipital bone[5], and a syrinx was defined as a fluid-containing cavity within the spinal cord parenchyma with a transverse diameter of greater than or equal to 2 mm[4].

Assessment of clinical signs

A questionnaire was used to assess the following factors in a face-to-face interview at the initial visit, and then by telephone for the follow up: name of the animal and owner, sex of the animal and neutering status, date of birth and death, if applicable, whether it was euthanasia or natural death, cause of death, general health status, history of brain or spinal cord diseases other than CM/SM, if the dog had evidence of inflammation in the external ear canal, history and clinical signs of skin disease, seizures, exercise intolerance, whether there was another MR scan performed following their initial visit, if the animal underwent craniocervical decompression, if the animal had been diagnosed with a heart murmur, and what grade it was. The owners were also asked to confirm the presence of the following signs: phantom scratching of shoulder and/or neck, facial and ear rubbing, vocalisation and spinal pain. At the initial consult, all dogs were examined by a board-certified neurologist, and had unremarkable complete blood cell count and serum biochemistry profile.

A VAS was used by the authors to assess the frequency and intensity of clinical signs suggestive of NeP. A 100 mm line ranging from 0 mm (asymptomatic dogs exhibiting normal exercise ability, no scratching, no facial rubbing and no vocalisation) to 100 mm (dogs with severely compromised exercise activity, scratching more than five times a day and vocalising more than five times a week) was used for the VAS assessment by intersecting the line with a second perpendicular line drawn by the observer, based on the subjective severity of the signs reported. To ensure consistency as much as possible between scorers, two of the authors[i,ii] responsible for the initial scoring underwent training, which involved independent scoring of clinical signs until their score varied less than 3%. The follow-up information gathered by calling the owners was cross-referenced to the history from the referring veterinarian. The owners were asked what medication the animal received, and what was their perception of the progress of their animal's condition (worse, unchanged, better) and QOL (acceptable or not acceptable). The follow-up information was subsequently given a second score (VAS) by one of the authors.[ii] The author who assessed the VAS at the follow up was blinded to the initial score. An assessment of the success of individual treatments is not attempted in this study; our purpose is to document the progression of clinical signs in non-surgically treated dogs.

In addition to the above, the volume of the caudal cranial fossa, the parenchyma within the caudal cranial fossa, and the sizes of the ventricles and syringes were measured at the time of

diagnosis using previously described methodology[4] and compared with the progression of the assessed clinical signs.

Statistical analysis

Statistical analysis was performed with a commercial software package[iii]. Paired nominal categorical data were compared using the McNemar's $\chi 2$ test. All quantitative data were assessed for normality of distribution with the D'Agostino and Pearson omnibus normality test and graphically. Means and SDs were calculated for normally distributed continuous data (means (±SD)), and medians and interquartile ranges (IQR) were determined for non-parametric data (median (IQR)). A Wilcoxon's signed rank test was used for statistical evaluation of paired, non-parametric data. The unpaired t-test (parametric data) or Mann-Whitney U test (non-parametric data) were used for comparing the morphometric values between dogs with and without deterioration of clinical signs assessed by VAS as appropriate. The association between owner's perception of their animal's progress (worse, unchanged, better) and progress based on VAS, was evaluated with Spearman's rank correlation. A P value of 0.05 or below was considered significant.

Results

Sixty-one CKCS were initially considered for this study. Of these, eight were excluded due to the absence of clinical signs attributable to CM/SM (n=3), craniocervical decompression (n=1), grade III/VI systolic heart murmur (n=2), generalised pyoderma (n=1), and otitis externa (n=1). Fifty-three dogs underwent MRI of the brain and the whole spinal cord. Of these, three were excluded because MRI studies were incomplete and two were excluded because of evidence of disc extrusion at C2–C3. Therefore, 48 dogs with MRI confirmed CM and clinical signs suggestive of NeP were included and followed up for a period of 39 (±14.3) (mean (±SD)) months from diagnosis, prospectively. There were 25 male dogs (17 neutered) and 23 female (16 neutered). Thirty-nine dogs (81%, 95% CI 69.9 - 92.1) had SM at the time of diagnosis.

At the end of the study, 36 dogs (75%, 95% CI 62.75 - 87.25) were still alive, four dogs died naturally at home with signs of suspected congestive heart failure (a postmortem examination was not performed), one dog was euthanased due to an aggressive ovarian tumour that had metastasised to other organs, and seven were euthanased due to severe signs of NeP. The mean age at diagnosis was 53.2 (±33.1) months, and the mean age at the end of the study, or death, 92.3 (±30.5) months.

Overall, 39 dogs were treated medically with gabapentin[iv], pregabalin[v] and/or intermittently carprofen[vi] and nine dogs were not on any medication for the management of NeP. Nine out of 48 dogs stopped scratching ($P < 0.001$), but there was no statistically significant change in the number of dogs exhibiting compromised exercise ability, vocalisation or facial rubbing behaviour. The median VAS of the clinical signs increased in the study population significantly ($P < 0.001$) from the initial median VAS of 75 mm (IQR 68–84 mm) to follow up VAS 84 mm (IQR 71.5–91 mm) (Figure 19). The severity of clinical signs based on VAS deteriorated in 36 (75%, 95% CI 62.75 - 87.25) dogs. From these dogs, 31 (86%, 95% CI 76.18 - 95.82) were treated with gabapentin or pregabalin, and carprofen. The remaining five dogs did not receive any treatment. A quarter of the dogs (25%, 95% CI 12.75 - 37.25) were static (n=7, 14.5%) or improved (n=5, 10.5%).

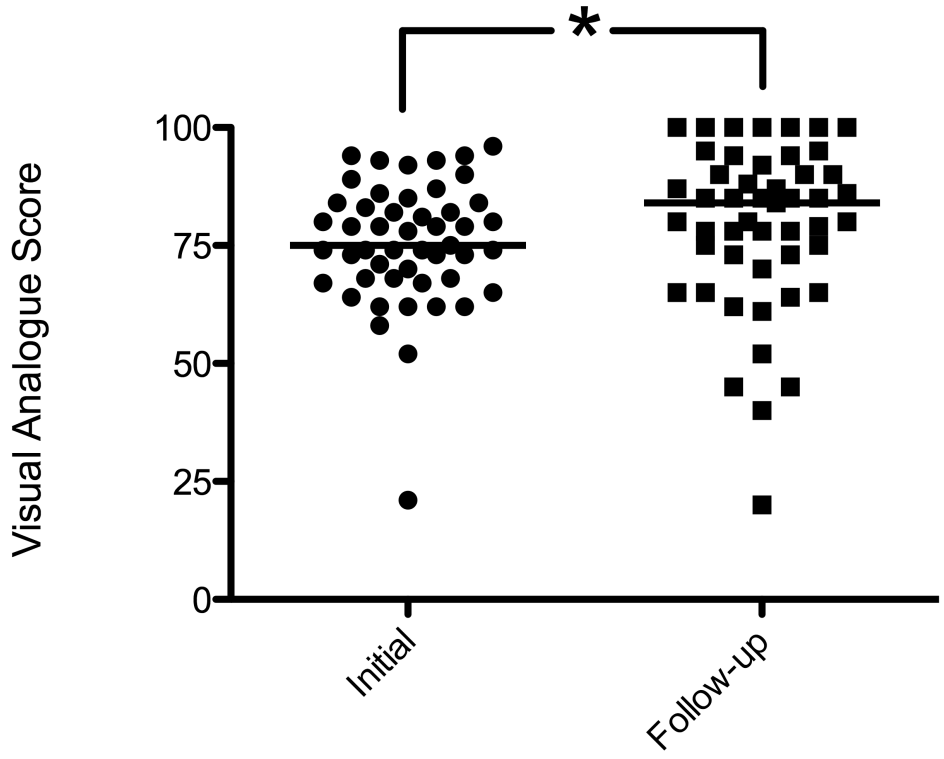

Figure 19. Scatter plots showing the distribution of Visual Analogue Score (%) in Cavalier King Charles Spaniel dogs with clinical signs at the beginning of the study (Initial) and 39 (± 14.3 months) later (Follow-up) (Bar represents median; Wilcoxon rank sum test, * represents P<0.05).

The owner's perception of their animal's progress, and progress based on VAS, was strongly positively correlated (Spearman's rank correlation (sr)=0.74, P<0.001). All the owners of the dogs that were alive at the end of the study reported that their dog's QOL was acceptable; most said had that if QOL were unacceptable they would have opted for euthanasia. There was no significant association between morphometric values (volume of the caudal cranial fossa, the parenchyma within the caudal cranial fossa, and the sizes of the ventricles and syringes) at the time of diagnosis between dogs with and without deteriorating clinical signs.

In three out of eight female entire bitches, the owners reported periodical aggravation of the CM/SM-related clinical signs during oestrus. Six dogs developed seizures during the study.

Discussion

To the authors' knowledge, this is the first report of clinical signs suggestive of NeP associated with CM/SM being progressive in the majority of dogs when treated non-surgically. In this study, morphometric values did not seem to play a significant role in the progression or improvement of the clinical signs. Three-quarters of the dogs displayed progression of clinical signs, whereas, one-quarter remained static or improved. Despite the deterioration of the clinical signs in the majority of these dogs, 75% were still alive 39 (±14.3) months later, with an acceptable QOL for the owners. All the owners of these dogs indicated that if their dogs' QOL was severely compromised, then they would opt for euthanasia. This study shows that non-surgical management of this condition can be an acceptable option considering that there is no evidence for better management in dogs.

The clinical course of the clinical signs seen in our study is similar to what has been reported in human beings with unoperated SM associated with type I Chiari malformation, and/or underdevelopment of posterior fossa.[28] It has been suggested that the natural history of symptomatic SM associated with type I Chiari malformation, and/or signs of posterior fossa underdevelopment, is characterised by an initial relatively rapid clinical progression accompanied with distended cavities. If surgery is not performed, then a state of syrinx equilibrium may be reached, that is, no further expansion of the syrinx, although this may be associated with irreversible spinal cord damage. Eventually, in some cases, there may be MRI signs of cavity collapse.[29] A possible explanation is that there was destruction of spinothalamic and lemniscal tracts by the syringes, so that pain could no longer be perceived.[30] A further possibility is that the caudal cranial fossa and brain parenchyma adjust to compensate for the overcrowding of the caudal cranial fossa.[31]

Rusbridge[26] followed up 15 CKCSs that underwent craniocervical decompression for 0.2–2.3 years after surgery, and despite an initial clinical improvement in 80% of the dogs, 47% deteriorated in a mean time of 1.3 years. Ten out of these 15 dogs had severe signs of NeP, and medical treatment was not successful. Despite the initial improvement though, all dogs continued to exhibit signs of NeP, and surgery did not change the size of the cervical syringes. Similar findings were described by Vermeersch and others[23] and Dewey and others.[24,25] One cause of deterioration was attributed to scar tissue adhering to exposed neural tissue and

preventing adequate CSF flow. This study does not allow for direct comparison of medical versus surgical treatment, nor does it determine whether the specific treatments used influence outcome. Our purpose was do document clinical progression in non-surgically treated dogs, irrespective of the choice of medical treatment. Whilst the choice of medical management could affect outcome, the majority of dogs in this study were treated with neuropathic painkillers commonly prescribed for this condition. Further studies that follow up cases for a longer period of time are needed to be able to compare the long-term outcome of conservative and surgical treatment.

Nine of the 48 dogs had CM only. Traditionally, clinical signs suggestive of NeP in CKCS have been associated with CM and SM, but from our study, we found that CM only may contribute to these signs, too. The pathophysiology of these clinical signs in dogs with CM is not well understood, but the overcrowding of the foramen magnum might be applying pressure onto brainstem nuclei causing signs suggestive of NeP.[14] Unfortunately, there is no evidence for this theory, but in the human literature, there are several reports associating Chiari malformation with trigeminal neuralgia that resolves after craniooccipital decompression or placement of a ventriculoperitoneal shunt.[32,33]

Interestingly, there was a significant improvement in scratching (nine dogs stopped scratching), whereas, the VAS got significantly worse. New research findings in the area of pruritus may give an explanation for this. Pruritus, traditionally, has been associated with a submodality or subquality of pain.[34] Advances in this area have elucidated differences between pruritus and pain, but have also obscured the distinction between them. Pruritus and pain appear to be independent sensations because nociceptive and pruriceptive stimuli each elicit unique behavioural responses.[35] Sun and Chen[36] reported that they have identified the first gene in the spinal cord of mice, linked with pruritus, and that it is responsible for the expression of gastrin-releasing peptide receptors (GRPR). These GRP receptors were found in a group of spinal cord cells called lamina-I neurones that relay both itch and pain sensation to the brain. However, Sun and others[34] believe that there is a specific subpopulation of GRPR neurones, located in the superficial dorsal horn within the lamina-I, which are specific only for the pruritic sensation (labelled-line hypothesis). Destruction of the described neurones in mice and stimulation afterwards with various pruritic agents showed up to an 85% reduction of pruritus compared with the controlled group, but nociception and motor function appeared to be unaffected.[34] Many attempts to localise the pathway of these GRPR neurones all the way

up to the thalamus (where they finally project) failed, but it is still believed that the pruriceptive pathway is different to the nociceptive pathway in the spinothalamic tract neurones that project to the posterior and ventral posterior region of the thalamus.[34] Further research is needed to elucidate the difference between these two pathways.

Another interesting finding of this study is that despite the fact that history of seizures at the time of diagnosis was an exclusion criterion, six dogs (12.2%) developed seizures in the investigated period of time. It was suggested that seizures may be related to ventriculomegaly secondary to overcrowding of the caudal cranial fossa[10], however, a recent study could not show a relationship between ventroculomegaly and seizures.[37] This finding is more likely related to idiopathic epilepsy, which is common in this breed[38] or other unknown pathologies. In human beings, epilepsy, in conjunction with type I Chiari malformation, is occasionally reported. Two subtypes are described: the first as an incidental finding in the diagnostic work-up of patients with idiopathic epilepsies, and the second where both type I Chiari malformation and epilepsy occur as part of a more widespread developmental disorder.[39]

In three out of eight female entire bitches, the owners reported periodical aggravation of the CM/SM-related clinical signs during oestrus. Hubscher and others[40] reported that oestrogen (17b-estradiol) administration reduces experimentally induced allodynia in rats, but there is no literature to support that oestrogens can deteriorate the signs of NeP. To the authors' knowledge, there is no involvement of oestrogens in the pathophysiology of NeP, and this may be an interesting finding that requires further investigation. Perhaps the stress associated with the oestrus can aggravate the perception of NeP, in a similar way that mood affects the pain perception in human beings.[41]

There are limitations to this study, considering the selection criteria. Only dogs with VAS of 50 or more underwent MRI, and were included in the study. However, we do not feel that selecting for dogs with prominent clinical signs is a significant limitation of the study as they reflect the general population of dogs that are presented to a neurologist for investigation of this condition. There is a possible bias from the owners and the veterinary surgeons when assessing the clinical signs. Also, one of the most important problems is that pain is a subjective variable and may have been inappropriately assessed considering that the dogs cannot verbally communicate their level of discomfort. Moreover, we can only assume that CM/SM is the cause of the described clinical signs and that these signs are suggestive of

neuropathic pain. A follow-up MRI was not performed, so we cannot exclude the possibility of development of other spinal diseases in the study period that may affect the progression of the clinical signs. The VAS itself, as with other scoring systems, is subject to bias, and its reproducibility can be questioned; however, the scorers in this study were trained to be as consistent as possible in their observations. The VAS is a recognised tool for measuring subjective phenomena, such as anxiety, pain, QOL[42-44], and it has been used extensively in people, and increasingly in veterinary literature. It seems to be reliable[45,46], more responsive[47], and easy to use, compared with other pain-scoring systems. When reviewing the length of this cohort study, euthanasia was a factor. The ability for human owners to eliminate their animals' suffering through euthanasia is not a shared ethical dilemma with human sufferers of CM/SM complex. For each owner, this will be an individual decision, and based on the bond they have with their pets, and also the potential economic implications. Finally, it is important to mention that this study is documenting the progression of a specific set of clinical signs in a group of dogs with CM and/or SM, and not the progression of the disease itself. Radiological progression of the disease has not yet been reported. 15% (95 % CI 4.9 to 25.1) of the study population was euthanased at the request of the owner due to the severity of the clinical signs. However, in the remaining surviving dogs, despite the progression of the clinical signs, the majority of the owners felt that QOL of their dogs was acceptable.

Conclusions

The findings of this study show that clinical signs of NeP are progressive in the majority (75%) of CKCS with CM/SM. Despite this, most owners considered their pets to maintain an acceptable QOL. Therefore, medical management of CM/SM with gabapentin, pregabalin and/or NSAIDS has a similar outcome to craniocervical decompression; however, a direct comparison is yet to be made and this requires further study. Morphometric features of CM associated with SM did not appear to correlate with the severity of clinical signs in this study.

Footnotes:

[i] Dr Kate Chandler

[ii] Dr Holger A Volk

[iii] Prism 5 for Mac, Graphpad Software 2007

[iv] Neurontin, 10 mg /kg every 8–12 hours

[v] Lyrica, 2–4 mg /kg every 8 hours

[vi] Rimadyl, 2 mg/kg every 24 hours

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Chapter 3

THE NATURAL PROGRESSION OF SYRINGOMYELIA IN DOGS

Chapter 3

CHANGES OVER TIME IN CRANIOCEREBRAL MORPHOLOGY AND SYRINGOMYELIA IN CAVALIER KING CHARLES SPANIELS WITH CHIARI-LIKE MALFORMATION

CHANGES OVER TIME IN CRANIOCEREBRAL MORPHOLOGY AND SYRINGOMYELIA IN CAVALIER KING CHARLES SPANIELS WITH CHIARI-LIKE MALFORMATION

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Summary

The purpose of this study was to determine the natural progression of canine Chiari-like malformation (CM) and syringomyelia (SM), which has not yet been described.

The objectives of this study were to i) determine if SM progresses with time ii) determine if features of craniocerebral morphology previously associated with CM are progressive (including caudal cranial fossa volume, caudal cranial fossa parenchymal volume, ventricular dimensions, height of the foramen magnum and degree of cerebellar herniation). A retrospective morphometric analysis was undertaken in 12 CKCS with CM for which repeat magnetic resonance images were available without surgical intervention.

The maximal syrinx width, height of the foramen magnum, length of cerebellar herniation and caudal cranial fossa volume increased over time. Ventricular and caudal fossa parenchymal volumes were not significantly different between scans.

The results of this study suggest that SM progresses with time. Increased caudal cranial fossa volume may be associated with active resorption of the supraoccipital bone, which has previously been found in histology specimens from adult CKCS. We hypothesise that active resorption of the supraoccipital bone occurs due to pressure from the cerebellum. These findings have important implications for our understanding of the pathogenesis and variable natural clinical progression of CM and SM in CKCS.

Introduction

Chiari-like malformation (CM) is a malformation of the hindbrain and the surrounding caudal cranial fossa (CCF) reported in small breed dogs. The condition is named after its analogous human counterpart, Chiari-type 1 malformation.[1] In Cavalier King Charles Spaniels (CKCS) the condition has a complex oligogenic trait of moderately high heritability.[2,3,4,5] CM is characterised by herniation of part of the cerebellar vermis through the foramen magnum.[6,7] Other reported abnormalities include occipital bone hypoplasia/dysplasia or a 'shallow' occipital bone[8], kinking of the medulla and malformations of the craniocervical junction[8,9,10], ventriculomegaly or hydrocephalus[11] and syringomyelia (SM).[6] SM is a single or series of non-cerebrospinal fluid filled cavities within the spinal cord parenchyma, the formation of which is most likely associated with alteration of cerebrospinal fluid (CSF) flow.[12] SM is responsible for clinical signs of neurological disease in dogs including pain, cervical scoliosis and ataxia.[6,13] There is a high prevalence of SM in symptomatic CKCS with CM.[3] The pathogenesis of CM/SM is therefore often investigated concurrently.

The relationship between CM and SM in CKCS is thought to be compression of subarachnoid CSF pathways and alteration of CSF flow at the level of the foramen magnum.[14] In humans with Chiari-type 1 malformation, this alteration is associated with hypoplasia of the bones of the posterior fossa (analogous to the canine CCF), with overcrowding of a normally developed hindbrain and consequential cerebellar tonsillar herniation.[15] Studies of CKCS cerebral and cranial morphology have revealed conflicting evidence regarding the association between SM and hypoplasia of the bones of the CCF, CCF volume and cerebellar herniation.[7,8,9,11,16] A possible shared limitation of these studies is a failure to identify an appropriate control group given that SM may be progressive; the prevalence of SM in asymptomatic CKCS scanned for breeding purposes is 25% at 12 months and 70% in dogs aged 72 months or more.[17] A subsequent study found an association between CCF volume and SM when comparing age-matched groups.[18] Changes in CCF volume with time may be an aetiological factor in CM/SM progression. A previous histological study of CCF bones from adult CKCS has found evidence of active remodelling of the supraoccipital bone, with replacement of bone with cartilage.[19] There may be a connection between this active remodelling and CCF volume if it occurs such that the supraoccipital bone thickness is reduced from within, thus increasing the inner CCF volume without changing the outer CCF dimensions.

There is a need for better understanding of the progression of CM and SM in dogs. We hypothesised that syrinx width increases with time in CKCS. In addition, we hypothesised that CCF volume, foramen magnum height and cerebellar herniation would progressively increase with time in CKCS with CM. We present a retrospective morphometric study of magnetic resonance images (MRI) from CKCS with CM. Analysis of craniocerebral morphometry from CKCS with CM for which two separate MRI studies were compared.

Materials and Methods

Subject selection

Clinical database software of three institutions was retrospectively reviewed between May 2004 and May 2010; Royal Veterinary College, Animal Health Trust and the Stone Lion Veterinary Hospital. CKCS were selected for inclusion if they had radiological findings consistent with CM (indentation of the cerebellum and herniation of the cerebellar vermis into the foramen magnum) that underwent two cranial MRI studies, both of which included sagittal and transverse slices from the cribiform plate rostrally to the third cervical spinal cord segment caudally. For each case selected, the presence of CM was confirmed by a European neurology or diagnostic imaging specialist. CKCS with a concurrent diagnosis of space occupying lesions or lesions associated with raised intracranial pressure were not included.

CKCS that were included in the study were assigned a case number. Descriptive data recorded included patient sex, age and clinical complaint at first and second presentation for MRI scan. The scan interval and treatments were additionally recorded and described. MRI scans were randomly assigned an image number such that analysis could be blinded to all descriptive data including whether it was the first or second scan. MRI scans were subsequently grouped into first and second scans prior to statistical analysis. A Fishers exact test comparing the presenting complaints (attributable to CM/SM or not) was performed between the two groups.

Magnetic Resonance Image analysis

All dogs in this study had been positioned for MRI in dorsal recumbency with the cervical spine in an extended position. The presence of SM was assessed using sagittal and transverse T2-weighted MRI of the cervical spinal cord. Only the cervical spinal cord was assessed as SM most commonly affects this region.[32] SM was defined as well-demarcated intramedullary lesions associated with the central canal, hyperintense on T2 weighted and hypointense on T1 weighted images. Lesions greater than 2 mm diameter in transverse plane were described as syringes. Lesions of 0-2 mm diameter were described as central canal dilation. For statistical analysis, the maximal transverse width of the central canal/syrinx (central canal/syrinx width) was recorded using commercially available imaging software[i] using a technique previously described.[13]

Cerebellar herniation length was assessed from mid-sagittal T2 weighted images using the same imaging software, with a previously described technique (Figure 20).[9] Foramen magnum height was first assessed by measuring a line from the most dorsal aspect of the basioccipital bone to the ventral most aspect of the supraoccipital bone. Cerebellar herniation length was measured from the tip of the cerebellar vermis to the point of bisection of the line used to measure foramen magnum height.[9]

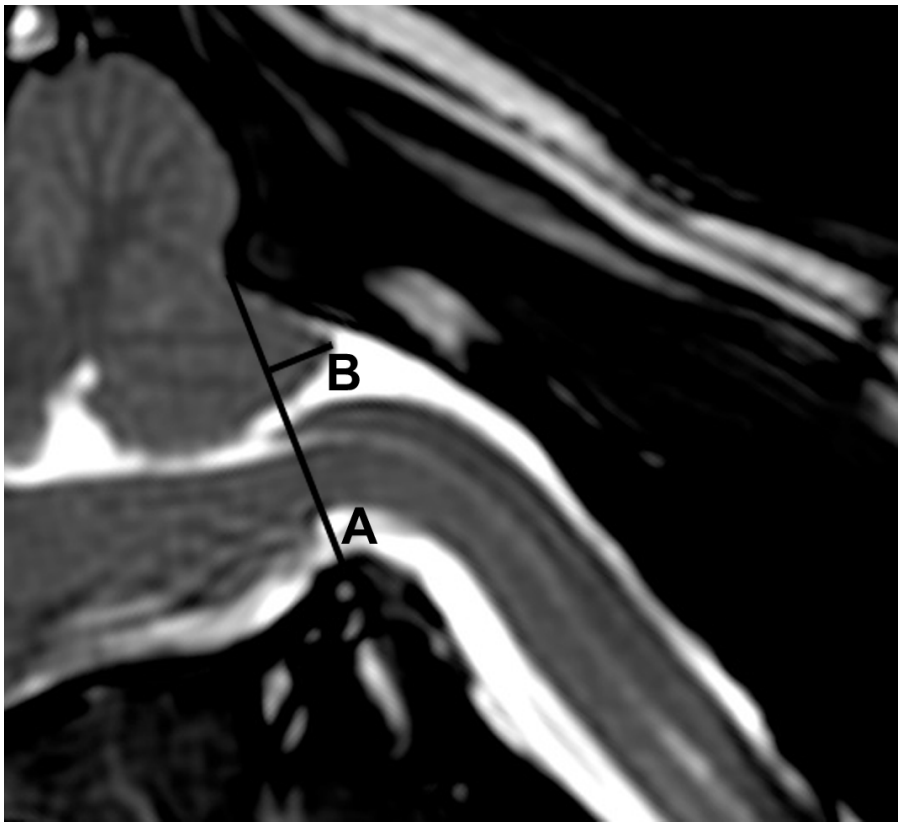


Figure 20. Measurements made on sagittal T2-weighted images: A: Foramen magnum, measured from the ventral most aspect of the supraoccipital bone to the caudal basisphenoid bone. B: Cerebellar herniation length, measured perpendicular from the line of the foramen magnum to the tip of the cerebellar vermis.

Craniocerebral volumetric data was obtained using a method previously described in detail.[11,18,24] Transverse T2-weighted images were exported to a commercially available 3D modelling software programme[ii]. The cranial fossa was divided into the rostral/middle cranial fossa and the caudal cranial fossa by the tentorium cerebelli. Following manual tracing of a

series of two-dimenstional masks from individual slices[11,18,24], the following were calculated by the software:

- CCF volume, expressed as a percentage of total cranial cavity volume (total cranial cavity volume is the summation of rostral, middle and caudal cranial fossae volumes as previously defined)
- CCF parenchymal volume, expressed as a percentage of CCF volume
- Ventricular system volume, expressed as a percentage of the total brain parenchymal volume

Individual masks were drawn by the same observer[iii].

Statistical analysis

Commercially available statistical software was used for data analysis[iv]. Data sets were assessed for normality of distribution with the Shapiro-Wilk W test. Where data sets for the two groups were normally distributed, a paired one-tailed t-test was used to analyse the statistical significance of data. Where data sets were not normally distributed, a one-tailed Wilcoxon signed rank test was used. One-tailed tests were used considering our prediction of the direction of effect based on the described observations of changes to the supraoccipital bone and the general assumption that SM is a progressive disease.

Parametric data is presented as the mean ± the standard deviation and non-parametric data is presented as the median with range. A p value <0.05 was considered significant.

Results

Twelve CKCS were included in the study, of which eight were male (67%) and four were female (33%). The mean age when first scanned was 44.3 months ± 30.27 months. The median scan interval was 9.5 months (3 – 83 months). Ten of twelve dogs presented for the first scan with at least one clinical sign that could be attributable to CM/SM (83.3%). Presenting clinical complaints and treatments are summarised in table 7. The majority of dogs (58%, 95% CI 31.95 to 80.67%) presenting for a second scan did so due to clinical complaints that were considered unrelated to the initial presenting condition and thus were suspected to represent a new condition. The difference in presenting complaints (attributable to CM/SM or not) between scans was significant (p=0.04). Four dogs were re-scanned due to poor control of the original complaint and one dog due to the development of dysaesthetic behaviour.

SM progression

Two CKCS (17%) had syringes at the time of the first scan, this increased to five (42%) with syringes at the time of the second scan. Three dogs initially had central canal dilation (25%), which increased to four dogs (33%) following the interval. Three of five dogs re-presenting due to clinical signs attributable to CM/SM had increased central canal width in the scan interval (table 7). There was a statistically significant (p=0.01) increase in central canal/syrinx width between the first (0.08cm ± 0.14cm) and second (0.18cm ± 0.16cm) scans (figure 21).

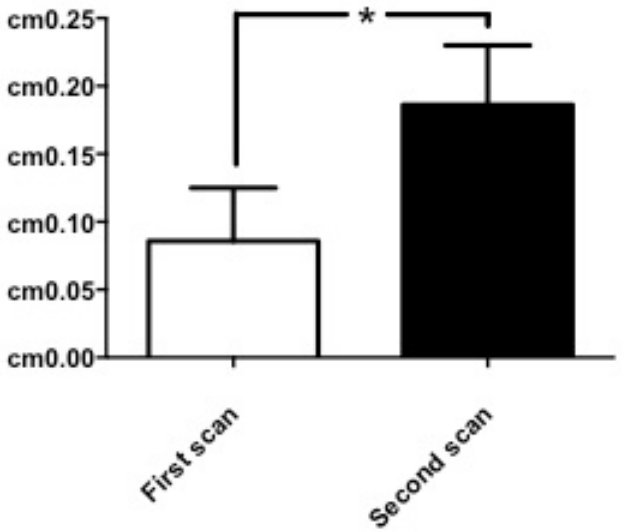


Figure 21 - Progression of central canal/syrinx width. Bar graph displaying distribution of central canal/syrinx width for first and second scans. There was a statistically significant difference in maximal transverse width between first and second scans (0.08cm ± 0.14cm vs 0.18cm ± 0.16cm, p=0.01). Bar represents mean (SD), * represents *P<0.05*.

Table 7 Part 1 - Summary of clinical complaint at presentation and treatments after first scan

Case Details		**First Scan**		**Scan interval (months)**
No.	**Age (months)/sex**	**Clinical complaint**	**Treatment**	
1	78/female neutered	Cervical hyperaesthesia Ataxia	Carprofen	42
2	59/male neutered	Dysaesthetic behaviour[1]	Gabapentin	5
3	13/male neutered	Dysaesthetic behaviour[1]	Gabapentin	17
4	74/male neutered	Cervical hyperaesthesia	Gabapentin	3
5	6/female	Dysaesthetic behaviour[1] Ataxia	Meloxicam Gabapentin	83
6	51/male neutered	Cervical hyperaesthesia	Acetazolamide Meloxicam	6
7	15/male	Seizures	Phenobarbitone	20
8	67/male neutered	Cervical hyperaesthesia Head tilt Ataxia	Clindamycin	4
9	24/female	Cervical hyperaesthesia	None	11
10	6/male	Cervical hyperaesthesia	Carprofen	37
11	90/male neutered	Facial paresis	Potentiated amoxcillin Meloxicam	3
12	48/female neutered	Cervical hyperaesthesia	Gabapentin	8

Table 7 Part 2 - Summary of clinical complaint at presentation and treatments after second scan

Case Details		**Second Scan**	
No.	**Age (months)/sex**	**Clinical complaint[2]**	**Treatment**
1	78/female neutered	Poor control of original complaint	Prednisolone
2	59/male neutered	Poor control of original complaint	Gabepentin
3	13/male neutered	Poor control of original complaint	Prednisolone
4	74/male neutered	Dysaesthetic behaviour[1]	Pregabalin
5	6/female	Recurrent generalised seizures	Phenobarbitone Gabapentin Meloxicam
6	51/male neutered	Poor control of original complaint	Acetazolamide Gabapentin
7	15/male	Recurrent generalised seizures	Phenobarbitone Levetiracetam
8	67/male neutered	Complex partial seizures	Phenobarbitone
9	24/female	Recurrent generalised seizures	Potassium Bromide
10	6/male	Migrating foreign body	Carprofen Prednisolone Trimethoprin-sulphonamide
11	90/male neutered	Head tilt	Enrofloxacin
12	48/female neutered	Complex partial seizures	Potassium Bromide

1. Dysaesthetic behaviour included phantom scratching and facial rubbing
2. Clinical complaints considered not attributable to CM/SM included seizures, head tilt, facial paresis and migrating foreign bodies.

Foramen magnum height and cerebellar herniation progression

Foramen magnum height increased in eleven (92%) dogs in the scan interval. Foramen magnum height increased significantly (p=0.025) between the first (1.52cm ± 0.08cm) and second (1.59cm ± 0.09cm) scans (figure 22). Cerebellar herniation length increased in eight (73%) dogs in the scan interval. There was a statistically significant (p=0.021) increase in the length of cerebellar herniation between the first (0.17cm ± 0.05cm) and second (0.22cm ± 0.09cm) scans (figure 22).

Craniocerebral volumetric progression

There was a significant difference (p=0.018) in CCF volume between the first (13.46%, ± 1.45%) and second (13.73% ± 1.59%) scans (figure 23). There was no significant difference (p=0.188) in CCF parenchymal volume (87.20% ± 3.09 vs 88.59% ± 1.97%) or volume of the ventricular system (p=0.188) between the first and second scans (3.21%, 2.00 - 14.34 vs 2.51%, 1.602 - 17.20%, figure 23).

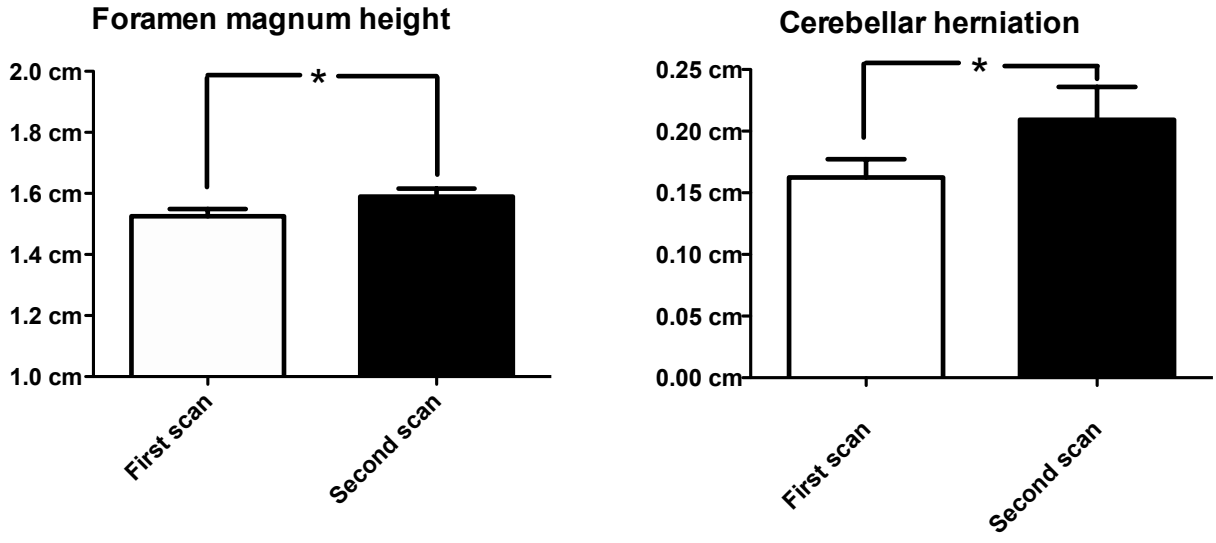
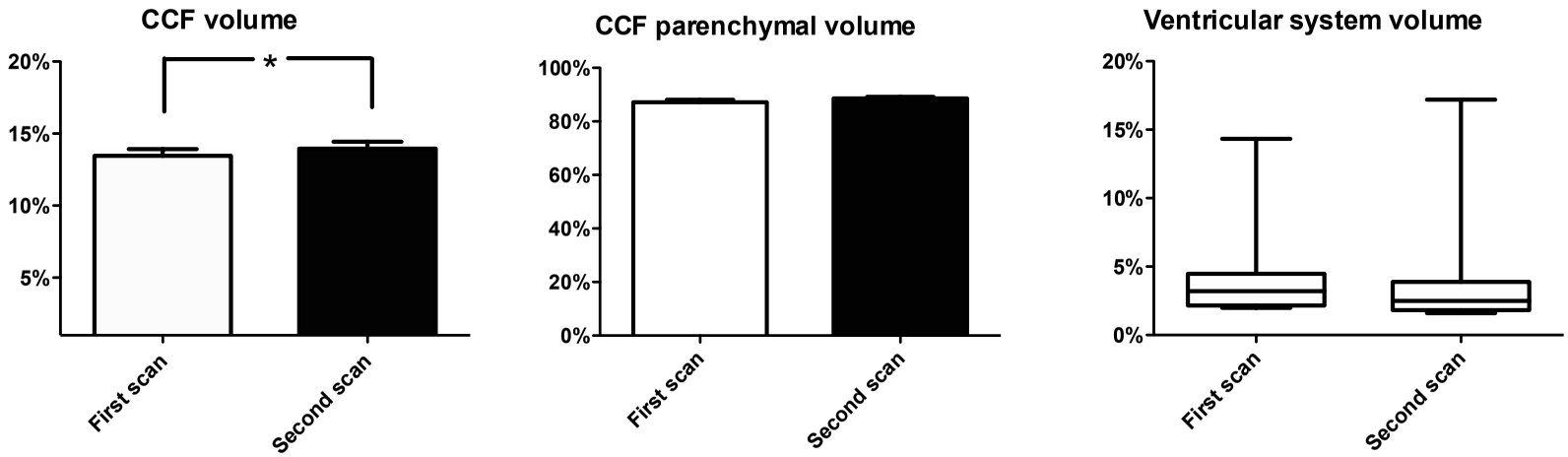


Figure 22 – Progression of foramen magnum height and length of cerebellar herniation.
Bar graphs displaying distribution of foramen magnum height and cerebellar herniation for first and second scans. There was a statistically significant difference between first and second scans for foramen magnum height (1.52cm ± 0.08cm vs 1.59cm ± 0.09cm, p=0.025) and length of cerebellar herniation (0.17cm ± 0.05cm vs 0.22cm ± 0.09cm, p=0.021). Bar represents mean (SD), * represents *P<0.05*.

Figure 23 – Progression of craniocerebral volumes. Bar graphs displaying distribution of caudal cranial fossa (CCF) volume, CCF parenchymal volume, and box and whisker plot displaying distribution of ventricular system volume between first and second scans. There was a statistically significantly difference between first and second scans for CCF volume (13.46%, ± 1.45% vs 13.73% ± 1.59%, p=0.018). There was no statistically significant difference between first and second scan for CCF parenchymal volume (87.20% ± 3.09 vs 88.59% ± 1.97%, p=0.065) and the ventricular system volume (3.21%, 2.00 - 14.34 vs 2.51%, 1.602 - 17.20%, p=0.188). Bar represents mean (SD) or median (range), * represents *P<0.05*.



Discussion

These results suggest that central canal/syrinx width increases in CKCS with CM with time. Further, the height of the foramen magnum, extent of cerebellar herniation and CCF volume significantly increased with time. These findings have important implications for our understanding of the pathogenesis and natural progression of medically managed CM/SM in CKCS.

Central canal/syrinx width increased significantly in the scan interval. The number of CKCS with syringes (lesions with transverse width of greater than 2mm) increased from 2/12 to 5/12 in the scan interval. Although not all CKCS developed syringes, there was progressive central canal dilation, which is a precursor of syrinx formation.[20,21] Thus this is the first report of SM progressing radiologically in CKCS with CM with time. As maximal syrinx width is associated with pain[13] this increase may also be clinically significant. Clinical progression was not determined in this study due to its retrospective nature and case selection criteria. Progression of clinical signs in medically treated CM/SM has recently been reported elsewhere.[22] Severe progression of clinical signs in our study population may have biased our case selection; CKCS with a more severe clinical phenotype prompting repeat MRI may have been more likely to have increased SM lesion width. In this study, significantly more CKCS presented for the second scan with clinical complaints considered not attributable to CM/SM.

Several studies have suggested a link between craniocerebral volumes, cerebellar herniation and the development of SM in CKCS. Reduced CCF volume as a result of impaired occipital bone development has been implicated as the cause of cerebellar herniation.[6,8,9] The adult occipital bone develops from the basioccipital, exoccipital, and supraoccipital bones which are derived from distinct somatic mesodermal derived cartilages.[23] A mesodermal insufficiency has therefore been proposed as a mechanism for mis-match between cerebral and cranial volumes and their association with SM[24], however, morphometric studies have not found this association in dogs.[8,11,16] CKCS have similar CCF volume to other small breeds such as the pug.[24] Conversely, CKCS have similar volumes of parenchyma within the CCF to larger Labrador retrievers.[24] In CKCS, small but significant increases in parenchymal volume within the CCF are associated with more severe SM.[11] Furthermore, in contrast to other small breed dogs, CKCS exhibit correlation between increased cerebellar volume and cerebellar crowding within the caudal aspect of the CCF (indicating that relative increases in cerebellar

volume are more severe in the caudal aspect of the bone cavity).[25] The findings of this study suggest that CCF volume increases significantly with time and therefore may play a role in progression of the disease.

The height of the foramen magnum was significantly increased in the scan interval. Our study also suggests the length of cerebellar herniation is increased. A positive association has previously been found between foramen magnum size and cerebellar herniation in CKCS.[9] This may represent a dynamic change occurring to the occipital bones that form the foramen magnum in response to the previously discussed parenchymal overcrowding of the CCF. This is supported by the previous finding that a large part of the supraoccipital bone of adult CKCS is cartilage, suggesting active remodelling.[19] We hypothesise that the increased CCF volume may be a result of inner resorption of the overlying occipital bones rather than an increase in its dimensions. The occipital bones in adults and children show a resorbtive pattern of the bone around the cerebellar hemispheres.[26] Bone remodelling occurs as an adaptation to mechanical load according to Wolff's law. The pulsatile movements of the cerebellum occurring during systole could exert a mechanical pressure on the caudal aspect of the CCF leading to occipital bone resorption, hence increasing the height of the foramen magnum and possibly changes in the extent of cerebellar herniation. In support of this theory, intra-operative and post-mortem findings of CKCS with CM have revealed that the supraoccipital bone overlying the cerebellar vermis can be remarkably thin and sometimes eroded so that the foramen magnum is enlarged dorsally.[27] A thick cartilagenous band is frequently found in surgery in place of the supraoccipital bone [C. Rusbridge, personal communication]. Importantly, the reduced cerebrospinal flow velocity as a result of CCF remodelling may alter the rate of progression of SM, which may explain the high prevalence of asymptomatic SM in CKCS and the variable response to medical treatment.

An alternate mechanism for the failure of adaptation of the CCF in CKCS previously proposed is early closure of bone sutures, similar to craniosynostosis in children.[24] Early suture closure may affect the cranial foramina enclosing the emissary vein at the retroarticular foramen; the result of compression of the inferior petrosal and sigmoid sinuses is increased intracranial pressure and reduced resorption of CSF in children.[28] This is a proposed mechanism for the development of SM in the Griffon Bruxellois that do not display cerebellar herniation.[29]

There are limitations to the measurements made in this study. Firstly, it should be noted that variations in cerebellar herniation might occur throughout the cardiac cycle, which were not accounted for. However, as noted in previous human studies, this limitation should be relatively minor and the variation is unlikely to significantly affect our measurements or conclusions.[30] Secondly, cerebellar herniation is increased by flexion of the neck.[31] The effect of positioning was minimised in this study. Some medications used to treat CM/SM, including corticosteroids, may influence the progression of CM/SM on MRI by altering cerebrospinal fluid production. None of the dogs in this study received corticosteroids in the scan interval (table 1). The medications prescribed were not considered likely to affect our measurements.

Conclusions

CCF volume, foramen magnum height, length of cerebellar herniation and central canal/syrinx width increased significantly over time. The CCF parenchymal volume and ventricular volume did not change significantly. This enhances our understanding of the pathogenesis of CM/SM in CKCS. We hypothesis that dynamic remodelling may occur to the occipital bone. This could explain the varied phenotype of the disease.

Footnotes

[i] OsiriX Medical Imaging Software 3.8.1

[ii] Mimics ® 13.10, Materialse n.v., 2009

iii Sarah Hamilton

iv Prism® for Windows Version 5.00, Graphpad Software Inc, 2007

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Chapter 4

NOVEL DYNAMIC ASSESSMENT OF CEREBELLAR PULSATION IN DOGS WITH CHIARI-LIKE MALFORMATION

Chapter 4

ASSESSMENT OF CEREBELLAR PULSATION IN DOGS WITH AND WITHOUT CHIARI-LIKE MALFORMATION AND SYRINGOMYELIA USING CARDIAC-GATED CINE MRI

ASSESSMENT OF CEREBELLAR PULSATION IN DOGS WITH AND WITHOUT CHIARI-LIKE MALFORMATION AND SYRINGOMYELIA USING CARDIAC-GATED CINE MAGNETIC RESONANCE IMAGING

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Summary

Canine Chiari-like malformation (CM) is characterised by herniation of part of the cerebellum through the foramen magnum. In humans with Chiari type I malformation (CM-I), abnormal pulsation of the cerebellum during the cardiac cycle has been documented and is pivotal to theories for the pathogenesis of syringomyelia (SM). The purpose of this study was to determine whether cerebellar pulsation occurs in dogs with CM and to investigate an association between cerebellar pulsation and SM.

We used retrospectively cardiac-gated cine balanced fast field echo (bFEE) magnetic resonance imaging sequences to assess pulsation of the brain in dogs and to objectively measure the degree of cerebellar pulsation with the neck in a flexed position. Seventeen cavalier King Charles spaniels (CKCS) with CM, eight with SM and nine without, were compared with six small breed control dogs. Linear regions of interest were generated for the length of cerebellar herniation from each phase of the cardiac cycle and the degree of cerebellar pulsation was subsequently calculated. Subjects' age, body weight and angle of neck flexion were also compared.

CKCS with CM and SM had significantly greater pulsation of the cerebellum than control dogs ($P = 0.003$) and CKCS with CM only ($P = 0.031$). There was no significant difference in age, body weight and angle of neck flexion between the three groups. Cardiac-gated cine bFEE MRI permitted the dynamic visualisation of cerebellar pulsation in dogs. These findings support the current theories regarding the pathogenesis of SM secondary to CM and further highlight the similarities between canine CM and human CM-I.

Introduction

Canine Chiari-like malformations (CM) of the craniocervical junction are noted for their similarity to their human counterpart, Chiari type I malformation (CM-I). The conventional definition of CM-I is reduced posterior cranial fossa volume with caudal descent of the cerebellar tonsils past the foramen magnum into a region occupied by the cervical spinal cord. Despite lacking tonsils, decreased caudal cranial fossa volume and herniation of the cerebellar vermis past the foramen magnum are defining features of canine CM.[1,2]

CM is a complex oligogenic trait in the Cavalier King Charles Spaniels (CKCS)[3] with one study finding 92% of CKCS to have at least one morphologic feature on magnetic resonance imaging (MRI) consistent with this diagnosis.[4] Syringomyelia (SM) occurs secondary to CM.[5] Clinical signs including cervical hyperaesthesia and dysaesthetic behaviour are most often associated with syringes in the dorsal horn of the spinal cord.[6]

The pathogenesis of SM secondary to CM remains contentious, in particular the mechanism of syrinx formation. Current theories have been summarised by Rusbridge.[7] The piston theory proposed by Oldfield and others[8] suggests the cerebellar tonsils (notably absent in dogs) act like a piston during systole, forcing high pressure CSF down the subarachnoid space and into the spinal cord. However, this theory does not adequately explain why a syrinx is at a higher pressure than the subarachnoid space. The intramedullary pulse pressure theory by Greitz[9] accounts for this by suggesting that extra-cellular fluid forming a syrinx originates from the high-pressure system in the spinal cord microvasculature. The source of this high pressure is CSF that is driven into the spinal cord during systole, close to an obstruction of the subarachnoid space. Extracellular fluid then forms a syrinx due to the differential between the pressure of the subarachnoid space caudal to the obstruction (low pressure) and the spinal cord in this region (high pressure). Both theories share the belief that herniation of the cerebellum creates a high-pressure pulse of CSF during systole.[9]

Motion of the cerebellar tonsils has been visualised in humans using ultrasound and magnetic resonance imaging (MRI) techniques.[8,10] Human patients with CM-I have greater movement of the cerebellar tonsils during the cardiac cycle when measured using cardiac-gated phase contrast cine MRI[10] and steady-state acquisition cine MRI.[11,12] The pulsation effect of the cerebellar tonsils is increased by up to ten times from normal in humans with CM-I.[13] Phase

contrast cine MRI has been used to study the alteration of CSF flow at the level of the foramen magnum in CKCS with CM/SM.[14] Movement of the caudal medulla and cerebellum has also been described in dogs with CM using that technique.[15] Currently, movement of the neuraxis during the cardiac cycle has not been visualised in dogs using steady-state acquisition cine MRI and the relationship of cerebellar pulsation to the presence and severity of SM has not been investigated in dogs.

The primary objective of this study was to use cardiac-gated cine MRI to compare the degree of cerebellar pulsation during the cardiac cycle between three groups; small breed dogs without CM, CKCS with CM alone and CKCS with CM and SM. A secondary objective was to assess the correlation between cerebellar pulsation and the width of the syrinx, when present.

Materials and Methods

Subject selection

Seventeen client-owned CKCS and six control dogs undergoing cranial and spinal MRI at the Royal Veterinary College (RVC) between April 2011 and November 2012 were recruited for inclusion in this study. Inclusion criteria were age of greater than eight months, with a presenting complaint of cervical spinal pain, seizures or peripheral vestibular disease. The absence of paresis and postural reactions deficits (determined by a board-certified neurologist) was also an inclusion criterion. These criteria were used to identify subjects that would undergo advanced imaging but were unlikely to suffer from space-occupying CNS lesions that may influence the assessment of cerebellar pulsation. Suffering clinical signs solely attributable to CM/SM was not an inclusion criterion, given that the absence of clinical signs does not exclude the diagnosis of SM.[16] Control dogs were brachycephalic toy or utility breeds according to the UK Kennel Club (two Pugs, two French bulldogs and two Yorkshire terriers). Descriptive data was recorded including age, sex, body weight and diagnosis.

Imaging technique

Imaging was performed using a 1.5T MRI unit[i] and a 5-element phased-array spinal coil. Patients were positioned in dorsal recumbency with the head in a flexed position to mimic a standing position.[14,17] Compression of the jugular veins was avoided during positioning. Vector electrocardiography was recorded during the examination to allow for retrospective cardiac gating. The heart rate was stabilised between 60 and 80 bpm prior to scanning to facilitate cardiac triggering of image acquisition. Initially, a standard imaging protocol was performed sufficient to obtain a clinical diagnosis and exclude the presence of CNS lesions and craniocervical junction abnormalities other than CM/SM that may affect cerebellar pulsation.

The protocol included sagittal and transverse T1 and T2-weighted turbo spin echo (TSE) sequences of the brain and cervical spinal cord, transverse T2-weighted fluid attenuated inversion recovery (FLAIR) and post-gadolinium enhanced T1-weighted TSE of the brain. Subsequently, a sagittal balanced fast-field echo (bFFE) sequence was performed of the brain and cervical spinal cord with a time to repeat (TR) of 12 ms, a time to echo (TE) of 6.0 ms,

slice thickness 2mm, flip angle 50**°,** field of view 220 mm x 126 mm, voxel size 0.7 mm (FH) x 0.7mm (AP), in 20 heart phases. The acquisition time was approximately 3 minutes.

Imaging analysis

Prior to analysis, sagittal T2-weighted bFFE and TSE sequences were loaded into open source imaging software[ii]. CM was defined as evidence of caudal cerebellar herniation into the foramen magnum or indentation by the supraoccipital bone (Lu et al., 2003). A syrinx was defined as a fluid-containing cavity within the spinal cord parenchyma with a diameter of greater than or equal to 2 mm.[6] Subjects were subsequently allocated into three groups: controls, CKCS+CM and CKCS+CM/SM.

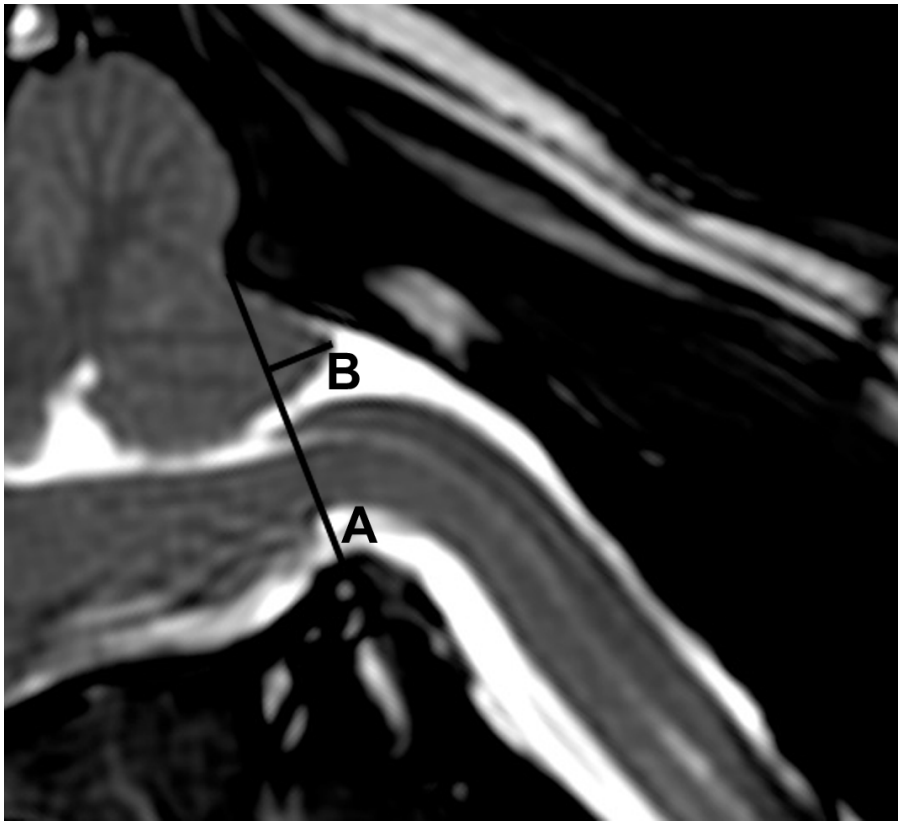

Figure 24. Measurements made on sagittal bFFE sequences for each phase of the cardiac cycle: A: Foramen magnum, measured from the ventral most aspect of the supraoccipital bone to the caudal basisphenoid bone. B: Length of cerebellar herniation, measured perpendicular from the line of the foramen magnum to the tip of the cerebellar vermis. The degree of cerebellar pulsation was calculated by subtracting the minimal herniation length from the maximum.

All scans had objective measurements made by the same observer[iii]. For each of the 20 bFFE images generated during the cardiac cycle, linear measurements were made using a similar technique to that described.[4,17,18] Measurements included the height of the foramen magnum (measured from the ventral most aspect of the supraoccipital bone to the caudal basisphenoid bone) and the length of cerebellar herniation (measured perpendicular from the line of the foramen magnum to the tip of the cerebellar vermis, figure 24). The degree of cerebellar pulsation was calculated as the minimum length of cerebellar herniation subtracted from the maximum length of cerebellar herniation found during the cardiac cycle. When present, the maximal diameter of a syrinx was recorded using a linear measurement.[6] Finally, the degree of neck flexion was determined for each subject using a method previously described.[11]

Statistical analysis

A commercial statistical software package[iv] was used for data analysis. Data sets for age, body weight, neck flexion angle and cerebellar pulsation were initially assessed for normality using the D'Agostino and Pearson normality test. Data sets for the three groups were subsequently analysed using a one-way ANOVA. Post-hoc analysis with a Bonferroni multiple comparison test was performed if a significant difference was detected. Correlation between cerebellar pulsation and SM width was tested with the Pearson r correlation test. Data are presented as mean ± SEM and $P<0.05$ was considered significant.

Results

Of seventeen CKCS included in the study, the majority (thirteen) presented for spinal hyperaesthesia (76%, 95% CI 0.53 – 0.90). Two CKCS presented for seizures and were diagnosed with idiopathic (primary) epilepsy. Two CKCS presented for peripheral vestibular disease and were diagnosed with otitis media. Three control dogs were diagnosed with idiopathic epilepsy and three with otitis media. No control dogs had CM or SM. All CKCS had imaging features consistent with CM. Nine CKCS had CM only, eight (47%) were also diagnosed with SM.

Males were over-represented in the study with 4 of 6 (67%), 6 of 9 (75%) and 5 of 8 (63%) subjects being male in the control, CKCS+CM and CKCS+CM/SM groups respectively. Overall, there was no significant difference (P = 0.74) in the mean ages of the groups (58.67 ± 18.20, 69.38 ± 9.73, 77.33 ± 10.25 months, respectively). Similarly, there was no overall significant difference (P = 0.1) in mean body weight of the groups (8.67 ± 1.53, 11.47 ± 0.89, 12.08 ± 0.83 kg, respectively).

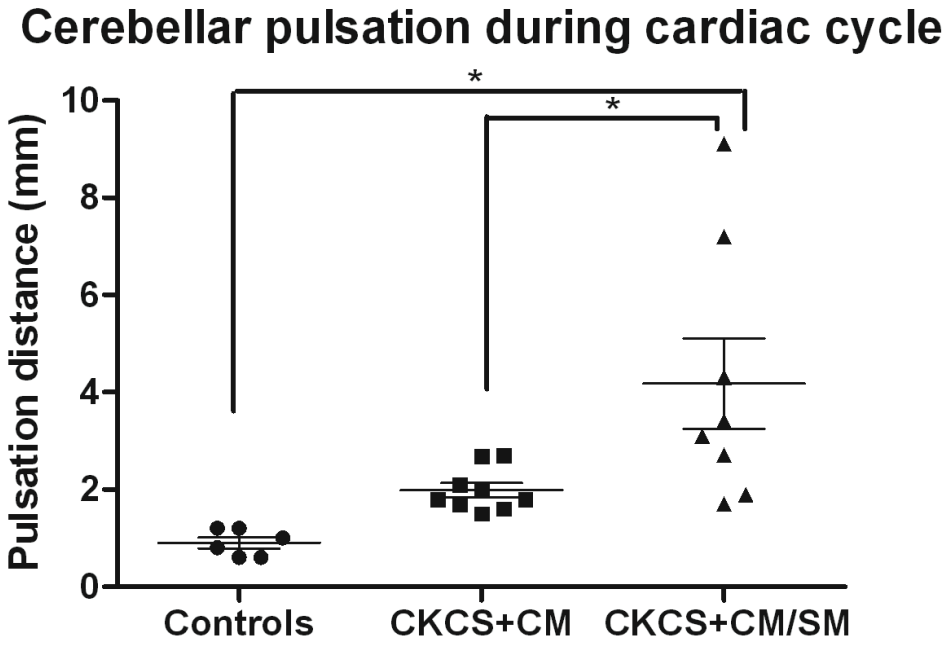

Fig. 25. Scatter plots showing distribution of cerebellar pulsation during the cardiac cycle between control dogs, CKCS+CM and CKCS+CM/SM. Error bars display mean ± SEM (one-way ANOVA with Bonferroni multiple comparisons test, *P<0.05).

Pulsation of the entire neuraxis during the cardiac cycle was evident in all study subjects. Following image analysis, a statistically significant difference in mean cerebellar pulsation was evident between the three groups (P = 0.003). Post-hoc comparisons indicated that mean cerebellar pulsation differed significantly between controls vs CKCS+CM/SM (P = 0.003, 0.90 ± 0.11 vs 4.18 ± 0.93 mm), and CKCS+CM vs CKCS+CM/SM (P = 0.03, 1.99 ± 0.14 vs 4.18 ± 0.93 mm), but not controls vs CKCS+CM (P = 0.63, figure 25). There was no overall significant difference (P = 0.11) in the angle of neck flexion between the groups (40.42 ± 4.45, 41.12 ± 3.92, 51.14 ± 3.19 degrees, respectively). The correlation between the degree of cerebellar pulsation and syrinx width was found to be significant in the CKCS+CM/SM group (r = 0.763, P = 0.03). The approximate extent of cerebellar pulsation is displayed in figure 26.

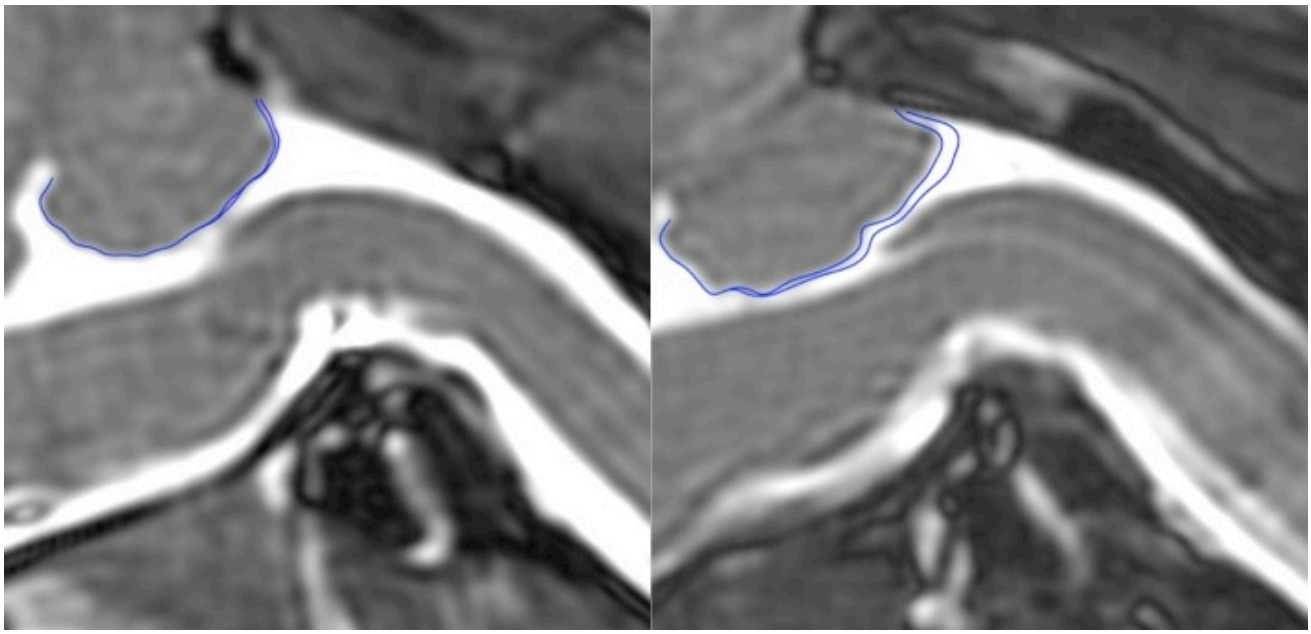

Figure 26. Sagittal balanced fast field echo cine MRI scans of the brain and cervical spinal cord, from a single phase of the cardiac cycle, from cavalier King Charles spaniels with mild Chiari-like malformation only (left) and severe Chiari-like malformation and syringomyelia (right). The maximum and minimum extent of cerebellar position is traced in blue. The syringomyelia case has more marked pulsation of the cerebellum during the cardiac cycle.

Discussion

Sagittal cardiac-gated bFFE MRI sequences facilitated the visualisation of neuraxial movement in dogs. During the systolic phase of the cardiac cycle the brain expands as extracerebral arteries engorge.[19] This imparts a systolic pressure wave to the intracranial CSF that is accommodated in normal subjects by sudden movement of CSF from the basal cisterns to the cranial portion of the vertebral canal.[8] In normal patients there is also movement of the hindbrain towards the foramen magnum, previously termed the piston action of the brain.[20] During the last part of systole the brain and spinal cord recoil due to the elastic properties of the thecal sac.[20] This effect was visible in all the study subjects, but pulsation of the cerebellum was quantitatively most pronounced in CKCS with CM and SM.

This study identified a quantitative difference in the degree of cerebellar pulsation between controls and CKCS with CM, which did not reach significance. A possible explanation for the inability to detect a significant difference is that overcrowding of the caudal cranial fossa and cerebellar herniation is not associated with a larger degree of cerebellar pulsation. However, as it is well documented that humans with CM-I have increased cerebellar pulsation[13], another explanation for the failure to identify a significant difference is probable. It is possible that an inappropriate control group was selected; clinically normal CKCS could not be recruited due to the extremely high prevalence of CM in the breed.[4] However, given their similar craniocerebral morphology, brachycephalic dogs are an appropriate control group for the study of CM.[21] Alternatively, as CKCS with CM and SM had significantly greater cerebellar pulsation than controls, it may be that pulsation is associated with a more severe disease phenotype and the current study was inadequately powered to identify a difference between cases with a mild phenotype (CM only) versus controls using the Bonferroni correction. Further study including a larger population of clinically affected CKCS may be required in the future.

The findings of this study suggest that cerebellar pulsation may be a more important factor in the development of SM than herniation alone. Pulsation of the cerebellum better supports the pulse pressure theory of syrinx formation.[9] Dynamic obstruction of the subarachnoid space may uncouple the normally even transmission of systolic CSF pressure between the subarachnoid space and the spinal cord, resulting in a centrifugal force and mechanical distension of the spinal cord. Repetitive distension of the spinal cord favours the accumulation

of extracellular fluid within the damaged tissue, eventually forming syringes.[9] The magnitude of the pressure differential may be directly related to cerebellar pulsation. In support of this theory, we found a correlation between the degree of cerebellar pulsation and maximum syrinx width, but a causative relationship remains unproven.

Given that subjects were client-owned patients examined at our institution by varying clinicians, an appropriate clinical severity scale was not recorded for subjects. No meaningful statistical comparison could therefore be performed to ascertain the relationship between clinical signs and imaging findings. Excluding neurologically abnormal dogs could have selected subjects with a mild phenotype in the SM group, including syringes of reduced volume. However, there is currently no reported association between syrinx width and the presence or severity of paresis and postural reaction deficits. A strong positive correlation between the degree of cerebellar pulsation and syrinx width was identified, which has previously been associated with the severity of spinal hyperaesthesia.[6] Nevertheless, the clinical relevance of cerebellar pulsation cannot be determined in the current study.

The measurements described cannot currently be considered applicable to clinical cases considering that this study does not provide evidence concerning their repeatability or reliability. However, measurement of the inferior edge of the cerebellar tonsils using this technique in humans was recently reported to have substantial to perfect intra- and inter-observer agreement.[22] Steady-state free precession imaging is favoured over phase contrast imaging that suffers from low resolution and limited ability to assess tissue margins, forcing previous studies to utilise a tonsillar motion index.[10] A similar technique has been used to assess arachnoid adhesions in humans[23] and further applications may be found in veterinary medicine, however, further study to establish the repeatability and reliability should be made. It should be noted that large magnetic susceptibility artefacts associated with subcutaneous microchips might limit the usefulness of this technique in the cervical region of dogs, which is why standard T2-weighted turbo spin echo sequences were used to measure syrinx dimensions. Flexion of the cervical spine was deemed necessary to better mimic the normal standing physiology of a dog, as previously described for assessment of CSF flow dynamics.[14] In addition, extension of the cervical vertebral column alters the degree of cerebellar herniation.[17]

Several morphometric features have been identified in CKCS that are significantly different between those with and without SM, including the length of the ventral aspects of the occipital bones[24], the volume of parenchyma within the caudal cranial fossa[2], overcrowding of the caudal aspect of the caudal cranial fossa by the cerebellum[25], reduced CSF space between the cerebellum and brainstem[17] and reduced volume of the jugular foramina.[26] It is currently unclear if some of the previously identified morphometric differences between CKCS with and without SM are directly causative or are epiphenomena of the disease. Despite probable differences in the pathogenesis of SM between humans and dogs, such as a reduced effect of gravity on CSF flow, both human CM-I and canine CM patients share cerebellar pulsation as a correlate of the disease. We propose that morphometric abnormalities affecting the soft tissues and osseous components of the craniocervical junction in CKCS act synergistically to reduce craniospinal compliance (the ability of the neuraxis-CSF system to accommodate for changes in CSF volume per unit pressure change created by cerebellar pulsation). A prior study found that the height of the foramen magnum and the length of cerebellar herniation appear to increase with time in CKCS with CM.[27] Speculatively there may also be an effect on cerebellar pulsation, either increasing due to unrestricted movement, or decreasing due to reduced congestion of the craniocervical junction. There may therefore be naturally occurring changes in craniospinal compliance that directly affect or at least contribute to the variable progression of canine CM/SM.

Conclusions

T2-weighted bFEE cine MRI scans with cardiac gating can be used to visualise movement of the canine neuraxis during the cardiac cycle. We found CKCS affected with SM to have significantly greater cerebellar pulsation during the cardiac cycle than control dogs and CKCS with CM only. This finding suggests cerebellar pulsation plays a role in the pathophysiology of SM secondary to CM in dogs, as it does in human CMI patients.

Footnotes

[i] Phillips Intera, Amsterdam, The Netherlands

[ii] OsiriX Medical Imaging Software, http://www.osirix-viewer. com

[iii] Colin Driver

[iv] Prism for Windows Version 5.00, Graphpad Software Inc, 2007

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SECTION IV

GENERAL DISCUSSION

General discussion

The main goal of this thesis was to gain new insights into the pathogenesis of syringomyelia (SM) secondary to Chiari-like malformation (CM) in dogs. For this purpose, we initially utilised a three-dimensional volumetric technique based on magnetic resonance images (MRI) of dogs with CM, to examine the relationship of potentially abnormal morphology of the brain and skull to the presence of SM. In subsequent chapters we examined the relationship of abnormal craniocerebral morphology to clinical signs of CM/SM in dogs. In addition, the progression of these morphologic abnormalities with time was assessed, as was a new imaging technique for quantifying the degree of cerebellar pulsation during the cardiac cycle.

SM has been recognised as a clinical entity in dogs since the late 1990's; clinical signs were reportedly consistent with a central spinal cord lesion.[1] A familial basis for SM was suspected in Cavalier King Charles Spaniels (CKCS)[2-4], which has been more recently described as a complex oligogenic trait with moderate hereditability.[5] SM was originally postulated to be a consequence of abnormal cerebrospinal fluid (CSF) hydrodynamics associated with an occipital bone malformation resulting in a small caudal cranial fossa (CCF) and subsequent cerebellar herniation.[1] This theory was based on the fact that several features of the analogous disease complex in humans (Chiari-type 1 malformation, CMI), such as cerebellar herniation, were present in CKCS with the disease.[1,6] However, a study by Lu and others[7] failed to identify a relationship between the severity of cerebellar herniation and SM in dogs. Subsequently, several morphometric studies in CKCS examined the relationship between the length of the occipital bones and the volume of the CCF and the presence of SM, with variable results.[8-11] Whilst CKCS appeared to have abnormal supra- and basi-occipital bones with an abnormally shallow CCF[9], CCF area or volume did not appear to be associated with SM.[10,11] Cross and others[12] found CKCS to have a similar sized CCF to small breed dogs, but had a parenchymal volume within the CCF that was similar to Labradors, suggesting parenchymal overcrowding of the CCF may exist, which has been found in children with CMI.[13]

Relationship of abnormal craniocerebral morphology to SM in dogs

To answer whether abnormal CCF morphology is associated with SM in this thesis, we utilised MRI studies from CKCS with and without SM. CKCS were used as study subjects as

the prevalence of morphological abnormalities typical for CM, as defined by an international panel of veterinary neurologists[14], are almost ubiquitous in this breed[9] and SM is often co-existant[2]. In chapters 1.3 and 4.1, additional small breed brachycephalic and mesaticephalic control dogs were used as they are considered medium (CM reported[15]) and low risk (CM not reported), respectively, when considering the development of SM.

A three-dimensional volumetric technique using commercially available imaging software, as developed by Cross and others[12], was used for accurate quantitative assessment of the parenchymal and osseous components of the CCF. These volumes are more accurate and reproducible than two-dimensional methods from mid-sagittal scans as used in previous studies[9,10] , indeed, morphometric measurements have been made in three dimensions in human studies.[6,13]

In **chapter 1.1** of this thesis, our study found that the volume of parenchyma within the CCF was significantly greater in CKCS with SM compared to those without. Further, there was a strong positive correlation between parenchymal overcrowding and SM severity, defined as maximal syrinx width. An association was also discovered between the volume of the ventricular system and SM severity, suggesting a shared pathogenesis. A proposed cause for this is an overall reduction in cranial and spinal compliance; the ability of both compartments to accommodate changes in CSF pressure transmission caused by compression of the subarachnoid space. An inability to accommodate normal CSF hydrodynamics could lead to CSF being retained in the ventricles. Surprisingly, a significant difference was not found for CCF volume between CKCS with and without SM. This finding is consistent with previous studies concerning CCF volume in CKCS.[9-11] However, whilst the age of CKCS recruited for our study was not significantly different between groups, a potentially shared limitation of these studies was the failure to provide an adequate control group by potentially including young, SM-free CKCS that may have progressed to develop SM with time. Indeed, the prevalence of SM in CKCS is significantly higher when scanned for breeding purposes when over five years of age[14]. In **chapter 1.2**, we applied our volumetric technique for the comparison of CCF morphology in two populations of CKCS; young (less than two years of age) SM-affected dogs versus old (greater than five years of age) SM-free dogs, whom might be expected to never develop SM and thus represent a more rigorous control population. This study not only supported our previous finding that CCF parenchyma percentage was greater in SM-affected CKCS, but also highlighted a significantly smaller CCF volume in SM-

affected CKCS as well. This finding is more in line with the current theory for SM development in children with reduced posterior fossa volume.[6,16,17]

CKCS have a relatively greater volume of CCF parenchyma compared to other small breed dogs.[12] As CCF parenchyma should be in a consistent ratio to other brain regions in normal dogs[18], in **chapter 1.3** we examined which sub-divisions of CCF parenchyma were enlarged in CKCS with SM, relative to CKCS without SM, small breed dogs and Labradors. We hypothesised that CKCS, unlike small breed dogs and Labradors, would have a relatively larger cerebellar volume with overcrowding of the CCF. We found CKCS to have a significantly higher CCF cerebellar percentage than small breed dogs and Labradors. Further, analysis of CKCS alone found those with SM to have a significantly higher CCF cerebellar percentage than those without. Partitioning of the CCF into rostral and caudal parts allowed us to determine that the degree of parenchymal overcrowding was more sensitive to cerebellar volume in the caudal part, suggesting that this part of the skull of CKCS does not accommodate the brain in the same way it would in other small breed dogs and Labradors.

Impaired development of the occipital bones forming the CCF (as defined in this thesis) may be caused by a failure of communication between one or more of the cartilaginous progenitors of the CCF (segmented mesoderm and unsegmented paraxial mesoderm) and the developing neural tube, specifically, rhombomere 1, which gives rise to the cerebellum.[6,19,20] Another possibility is overgrowth of the cerebellum, which normally develops late in gestation.

Relationship of abnormal craniocerebral morphology to clinical signs in dogs

Unlike in humans, it is contentious in the veterinary literature as to whether CM alone causes clinical signs.[7,10] Conversely, the development of SM secondary to CM has been associated with neurologic signs and neuropathic pain in CKCS[7,21], with large and asymmetrical syringes being the strongest predictors of pain[7.21] In one report concerning CM in CKCS, 32% of the study population had seizures.[7] There is a heritable basis for idiopathic epilepsy in CKCS, however this appears to be a separate genetic subset from dogs with occipital bone hypoplasia.[3] Hydrocephalus has previously been associated with seizures in dogs.[8] Rusbridge and others[9] postulated that ventriculomegaly in CKCS, associated with CM, may result in seizures. To answer whether abnormal craniocerebral morphology is associated with seizures, in **chapter 2.1** of this thesis we performed a retrospective case-controlled morphometric study

in CKCS with and without seizures. We found no significant difference in CCF volume, the CCF parenchyma percentage or the ventricular volume between CKCS with and without seizures. 61% of dogs in this study had seizures that were classified as having partial onset, which is similar to previous studies regarding the general canine population.[10] Inter-ictal electroencephalographic studies performed when possible revealed paroxysmal abnormalities that were mainly located over the frontal and temporal regions, rather than the cerebellum, suggesting they are not correlated with CM. As electrodes were not placed over the cerebellum, inter-ictal abnormalities in this region could not be completely excluded. These findings are similar to those found in a small series of humans with Chiari-I malformation.[24] Therefore, another cause of recurrent seizures in CKCS is suspected, such as familial epilepsy as has been previously reported.[4]

Clinical signs typically associated with CM and/or SM include cervical scoliosis, thoracic and pelvic limb ataxia, thoracic limb paresis and behaviour suggestive of neuropathic pain (NeP).[1,25,26] The prevalence of CKCS with SM that suffer clinical signs suggestive of NeP is unknown, as is the response of CKCS to medical therapy for NeP. In **chapter 2.2** of this thesis we described a prospective cohort study following 48 CKCS with clinical signs suggestive of NeP for a period of 39 months from diagnosis, using a visual analogue scale (VAS) for determination of NeP severity. We also used our volumetric technique to determine whether abnormal craniocerebral morphology could predict a progression of clinical signs. At the end of the study, 75% of CKCS were available for follow up. We found that the overall severity of clinical signs increased significantly in 75% of CKCS despite medical therapy. A quarter of the dogs were static or improved. There was no statistically significant change in the number of dogs exhibiting compromised exercise ability, vocalisation or facial rubbing behavior. Despite these findings, the majority of owners felt that the quality of life of their dog was acceptable. The CCF volume, the CCF parenchyma percentage and the volume of the ventricular system were not significantly different between those dogs that did and did not have deteriorating clinical signs. The progression of the clinical signs seen in our study is similar to those previously reported in humans with unoperated SM associated with CMI.[27] Several reports of craniocervical decompression for the treatment of CM/SM have been described in dogs.[28-31] Despite initial improvement following surgery, many dogs continued to exhibit signs of NeP, and surgery did not necessarily change the size of the cervical syringes.[28-31] A direct comparison of medical and surgical treatment cannot be made in this study. As we did not control for the medical treatments used in the study, an assessment of

outcome with specific treatment modalities cannot be assessed. Further studies with longer follow up periods are needed to compare the long-term outcome of conservative and surgical treatment. At this time there is no evidence to support that surgery or medical treatment has a more favourable long-term outcome for the management of NeP in CKCS.

The natural progression of chiari-like malformation and syringomyelia in dogs

It has long been suspected that SM is a late onset disease. As previously mentioned, the prevalence of asymptomatic SM is higher in older dogs scanned for breeding purposes.[14] We hypothesised that SM would progress in appearance on MRI with time in CKCS, and that features of abnormal craniocerebral morphology associated with CM would change with time in CKCS. In **chapter 3**, we described a retrospective morphometric study in 12 CKCS that had two MRI scans at a median interval of 9.5 months. The majority of dogs presenting for a second scan did so due to clinical complaints that were considered unrelated to the initial presenting condition and thus were suspected to represent a new condition. The number of dogs with SM increased in the scan interval from 17 to 42%. There was a statistically significant increase in central canal or syrinx width between the first and second scans. Although not all CKCS developed central canal dilation > 2mm (in order to classify as a syrinx), there was progressive central canal dilation, which is a precursor of syrinx formation.[32,33] Clinical signs were not assessed in this study, but given that syrinx width is associated with pain[21] the increase in width could explain the progression in clinical signs we described in chapter 2.2. In addition, foramen magnum height, the length of cerebellar herniation and CCF volume were significantly increased. There was no significant difference for CCF parenchymal percentage or ventricular system volume between the first and second scans. A positive association has previously been found between foramen magnum size and cerebellar herniation in CKCS.[10] This may represent a dynamic change occurring to the occipital bone that forms the foramen magnum in response to the parenchymal overcrowding of the CCF described in chapter 1.1. This is supported by the previous finding that a large part of the supraoccipital bone of adult CKCS is cartilage, suggesting active remodelling.[34] Giejda et al.[34] recently used general histology techniques to describe features of the supraoccipital bones from 11 CKCS fetuses and compared them to 6 control fetuses (brachycephalic dogs). They found CKCS fetuses to have significantly reduced numbers of chondrocytes and increased numbers of apoptotic chondrocytes to controls. They commented that reduced cellular capacity at birth could contribute to the formation of a smaller occiput, lending

credence to the mesodermal insufficiency theory. In addition, histologic examination of adult supraoccipital bones revealed active remodeling with a large portion of the bone being cartilaginous tissue. It was theorized that an imbalance between production and remodeling of the bone could alter the capacity to accommodate the mechanical pressure from the growing brain.[34] It is conceivable that the increase in CCF volume documented here could represent resorption of the inner surface of the overlying occipital bones, rather than an increase in its dimensions. In support of this, the occipital bones in adults and children normally display a resorptive pattern of the bone around the cerebellar hemispheres.[35] The dynamic changes to the bones of the CCF could explain the variable natural progression of SM in dogs.

Assessment of cerebellar pulsation in dogs

Abnormal CSF hydrodynamics are suspected to result in SM.[36] In dogs, experimental injection of kaolin at the foramen magnum resulted in SM.[37] Dynamic obstruction of the foramen magnum occurs in CMI patients due to pulsation of the cerebellum.[38-40] In **chapter 4**, our assessment of cerebellar pulsation in dogs was made using cardiac-gated balanced fast field echo scans, a novel MRI technique for use in dogs. Movement of the entire neuraxis was documented. The degree of cerebellar pulsation was significantly greater in CKCS with SM compared to those without SM and small breed brachycephalic control dogs. In addition, a strong positive correlation was found between degree of cerebellar pulsation and syrinx width.

Limitations of the study and future perspectives

The ideal control population for the study of SM secondary to CM in CKCS would be CKCS without CM. Unfortunately the prevalence of CM in CKCS is very high, in one study 92% of CKCS had at least one morphologic abnormality consistent with this diagnosis, such as occipital hypoplasia, cerebellar identation and medullary kinking.[10] As we were predominantly interested in the relationship of individual morphometric features of CM to the presence of SM within this breed, we did not consider this a major limitation. The studies described in chapter 1.3 and 4 used small brachycephalic dog breeds as controls, which have been described as an appropriate control population for the study of CM[41] especially as CM is sporadically reported in these breeds.[30] None of the dogs selected for these studies had imaging features of CM. The study described in chapter 3 is the first to document that SM is a

progressive disease in CKCS. It was important, therefore, to include age-matched control groups in chapters 1.2 and 1.3.

In our studies three-dimensional volumes were constructed using a manual image segmentation technique. In each case, approximately two hours was required to generate the individual masks from each 2 mm MRI slice. Whilst this meticulous task was considered accurate, we were not able to assess intra or inter observer variability. Whilst acceptable in an experimental setting, the clinical application of this volumetric technique is therefore highly restricted. In addition, this technique is not applicable to alter breeding standard guidelines, which have been generated on the basis of MRI features of the disease.[42,43] This is also true for our assessment of cerebellar pulsation, which was a much quicker technique to perform. However, measurement of the inferior edge of the cerebellar tonsils using this technique in humans was recently reported to have substantial to perfect intra- and inter-observer agreement.[44]

Although the study described in chapter 2.2 evaluated the medical treatment of dogs with CM/SM, our study design does not permit comparison of medical and previously reported surgical outcomes. This is only possible with a randomized and blinded study design. However, ethical objections can be made by such an approach, since blinding for conservative or surgical treatment could result in withholding the most appropriate treatment for an individual patient. For example, it does appear that medical management provides dogs with acceptable quality of life despite the progression in clinical signs.

Although this thesis has provided new information about the pathogenesis of SM secondary to CM in dogs, the exact mechanism by which fluid accumulates in the spinal cord secondary to obstruction of the foramen magnum remains undetermined. There is compelling evidence both histologically[34] and now morphologically that the CCF and associated soft tissue structures are abnormally formed in CKCS with CM. However, it remains unclear if some of the morphometric differences between CKCS with and without SM identified in this thesis are directly causative or are epiphenomena of the disease. Despite probable differences in the pathogenesis of SM between humans and dogs, such as a reduced effect of gravity on CSF flow, both CM-I and CM patients share cerebellar pulsation and abnormal CSF hydrodynamics[45] as a correlate of SM.

A unifying theory for hydrocephalus, CMI and SM has been proposed based on reduced venous drainage and altered compliance.[46] Mechanical compliance determines the ability of a system to accommodate a change in volume in the face of pressure changes. Craniospinal compliance is the sum of the ability of both the cranial and spinal compartments to accommodate changes in parenchymal, blood or CSF volumes which all exist in a state of dynamic equilibrium.[47] Compliance is primarily concerned with the dimensions of the skull, but is influenced by several other factors including CSF volume, free CSF flow, the integrity of vasculature, space occupying lesions and autonomic regulation of blood flow. We propose that CM establishes a situation of reduced compliance. Further, reduced cranial compliance may result in a larger pressure gradient between the cranial and spinal systems. The studies in this thesis suggest CCF overcrowding; hence the volume of CSF in the subarachnoid space surrounding the cerebellum is reduced. A recent study by Fenn and others[48] has found CKCS to have a reduced volume of blood in the venous sinuses. There are two observable outcomes of reduced compliance. Firstly, reduction of the CSF space at the foramen magnum may lead to retaining of CSF in the head, which would otherwise be free to move through the foramen magnum. Ventriculomegaly is a common observation in CKCS with CM[1,49] and in our study described in chapter 1.1 the volume of the ventricular system was positively correlated with the severity of SM, suggesting a shared pathogenesis. Secondly, reduced cranial compliance may affect the transition of pressure between the cranial and spinal systems' blood and CSF compartments. This could explain the turbulent CSF flow patterns that were observed by Cerda-Gonzalez and others[45] at the foramen magnum. The increased cerebellar pulsation observed in this thesis might also be associated with altered pressure and compliance; however, it remains unclear as to whether this is a cause or effect of this change. Phase contrast cine MRI has been used to assess craniospinal compliance in humans[50], a technique that could be applied in dogs in the future. Further research could assess the clinical correlates of altered CSF hydrodynamics, which might be a better determinant of clinical outcome than abnormal craniocerebral morphology.

Conclusions

In conclusion, this thesis demonstrated that overcrowding of the CCF of CKCS with CM is associated with SM. When a stringent age-matched control group of CKCS is used (accounting for the finding of this thesis that SM progresses with time), an abnormally small CCF is also found to be associated with SM. We found the subdivision of hindbrain parenchyma responsible for this overcrowing to be the cerebellum, particularly in the caudal aspect of the CCF, which may not accommodate the cerebellum in the same way it would in other small breed brachycephalic or mesaticephalic dogs. The progression of clinical signs associated with CM/SM, including seizures, does not appear to be associated with individual features of CM such as CCF volume. Our inability to find an association between morphology and clinical progression could be related to our finding that certain features of CM, such as CCF volume, change with time. Unfortunately, the majority of dogs undergoing medical therapy for CM/SM will display a progressive clinical syndrome. Despite this finding, the majority of CKCS with CM/SM will maintain a good quality of life and the best treatment modality remains undetermined. The exact mechanism of syrinx development secondary to CM is not determined in this thesis, but in similarity to human CMI, cerebellar pulsation during the cardiac cycle is associated with the disease.

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SUMMARY

Summary

Syringomyelia (SM) is an enigmatic spinal cord disease in dogs. SM is frequently co-existent with canine Chiari-like malformation (CM), which occurs at the craniocervical junction and involves osseous and soft-tissue elements of the caudal cranial fossa (CCF); however, the association between SM and CM is poorly understood. In this thesis several aspects of the association between the morphological and clinical features of CM were evaluated in relation to SM.

It can be divided in the following sections:

As a general introduction **(Section I)** a brief review is given about the current literature concerning the pathogenesis, diagnosis, and treatment of SM secondary to CM. Additionally, points of controversy and scope for further research are discussed.

The scientific aims **(Section II)** of this thesis were to gain more information about the pathogenesis, natural progression and treatment of canine CM/SM using Cavalier King Charles Spaniels (CKCS) as a disease model, given the high prevalence of the disease complex in this breed. Furthermore, we wished to evaluate whether cerebellar pulsation occurs in dogs with CM and whether cerebellar pulsation is related to SM presence and severity.

The materials and methods **(Section III)** describes the selection criteria used for identifying study subjects and diagnostic imaging studies used in the prospective and retrospective elements of the thesis.

The results of this thesis are presented in **Section IV**, which is divided in **4 chapters**.

Chapter 1 describes the relationship of abnormal craniocerebral morphology, associated with CM, to SM. A novel volumetric technique by manual segmentation of magnetic resonance images (MRI) was developed for this purpose. In the **first part of chapter 1**, the relationship of brain parenchyma in the CCF and ventricle size to SM was investigated. CCF parenchymal percentage and ventricular volume were significantly greater in CKCS with SM compared to those without. In the **second part of chapter 1**, age-matched CKCS were used as SM is

suspected to be a late onset disease. Examining two extremes of disease phenotype identified that CCF volume was also significantly smaller in young CKCS with SM compared to older CKCS without SM. In the **third part of chapter 1**, volumes of the subdivisions of the hindbrain were assessed in relation to SM. The cerebellum was found to be significantly larger in CKCS, compared to small breed dogs and Labradors. Overcrowding of the caudal part of the CCF was found to be sensitive to changes in cerebellar volume in CKCS, unlike small breed dogs and Labradors, suggesting this part may not adapt to cerebellar volume as well in CKCS.

Chapter 2 describes the relationship of abnormal craniocerebral morphology to clinical signs in dogs. The **first part of chapter 2** evaluates the association between CM and seizures in CKCS. No significant association was found between CCF volume, CCF parenchymal percentage or the volume of the ventricular system and seizures. Electroencephalographic studies found paroxysmal activity to be present over the frontal and parietal regions, suggesting CM was unrelated. A familial basis for the seizures is suspected. The **second part of chapter 2** described a prospective cohort study, determining the progression in clinical signs of CKCS undergoing medical treatment for several months following the clinical diagnosis of CM/SM. A visual analogue scale was used in order to assess the severity of each patient's neuropathic pain. The majority of CKCS displayed a progressive clinical syndrome in the study period. Despite this, most patients were determined to maintain a good quality of life by their owners. No significant association was found between CCF volume, CCF parenchymal percentage or the volume of the ventricular system and whether clinical signs were progressive in this study.

Chapter 3 describes the natural progression of CM and SM in dogs. Changes over time in craniocerebral morphology and SM were determined in CKCS with CM by volumetric analysis of sequential MRI scans. The number of CKCS with SM increased in the study period and SM lesions already present progressed in severity. Several features of abnormal craniocerebral morphology associated with CM, including CCF volume, the height of the foramen magnum and the length of cerebellar herniation, increased in the study period. Dynamic changes occur in the CCF, which might reflect active remodeling of the occipital bones. This could explain the variable natural progression of CM/SM in dogs and why an association cannot be determined between morphology and clinical signs.

Chapter 4 describes the use of a novel MRI technique for the dynamic assessment of cerebellar pulsation in dogs. Movement of the neuraxis during the cardiac cycle was visualised in dogs made using a novel cardiac-gated cine MRI technique; steady state free precession imaging. Quantification of the degree of cerebellar pulsation in dogs with and without CM and SM was made using linear measurements at each phase of the cardiac cycle. CKCS with CM and SM had a significantly greater degree of cerebellar pulsation during the cardiac cycle than did small breed brachycephalic controls dogs without CM. CKCS with CM only had a greater degree of cerebellar pulsation than control dogs, but this did not reach significance, possibly representing a milder phenotype of the disease.

In conclusion, the studies presented in this thesis provide new information concerning the pathogenesis of SM secondary to CM in dogs. Overcrowding of the CCF of CKCS with CM by brain parenchyma is associated with SM. When a stringent age-matched control group of CKCS is used (accounting for the finding of this thesis that SM progresses with time), an abnormally small CCF is also found to be associated with SM. The sub-division of hindbrain parenchyma responsible for this over-crowing appears to be the cerebellum, particularly in the caudal aspect of the CCF, which may not accommodate the cerebellum in the same way it would in other dogs. The progression of clinical signs associated with CM/SM, including seizures, does not appear to be associated with individual features of CM such as CCF volume. Our inability to find an association between morphology and clinical progression could be related to our finding that certain features of CM, such as CCF volume, change with time. Unfortunately, the majority of dogs undergoing medical therapy for CM/SM will display a progressive clinical syndrome. Despite this finding, the majority of CKCS with CM/SM will maintain a good quality of life and the best treatment modality remains undetermined. The exact mechanism of syrinx development secondary to CM is not determined in this thesis, but in similarity to human Chiari type I malformation, cerebellar pulsation during the cardiac cycle is associated with SM.

SAMENVATTING

Syringomyelie (SM) is een ruggenmergaandoening bij honden waarover nog steeds niet alles bekend is. Deze aandoening komt vaak gelijktijdig voor met caniene "chiari-like"-malformatie (CM) van de craniocervicale overgang; zowel botstructuren als wekedelenstructuren van de caudale craniale fossa (CCF) zijn betrokken bij CM. Het verband tussen SM en CM is echter nog niet volledig duidelijk. In dit doctoraal proefschrift wordt het verband tussen de morfologische en klinische bevindingen van CM en SM beschreven.

Het proefschrift is onderverdeeld in de volgende secties en hoofdstukken:

In de introductie **(Sectie I)** wordt een kort overzicht gegeven van de reeds beschikbare literatuur met betrekking tot de pathogenese, diagnose en behandeling van SM ten gevolge van CM. Daarenboven worden discussiepunten rond deze aandoening aangehaald en mogelijkheden voor toekomstgericht onderzoek besproken.

Het wetenschappelijk doel van dit proefschrift **(Sectie II)** was het verkrijgen van meer informatie betreffende de pathogenese, natuurlijke progressie en behandeling van caniene CM/SM bij cavalier-king-charles-spaniëls (CKCS), gezien de hoge prevalentie van CM/SM bij dit ras. Bijkomend werd nagegaan of cerebellaire pulsatie voorkomt bij honden met CM en of deze pulsatie gerelateerd is aan de aanwezigheid en ergheid van SM.

Materiaal en methoden **(in Sectie III)** beschrijft de selectiecriteria die werden gebruikt voor de identificatie van de studieobjecten en de diagnostische beeldvormingsstudies die werden gebruikt voor de prospectieve en retrospectieve onderdelen van deze thesis.

De resultaten van dit doctoraat worden weergegeven in **Sectie IV**, die onderverdeeld is in **4 hoofdstukken**.

In hoofdstuk 1 wordt het verband beschreven tussen de abnormale craniocerebrale morfologie bij CM en SM. Een nieuwe volumetrische MRI-techniek, waarbij gebruik gemaakt wordt van manuele segmentatie van beelden, werd ontwikkeld voor dit doel. In het eerste deel van **hoofdstuk 1** werd het verband tussen het percentage hersenparenchym in de CCF en de hersenventrikelgrootte enerzijds en SM anderzijds onderzocht. Hieruit bleek dat

het percentage hersenparenchym in de CCF en het ventrikelvolume significant groter waren bij de CKCS met SM dan bij de CKCS zonder SM.

In het tweede deel van **hoofdstuk 1** werden de CKCS onderverdeeld in leeftijdsklassen, aangezien vermoed wordt dat SM een aandoening is die zich ontwikkelt op oudere leeftijd. Het onderzoek van twee extreme fenotypische uitingen van de aandoening toonde aan dat het volume van de CCF significant kleiner was bij de jonge CKCS met SM dan bij de oude CKCS zonder SM.

In het derde deel van **hoofdstuk 1** werd het volume van de verschillende onderdelen van de achterhersenen bekeken in verhouding tot SM. Het cerebellum bleek significant groter te zijn bij de CKCS dan bij de andere kleinere hondenrassen en labradors van de studie. In tegenstelling tot de andere kleine hondenrassen en labradors bleek overvulling van het caudale deel van de CCF gevoelig te zijn voor veranderingen in het cerebellaire volume bij de CKCS van de studie. Dit suggereert dat het caudale deel van de CCF bij CKCS zich niet zo goed aanpast aan het cerebellaire volume.

In hoofdstuk 2 wordt de relatie beschreven tussen afwijkende craniocerebrale morfologie en klinische symptomen bij de hond. In het eerste deel van **hoofdstuk 2** wordt ingegaan op het verband tussen CM en het voorkomen van epilepsie bij CKCS. Er werd geen significant verband gevonden tussen het volume van de CCF, het percentage hersenparenchym in de CCF en het volume van het ventriculaire systeem enerzijds en epilepsie anderzijds. Met elektro-encefalografie werd paroxysmale activiteit aangetoond ter hoogte van de frontale en pariëtale gebieden. Deze bevinding suggereert dat CM niet betrokken was. Een genetische factor voor epilepsie wordt hier vermoed. In het tweede deel van **hoofdstuk 2** wordt een prospectieve studie beschreven van de progressie van de klinische symptomen gedurende verscheidene maanden na de diagnose van CM/SM bij CKCS die een medicamenteuze behandeling ondergingen. Een ‘visueel analoge’ schaal werd gebruikt om de ergheid van neuropathische pijn te beoordelen bij elke patiënt. Het overgrote deel van de CKCS vertoonde een progressieve verslechtering van de symptomen gedurende de studieperiode. Ondanks deze bevinding behielden deze honden een goede levenskwaliteit volgens de eigenaars. In deze studie werd geen significant verband gevonden tussen het volume van de CCF, het volume hersenparenchym in de CCF of het volume van het ventriculaire systeem enerzijds en progressieve klinische achteruitgang anderzijds.

In hoofdstuk 3 wordt het natuurlijke verloop van CM en SM bij honden beschreven. Veranderingen in de craniocerebrale morfologie over de tijd en SM werden nagegaan bij CKCS met CM door middel van de volumetrische analyse van sequentiële MRI-scans. Het aantal CKCS met SM nam toe gedurende de studieperiode en de reeds aanwezige SM-laesies werden erger. Verschillende kenmerken van abnormale craniocerebrale morfologie bij CM, zoals onder andere het toenemende volume van de CCF en de toenemende lengte van de cerebellaire herniatie werden gedurende de studieperiode waargenomen. Dynamische veranderingen kwamen voor ter hoogte van de CCF, die waarschijnlijk de oorzaak zijn van een actieve remodellering van de occipitale beenderen. Dit is mogelijk een verklaring van de variabele natuurlijke progressie van CM/SM bij honden en van het feit dat er geen verband kan gelegd worden tussen morfologie en klinische symptomen.

In hoofdstuk 4 wordt het gebruik van een nieuwe MRI-techniek toegelicht voor de dynamische beoordeling van cerebellaire pulsatie bij de hond. Beweging van de neuraxis gedurende de cardiale cyclus werd in beeld gebracht bij honden gebruik makend van een nieuwe 'cardiac-gated cine MRI'-techniek. Het kwantificeren van de graad van cerebellaire pulsatie bij honden met en zonder CM en SM gebeurde aan de hand van lineaire metingen op verschillende momenten tijdens de cardiale cyclus. De CKCS met CM en SM vertoonden een significant grotere graad van cerebellaire pulsatie gedurende de cardiale cyclus dan de kleine brachycefale honden zonder CM uit de controlegroep. De CKCS met alleen CM vertoonden een grotere graad van cerebellaire pulsatie dan de honden van de controlegroep, die echter statistisch niet significant was. Dit weerspiegelt mogelijkerwijs een mildere fenotypische uiting van de aandoening.

We kunnen concluderen dat de studies weergegeven in het voorliggend proefschrift nieuwe informatie geven betreffende de pathogenese van SM secundair aan CM bij de hond. Overvulling van de CCF met hersenparenchym bij CKCS met CM is geassocieerd met SM. Bij een strikte 'age-matched' controlegroep van CKCS (rekening houdend met de bevinding in dit proefschrift dat SM verergert met de tijd), wordt een abnormaal kleine CCF ook gerelateerd aan het voorkomen van SM. Het deel van de hersenen dat verantwoordelijk is voor de overvulling van de CCF blijkt het cerebellum te zijn, voornamelijk ter hoogte van het caudale aspect van de CCF. Dit deel van de CCF zou zich niet aanpassen aan het volume van het cerebellum zoals bij andere rassen. De verslechtering van de klinische symptomen geassocieerd met CM/SM, alsook epilepsie, blijken niet geassocieerd te zijn met de

individuele kenmerken van CM, zoals het gestegen volume van de CCF. Er werd geen associatie gevonden tussen morfologie en klinische achteruitgang. Dit kan verband houden met onze bevinding dat bepaalde kenmerken van CM, zoals het gestegen volume van de CCF, veranderen met de tijd. Jammer genoeg vertonen de meeste honden die een medicamenteuze behandeling toegediend krijgen voor CM/SM een progressieve klinische achteruitgang. Desondanks behouden de meeste CKCS met CM/SM een goede levenskwaliteit. De optimale behandeling blijft tot op heden echter onbekend. Het exacte mechanisme hoe een syrinx zich ontwikkelt ten gevolge van CM werd niet achterhaald in dit proefschrift, maar zoals bij humane chiari-type I-malformatie is cerebellaire pulsatie gedurende de cardiale cylcus geassocieerd met SM.

CURRICULUM VITAE

Curriculum Vitae

Colin John Driver was born on 24th June 1983 in Chertsey, United Kingdom. He attended High School in nearby Shepperton. After School he enjoyed brief employment with the Veterinary Laboratories Agency (VLA), part of the UK government department of environmental, farm and rural affairs. Within the VLA Colin worked for the neuropathology department investigating the pathogenesis of bovine spongiform encephalopathy and other transmissible spongiform encephalopathies. Colin gained an interest in neurology, neuropathology and scientific study during this period.

Colin started his veterinary studies at the Royal Veterinary College (RVC), University of London, in 2002. In 2005, he left for a year long intercalated BSc in Immunology and Oncology at Kings College, University of London, for which he was awarded a prestigious Wellcome Trust fellowship. He obtained a first class honours degree. Returning to the RVC, he graduated as a vet in 2008 with honours; an achievement based on consistently high examination results during the degree.

After graduation, he completed a one-year rotating internship in small animal medicine and surgery at the Queen Mother Hospital for Animals (QMHA), part of the RVC's clinical campus in Potters Bar, Hertfordshire. During this year he gained an interest in the canine and feline neurology, particularly spinal cord diseases including Syringomyelia (SM). This led to a successful application for a residency in veterinary Neurology and Neurosurgery at the QMHA in 2009. During the residency, Colin had several successful research projects and publications regarding the pathogenesis of SM secondary to Chiari-like malformation in dogs, resulting in the successful application of a cumulative PhD scholarship to be performed between the RVC and the Department of Medicine and Clinical Biology of the Small Animals, Ghent University, Belgium, under the combined supervision of Prof. Dr Holger Volk (RVC) and Prof. Dr. Luc Van Ham (Ghent).

Colin finished his residency in July 2012 and successfully passed the diploma examination of the European College of Veterinary Neurology (ECVN), becoming an ECVN diplomate and thus a European specialist in veterinary neurology and neurosurgery.

Colin spent a further 15 months at the RVC as a staff clinician; during this period Colin trained several residents in medical and surgical neurology and completed his PhD thesis. In October 2013 Colin left the RVC for a career in private practice; he is currently employed as a neurologist at Anderson Moores Veterinary Specialists, Winchester, Hampshire, UK.

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