UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

RELAPSING MENINGOENCEPHALOMYELITIS OF UNKNOWN ORIGIN: NEUROLOGICAL PRESENTATION, DIAGNOSTIC CLINICOPATHOLOGICAL FINDINGS, IMAGING CHARACTERISTICS AND THERAPEUTIC MANAGEMENT OF DOGS WITH AND WITHOUT RELAPSE

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ANA RITA VAZ DE ARAGÃO

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Resumo

Meningoencefalomielite de Origem Desconhecida Recidivante: Apresentação neurológica, Sinais clinicopatológicos, Características imagiológicas e Gestão terapêutica de cães com e sem recidiva

A meningoencefalomielite de origem desconhecida (MUO) é uma condição difícil de tratar e muitos cães acabam por recidivar, morrer ou ser eutanasiados. Assim, para descrever e comparar sinais, apresentação neurológica, resultados de ressonância magnética (RM), análise do líquido cefalorraquidiano (LCR), e protocolo de tratamento, foi realizado um estudo retrospetivo que incluiu 52 cães com um diagnóstico presuntivo de MUO apresentados numa instituição entre Maio de 2017 e Dezembro de 2021, agrupados de acordo com o seu desfecho: recuperados (grupo 0), com recidiva (grupo 1) ou mortos (grupo 2).

Vinte e quatro cães sobreviveram à MUO (46%), 13 recidivaram (25%) e 15 morreram ou foram eutanasiados em consequência da MUO (29%). O desenvolvimento dos sinais clínicos e a presença de convulsões e/ou hiperestesia não foram sugestivos do desfecho. A maioria dos cães tratados apenas com corticosteroides foram eutanasiados ou morreram (67%) [$p < 0,001$] e os cães que recidivaram foram mais frequentemente tratados com 3 ou mais fármacos (62%) [$p < 0,001$]. As localizações de lesões mais frequentes em RM são os hemisférios cerebrais (63%), tálamo (40%) e medula espinal (44%). Lesões multifocais, hérnia cerebral, presença de contraste meníngeo e parenquimatoso, e tipo e total de células no LCR não estavam associados a um maior risco de recidiva ou morte.

Assim que o diagnóstico é alcançado, parece vantajoso iniciar o tratamento com um segundo agente imunossupressor (para além dos corticosteroides) e muitas vezes a doença só é gerida através de poliquimioterapia, uma vez que a taxa de recidiva é elevada. Sinais clínicos anteriormente reportados, como convulsões, lesões multifocais, hérnia cerebral, presença de contraste meníngeo e parenquimatoso, e tipo e total de células no LCR não estão associados a um maior risco de recidiva e morte.

Palavras-chave: Cão, Meningoencefalite, Recidiva, Tratamento imunossupressor

Abstract

Relapsing Meningoencephalomyelitis of Unknown Origin: Neurological presentation, Diagnostic clinicopathological findings, Imaging characteristics and Therapeutic management of dogs with and without relapse

Meningoencephalomyelitis of Unknown Origin (MUO) is a challenging condition to treat and many dogs eventually relapse, die or are euthanised. Hence, to describe and compare signalment, neurological presentation, magnetic resonance imaging (MRI) findings, cerebrospinal fluid (CSF) analysis and treatment protocol, a retrospective study was conducted including 52 client-owned dogs with a presumptive diagnosis of MUO presented at one institution from May 2017 to December 2021, grouped according to their outcome: recovered (group 0), relapsed (group 1) or deceased (group 2).

24 dogs survived MUO (46%), 13 relapsed (25%) and 15 died or were euthanised because of MUO (29%). The onset and presence of seizures and/or hyperesthesia were not predictive of the outcome. Most dogs treated with corticosteroids alone were euthanised or died (67%) [$p < 0.001$] and the relapsing dogs were more often treated with 3 or more drugs (62%) [$p < 0.001$]. The most prevalent MRI abnormalities locations were in the cerebral hemispheres (63%), thalamus (40%) and spinal cord (44%). Multifocal lesions, brain herniation, presence of meningeal and parenchymal contrast enhancement, and type and cell count of CSF pleocytosis were not associated with a higher risk of relapse or death.

As soon as the diagnosis is achieved, it seems advantageous to start the treatment with a second immunossupressive agent (other than corticosteroids) and often the disease is only managed using a multi-drug therapy protocol, since the rate of relapse is high. Previously reported findings, as seizures, multifocal lesions, brain herniation, presence of meningeal and parenchymal contrast enhancement, and type and cell count of CSF pleocytosis aren’t associated with a higher risk of relapse and death.

Keywords: Dog, Meningoencephalitis, Relapse, Immunosuppressive treatment

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List of Abbreviations and Symbols

1H MRS - Single Voxel Proton Magnetic Ressonance Spectroscopy

BBB – Blood-brain barrier

CA - Cytosine arabinoside

CD3 - Cluster of Differentiation 3

CNS – Central Nervous System

COP - Cyclophosphamide, vincristine, prednisone

CRI - Constant Rate Infusion

CSF – Cerebrospinal fluid

CT – Computed tomography

DLA II - Dog leukocyte antigen II

DNA - Deoxyribonucleic acid

DVM – Doctor of Veterinary Medicine

EME - Eosinophilic meningoencephalitis

FDG-PET - Fluorodeoxyglucose PET

GME - Granulomatous meningoencephalomyelitis

IGTS - Idiopathic generalized tremor syndrome

IV – Intravenous

IVDD - Intervertebral disk disease

MHC - Major Histocompatibility Complex

MRI – Magnetic resonance imaging

MS - Multiple sclerosis

MUO - Meningoencephalomyelitis of unknown Origin

NIMEs – Non-infectious meningoencephalomyelitides

NLE - Necrotizing leucoencephalitis

NME - Necrotizing meningoencephalomyelitis

PACS - Picture Archiving Communication System

PCR - Polymerase Chain Reaction

PEE - Postencephalitic epilepsy

PET - Positron emission tomography

PLR - Pupillary light response

SC – Subcutaneous

SRMA - Steroid-responsive meningitis-arteritis

T1W - T1-weighted image

T2-FLAIR - T2-weighted fluid-attenuated inversion recovery

T2W - T2-weighted

TNCC - Total nucleated cell count

WBC - White blood cells

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I. Internship Report

The curricular internship was initiated November 2nd 2021 in Clinica Veterinaria San Marco (Padua, Italy), under the supervision of ECVN Diplomate Dr. Marika Menchetti. Although the official internship was spent in the Department of Neurology and Neurosurgery (started in November), a previous period of one month was spent rotating through all the other services as a way of adaptation to the hospital and gaining practical experience in other fields (starting October 4th 2021). I finished my internship on July 5th 2022, completing a total of 9 months and 1 day in that same hospital, 8 months and 3 days of which spent as a Neurology and Neurosurgery intern. The Neurology and Neurosurgery department consists of two ECVN diplomates, one resident, three DVM and one intern. On a day-to-day basis, one was responsible for the appointments of the day, two were responsible for the emergencies (one in the morning and one in the afternoon) and one was responsible for the hospitalization yard. I usually rotated through all areas, since I was given the freedom to be wherever I felt was most interesting.

During this period, I had the opportunity to spend most of my time in three different environments: hospitalization yard, consult room or surgery room. The ordinary day started in the hospitalization yard with assessment of the neurological condition of our recovered neurological patients (performing physical and neurological examinations), evaluating the treatment that was being done and need for alterations, and performing routine medical procedures (placing catheters, collecting blood, doing bladder controls, etc). I was also responsible to take all our dogs out in the morning to evaluate the ability to urinate and defecate normally (specially, after spinal surgery) and to evaluate their gait and mental status. In days of scheduled subcutaneous Cytarabine administrations, I was also responsible for receiving the animals, prepare the Cytarabine injections and administer the drugs to the animals.

When all our recovered animals were taken care of and all their progress was registered in our medical data records, I would go to the scheduled appointments for the day. These consisted of first visits or neurological check-ups and were the responsibility of one of the six Neurology staff members. During the consults I was given the opportunity to learn how to deal with clients and to perform a thorough anamnesis, as well as perform and assist physical and neurological examinations, diagnostic procedures (taking blood from the patients, X-rays), analyse advanced imaging results (MRI, CT and/or electrodiagnostics) and discuss differential diagnosis with the staff members and possible treatment options for those cases. When in need to recover the patient, I was responsible for preparing the admission. The process of placing catheters, creating a line of fluidotherapy and preparing the hospital bed was my responsibility daily.

In days of booked or emergency surgeries, I was responsible for preparing the surgical room, placing the animals in the surgery table, performing the trichotomy and scrubbing. I watched numerous surgeries (hemilaminectomy, ventral slot, dorsal laminectomy, Wobbler surgery, spinal stabilization surgery, etc) and was given the opportunity to enter as second surgeon in a few of them. Regarding surgery, my supervisor gave me the opportunity to participate in two surgery workshops of spinal surgery only for Neurology staff members, where I learned and practiced the basics of a thoracolumbar hemilaminectomy in a cadaver. To add, in a few occasions, I was conceived the opportunity of performing the CSF tap myself in cadavers to practice. I watched and assisted numerous procedures of eletrodiagnostics and muscle biopsies in suspected peripheral nervous system patients.

Being San Marco a Clinic with a high educational component, during this time I attended numerous lectures, webinars and congresses in all fields of Veterinary Medicine.

Every Wednesday at 7.30 am, I attended the Neurology Book reading and Journal Club, an opportunity to study and be updated on the latest news in this field. I was responsible for presenting a few of the articles from the Journal Clubs myself but took part in all the Book readings. Overall, it was a space to further the theory of what I saw in practice daily.

II. Literature review

1. Introduction on Inflammatory diseases affecting the Central Nervous System

Inflammatory central nervous system (CNS) disorders are one of the most common causes of neurological dysfunction in dogs and cats and comprise a large spectrum of infectious and immune-mediated conditions. They can affect the brain, the spinal cord and/or the meninges. Based on the affected tissues, encephalitis, myelitis, meningitis or meningoencephalomyelitis are the accurate terms to use if the brain, spinal cord, meninges or all the latter are affected, respectively.

The infectious CNS diseases are caused by Viruses (Canine distemper virus, feline coronavirus), bacteria (*Staphylococcus* species, *Streptococcus* species, *Pasteurella*, coliforms, *Actinomyces*, *Nocardia* species), fungi (*Cryptococcus*, *Coccidioides* species, *Blastomyces*, *Histoplasma*), rickettsiae (*Ehrlichia*, *Rickettsia*, Rocky Mountain spotted fever), protozoa (*Toxoplasma gondii*, *Neospora caninum*), parasites (*Dirofilaria immitis*, *Cuterebra*, *Angiostrongylus vasorum*) and algae (*Prototheca wickerhamii*, *Prototheca zopfii*). Opposing to human medicine where infectious agents are the most common causes of encephalitis and meningitis, in Veterinary Medicine they are relatively uncommon, as in a recent study it was suggested that the infectious conditions account for only 16,4% of the total CNS inflammatory diseases (Gonçalves et al. 2022).

Meningoencephalomyelitis may also be non-infectious in nature, caused by an immune-mediated inflammatory response in which the body's immune system attacks its own tissues. The non-infectious meningoencephalomyelitides (NIMEs) are the most common causes of inflammatory CNS diseases and include Steroid-responsive meningitis-arteritis (SRMA), Eosinophilic meningoencephalitis, Granulomatous meningoencephalomyelitis (GME), Necrotizing meningoencephalomyelitis (NME), Necrotizing leucoencephalitis (NLE), Eosinophilic meningoencephalitis (EME), Idiopathic generalized tremor syndrome (IGTS) and Idiopathic hypertrophic pachymeningitis (Dewey CW and da Costa RC 2016). When histopathology is lacking, a wide term has been proposed to include all clinically diagnosed cases of suspected GME, NME and NLE: Meningoencephalomyelitis of Unknown Origin (MUO). Eosinophilic meningoencephalitis, SRMA, IGTS and Idiopathic hypertrophic pachymeningitis are not generally referred to as "MUO" due to the different clinical presentation, Magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) abnormalities.

2. Meningoencephalomyelitis of Unknown Origin (MUO): Signalment, Neurological signs and Histopathological features

Meningoencephalomyelitis of Unknown Origin or MUO is a common group of idiopathic, non-infectious, immuno-mediated inflammatory diseases affecting the Central Nervous System, that emerged as one of the most challenging neurological entities for the clinicians to diagnose and treat in the field of Veterinary Medicine. Although it has long been described (as early as 1972) and extensive information regarding the clinical and pathological features exists, much about the pathogenesis of this CNS entity remains unknown.

MUO was considered to be the underlying disease in more than 25% of the inflammatory CNS diseases (Tipold 1995). However, in a recent epidemiological study in a large population of dogs with CNS inflammatory diseases, almost half of them (47.5%) had a suspected diagnosis of MUO (Gonçalves et al. 2022), representing, for that reason, one of the most important inflammatory diseases of the canine CNS.

With the aim of identifying an infectious agent as a possible trigger of MUO, investigations using different diagnostic techniques were performed. Nonetheless, Polymerase Chain Reaction (PCR), Serology, Culture, Histopathologic examinations, Immunohistochemistry and, more recently, Next-generation sequencing (or Deep Sequencing), all failed to detect a consistent suspected pathogen (Tipold 1995; Schatzberg et al. 2005; Barber et al. 2012; Hoon-Hanks et al. 2019; Nessler et al. 2021). MUO is thought to be the result of a deviant immune response against the CNS tissues, most likely of multifactorial etiology, including an underlying genetic predisposition and an additional unknown external trigger. Persists the doubt whether this trigger is an infectious agent or an environmental factor (external and/or internal) (Cornelis et al. 2019; Nessler et al. 2021).

MUO includes three entities (GME, NME and NLE) only differentiated by sampling CNS material through a biopsy, a procedure cost prohibitive, extremely invasive, dangerous, and highly risky of patient morbidity. Hence, it is practically not recommended in a live patient and a necropsy is frequently the only way to achieve a definitive diagnosis. Histopathology provides valuable information regarding the major populations of cells and their distributions, and characteristic lesions of these subtypes of MUO, allowing to differentiate between them with a high certainty. It has been suggested before that each one of them has its unique typical lesions, localization in the CNS and breed prevalence (Park et al. 2012), though intersecting in these features might occur.

2.1. Granulomatous meningoencephalomyelitis (GME)

Granulomatous meningoencephalomyelitis (GME) is the current term used to describe the idiopathic granulomatous inflammation taking part in the CNS and is considered to be the most prevalent subtype of MUO (Coates JR and Jeffery ND 2014). Throughout the years, it has been described by several definitions (eg. inflammatory reticulosis, lymphoreticulosis, neoplastic reticulosis), but all reflect the same entity, probably first described in 1936 (de Lahunta et al. 2015).

GME might appear as 3 different forms according to the distribution of the granulomatous lesions: disseminated (or multifocal), focal and ocular. The disseminated form is the most common pattern, with the lesions happening mainly in the cranial cervical spinal cord, brainstem and forebrain; the focal form is more rare and characterized by single mass lesions localized in the spinal cord, brainstem, thalamus, optic nerves or cerebral hemispheres; the ocular form manifests as a optic nerve inflammation and is considered to be a focal form of GME or a part of a multifocal GME process (Talarico et al. 2010; Uchida et al. 2016; Posporis et al. 2019). Even though it is rare, there's also a report of GME with Peripheral Nervous System involvement (Fliegner et al. 2006).

Histopathologically, GME is characterised by nonsuppurative angiocentric granulomatous lesions in the white matter and meninges of the brain and spinal cord consisting of macrophages mixed with lymphocytes and plasma cells. Perivascular cuff lesions may be recognizable as the earlier signs of the GME, seen as accumulation of inflammatory cells around blood vessels. Astrogliosis and microgliosis are frequently detected and malacic changes can also be observed. In the acute phase of the disease, gray and white matter are equally affected, whereas in the chronic phase the white matter is predominantly affected (Talarico et al. 2010; Vandevelde et al. 2012; Park et al. 2012).

It had been suggested that GME is the result of a delayed type of hypersensitivity with an autoimmune basis since there is a prevalence of MHC class II antigen-positive macrophages and CD3 antigen-positive T-lymphocytes (Kipar et al. 1998). Some authors describe that GME might also be the result of a "lymphoproliferative disorder" with an inflammatory and neoplastic basis, with anomalous lymphocytes being found within brain lesions (Fankhauser et al. 1972). Whether GME is the result of a T-cell mediated delayed hypersensitivity mechanism or has a neoplastic process underneath, remains unclear.

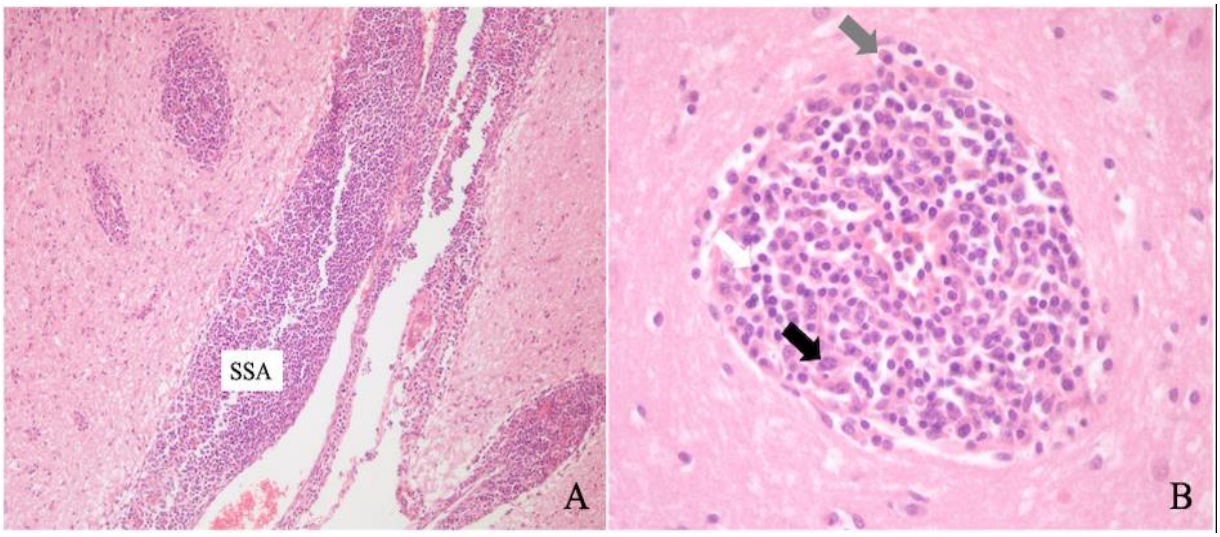


Figure 1. [Property of San Marco Veterinary Clinic] (A) The subarachnoid space (SSA) and gray matter of the cerebral cortex show marked angiocentric infiltration of mononuclear cells along Virchow-Robin spaces. Are accompanied by a moderate spongy state of the nerve parenchyma, moderate astrocytosis and astrogliosis, and moderate microglia activation. Picture indicative of diffuse form of granulomatous meningoencephalitis. Hematoxylin and eosin staining, 10x magnification. (B) Detail of an inflammatory infiltrate in cerebral gray matter; a mixed angiocentric cellular infiltrate composed of epithelioid-type macrophages (black arrow), lymphocytes (white arrow) and plasma cells (gray arrow) is appreciated. Picture indicative of granulomatous meningoencephalitis. Hematoxylin and eosin staining, 40x magnification.

Middle-aged toy and terrier breeds are predisposed to GME most commonly affecting dogs ranging from 4 to 8 years old, though large breed dogs are thought to account for 25% of all MUO cases (Cornelis et al. 2016b; Talarico et al. 2010). A female predisposition has been reported in previous studies, but recently this has been refuted (Cornelis et al. 2019).

Disseminated GME has frequently an acute onset and rapid progression of clinical signs, often with signs of involvement of the forebrain and brainstem. Focal GME signs suggest the growth of a space-occupying lesion (with a differential diagnose of neoplasia), manifesting as an insidious or slow progressive disease with predominantly forebrain signs alone. Ocular GME clinically manifests as acute signs of visual dysfunction. The clinical signs reflect the focal or multifocal nature of the lesions and their localization within the CNS. Extraneural signs are rare and laboratorial tests are usually within limits; however, pyrexia and systemic leukocytosis can occasionally accompany CNS inflammation (Talarico et al. 2010).

Either focal or multifocal GME present commonly as seizures, cerebellovestibular dysfunction, altered mental status, visual deficits and hyperesthesia (usually cervical) (Coates JR and Jeffery ND 2014; de Lahunta et al. 2015; Dewey CW and da Costa RC 2016; Cornelis et al. 2019). Large breed dogs present more commonly with signs of decreased mentation, compared to small breeds (Cornelis et al. 2016b). Signs of myelopathy alone are rarely recognized (Griffin et al. 2008; Wong et al. 2010; Cornelis et al. 2017a).

2.2. Necrotizing encephalitis

The Necrotizing encephalitis (NE), a term that includes Necrotizing Meningoencephalitis (NME) and Necrotizing Leukoencephalitis (NLE), were historically reported as a breed-specific diseases, as Pug Dog Encephalitis and Necrotizing Encephalitis of Yorkshire Terriers, respectively. In the meantime, these diseases have been reported in other breeds, predominantly toy and small breed dogs, as Pug, Yorkshire Terrier, Maltese, Chihuahua, Pekingese, West Highland White Terrier, Boston Terrier, Japanese Spitz, Miniature Pinscher, Papillons, Shih Tzus, Coton de Tulears, Brussels Griffons and French Bulldog (Cordy DR and Holliday TA 1989; Tipold et al. 1993; Stalis et al. 1995; Cantile et al. 2001; Aresu et al. 2007; Timmann et al. 2007; Talarico et al. 2010; Cooper et al. 2014). A familial predisposition is suspected in these inflammatory disorders, especially in Pug dogs, where a strong familial association has been found (Greer et al. 2009). In this same breed, fawn females with NME are overrepresented compared to black males (Greer et al. 2010).

NE mainly affects young dogs, usually under 4 years old. In NME there is a meningeal and neocortical location with extension into the corona radiata and in NLE there is a deep cerebral white matter location with extension into the adjacent gray matter. NME and NLE, similarly to GME, have an unclear underlying pathogenesis, but appear histopathologically distinct from the latter. The characteristic lesions of both NME and NLE include non-suppurative, asymmetrical, multifocal, bilateral areas of cerebral necrosis. Frequent overlap in the topography of the lesions, clinical signs and breeds between them, gives space for suspicion where they are two different entities or are actually just one in two different stages (de Lahunta et al. 2015). To avoid confusion resulting from the intersection of breeds, it is suggested to use the term NE until histopathology enables us to separate them in these two different entities, NME and NLE.

It was proposed that NME and NLE result from autoimmune inflammations with involvement of possibly two different antigenic agents. The epitopes of these agents have components (proteins) also expressed by CNS cells (like oligodendroglial cells), that, in turn, are also attacked when the immune system responds to the infectious agent. As a result, the body generates an autoimmune inflammation and massive destruction of the CNS tissue. This molecular mimicry has also been proposed as an explanation for the pathogenesis of Multiple sclerosis (MS) in humans (de Lahunta et al. 2015), a disease with suspected genetic and pathogenic similarities to NE.

Generalized seizures, behavioural changes, vestibulocerebellar dysfunction and central visual deficits are the most common clinical signs. Most dogs have an acute onset while others have a more insidious and slower progression of clinical signs. Many dogs die or are

euthanized due to the frequency and severity of seizures and rapid deterioration of quality of life.

2.2.1. Necrotizing Meningoencephalitis (NME)

NME lesions are located commonly in the cerebral hemispheres and subcortical white matter and consist of perivascular and parenchymal infiltration of lymphocytes and macrophages and extensive necrosis. The severity of the necrosis varies according to the stage of the disease, changing from neuronal necrosis and gliosis (early disease) to cavitation of the parenchyma (advanced disease). Inflammatory lesions extend from the leptomeninges, through the cerebral cortex, corona radiata and subcortical white matter, rarely affecting the cerebellum, brainstem and spinal cord. Lesions may be bilateral but are usually worse in one cerebral hemisphere. Often, the demarcation between gray and white matter is lost (Talarico et al. 2010; Park et al. 2012; Vandevelde et al. 2012).

NME in dogs is thought to share clinical similarities with the acute fulminant or nonprototypic forms of Multiple sclerosis (MS) in humans. There is the same genetic modification in the human and dog leukocyte antigen II (DLA II) that increases the risk of developing these diseases, a finding that was registered in toy breeds, like Pugs and Maltese. However, in humans, MS manifests as demyelination while, in NME, this is not observed (Greer et al. 2010; Schrauwen et al. 2014).

2.2.2. Necrotizing Leukoencephalitis (NLE)

NLE is characterized by profound asymmetrical malacic changes that often involve the cerebral white matter and thalamus, with less involvement of the leptomeninges and cerebral cortex. Extensive edema and cavitations are common in the white matter lesions (Uchida et al. 2016).

3. Diagnosis

MUO definitive diagnosis, as previously reported, can only be accomplished through histopathology of the CNS lesions. Since brain biopsy is likely to cause gross procedure-related brain damage, several minimal invasive techniques have been developed for ante-mortem tissue sampling. CT or MRI-guided biopsy, stereotactic CT or MRI-based systems, ultrasound-guided biopsy, endoscopic-guided biopsy, and free-hand CT-guided biopsy have all been investigated, though their rates of morbidity and fatality are considerably high. A study concluded that the benefits of sampling a piece of the lesions outweighs the potential case fatality rate and transient neurological deficits, allowing the development of a specific treatment directed towards the subtype of MUO. However, this same study had a fatality and morbidity

rate of 6 and 29%, respectively (Flegel et al. 2012). Until a safer procedure is developed, most clinicians don't recommend CNS biopsies.

As a result, when histopathology is lacking, the antemortem diagnosis can only be presumptive and is usually challenging to achieve for the clinician. Granger et al. (2010), via a multimodal approach to diagnosis, proposed that the terminology MUO should only be used in:

- Dogs older than 6 months;
- Suspected focal, multifocal or diffuse lesions based on the neurologic examination findings;
- Evidence of multiple, single or diffuse intra-axial hyperintense lesions on T2-weighted (T2W) MR images;
- CSF analysis should have pleocytosis with more than 50% mononuclear (monocytes/lymphocytes) cells and increased protein concentration;
- Absence of identifiable geographical infectious diseases.

The tested infectious diseases should take in consideration the prevalent diseases in the area, as these vary with geographical location. CSF and/or serum can be tested with PCR and/or serology for *Neospora caninum*, *Leishmania infantum*, *Toxoplasma gondii*, Canine Distemper Virus, *Bartonella spp*, *Borrelia burgdorferi*, *Cryptococcus spp*, *Ehrlichia spp*, *Anaplasma spp*, *Rickettsia rickettsia* and *Coccidioides immitis*. Considering that the treatment lays on immunossupression, in dogs inhabiting in endemic zones of infectious diseases (e.g., canine leishmaniosis), these agents should be ruled out whenever possible (Portero et al. 2021).

3.1. Advanced Imaging

Although Computed Tomography (CT) scan in some cases can help diagnose inflammatory brain diseases (particularly when combined with analysis of the CSF), Magnetic Resonance Imaging (MRI) is considered to be the imaging gold standard for inflammatory CNS diseases.

MRI has been reported to be 94,4% sensitive and 95,5% specific in overall brain lesion detection, particularly when detecting a neoplastic or inflammatory lesions (Wolff et al. 2012). However, the complete agreement between neurological and imaging findings are thought to be quite low. Overall, the sensitivity of imaging in identifying all inflammatory lesions suspected from the neurological examination is of less than 60% (Granger et al. 2010). Moreover, Lamb et al. (2005) described that approximately 25% of dogs with MUO with an inflammatory CSF, have a completely normal MRI.

The 3 different subtypes of MUO have usually characteristic features, as the typical topographic distribution or the presence of necrosis. This knowledge could help direct a presumptive antemortem diagnosis, though none of these imaging features are considered pathognomonic of the disease process underneath. Thus, MRI cannot be used to differentiate between GME, NME and NLE, a diagnosis only achieved with histopathology.

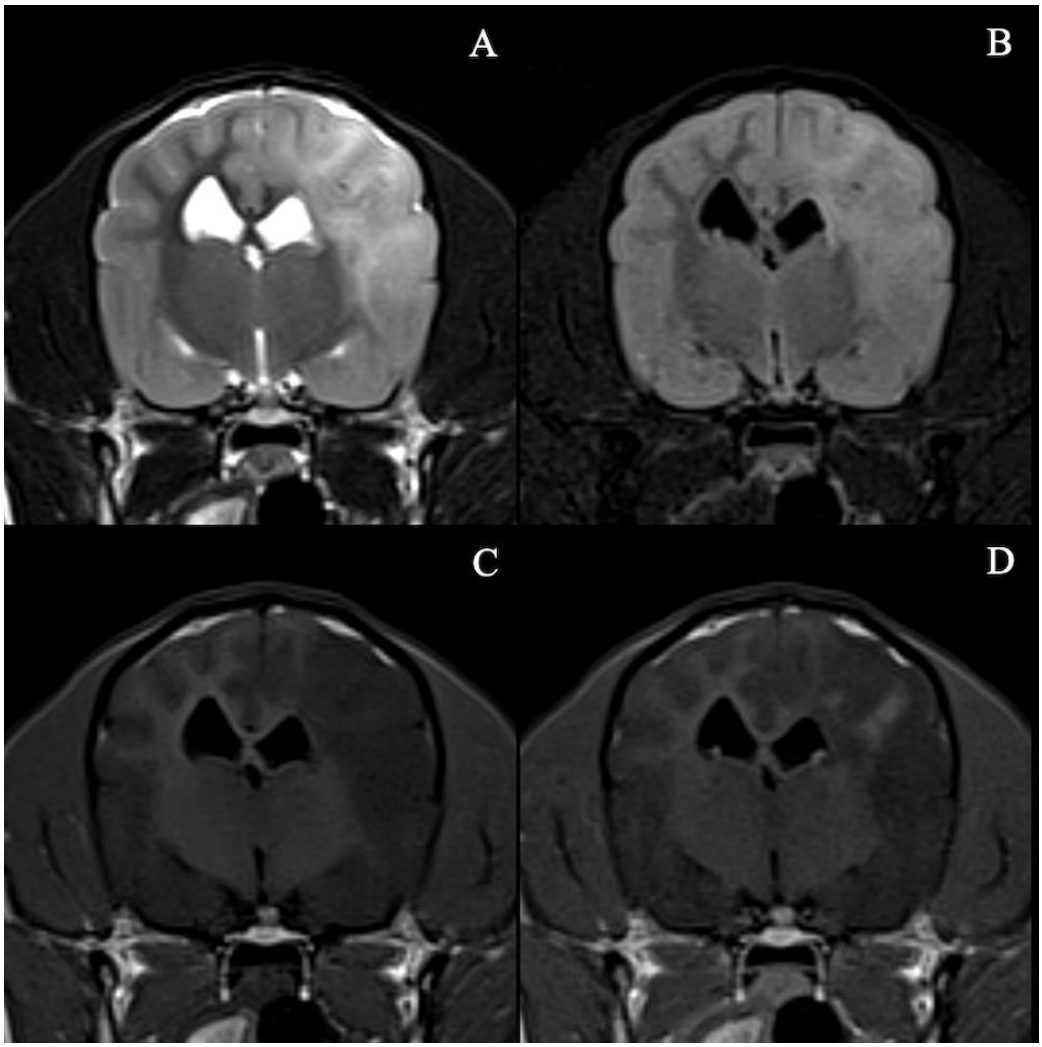


Figure 2. [Property of San Marco Veterinary Clinic] Four-year-old female pinscher with MUO; transverse T2-weighted (A), FLAIR (B), T1 pre-contrast (C) and T1 post-contrast (D) MRI images at the level of thalamus, showing a large area with poorly defined margins in the left temporal region, which appears inhomogeneously hyperintense in T2 (A) and FLAIR (B), hypointense in T1 (C) with focal and inhomogeneous paramagnetic contrast-enhancing (D) intake of the encephalic and mild meningeal parenchyma; mass effect with rightward "midline shift" and partial obliteration of the left lateral ventricle is observed.

In GME, the most common MRI findings include one or more contrast-enhancing masses, hyperintense on T2W and T2-FLAIR (T2-weighted fluid-attenuated inversion recovery) sequences and hypo- or isointense in T1W (T1-weighted image). They can be in the

white or gray matter, involving primarily the forebrain, brainstem and cerebellum (Thomas JB and Eger C 1989; Cherubini et al. 2006, Mai 2018). The margins of the lesions are commonly infiltrative or irregular, though reports of neoplastic-like lesions were also described (Lobetti RG and Pearson J 1996; Cherubini et al. 2006). When compared with a neoplasia, MUO is less likely to have a strong contrast-enhancement, to have heterogenous T2W or T2-FLAIR signal intensity and to have mass effect. However, all these characteristics are variable and, in many cases, the differentiation between these two entities is only achieved with the aid of CSF analysis.

The most common findings in NME are multifocal, asymmetric with variable contrast-enhancement lesions affecting the cortical gray and white matter, causing loss of gray-white matter distinction. The lesions are iso- or hypointense on T1W images and hyperintense on T2W and T2-FLAIR images and affect primarily the cerebral hemispheres, hippocampus, thalamus, caudal brainstem, and cerebellum. Mass effect (causing falx cerebri shift) and variable degrees of ventriculomegaly can also be present (Coates JR and Jeffery ND 2014; Mai 2018).

In the case of NLE, the most common MRI findings are multifocal, asymmetric irregularly shaped lesions, hyperintense on T2W and T2-FLAIR images, and hypointense in T1W. The lesions have variable contrast-enhancement and predominantly affect the subcortical white matter of the cerebral hemispheres but also the cortical gray matter, thalamus and brainstem. Lateral ventriculomegaly and cyst-like lesions can also be apparent (Coates JR and Jeffery ND 2014; Mai 2018).

The presence or absence of the contrast enhancement in CNS inflammatory diseases was suggested to be the result of a disruption of the blood-brain barrier (BBB) and different patterns of vascularization. The BBB is a dynamic interface between the CNS and the circulating blood responsible for the CNS homeostasis. When an inflammatory disease develops, a dysfunction of this physical barrier happens, which leads to different grades of breakdown of the BBB. The image enhancement seen in the MRI is not an “all or nothing” phenomenon but depending on the chronicity of the process. As a result, in early inflammatory processes the MRI may be negative, which doesn’t rule out meningeal disease (Cherubini et al. 2006).

Positron emission tomography (PET), Fluorodeoxyglucose PET (FDG-PET), Single Voxel Proton Magnetic Ressonance Spectroscopy (1H MRS) and Transcranial Sonographic were used in previous studies to evaluate their potential use as imaging diagnostic tools, though further studies on their utility are necessary.

3.2. Cerebrospinal fluid (CSF) analysis

CSF evaluation usually provides the most important information in the antemortem diagnosis of MUO. A predominantly mononuclear (monocytes/lymphocytes) pleocytosis with more than 50% mononuclear cells is one of the suggested guidelines for the presumptive diagnosis of MUO since it is a specially common finding in these patients. A pleocytosis is defined as an increase in the total nucleated cell count (TNCC) of the CSF, considering that it is elevated when there are more than 5 TNCC/µL. Infrequently, in less than 10% of cases MUO patients will have predominantly a neutrophilic pleocytosis. An increase protein concentration in the CSF can also be present, but it is a nonspecific indicator of CNS disease, caused by disruption of the BBB or albuminocytological dissociation (Granger et al. 2010).

Although CSF analysis is more sensitive than MRI in identifying abnormalities consistent with inflammatory disease, normal CSF analysis has been described in 3 to 57% of MUO cases. In cases where from MRI comes a suspected raised intracranial pressure, this crucial part of diagnosis is lacking, due to the risk of performing the CSF tap. This could create a possible bias towards less severe cases or could lead to cases of misdiagnosis (Cornelis et al. 2019).

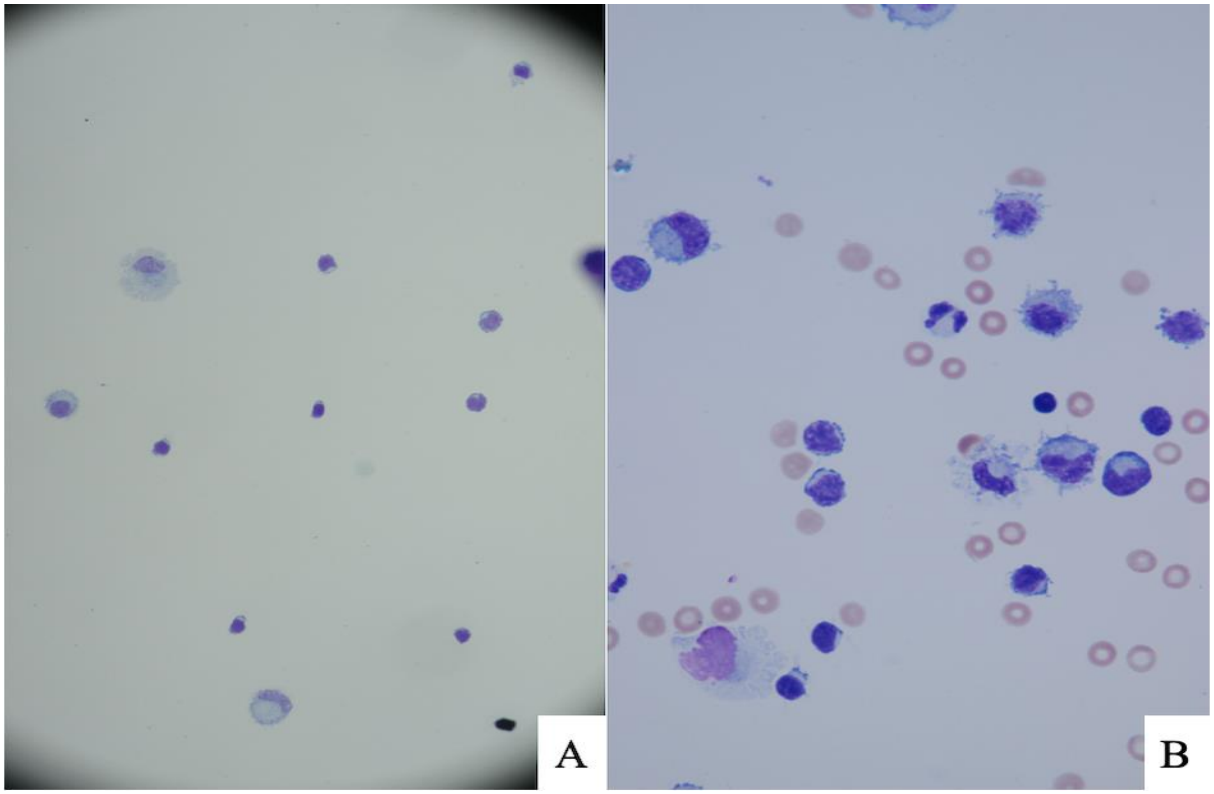


Figure 3. [Property of San Marco Veterinary Clinic] Cerebrospinal fluid with evidence of predominantly lymphocytic pleocytosis (A) (10x) and predominantly mononuclear pleocytosis (B) (50x); Diff Quick staining.

4. Treatment

In the past two decades multiple investigations searched to uniformize and optimize the treatment protocol for MUO. However, a favourable and universally accepted regimen within the scientific committee has not been discovered yet and, consequently, the treatment of MUO in dogs remains a matter of clinical preference and experience.

Some clinicians first approach to the disease is to introduce therapy with an anti-inflammatory dosage of corticosteroids and an antibiotic (clindamycin and/or doxycycline) while waiting for the results of serology and PCR to the geographical infectious agents. After these have been excluded, being MUO a disease with a suspected auto-immune basis, the antibiotics are discontinued, and the corticosteroids are increased to an immunosuppressant dosage. The corticosteroids most commonly used are Prednisone, Prednisolone and Dexamethasone and, in the case of Prednisolone, the daily dose varies from 0,5 to 30 mg/kg (Cornelis et al. 2019). The Prednisolone treatment schedule has not been established, but three previous studies (Munana KR and Luttgen PJ 1998; Smith et al. 2009; Lowrie et al. 2013) used this same (or a similar) treatment protocol:

- Prednisolone 1 mg/kg every twelve hours, *per os*, for four weeks;
- Prednisolone 0,5 mg/kg every twelve hours, *per os*, for six weeks;
- Prednisolone 0,25 mg/kg every twelve hours, *per os*, for six weeks;
- Prednisolone 0,25 mg/kg every twenty-four hours, *per os*, for six weeks;
- Prednisolone 0,25 mg/kg every forty eight hours, *per os*, for six weeks;
- Prednisolone 0,25 mg/kg every seventy two hours, *per os*, for six weeks.

The immunosuppressant mechanism of action of this drug relies on its ability to supress and induce apoptosis of T-cells, decrease B-cell antibody production, and disrupt the intercellular communication of leukocytes via the interference with lymphokine production, biologic action, or both (Plumb 2011).

Corticosteroids monotherapy may solve the clinical signs in some dogs with MUO, though, in others the response to corticosteroids alone is variable and often temporary (Zarfoss et al. 2006). Additionally, long-term, high-dose corticosteroid therapy often causes adverse side effects as polydipsia, polyphagia, polyuria, weight gain, gastrointestinal signs, hepatotoxicity, behavioural changes, panting, muscle weakness and/or atrophy, dermatological issues, predisposition to infections, pancreatitis (Plumb 2011; Cornelis et al. 2019). Thus, most clinicians choose to adjunct an immunomodulatory agent early after diagnosis.

Currently, the most used treatment protocol for MUO is a combination of Corticosteroids and Cytosine arabinoside (CA) or Cytarabine. CA is a chemotherapeutic agent used in Veterinary Medicine to treat several neoplastic conditions, as lymphosarcomas (lymphomas), and as an immunosuppressant. It is a synthetic nucleoside analogue that crosses the BBB in dogs, undergoes enzymatic activation and is converted intracellularly into cytarabine triphosphate (a compound responsible for inhibition of DNA polymerase) resulting in the inhibition of DNA synthesis in mitotically active cells (Scott-Moncrieff et al. 1991). It also prevents DNA repair, inhibit ribonucleotide reductase and membrane glycoprotein synthesis, and causes topoisomerase dysfunction (Zarfoss et al. 2006). The side effects are dose dependent and the most commonly seen include myelosuppression, gastrointestinal issues (vomiting, diarrhoea) and hair loss (Scott-Moncrieff et al. 1991).

Since CA has a low systemic bioavailability when administered *per os*, it is only used parenterally, being intravenous (IV) or subcutaneously (SC) the most used routes of administration. In the latter, various protocols have been investigated but the most frequently used is a subcutaneous injection of CA at the dose of 50 mg/m^2 every twelve hours for two consecutive days and repeated every three to six weeks, indefinitely (Zarfoss et al. 2006). The IV or Constant Rate Infusion (CRI) protocol usually delivers 100 to 300 mg/m^2 of CA to the patient in a period varying from 8 until 24 hours. Previous studies suggested that the absorbed concentration of CA achieved with the CRI protocol leads to a consistent and prolonged exposure of the plasma and serum to the drug compared with the SC protocol (Crook et al. 2012; Early et al. 2016). However, a recent report described that the SC protocol provided a higher concentration of the medicine when compared to the CRI protocol, exceeding its proposed therapeutic target, and having the benefits of reducing the hospitalization time, the repeated catheterization of the patient and the costs to the client (Levitin et al. 2021). Further investigations need to be done to compare the efficacy and advantages of the CRI instead of the SC protocols.

A third drug among the most commonly used treatment options available for MUO is Cyclosporine. Either alone or as adjunctive therapy, Cyclosporine is a potent immunosuppressive agent that supresses T-lymphocyte activation and proliferation, and blocks the transcription of cytokine genes in activated T-cells including interleukin-2, which indirectly leads to inhibition of T-cell proliferation (Viviano 2013).

Although Cyclosporine is considered to have poor BBB permeability in healthy animals, in dogs affected by MUO the dysfunction of this barrier may result in the accumulation of the drug in the affected areas of the CNS (particularly in the choroid plexus and cerebral endothelial cells) (Begley et al. 1990). On the other hand, since GME is suggested to be the

result of a T-cell-mediated delayed-type hypersensitivity and this response starts in the peripheral lymphoid organs, there may not even be a need for Cyclosporine to cross the BBB and supress the immune response to the CNS (Adamo et al. 2007).

An ideal dose to treat MUO has not been established yet, but aggressive high-dose therapy is recommended when the patient is in a life-threatening situation rather than a conservative approach. In previous studies, Cyclosporine was added to the steroid therapy at a dose ranging from 3 to 15 mg/kg *per os* every twelve hours (Adamo FP and O'Brien RT 2004; Gnirs 2006; Adamo et al. 2007). Treatment with Cyclosporine is considered to be much safer in dogs than in humans, with just a few adverse effects reported on literature, including gastrointestinal issues (diarrhoea, vomiting and nausea), hypertrichosis and transient lymphopenia, that are usually reversible when the drug is withdrawn (Robson 2003).

Azathioprine (Wong et al. 2010), Mycophenolate mofetil (Barnoon et al. 2016), Leflunomide (Gregory et al. 1998), Procarbazine (Coates et al. 2007), Lomustine (Uriarte et al. 2007, Flegel et al. 2011) and the COP protocol (Cyclophosphamide, vincristine, prednisone) (Smith et al. 2009) have all been studied as possible immunosuppressive treatments for MUO, though the optimal treatment is not defined until these days.

An important aspect in the treatment of MUO is that a great number of these animals will need anticonvulsant therapy, since they arrive to the hospital with seizures or eventually develop later epilepsy. In a recent study (Kaczmarska et al. 2020), in a large population of dogs with a presumptive diagnosis of MUO, 51% arrived at the hospital with acute symptomatic seizures and 23% developed postencephalitic epilepsy (PEE). All received antiepileptic drugs, usually Phenobarbital (after initial stabilization), followed by Levetiracetam. Considering the high prevalence of seizures in dogs with MUO, many dogs need to take antiepileptic drugs as an accessory therapy to the immunosuppressants. Also, to note that there are some interactions between immunosuppressants and anticonvulsants, as Phenobarbital and Cyclosporine. Cyclosporine is metabolised by cytochrome P-450, which is induced by Phenobarbital and as a result Cyclosporine blood levels are reduced. Thus, adjustment of the Phenobarbital dose in dogs receiving also Cyclosporine might be needed (Robson 2003).

The treatment with immunosuppressants and, often with anti-convulsant therapy, is long lasting (months, years or even for life), taking a huge effort, commitment, and financial burden for the owners to deliver a good quality of life to their dog.

5. Prognosis

MUO is considered to be a fatal disease as the prognosis for completely recover is often guarded to poor. It is an unpleasant disease to treat for the clinician, since most animals relapse, die or are euthanized within a few weeks to months after diagnosis, even with an

aggressive and appropriate treatment (Thomas JB and Eger C 1989). In some cases, MUO can be controlled and the animal can have a good quality of life, but will need to remain on medication for many months, or even years.

The median survival times with a prednisolone only treatment ranges from 28 to 602 days, increasing to 26 to 1063 days when added Cytosine arabinoside or to 236 to 930 days when added Cyclosporine (Cornelis et al. 2019). Furthermore, 15% of the cases die before being treated and approximately 25 to 33% of the cases dye or are euthanised within one week after diagnosis (Munana KR and Luttgen PJ 1998; Lowrie et al. 2013). When we evaluate this information, the survival rates are quite low and there is a need for further information that could help us predict the outcome with the aim of improving the treatment. For several years, investigations to discover and describe features that could help us access the severity of the cases have been made, but an universal *consensus* within the scientific committee regarding these prognostic indicators has not been achieved yet.

Munana KR and Luttgen PJ (1998), in a population of 42 dogs with GME, suggested that focal GME (particularly, focal forebrain) was associated with longer survival times than those with multifocal involvement. To add, focal forebrain lesions that underwent radiation therapy had better long-term survival, since radiation seems to be an effective treatment for dogs (particularly those with clinical signs suggesting focal involvement).

The following year, Bateman SW and Parent JM (1999), in a large population of dogs admitted for status epilepticus and cluster seizures for several different reasons, suggested that seizures resulting from GME may indicate a poor prognosis.

Coates et al. (2007), was unable to show that forebrain lesions were associated with longer survival times (as previously reported) but supported the evidence that seizures and an altered mentation were associated with shorter survival times.

In a group of Pugs with NME, Young et al. (2009), concluded that contrast enhancement is variable and not predictive of survival time, and greater MRI lesion burden didn't correlate to survival time. This latter feature was investigated due to its role in establishing prognosis and utility as a biomarker of disease severity in humans with Multiple Sclerosis (Mowry et al. 2009).

Lowrie et al. (2013), in dogs receiving a standard treatment protocol, concluded that survival was not affected by age at presentation, seizures, presence of postcontrast hyperintense lesions, rostral or caudal fossa involvements, transtentorial herniation, CSF cell count and/or protein concentration. An increase in the latter two factors weren't also associated with a higher risk of relapsing at a repeated CSF analysis at the three-month re-examination,

but an abnormal CSF at this time was predictive of relapse. On the other hand, signs suggestive of increased intracranial pressure (foramen magnum herniation, loss of cerebral sulci and mass effect) were all associated with mortality and survival to three months post-diagnosis was suggested as a good prognostic indicator for survival. Three years later, the same authors supported their previous findings, concluding that loss of cerebral sulci and foramen magnum herniation were risk factors predictive of mortality, and overcoming the three-month survival mark was associated with a good long-term outcome (Lowrie et al. 2016).

Barnoon et al. (2016), in a group of 25 dogs, suggested that early diagnosis and treatment might influence survival time, since significantly longer survival times were recorded in dogs that presented within 7 days of onset of clinical signs, relative to those presented after more than 7 days.

This same year, Cornelis et al. (2016a), in a search for prognostic indicators for 1-week survival, discovered that the presence of seizures, decreased mentation and higher neutrophil percentage (total nucleated cell count) on CSF analysis at the presentation were associated with death within 7 days from diagnosis, possibly due to a more severe clinical phenotype.

The year after, Oliphant et al. (2017), also proved the previous association between an elevated CSF total nucleated cell count (TNCC) and shorter survival times and added that older dogs had a worse prognosis compared to younger dogs.

Kaczmarska et al. (2020), reported that dogs with suspected increased intracranial pressure features on MRI (particularly loss of cerebral sulci and caudal transtentorial brain herniation) were at higher risk of death. However, acute symptomatic seizures due to MUO and a treatment protocol with corticosteroid monotherapy weren't associated with death.

In the most recent study available regarding prognostic indicators, Brady et al. (2020), refuted several of the former findings and concluded that multifocal or caudal fossa lesions on MRI, CSF cell count and/or total protein, or suspected intracranial pressure at time of diagnosis (loss of cerebral sulci and foramen magnum herniation) weren't associated with a higher risk of death or relapse and didn't affect the long-term outcome.

As seen, more than twenty years later, the prognostic indicators of relapse, death or good outcome aren't still defined. Acknowledgement of clinical and neurological findings, MRI and CSF characteristics and treatment protocols associated with poorer prognosis (relapse or death) might lead to institution of a more aggressive therapy and a closer follow-up of the patient. Currently, these prognostic indicators need further validation, so that they can be used to access the prognosis of these cases.

III. Relapsing Meningoencephalomyelitis of Unknown Origin: Neurological presentation, Diagnostic clinicopathological findings, Imaging characteristics and Therapeutic management of dogs with and without relapse

1. Introduction and Objectives

Inflammatory diseases comprise an important part of the disorders affecting the central nervous system (CNS). These include infectious (viral, bacterial, fungal, rickettsial, protozoal and algae) or immune-mediated diseases. The latter include non-infectious meningoencephalomyelitides (NIMEs), as Steroid-responsive meningitis-arteritis (SRMA), Eosinophilic meningoencephalitis, Granulomatous meningoencephalomyelitis (GME), Necrotizing meningoencephalomyelitis (NME), Necrotizing leucoencephalitis (NLE), Eosinophilic meningoencephalomyelitis (EME), Idiopathic generalized tremor syndrome (IGTS) and Idiopathic hypertrophic pachymeningitis (Dewey CW and da Costa RC 2016). Given the difficulty of achieving an ante-mortem definitive diagnosis, the term Meningoencephalomyelitis of Unknown Origin (MUO) was created to describe cases where GME, NME and NLE are suspected (and histopathology is lacking).

MUO presumptive diagnosis is based on a multimodal approach that includes signalment, clinical and neurological signs, advanced imaging [magnetic resonance imaging (MRI) or computerized tomography (CT)], CSF analysis suggestive of inflammatory disease and exclusion of geographical infectious agents. Middle-aged small and medium sized breeds, specially of toy and terrier breeds, are predisposed to MUO, but all breeds and sizes can be affected (Granger et al. 2010; Talarico et al. 2010; Coates JR and Jeffery ND 2014; de Lahunta et al. 2015; Dewey CW and da Costa RC 2016; Cornelis et al. 2019). Some authors suggested that there is female predisposition, but others found no difference between the female:male ratio (Munana KR and Luttgen PJ 1998; Talarico et al. 2010; Granger et al. 2010; Cornelis et al. 2019).

The clinical presentation of MUO is variable and correlates with the distribution of CNS lesions. Forebrain and brainstem locations were reported as most frequently affected in multifocal GME, whilst forebrain alone is more frequently involved in focal GME. NE lesions tend to have a predilection for the forebrain (Granger et al. 2010; Talarico et al. 2010; Coates JR and Jeffery ND 2014; Cornelis et al. 2019). Often, dogs present with seizures, visual deficits, disorientation, loss of balance, paresis and ataxia, and changes in behaviour (Talarico et al. 2010). Eight percent of the cases present with signs of myelopathy only (Granger et al. 2010). Commonly MUO is acute in onset (Talarico et al. 2010).

Treatment of this condition consists of immunosuppression, generally corticosteroids, and often other immunosuppressive drugs. Currently, possible adjunctive immunosuppressive

agents are cytosine arabinoside (cytarabine), cyclosporine, azathioprine, mycophenolate mofetil, leflunomide, lomustine, procarbazine and the COP protocol (cyclophosphamide, vincristine, prednisone). The dosages, routes of administration and possible combinations vary between them and the best treatment for MUO has not been established yet.

Typically, MRI findings and CSF analysis are used as an important piece of the *in vivo* diagnosis. MUO MRI abnormalities include focal or multifocal lesions, hyperintense in T2W and FLAIR and isointense in T1W, with variable contrast enhancement. However, in up to seven percent of the dogs affected by MUO, MRI can result normal (Granger et al. 2010). CSF pleocytosis is predominantly mononuclear with more than 50% of mononuclear cells present, but a mixed pleocytosis (no predominance of any cell type) or normal CSF can be present (Smith et al. 2009).

The overall prognosis for MUO is guarded to poor. Even with aggressive therapy, many dogs die and/or relapse. Just a few studies have previously investigated possible prognostic factors for MUO that could help predict a good or a bad outcome, though these remain dubious. Focal vs multifocal (Munana KR and Luttgen PJ 1998; Coates et al. 2007; Lowrie et al. 2013; Brady et al. 2020), seizures (Bateman SW and Parent JM 1999; Coates et al. 2007; Lowrie et al. 2013; Cornelis et al. 2016a; Kaczmarska et al. 2020; Brady et al. 2020), contrast-enhanced lesions (Young et al. 2009; Lowrie et al. 2013), features suggestive of raised intracranial pressure (Lowrie et al. 2013; Lowrie et al. 2016; Kaczmarska et al. 2020; Brady et al. 2020) and CSF cell count and/or protein concentration (Lowrie et al. 2013; Cornelis et al. 2016a; Oliphant et al. 2017; Brady et al. 2020), have all been previously investigated as possible predictors of survival, relapse and/or death.

Despite MUO being an entity recognized for several decades, its pathogenesis remains elusive and gold standard treatment protocols have yet to be established. Setting up a closer follow-up or a more aggressive treatment plan based on a number of patient variables at initial presentation, could provide better outcomes and survival rates. Hence, in the current study, we aimed to describe and compare the clinical presentation, MRI and CSF analysis findings, and treatment protocols used between dogs with MUO that survived, relapsed or died. These could provide valuable information and result in the improvement and guidance of possible treatments, while assess the effect of some variables on survival time and rate of relapse.

2. Materials and methods

2.1. Case selection

The electronic database of the San Marco Veterinary Clinic (Italy) was searched between May 2017 and December 2021 for dogs with presumptive diagnosis of MUO using the following key words: “meningoencephalitis”, “meningitis”, “myelitis”, “pleocytosis”, granulomatous meningoencephalomyelitis”, “necrotizing meningoencephalomyelitis”, “leucoencephalitis” and “encephalitis”. All records recovered from this search were reviewed and dogs satisfying the criteria for either confirmed or presumptive MUO were included in this report. Data gathered for each patient included: signalment (age, breed, sex, bodyweight), anamnesis (including the duration of clinical signs prior to arrival), physical and neurological examinations, complete blood count, serum biochemistry profile and urine examination, MRI findings, CSF analysis results (when available), PCR results for common geographical infectious agents (when available), treatment protocols used (doses and drugs of choice), date of death (euthanasia/death because of MUO or for reasons unrelated to MUO) and date(s) of relapse(s).

The size of the population in this study was determined by the number of cases in accordance with the inclusion criteria. The criteria followed the guidelines proposed by Granger et al. (2010). Thus, every dog included in the present study had:

- A complete medical history;
- Suspected focal, multifocal or diffuse lesion(s) based on neurologic examination findings;
- Complete MRI study of the affected CNS region;
- CSF analysis performed at diagnosis (cell count, cytological examination and total protein concentration determination);
- Absence of identifiable geographical infectious diseases;
- Follow-up of at least 6 months through medical records or telephone contact with the owner or referring veterinarian.

Cases that did not have CSF collected (due to signs of increased intracranial pressure), or where CSF TNCC was within normal limits, were still included if MUO was considered to be the most likely differential diagnosis taking in consideration the other plausible diagnosis and response to treatment. Increased intracranial pressure was suspected based on haemodynamic (bradycardia, hypertension) and/or MRI findings suggestive of increased intracranial pressure, as mass effect, caudal transtentorial herniation, subfalcine herniation, foramen magnum herniation and perilesional edema (Bittermann et al. 2014). Dogs without complete medical records and thorough neurological examination were excluded from the

study. Dogs that were submitted to a CT (instead of MRI) were excluded. Dogs that were lost to follow-up were excluded.

Dogs were, then, separated in three groups:

- Group 0: recovered from MUO;
- Group 1: relapsed of MUO during the period of study;
- Group 2: deceased because of MUO (spontaneous death or euthanasia).

Dogs were classified as recovered or healed if they were not showing the previously reported neurological signs or if improvement was seen according to the owner or referring veterinarian during the period of the study. Dogs were classified as relapsed if in any of the medical records was registered that they had a sudden deterioration of the neurological status following an initial improvement after diagnosis and initiation of treatment and/or had a repeated MRI/CSF compatible with relapse. Dogs were classified as deceased if they suffered euthanasia or spontaneous death because of the disease progression or if no change in neurological signs was seen.

2.2. Diagnosis

In all cases, the presumptive diagnosis of MUO was done based on signalment, anamnesis, clinical signs, neurological examination, MRI findings and CSF analysis obtained from the cerebellomedullary or lumbar cisterns. The most common geographical infectious agents in the North of Italy (*Toxoplasma gondii, Neospora caninum, Leishmania infantum, Canine Distemper Virus*) were ruled out whenever possible using PCR or CSF samples. A confirmed diagnosis of MUO was not achieved in any of these cases since histopathology was not available.

Regarding the onset the three different groups of dogs were separate in three categories: hyperacute, acute or chronic onsets. An hyperacute onset was considered if the dogs had clinical signs for less than 24 hours; an acute onset was considered if the dogs had clinical signs for a period of 24 to 48 hours; a chronic onset was considered if the dogs had clinical signs for more than 48 hours.

The neurological signs registered are consistent with those presented at first-time presentation at the Clinic. The dogs were first distributed to access the overall prevalence of an altered behaviour, an altered mental status, posture abnormalities, gait abnormalities, proprioceptive deficits, cranial nerves abnormalities, seizures and hyperesthesia, and, then, were compared between the three different groups of dogs. To further the investigation, the population was studied on the specific neurological abnormalities presented: dysphoria,

depression, compulsion, disorientation, stupor, head tilt, head turn, pleurothotonus, low head/neck, emprosthotonus, opisthotonos, kyphosis, monoparesis, paraparesis, hemiparesis, tetraparesis, non-ambulatory tetraparesis, hypometria, hypermetria, proprioceptive ataxia, vestibular ataxia, cerebellar ataxia, proprioceptive deficits, menace response deficits, pathological nystagmus, strabismus, nostrils insensibility, myosis, mydriasis, anisocoria, pupillary light response (PLR) deficits, facial nerve deficits, seizures, hyperesthesia (craniocervical, thoracolumbar, lumbosacral, diffuse).

MRI examinations were performed either using a 0.4 Tesla MRI (Aperto Lucent, SN, X418, Hitachi) from 2017 to 2018 or a 3 Tesla MRI (MAGNETOM Skyra, Siemens, Erlangen, Germany) from 2018 to 2021. All MRI images were retrieved from the Picture Archiving Communication System (PACS) and analysed using a dedicated freestanding workstation and postprocessing software (SyngoVia, Siemens, Erlangen, Germany). Pulse sequences varied but always included sagittal and transverse T2-weighted (T2W) images, transverse T2-fluid-attenuated inversion recovery (T2W-Flair) images and transverse T1-weighted (T1W) images before and after paramagnetic contrast injection and were evaluated for signal intensity and homogeneity. Signal intensities were given with respect to normal grey matter unless stated otherwise. All MRI studies were reviewed by the Head of the Diagnostic Imaging department or a DVM specialist in diagnostic imaging. The CNS was separated in 7 regions and studied for their prevalence in the population: Brain hemispheres, Thalamus, Mesencephalon, Pons, Medulla oblongata, Cerebellum and Spinal cord. Multifocal and focal lesions were studied for their prevalence and compared between the three groups. Multifocal lesions were considered when two or more of the CNS regions were affected. A focal lesion was considered when just one of these regions was affected. Brain herniation (transtentorial, subfalcine and transforaminal) was accessed for prevalence and compared between the groups. The dogs were studied for the prevalence and type of contrast enhancement (meningeal and parenchymal) and compared between the three groups of dogs.

Cerebrospinal fluid analysis was considered normal if total nucleated cell count (TNCC) was ≤ 5 cells/µl and abnormal if TNCC were ≥ 6 cells/µl. The TNCC and type of pleocytosis was compared between the three groups of dogs.

2.3. Therapeutical management

All dogs were treated with different combinations of immunosuppressive drugs, using corticosteroids, cytarabine, cyclosporine, mycophenolate mofetil, azathioprine and leflunomide, in accordance with guidelines from other studies (Gregory et al. 1998; Zarfoss et al. 2006; Adamo et al. 2007; Wong et al. 2010; Woolcock et al. 2016; Cornelis et al. 2019). It

was investigated the prevalence of dogs doing monotherapy, two drugs, or three or more drugs and these categories were compared between the three different groups of dogs. The different combinations of drugs were registered.

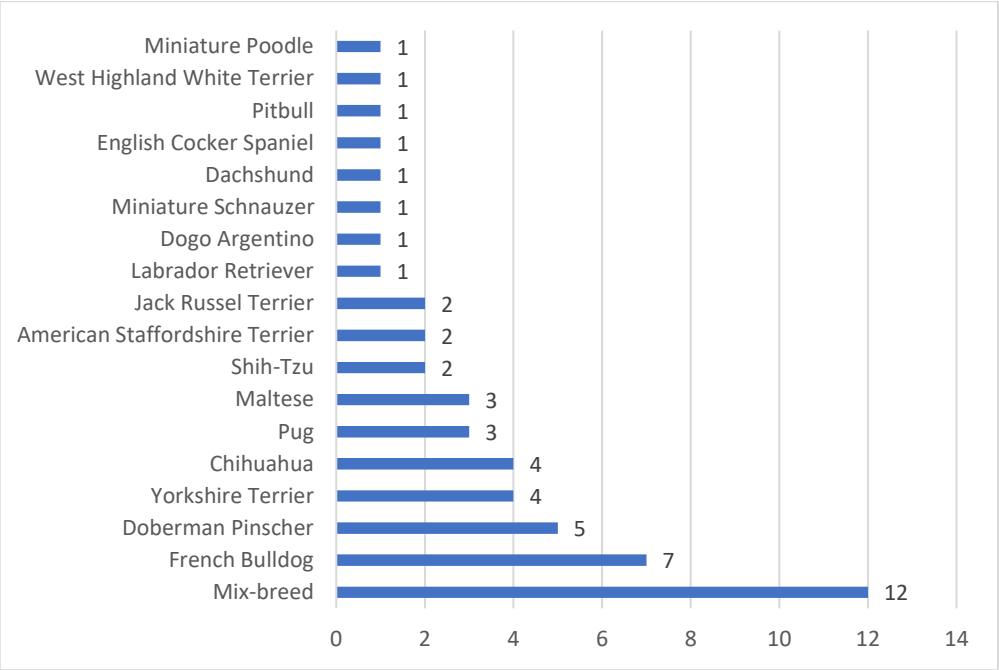
2.4. Statistical analysis

Data analysis was performed with the aid of a standard statistical software package (PAST 3.x The past of the future, Hammer and Harper, Natural History Museum, University of Oslo, Oslo, Norway), while calculations and graphs were obtained using an electronic spreadsheet (Microsoft Excel, Microsoft Corporation, Microsoft Redmond campus, Redmond, Washington, United States). A descriptive statistical analysis was performed to evaluate an association between the onset of presentation, seizures, hyperesthesia, therapy protocols, brain herniation (subfalcine, transtentorial or through foramen magnum), presence and type of contrast enhancement, cell count and type of CSF pleocytosis (mixed, mononuclear, neutrophilic), interval between presentation and the beginning of corticosteroid therapy and multifocal lesions on MRI, and the outcome (recovering, relapsing or dying). The Pearson's Chi-squared test was used to compare the onset (hyperacute, acute or chronic), presence of seizures, presence of hyperesthesia, therapy protocols (monotherapy, two drugs and three or more drugs), presence of brain herniation, presence and type of contrast enhancement, and type of CSF pleocytosis between recovered, relapsed and dead dogs. The Kruskal-Wallis rank sum test was used to compare the interval between presentation at the hospital and starting corticosteroid therapy, the number of regions of the CNS affected and the number of TNCC/µL, between the recovered, relapsed and dead dogs. p-values were considered significant when $p < 0.05$.

3. Results

In the period between May 2017 and December 2021, 54 dogs were diagnosticated presumptively with MUO by the Neurology and Neurosurgery Department of the San Marco Veterinary Clinic (Padua, Italy). One dog was diagnosticated with CT (instead of MRI) and one dog had less than six months of follow-up. Both dogs were excluded from this study and 52 dogs were included since they met the inclusion criteria.

The age at the onset ranged from 5 to 145 months (median of 65 months). Breeds included mixed-breeds (23%, n=12), French Bulldogs (13%, n=7), Doberman Pinscher (10%, n=5), Yorkshire Terriers (8%, n=4), Chihuahuas (8%, n=4), Pugs (6%, n=3), Maltese (6%, n=3), Shih-Tzus (4%, n=2), American Staffordshire Terriers (4%, n=2), Jack Russel Terriers (4%, n=2), and one case each of eight other breeds (2%, n=1) described in the Graphic 1.



Graphic 1. Absolute frequency of breeds of 52 cases with a presumptive diagnosis of MUO present in a population of 52 dogs presumptively diagnosed with MUO.

Dogs weighing >15 kg were considered large breed dogs, dogs < 15 kg were considered small/medium breed dogs (Cornelis et al. 2016). Six dogs were large breed (12%) and forty six were small/medium sized dogs (88%).

Females represent 60% of the population (n=31), of which 14 were neutered, and males represent 40% (n=21), of which 3 were neutered.

The study population included 52 dogs, of which 46% recovered (n=24), 25% relapsed (n=13) and 29% died or were euthanised because of MUO (n=15), being included in group 0, group 1 and group 2, respectively.

The registered deaths occurred from 1 to 86 days after the diagnosis, mainly occurring 6 days after (median of 6 days). The relapses occurred between 106 and 386 days from diagnosis (median 235 days).

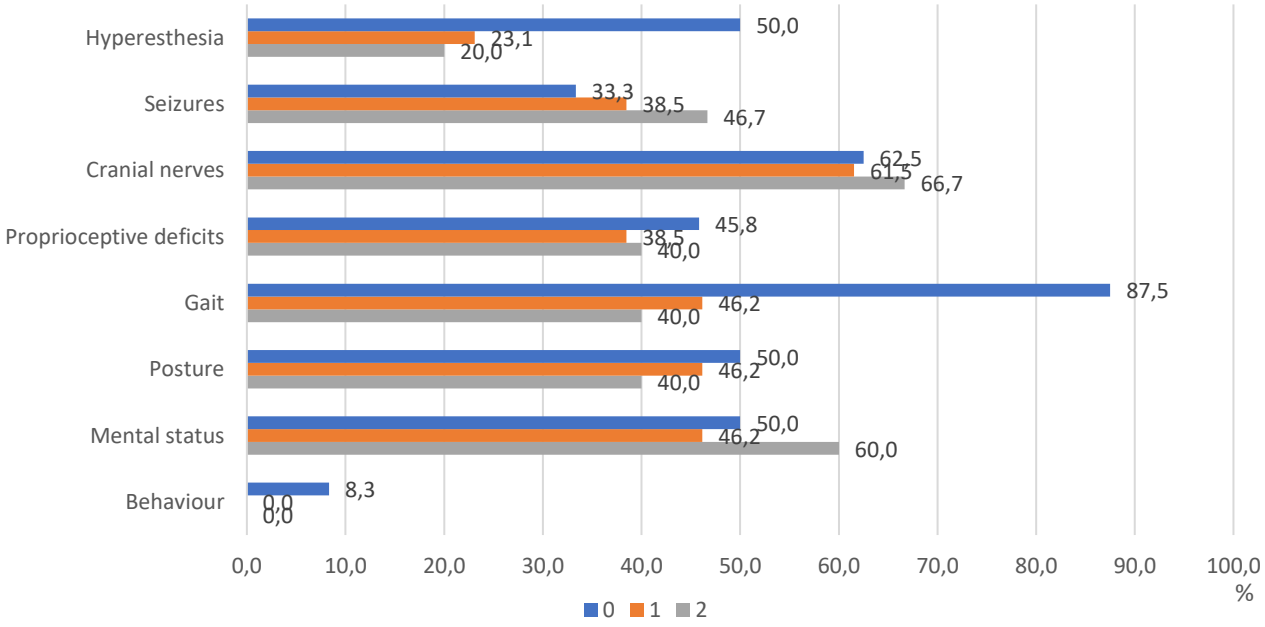
3.1. Clinical signs

Twenty six dogs presented with a chronic onset (50%), fifteen dogs with an acute onset (29%) and eleven with an hyperacute onset (21%). In group 0, 46% of dogs presented with a chronic onset (n=11), 38% with an acute onset (n=9) and 17% with an hyperacute onset (n=4). In group 1, 46% of the dogs presented with a chronic onset (n=6), 38% with an acute onset (n=5), and 15% with an hyperacute onset (n=2). In group 2, 60% of the dogs presented with a

chronic onset (n=9), 33% with an hyperacute onset (n=5) and 7% with an acute onset (n=1). The type of onset was not associated with the outcome (p=0.2614).

Regarding the neurological signs, the most common findings were an abnormal gait (n=33) and cranial nerves deficits (n=33), each one being present in 63% of the population, followed by an abnormal mental status (52%, n=27), an abnormal posture (46%, n=24), proprioceptive deficits (42%, n=22), seizures (38%, n=20), hyperesthesia (35%, n=18) and at last an abnormal behaviour (4%, n=2).

In group 0, the most common neurological finding at the time of presentation was an abnormal gait (88%, n=21), followed by cranial nerves deficits (63%, n=15), abnormal mental status (50%, n=12), posture (50%, n=12) and hyperesthesia (50%, n=12), and at last proprioceptive deficits (46%, n=11), seizures (33%, n=8) and an abnormal behaviour (8%, n=2). In group 1, the most common neurological finding at the time of presentation were cranial nerves deficits (62%, n=8), followed by abnormal mental status (46%, n=6), posture (46%, n=6) and gait (46%, n=6), and at last proprioceptive deficits (39%, n=5), seizures (39%, n=5) and hyperesthesia (23%, n=3). In group 2, the most common neurological finding at the time of presentation were cranial nerves deficits (67%, n=10), followed by an abnormal mental status (60%, n=9), seizures (47%, n=7), posture (40%, n=6), gait (40%, n=6), proprioceptive deficits (40%, n=6) and hyperesthesia (20%, n=3). The latter distribution of neurological findings is evidenced in the Graphic 2 below.



Graphic 2. Relative frequency of the neurological signs in dogs from the groups 0, 1 and 2.

Overall, the neurological signs found in this population of 52 dogs are detailed below (Table 1).

	Clinical signs	*Percentage (%)*
Behaviour	Dysphoria	3,85
Mental status	Depression	38,46
	Compulsion	13,46
	Disorientation	7,69
	Stupor	3,85
Posture	Head tilt	17,31
	Head turn	5,77
	Pleurothotonus	9,62
	Low head/neck	7,69
	Emprosthotonus	3,85
	Opisthotonus	1,92
	Kyphosis	3,85
Gait	Monoparesis	1,92
	Paraparesis	9,62
	Hemiparesis	11,54
	Tetraparesis	1,92
	Non-ambulatory tetraparesis	7,69
	Hypometria	1,92
	Hypermetria	1,92
	Proprioceptive ataxia	11,54
	Vestibular ataxia	15,38
	Cerebellar ataxia	3,85
Proprioceptive deficits	Proprioceptive deficits	30,77
Cranial nerves	Menace response deficits	26,92
	Pathological nystagmus	15,38
	Strabismus	21,15
	Nostrils insensibility	5,77
	Myosis	1,92
	Mydriasis	1,92
	Anisocoria	1,92
	Pupillary light response (PLR) deficits	3,85
	Facial nerve deficits	1,92
Seizures	Seizures	38,46
Hyperesthesia	Hyperesthesia	34,62

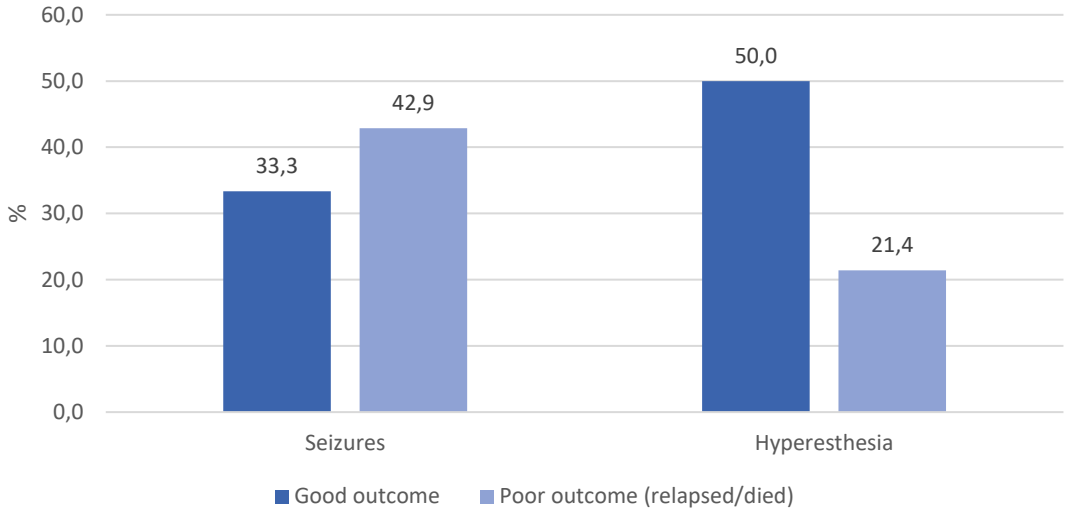
Table 1. Relative frequency of the neurological signs found in a population of 52 dogs presumptively diagnosed with MUO.

3.1.1. Seizures and Hyperesthesia

Twenty dogs showed seizures at the time of presentation (38%). Regarding the dogs with a good outcome (recovered), eight had seizures (33%). Among the dogs with a poor outcome (relapsed or died) twelve had seizures (43%). Seizures at the time of presentation weren't associated with a higher risk of relapsing or dying (p=0.6761).

Eighteen dogs presented with hyperesthesia (35%). The most common form of hyperesthesia was craniocervical pain (n=8), followed by diffuse pain (n=4) and at last thoracolumbar (n=3) and lumbosacral (n=3). Twelve dogs with good outcome (recovered) had pain (50%) and six dogs with a poor outcome (relapsed or died) had pain (21%). Hyperesthesia at the time of presentation wasn't associated with a higher risk of relapsing or dying (p=0.06196).

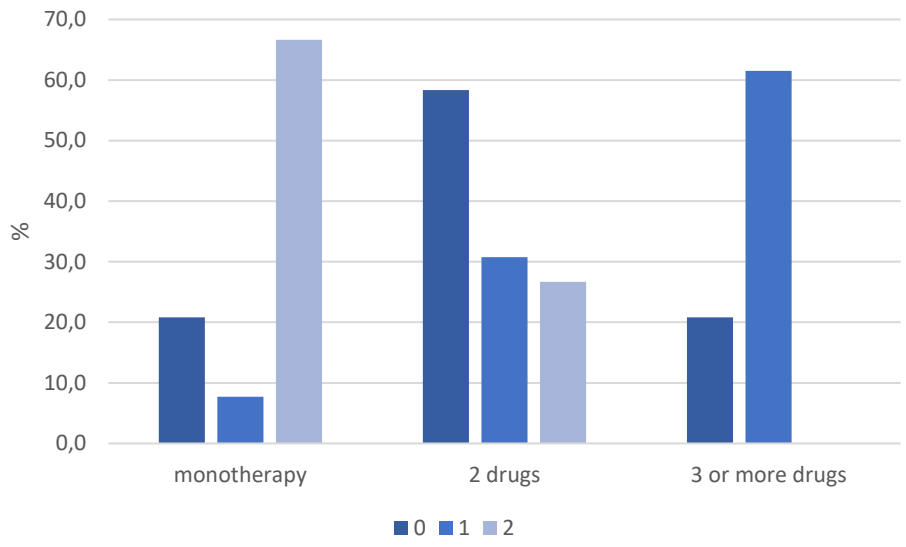
Only two dogs had both seizures and pain.



Graphic 3. Relative frequency of seizures and hyperesthesia in dogs with good and poor outcomes.

3.2. Therapeutical management

Fifty one dogs were treated with corticosteroids therapy. One dog died before introducing any kind of therapy. Thirty five had at least one other immunosuppressant agent introduced (67%) and sixteen did steroids alone (31%).

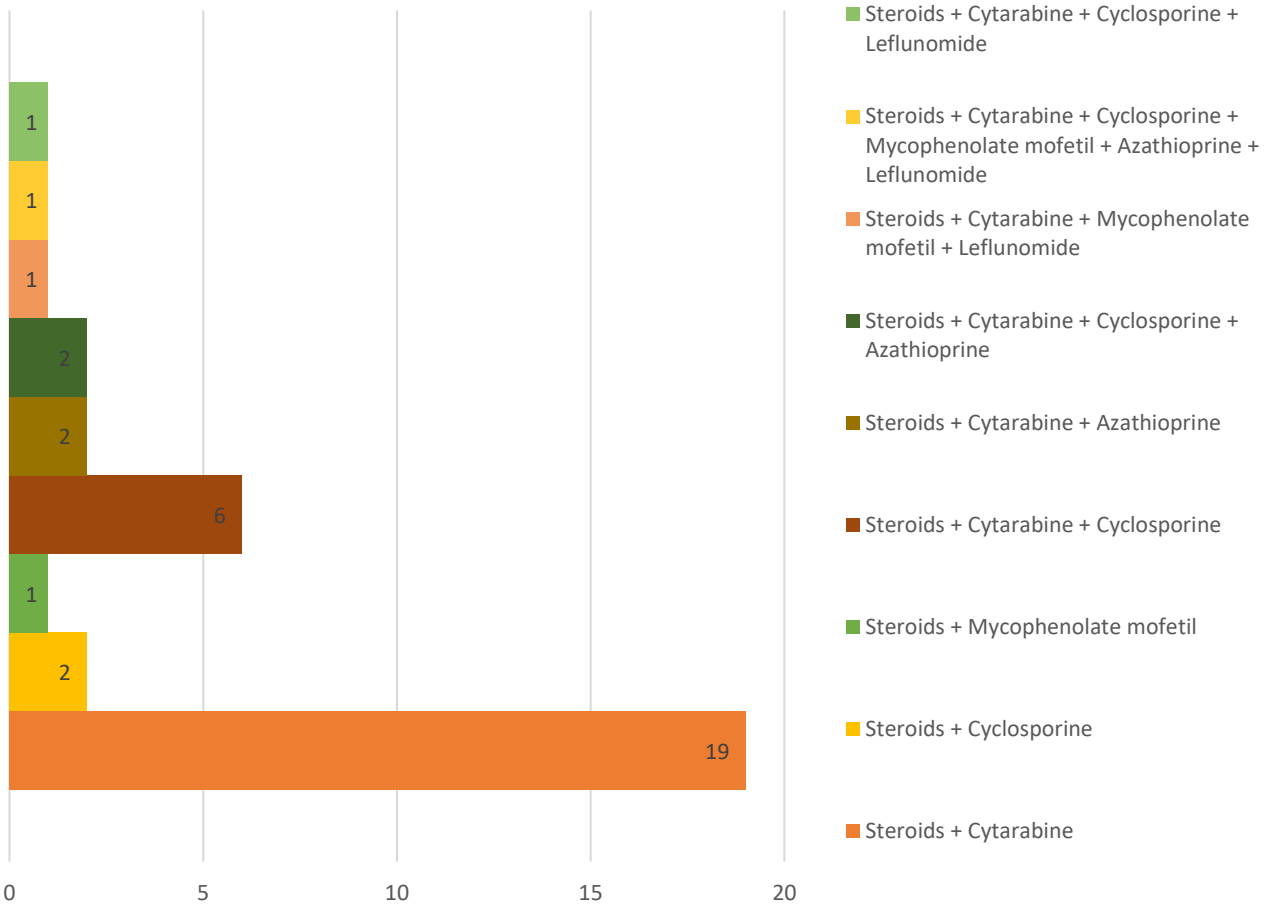


Graphic 4. Comparison of therapy regimen between dogs from groups 0,1 or 2

58% of the dogs from group 0 have been treated with two drugs (n=14), 21% did corticosteroids alone (n=5) and 21% have been treated with 3 or more drugs (n=5). The dogs from group 1 were more frequently managed with a multi-drug protocol with 3 or more drugs (62%, n=8), followed by 31 % doing therapy with 2 drugs (n=4) and just one dog being managed with corticosteroids alone (8%). 67% of the dogs from group 2 were doing corticosteroids monotherapy (n=10) and 27% were being treated with 2 or more drugs (n=4). Dogs that did corticosteroids monotherapy were at higher risk of dying compared with the other dogs ($p<0,001$). Of the 15 dogs that eventually died, 10 were doing corticosteroids alone. The relapsing dogs were more likely to have been treated with 3 or more drugs ($p<0,001$).

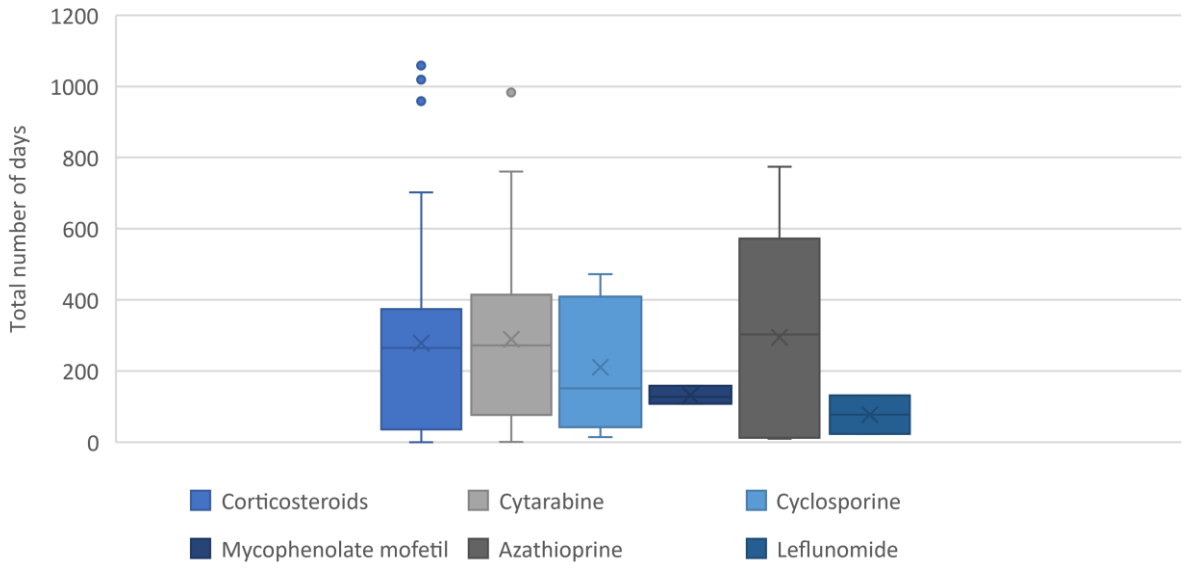
Five different immunosuppressant agents (cytarabine, cyclosporine, mycophenolate mofetil, azathioprine and leflunomide) were studied as adjunctive therapies to corticosteroids. Cytarabine was the preferred adjunctive therapy, used by 91% of the dogs treated with at least one other adjunctive therapy to corticosteroids (n=32), followed by cyclosporine in 34% of the dogs (n=12), azathioprine in 14% of the dogs (n=5), mycophenolate mofetil in 9% of the dogs (n=3) and leflunomide in 9% (n=3).

A protocol with two drugs was done in 22 dogs (42%): corticosteroids and cytarabine (n=19), corticosteroids and cyclosporine (n=2) or corticosteroids and mycophenolate mofetil (n=1). A protocol with three drugs was done in 8 dogs (15%): corticosteroids, cytarabine and cyclosporine (n=6) or corticosteroids, cytarabine and azathioprine (n=2). A protocol with 4 drugs was done in 4 dogs (8%): corticosteroids, cytarabine, cyclosporine and azathioprine (n=2), corticosteroids, cytarabine, mycophenolate mofetil and leflunomide (n=1) or corticosteroids, cytarabine, cyclosporine and leflunomide (n=1). A protocol with 6 drugs was done in just 1 dog (case 36) to manage the disease with all the immunosuppressants above (corticosteroids, cytarabine, cyclosporine, azathioprine, mycophenolate mofetil and leflunomide). The different protocols mentioned are elucidated in the Graphic 6.



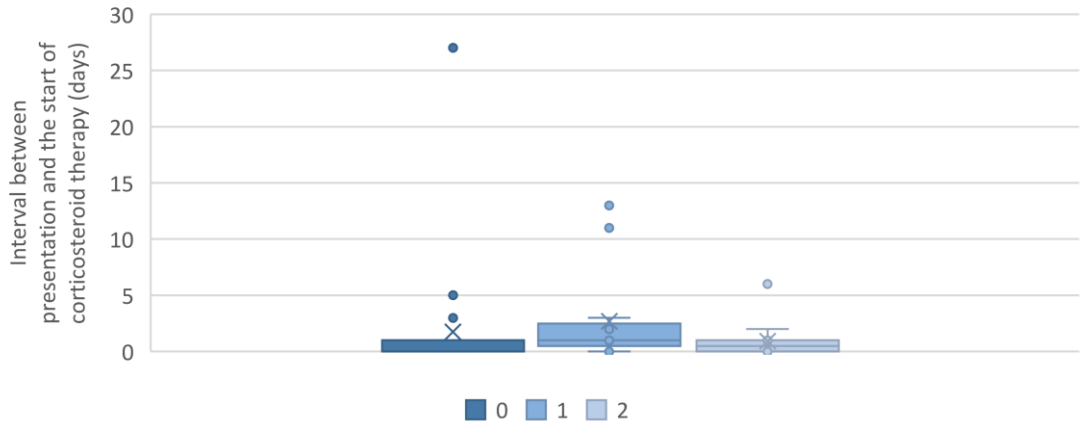
Graphic 5. Absolute frequency of the different multi-drug protocols used in a population of 52 dogs presumptively diagnosed with MUO.

Corticosteroids therapy ranged from 0 to 1059 days of duration (median of 265 days), cytarabine from 1 to 983 days (median of 272 days), cyclosporine from 14 to 472 days (median 151 days), mycophenolate mofetil from 108 to 159 days (median 128 days), azathioprine from 10 to 774 days (median 303 days) and leflunomide from 23 to 132 days (median of 78 days).



Graphic 6. Total number of days of the different immunosuppressive therapies in a population of 52 dogs presumptively diagnosed with MUO

Most dogs had Corticosteroids introduced at a median of one day after presentation at the hospital (0-27 days), Cytarabine at a median of two days after presentation at the hospital (0-216 days), Cyclosporine at a median of eighty days after presentation at the hospital (1-582 days), Mycophenolate mofetil at a median of one hundred days after presentation at the hospital (27-192 days), Azathioprine at a median of two hundred and seventy six days after presentation at the hospital (6-641 days) and Leflunomide at a median of one hundred and fifty five days after presentation at the hospital (150-160 days). It was seen that the dogs that recovered, the dogs that relapsed and the dogs that died did not have a statistically different interval between the first presentation at the hospital and the starting of the corticosteroid therapy. Therefore, the time of starting the corticosteroids wasn't relevant for the outcome (p=0.1091).

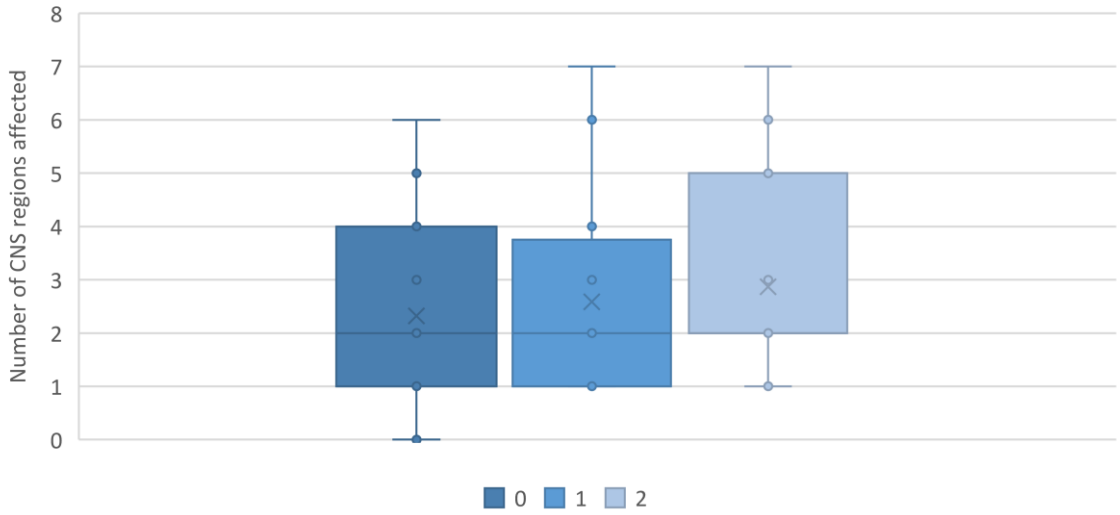


Graphic 7. Interval between first presentation at the hospital and the start of corticosteroid therapy in dogs from groups 0, 1 and 2 (days).

3.3. Magnetic resonance imaging (MRI)

A normal MRI was seen in 2 cases (4%). For the description purpose the CNS was separated in 7 regions: Brain hemispheres, Thalamus, Mesencephalon, Pons, Medulla oblongata, Cerebellum and Spinal cord. Lesions were most frequently found in the Brain hemispheres (63%, n=33), followed by co-involvement of the Spinal cord (44%, n=23), the Thalamus (40%, n=21), the Pons (33%, n=17), the Medulla oblongata (31%, n=16), the Mesencephalon (27%, n=14) and at last the Cerebellum (15%, n=8).

The majority of dogs had multifocal lesions (n=37; 69%). Two regions were affected in 19 cases (37%), three in 3 cases (6%), four in 6 cases (12%), five in 3 cases (6%), six in 3 cases (6%) and all the CNS regions were involved 2 cases (4%). The number of CNS regions affected was compared between the 3 groups to evaluate if it was predictive of the outcome. In all 3 groups the median of CNS regions affected was two and a multifocal distribution wasn't associated with the outcome (p=0.6728).



Graphic 8. Comparison of the number of CNS regions affected between dogs from groups 0, 1 and 2.

Brain herniation was seen in 13% of the dogs (n=7). All these were transforaminal brain herniations (n=6), except for one that was transtentorial brain herniation. Three dogs that recovered (13%), one that relapsed (8%) and three that died (20%), had brain herniations. The presence of brain herniation wasn't associated with a higher risk of poor outcome (p=0.6807).

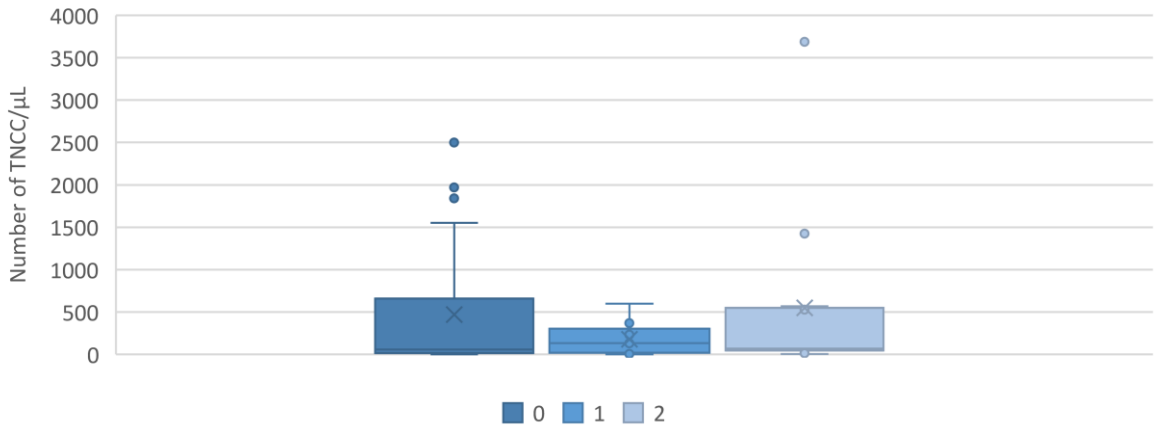
Thirty two dogs had parenchymal and/or meningeal contrast enhancement (62%). Ten had meningeal enhancement (19%), twenty seven had parenchymal enhancement (52%) and five had both (10%). The presence of contrast enhancement in MRI wasn't associated with the outcome (p=0.5707) and the presence of meningeal or parenchymal contrast enhancement wasn't also associated with the outcome (p=0.5252 and p=0.7676, respectively).

	0	1	2	p-value
Contrast enhancement	58,33	53,85	73,33	0.5707
Meningeal	20,83	7,69	26,67	0.5252
Parenchymal	50,00	46,15	60,00	0.7676

Table 2. Relationship (relative frequency) between contrast-enhancement (presence and types) and dogs from groups 0,1 and 2.

3.4. Cerebrospinal fluid (CSF) analysis

In two dogs the CSF was not collected due to suspected raised intracranial pressure. 87% of the dogs from this study had an abnormal CSF (n=45). The CSF results ranged from 0 to 3687 TNCC/µL, with a median of 70 TNCC/µL. The number of cells per µL was compared between groups and a higher number of cells wasn't associated with a higher risk of relapsed or death (p=0.8152).



Graphic 9. Comparison of CSF TNCC/µL between dogs from groups 0, 1 and 2.

Five dogs had a normal CSF at the time of diagnosis (10%): three recovered, one relapsed and one died. Twenty three had a mononuclear pleocytosis (47%); of those, eleven recovered, seven relapsed and five died. Twenty one had a mixed pleocytosis (43%); of those, nine recovered, five relapsed and seven died. The presence and type of pleocytosis was compared between groups and no association was evident for the outcome (p=0.8836).

4. Discussion

Immuno-mediated conditions of the CNS play a major role, comprising over 80% of the total CNS inflammatory disease (Gonçalves et al. 2022). MUO, particularly, is among the most common and the prognosis is often guarded to poor. With the present study we tried to further our knowledge on this disease, since much is still unknown. In veterinary literature, information regarding the predictors of progression and outcome of MUO are still lacking and this could eventually help achieve a more successful treatment. Hence, we tried to investigate the role of several variables on the outcome, while help characterize better this clinicopathological entity, regarding signalment, neurological signs, treatment protocols, MRI and CSF features.

MUO has been reported in several previous studies as a disease that mainly affects middle-aged small-breed dogs, particularly of toy and terrier breeds (Talarico et al. 2010; Cornelis et al. 2016b). However, any breed can be affected and large-breed dogs occasionally develop the disease. In fact, in the present study, the majority of the population is of small and medium-sized breeds (88%), with a median age of 65 months (5 years). A previous study with 111 dogs concluded that 25% of the dogs diagnosed with MUO were large-breed dogs (Cornelis et al. 2016b). Yet, in our study, only 12% of the dogs were large-breed, a finding that could reflect the dimension of our sample compared with the previous or could be the result of a difference in the demographic or geographical distribution of large-breed client-owned dogs in Italy. A female predominance (60%) was seen which is consistent with older reports (Munana 1998), but contrasts with more recent ones (Granger et al. 2010; Talarico et al. 2010; Cornelis et al. 2019). This could be an actual predominance or could reflect the clinical diagnostic bias applied to these cases.

The prevalence in the present report of French Bulldogs, Doberman Pinscher, Yorkshire Terrier, Chihuahua, Pug and Maltese is consistent with previous investigations (Cordy DR and Holliday TA 1989; Cantile et al. 2001; Aresu et al. 2007; Cooper et al. 2014). A genetic defect in DLA-II known to increase the risk of developing MUO was found in toy breeds, like Pugs and Maltese (Schrauwen et al. 2014; Greer et al. 2010). Thus, it is not surprising that these breeds are overrepresented here.

Commonly reported neurological signs are an altered mental status, vestibulocerebellar signs, seizures, visual deficits, tetraparesis and ataxia (Talarico et al. 2010; Coates JR and Jeffery ND 2014; de Lahunta et al. 2015; Dewey CW and da Costa RC 2016; Cornelis et al. 2019). In fact, in the present study, several patients presented with an altered mental status (mainly depression and compulsion), vestibular signs (head tilt, vestibular ataxia, nystagmus and strabismus), ataxia and seizures. Moreover, paresis, proprioceptive deficits and paraspinal hyperesthesia (specially craniocervical) are common in the present report and

when we think that a high percentage of the breeds enrolled in this study are also predisposed to spine problems, this finding takes a major importance. When we come across a middle-aged French Bulldog with an acute onset of paresis, our primary differential diagnosis is intervertebral disk disease (IVDD). Although considered a rare disorder, spinal MUO should always be taken in consideration when signs of myelopathy are present and put at the top of our differential diagnosis list (after IVDD), as the neurological examination is comparable to the latter (Griffin et al. 2008; Cornelis et al. 2017a).

It has been suggested before that the presence of seizures at presentation was associated with shorter survival times (Bateman SW and Parent JM 1999; Coates et al. 2007). However, in the present study, dogs presenting at the clinic with a history of seizures, weren't at higher risk of relapsing or dying. This finding was more according with more recent studies where no association was also found (Lowrie et al. 2013; Kaczmarska et al. 2020). Medical and research advances in the past decade have led to a better understanding of seizures and creation of ways to manage them more successfully. Nowadays, the veterinary hospitals are equipped with technological and pharmacological tools that provide better and quicker assistance to dogs with seizures, decreasing the rates of death and relapses that once existed. This improvement in medical assistance could be the reason why more recent reports are unable to corroborate the association of seizures with a poor outcome.

Similar to two other reports (Smith et al. 2009; Lowrie et al. 2013), all of the deaths related to MUO occurred within 3 months of diagnosis, mainly registered in the first 7 days post-diagnosis (median 6 days). This could reinforce a previous finding that suggested survival to three months as a good prognostic indicator for long-term survival (Lowrie et al. 2013).

Response to therapy and outcome of MUO in dogs are highly variable. In our study, 29% of the dogs died and 25% of the dogs relapsed, much less than in other reports. In 39 dogs, Lowrie et al. (2013), reported death and relapse rates of 56% and 65%, respectively. Brady et al. (2020), in 40 dogs, reported dead and relapse rates of 47,5% and 78%, respectively. This discrepancy between our and their studies could reflect a more aggressive or favourable treatment in our institution. A great number of our patients had a second immunosuppressant adjunct early on after diagnosis and almost all of them had cytarabine introduced. Brady et al. (2020) had a standardized treatment using cyclosporine and corticosteroids, while Lowrie et al. (2013) had a standardized treatment using cytarabine and corticosteroids. It is possible that, comparing cyclosporine and cytarabine, the latter could have a higher efficacy in dogs with MUO, explaining why our rates of poor outcome are so little compared with Brady et al. (2020)'s investigation. On the other hand, Lowrie et al. (2013) conducted a study with a similar protocol to ours. The only difference between our and Lowrie

et al. (2013)'s study is that, since the end of 2020, we delivered to most of our patients an initial dose of cytarabine in CRI (and not SC as the other report). This finding could support a more beneficial regimen with CRI instead of a SC protocol, though further studies are required to prove this claim.

Granger et al. (2010) suggested that 15% of dogs with GME die before being treated and in our population only one dog died spontaneously before being treated (2%). This could reflect the fact that the sample size in our study (52 dogs) is almost nine times smaller than the latter (457 dogs).

Except for this one dog that died before initiation of therapy, all the remaining died doing some type of immunosuppressive therapy. To add, the time of introduction of the corticosteroid therapy after presentation at the hospital, wasn't relevant for the outcome. A dog started immunosuppressive therapy as late as 27 days after the onset of clinical signs and still recovered. He first presented at the Clinic with paraparesis, proprioceptive ataxia, facial nerve paralysis and lumbosacral hyperesthesia. On MRI, he was one of the dogs that presented with the most CNS regions affected, with involvement of the mesencephalon, pons, medulla oblongata, cerebellum and spinal cord. This leads us to think that the response to therapy is uncertain, probably function of each individual, environment or other. Regardless the initiation of appropriate and aggressive immunosuppressive treatment and how many CNS regions are affected, some dogs seem more resistant to the immune-mediated process underneath and respond greatly to therapy, while others respond poorly or don't respond at all. Until further investigations, the cause for this discrepancy remains unknown.

Corticosteroid monotherapy was found to be associated with a higher risk of death. MUO is an immune-mediated disease that needs an aggressive immunosuppressive therapy and, still, many dogs die with the right treatment. In a recent publication, the three-year death rate of 182 dogs doing glucocorticoid monotherapy was 69,64% and if this period was increased to five years, the death rated peaked to 88,7% (Pausova et al. 2021). In our population, 67% of the dogs that did this same protocol (glucocorticoid monotherapy) died within the four-year period of this study. Corticosteroids have been the first-line therapy for the treatment of MUO, though from our report arises the urgent need for introduction of a second-line immunosuppressive drug as soon the diagnosis is accomplished. Despite several studies have been directed towards the search for the best adjunctive therapy to the corticosteroids, the optimal treatment is still controversial and there's no universally accepted protocol to treat MUO. Hence, the second immunosuppressor introduced remains a choice of every clinician, based on personal experience and current literature.

Though we didn't study the effectiveness of either treatment combination, many dogs in this population did as second-line immunosuppression cytarabine [either subcutaneously (SC) or in constant rate infusion (CRI)]. Previous investigations have compared the advantages and disadvantages of both cytarabine protocols (Menaut et al. 2008; Lowrie et al. 2016), but, at the end, the route of administration is not consensual among neurologists. In San Marco Clinic, the past few months, the patients have been treated with immunosuppressive doses of corticosteroids and, depending on the character of either dog or owner, do a first administration of cytarabine in CRI (after which continue with SC administrations). The choice of this protocol was based on the study by Lowrie et al. (2016) that suggested significantly better survival rates for dogs receiving cytarabine in CRI compared to SC administrations. We find this a good protocol until the time of writing of this report.

As evidenced, the treatment of MUO is relatively long, lasting months, years or being even for lifetime. The duration of corticosteroid therapy here had a median of 265 days (almost nine months), but one of the dogs did this same therapy for 1059 days (almost three years). Since relapses are common, the loss of owner's compliance/adherence to the treatment prescribed might have disastrous consequences, leading to failure in managing the disease and potential death. As previously reported, discontinuation of treatment before resolution of the lesions always results in relapse (Lowrie et al. 2013) and there's a natural tendency for owners to cease therapy once visible signs of improvement are seen. Consequently, it is beneficial to educate and maintain a regular contact with the owner to avoid reappearance of clinical signs.

Forebrain lesions (brain hemispheres and thalamus) were predominant here, as reported elsewhere (Cherubini et al. 2006). A normal MRI present in 4% of our cases is also consistent with Granger et al. (2010) that reported that normal MRI are found in 7% of MUO cases. Spinal MUO lesions were in high percentage in the current study (44%), though only five of these cases had lesions restricted to the spinal cord.

Multifocal lesions and brain herniation weren't associated with an increased death or relapse rate, contrary to what was reported before (Munana KR and Luttgen PJ 1998; Lowrie et al. 2013). Transforaminal herniation, which accounts for six of the seven cases of brain herniation recorded here, is characterized by shifting of the cerebellar vermis through the foramen magnum, which could raise the intracranial pressure and culminate in a poorer prognosis (Lowrie et al. 2013).

Post-contrast lesion enhancement wasn't associated with mortality. The presence and degree of enhancement might be reflective of the severity of lymphohistiocytic inflammation and of the age of the lesion (Flegel 2017). Lesions with moderate enhancement may reflect an

active lesion, whilst lesions without enhancement might reflect a more chronic process of the same disease. Therefore, some dogs may not show contrast enhancement at all, which doesn't rule out meningitis or encephalitis, and certainly can't be used to predict a good or poor outcome. Moreover, the contrast enhancement (type and distribution) present can also depend on the MUO subtypes (GME, NME and NLE) which weren't explored in the present study.

Complementary to the MRI studies, CSF is an important tool for diagnosis of MUO. CSF cell count and type of pleocytosis at initial diagnosis didn't seem to affect the survival and relapse rate, accordingly to what was reported before (Lowrie et al. 2013). Compared with MRI, previous reports stated that CSF is more sensitive for detection of inflammatory lesions (Lamb et al. 2005), though in this study five dogs had a normal CSF and just two had a normal MRI. This could reflect different and less sensitive magnetic fields used in the past compared to the increased diffusion of high-field MRI used more recently, that could rise the sensitivity for detection of CNS inflammations. Nonetheless, until this day, the combination of CSF analysis and MRI remains the best to way to achieve a diagnosis of MUO.

Several limitations exist in the current study. Histopathological confirmation for all cases lacked. The definitive diagnosis of MUO is only achieved with histopathology and, therefore, misdiagnosis in the present study is possible. A standard treatment protocol wasn't used in all cases included. With the purpose of comparing corticosteroid monotherapy and a multi-drug therapy protocol, the drugs and dosages should have been standardized. However, as an optimal treatment does not exist, setting up a standardized treatment study with the purpose of comparison between all three groups would be unethical. Cases with suspected MUO but with normal CSF analysis or a normal MRI were included in this study and, though both are a common finding (Lamb et al. 2005; Granger et al. 2010), we can't exclude that misdiagnosis may have happened.

5. Conclusions

The diagnosis Meningoencephalomyelitis of Unknown Origin (MUO) is only achieved with certainty when histopathological examination is performed. Given the highly possible harm of this procedure to the patient's well-being, the only ante-mortem diagnosis we can accomplish is a presumptive diagnosis. This is based on several previous papers that studied MUO's clinicopathological features and, nonetheless, it is still a challenging entity to diagnose and treat.

MUO is more frequently seen in middle-aged small and medium-sized breeds of breeds like Chihuahua, Pug, Maltese and French Bulldogs, though any size and breed may be affected. A female predominance has been established, but this remains controversial.

Frequently the lesions are in the forebrain and/or spinal cord, resulting in an acute, hyperacute or chronic onsets. Neurological signs compatible with MUO include seizures, gait and/or posture abnormalities, proprioceptive deficits, hyperesthesia, cranial nerve deficits and changes in behaviour and/or mental status.

Immunosuppressive treatment with more than one agent is advisable, since most animals receiving only corticosteroids unfortunately die. Sometimes treatment can be withdrawn, but lasting administrations of drugs are frequently needed. For this reason, the compliance of owners is the centre of a successful treatment. Even if the treatment is appropriate and aggressive, many dogs relapse or die. The relapses occur frequently when the immunosuppressant doses are being tapered or are suspended. As result to handle the disease and common relapses, three or more drugs are often needed.

It is possible that the patients enter full or partial remission from the disease and maintain a very good quality of life on the long-term. Survival to the 3-month mark after diagnosis could be used as good prognostic indicator. Seizures and/or hyperesthesia, multifocal lesions, brain herniation, presence of meningeal and parenchymal contrast enhancement, and type and cell count of CSF pleocytosis weren't associated with a higher risk of relapse and death. Overall, prognosis for MUO is highly variable and further investigations are needed regarding probable indicators of relapses and death, in order to improve treatment efficacy and survival rates.

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