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Case Report

Clinical and Pathological Findings of Necrotizing Meningoencephalitis in a Maltese Dog

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Abstract

A 2-year-old, intact female Maltese dog was presented to the veterinarian with a history of acute neurological signs. On neurological examination the dog showed deficit of mental status (apathy and depression), seizures, constant howling, head turn and compulsive circling to the right side and falls to the left side. The treatment protocol using prednisolone (for seizures remission) and cyclosporine (initiated in the chronic stage) did not stop the progression of the disease and euthanasia was elected 65 days later. Necropsy revealed mild cerebral asymmetry, and in the frontal (more affected) parietal and occipital lobes of the right hemisphere there were friable, depressed and yellowish areas characterizing malacia. The left contralateral frontal lobe was edematous and slightly yellowish. At histopathology, the lesions were characterized by marked, multifocal to coalescing necrotizing meningoencephalitis, characterized by focally extensive areas of malacia, especially in the cortex of the frontal and right parietal lobes. Extension of lesions to white matter was observed only in the caudal region of the right frontal lobe. Plasma cells and lymphocytes infiltration was observed around vessels, leptomeninges and in the neuroparenchyma. In addition, the non-cavitation areas were also characterized by neuropil vacuolization, neuronal necrosis, neuronophagia, astroglyosis with various gemistocytes, endothelial hyperplasia and hypertrophy. The immunohistochemical analysis showed predominance of CD3 positive T lymphocytes in proportion to CD79 positive cells. Clinical signs, character and distribution of neurological lesions were compatible with necrotizing meningoencephalitis (NME). This condition, initially reported only in Pugs, currently affects other breeds and attention should be given to the differential diagnosis with other neuropathies in dogs.

Key Words: brain, clinical signs, seizures, malacia, histopathology, immunohistochemistry, dogs.

Introduction

Necrotizing meningoencephalitis (NME) is a central nervous system (CNS) nonsuppurative inflammatory disorder of dogs, with poorly understood etiopathogenesis. Necrotizing leukoencephalitis (NLE) and granulomatous meningoencephalomyelitis (GME) are also CNS idiopathic inflammatory conditions. Nevertheless, each disease has unique histopathological features (18). An autoimmune pathogenesis has been suggested for NME based on the presence of anti-astrocytic and anti-glial fibrillary acid protein (GFAP) autoantibodies in the cerebrospinal fluid (CSF) of affected dogs (14, 21). However, similar antibody levels occur in the CSF of dogs with GME, brain tumors and even in some clinically normal dogs (20, 21). A genomic study in Pugs with NME showed a single strong association with dog leukocyte antigen (DLA) class II, and supports the role of the immune system in the disorder (6). Genetic predisposition has been also confirmed in Pug dogs with NME but it is believed that there are additional influences contributing to the phenotypic expression of the disease (3, 6). A recent study showed that viral pathogens are not common in the brain of dogs with NME. However, *Mycoplasma canis* was found in some cases of NME and

further investigation is warranted to determine the importance of *M. canis* in this condition (2).

NME has been reported in various toy breeds including Pug dogs, Yorkshire terrier, Maltese, Chihuahua (5, 18), Shih Tzu (5), West highland white terrier, Boston terrier, Spitz Japanese and Pinscher (18), Pekingese (8) and French bulldog (13). NME seems to be more common in females (5, 10) and, has been diagnosed in dogs with six months to seven years of age (18). However, the more common age range from two (18) to four years (5).

The clinical signs associated with NME are rapidly progressive and associate with the neuroanatomical localization (18). The most common signs include seizures, depression, circling, (5, 6, 22), visual deficit (5, 6), postural reaction deficits (6) and vestibule-cerebellar signs (5, 16).

Definitive antemortem diagnosis is challenging because histopathology is mandatory. For most cases, the clinical diagnosis is presumptive, associating clinical signs and neuroanatomic localization, CSF analysis and advanced imaging tests to exclude other causes (5, 16, 18). The prognosis of NME cases is poor due to the progressive course of the disease, with lower survival rate in animals with seizures (5). Clinical signs in correlation with the localization of CNS lesions and immunohistochemical findings of a NME case in a Maltese dog are described.

Case report

A 2-year-old, intact female Maltese dog was presented to the veterinarian with a history of acute neurological signs characterized by seizures, constant howling, and circling to the right side. The owner informed that the immunization schedule had been followed. On neurological examination, the dog presented deficit of mental status (apathy and depression), head turn, compulsive circling to the right side and falls to the left side. During ambulation the animal turned away from obstacles, indicating no visual deficit. Abnormal movements and proprioception deficit to the left side were detected. There was also left deficit to menace response and cervical sensibility. A multifocal intracranial lesion involving the telencephalon was suspected. The complete blood count and serum chemistry profiling were unremarkable. Magnetic resonance imaging (MRI) and CSF analysis could not be performed because of the client's refusal. According to breed, age, history and neuroanatomic localization of the lesions, an inflammatory neurological disease of unknown cause was suspected. On the basis of the suspicion, phenobarbital (6mg/kg bid), doxycycline (10mg/kg bid), prednisolone (2mg/kg bid) and A and B vitamins were prescribed. A re-check five days after the onset of therapy was performed and no changes were detected when thorough clinical examination was performed. On neurological examination, reduction of circling and seizures was observed but there was no improvement in posture, proprioception and menace response. Because clinical signs improved gradually, the initial prescription was maintained. Fifty three days after the first presentation the dog was checked again. The owner reported that the initial dosage of prednisolone was maintained but, arbitrarily the client did not maintained the medicine after three weeks, and the seizures episodes started again after discontinuing the corticoid therapy. The veterinarian detected that all clinical signs mentioned above worsened. A new treatment protocol adding cyclosporine (6 mg/kg bid) to prednisolone was prescribed. However, ten days later, the clinical signs worsened dramatically. The dog presented severe changes of the mental status (disorientation, aggression and apathy), increased postural deficit, and bilateral deficit to menace response and cluster seizures. Due to the poor prognosis, the owner elected euthanasia.

At necropsy, there was mild asymmetry between the cerebral hemispheres. In the right hemisphere there were multifocal to coalescing depressed, markedly friable and yellowish approximately 0.5 to 1.0 cm-diameter areas. In the frontal lobe there was a locally extensive 2.0 cm-diameter malacic area. The left contralateral frontal lobe was edematous, slightly yellowish and friable (Figure 1). On the corresponding cut surface of the right frontal lobe, there was partial loss of cortical parenchyma and demarcation between grey and white matter was not evident. The frontal lobes of both hemispheres were markedly affected followed by parietal and occipital lobes. No gross lesions were observed in the temporal lobe, hippocampus, cerebellum and brain stem. Extraneural gross lesions included moderate diffuse pulmonary congestion and edema. In the liver there was moderate and diffuse glycogen degeneration.

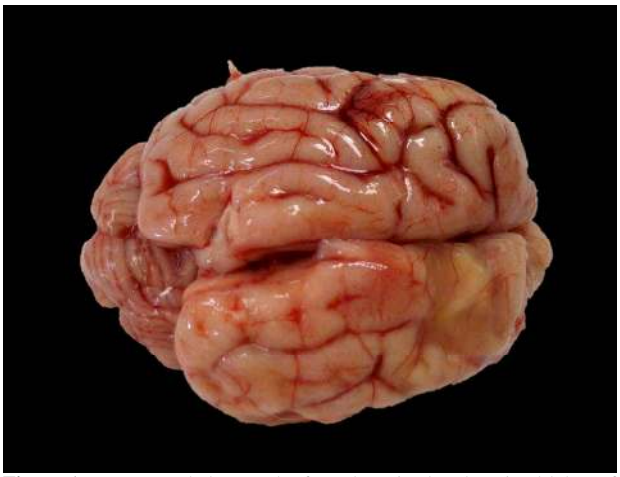

Figure 1. Dog, encephalon. In the frontal, parietal and occiptal lobes of the right cerebral hemisphere, depressed and yellowish areas can be observed characterizing multifocal to coalescing malacia.

The whole brain and samples of multiple organs were fixed in 10% buffered formalin and routinely processed for histopathology. Histologically, there was marked multifocal to coalescing, necrotizing

nonsuppurative meningoencephalitis. The cerebral cortex showed areas with markedly increased cellularity interspersed with multifocal cavitations, partially filled with numerous gitter cells, characterizing malacia (Figure 2). Moderate to marked infiltration of cells, such as plasma cells and lymphocytes were observed around vessels and diffuse in the leptomeninges. In addition, the non-cavitation areas were characterized by neuropil vacuolization, neuronal necrosis, neuronophagia, astroglyosis with various gemistocytes, endothelial hyperplasia and hypertrophy (Figure 3 and 4).

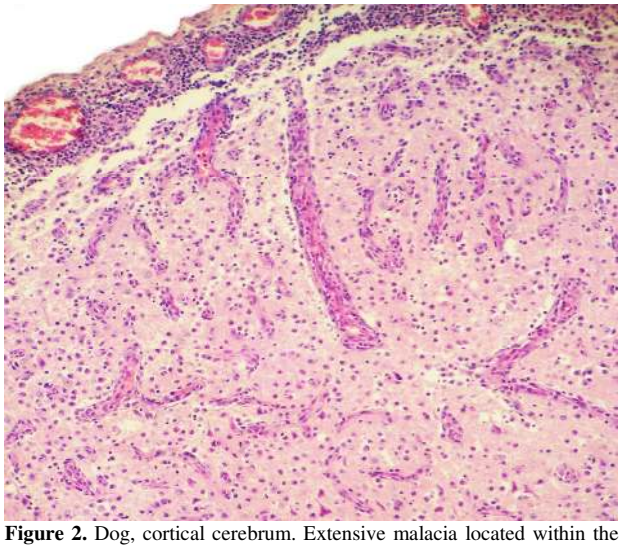

Figure 2. Dog, cortical cerebrum. Extensive malacia located within the grey matter. Nonsuppurative inflammatory infiltration can be observed in the leptomeninges, especially around blood vessels. Hematoxylin and Eosin, 10x.

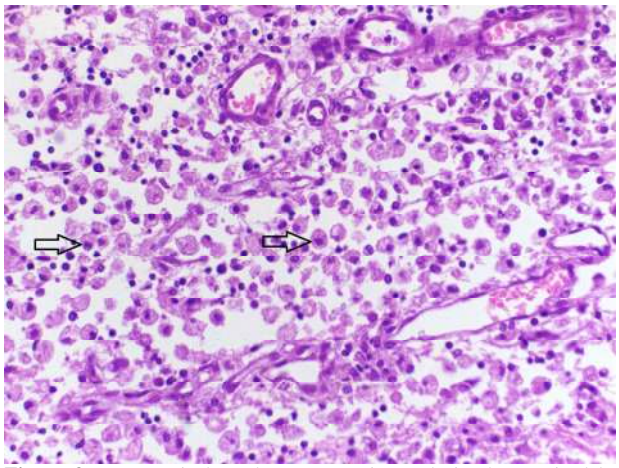

Figure 3. Dog, cortical cerebrum. Extensive malacia characterized by loss of parenchyma and replacement by numerous gitter cells (arrows) and few lymphocytes. Hematoxylin and Eosin, 400x.

Single or binucleate gemistocytes were more commonly seen adjacent to necrotic areas. These areas were more intense in the frontal and parietal lobes of the right hemisphere. In the white matter subjacent to the necrotic right frontal cortex there were plasma cells and lymphocytes perivascular cuffs associated with mild vacuolization and axonal degeneration. No lesions were found in the hippocampus, diencephalon (thalamus and hypothalamus), mesencephalon, cerebellum and medulla oblongata. Paraffin embedded cerebral sections were selected and immunohistochemistry for CD3 (lymphocytes T marker) and CD 79a (lymphocytes B marker) was performed as formerly described (1). The immunohistochemical analysis showed that positive CD3 cells were predominant in the perivascular cuffs, leptomeninges and also in the neuroparenchyma (Figure 5 and 6). Less numbers of positive CD79a cells were observed in the perivascular cuffs and leptomeninges but they were rarely observed in the neuroparenchyma.

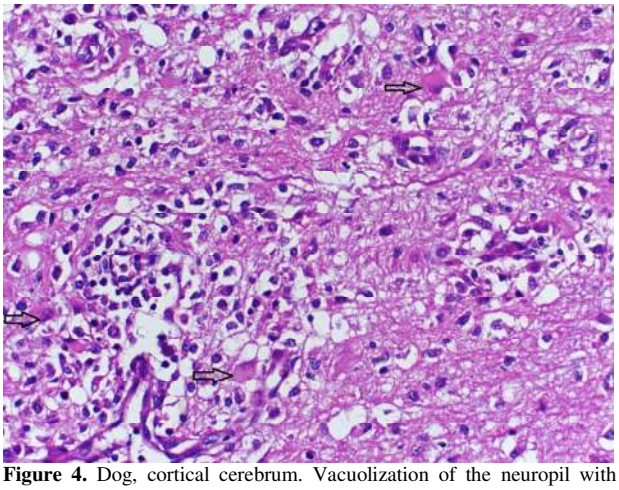

Figure 4. Dog, cortical cerebrum. Vacuolization of the neuropil with proliferation of glial cells including gemistocytes (arrows) and perivascular lymphocytes. Hematoxylin and Eosin, 400x.

Gross and clinical findings were consistent with a multifocal to coalescing malacia involving the cerebral cortex. The histopathology allowed the definitive diagnosis of NME characterized by multifocal to coalescing necrotizing nonsuppurative meningoencephalitis, affecting the grey matter of frontal, parietal and occipital lobes, predominating in the right cerebral hemisphere.

NME is an inflammatory and necrotizing disease with great tropism for cerebral hemispheres. The lesions occur preferentially in the telencephalon, especially in the grey matter (occasionally can be observed in the subcortical white matter) and leptomeninges with multifocal distribution (5, 13, 16, 22).

The dog of the present study showed clinical signs rapidly progressive and associated with the neuroanatomic localization of the lesions as observed in other studies (18). The lesions and clinical signs of most cases of NME are found exclusively in the cerebrum, distinctive characteristic for this condition (8, 13). Detailed neurological examination allows to determining the sites of the lesions, which are extremely important for presumptive diagnosis and treatment. In addition to neurological examination for supporting the clinical

suspicion, are fundamental the epidemiological data, CSF analysis, cross-sectional imaging via computed tomography (CT) scan or magnetic resonance imaging (MRI) of the CNS and infectious diseases testing. Ct-guided brain biopsy and histopathological evaluation of brain tissue may be considered in cases of suspected NME (5, 18).

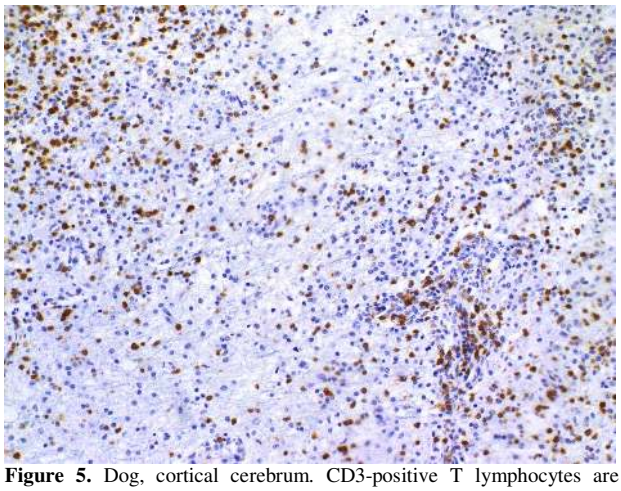

Figure 5. Dog, cortical cerebrum. CD3-positive T lymphocytes are observed around blood vessels and within the cortical neuroparenchyma. Chromogen DAB. Harris hematoxylin counterstain, 200x.

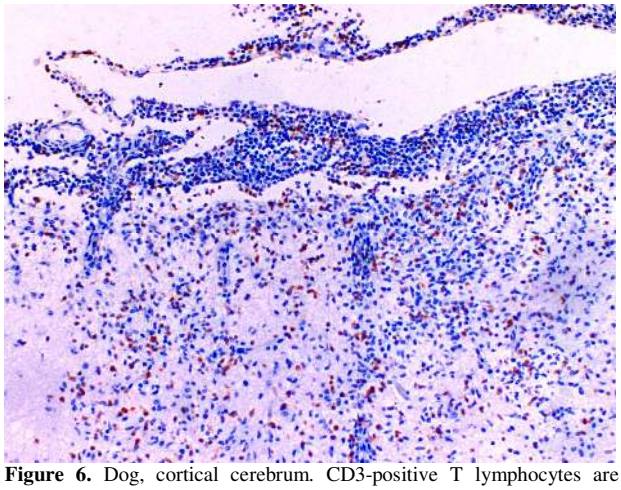

Figure 6. Dog, cortical cerebrum. CD3-positive T lymphocytes are observed in the leptomeninges and in the cortical neuroparenchyma. Chromogen DAB. Harris hematoxylin counterstain, 200x.

Signs of depression, disorientation, aggression, circling, menace response and frequent seizures were the main neurological signs in the dog of this report. The clinical signs observed are related to lesions in the cerebral cortex and/or thalamus (12). The mental depression could be also associated with lesions in the brain stem, particularly in the mesencephalon (11, 12). Seizures and circling could be also related to lesions in the hypothalamus (12). Nevertheless, no lesions were seen in the mesencephalon and hypothalamus of this dog, and no explanations for those signs are found at this moment Initially, the clinical signs in this dog (proprioceptive deficit of the left thoracic and pelvic limbs) suggested that the lesion was worse in the right cerebral hemisphere. Unilateral or asymmetric lesions that affect the frontal lobe of the cerebrum can determine walking in circles, generally to the same side as the lesion and head turn (11). Most clinical signs noted in this dog could be related to the gray cerebral matter, especially of the frontal lobe. Lesions were not found in the cerebellum and brain stem; the eyes, inner ear, and the vestibular nerve were not examined. The vestibular system is constituted of neural cells in the inner ear and their nuclei are in the brain stem (11). Lesions in this system can cause nistagmus, ataxia and proprioceptive deficits (12). Thus, in this dog, proprioception deficit could not be definitely related to the vestibular system. Most cases of NME in Pug dogs showed lesions in the cerebrum only. Nevertheless, a high proportion of affected dogs showed lesions also in the brain stem and in the cerebellum (10).

The treatment protocol initiated after presumptive diagnosis of meningoencephalitis improved the neurological clinical picture (remission of seizures), however, some signs persisted and discontinuous prednisolone protocol allowed the return of seizures and all other clinical signs worsened. The resumption of therapy (prednisolone and cyclosporine) did not avoid the clinical deterioration. Clinical signs remission and long-term survival after prednisolone and cyclosporine therapy was reported in a Pekingese dog (8). In the present report, the discontinued prednisolone therapy by the client and the later administration of cyclosporine, an immunosuppressive agent directly suppressing T lymphocyte activation and proliferation (4), possibly determined the failure of the treatment.

The differential diagnosis of NME using histopathology must include other CNS disorders: NLE, GME, canine distemper, as well as tumors (13). NLE is characterized by inflammatory and necrotic lesions similar to NME, however, the lesions are predominately observed in the white matter. Inflammatory cells infiltration is prominent in the ependyma and mild in the choroid plexus. GME is another idiopathic canine disorder affecting mainly the cerebellum and brainstem. The disease is characterized by nodular granulomatous lesions containing macrophages and epithelioid cells, mainly in subcortical regions. In addition, there are perivascular cuffs constituted of lymphocytes, plasma cells, macrophages, and some neutrophils (13, 18). Neurological cases of canine distemper are characterized by demyelination of axons in the white matter of the cerebellum and brainstem; most cases are associated with nonsuppurative encephalitis, malacia and in some cases, inclusion bodies in glial cells. Occasional cases are characterized by laminar cortical necrosis of the telencephalon (15). Co-infection of canine distemper and toxoplasmosis may occur, determining malacia associated with parasitic cysts (9,15). The dog of this report did not present any changes for diseases included in the

differential diagnosis and also had been appropriately vaccinated.

The immunohistochemical study showed predominance of T lymphocytes in the leptomeninges, around vessels and in the neuroparenchyma similar to the observed in other studies in dogs with NME (7, 13). For viral encephalites like canine distemper, T lymphocytes are found diffusely around blood vessels and predominately in the white matter (19). For protozoans and bacterial diseases, B lymphocytes are found predominately around blood vessels (17). A study using double-labeling immunofluorescence antibody demonstrated predominance of CD3+ T lymphocytes in close proximity or attached to astrocytes, and the cytoplasm and astrocytic processes were positive for IgG in the NME and NLE lesions (13). The involvement of the autoantibody to astrocytes in the NME cases supports the immune-mediated pathogenesis hypothesis however, does not confirm if anti-astrocytic and anti-GFAP antibodies are a primary cause or a secondary consequence of NME (13, 21).

In conclusion, this study shows that neurological examination related to neuroanatomical localization can be useful for clinical presumptive diagnosis of NME. This condition, initially reported as a Pug disease, currently affects other breeds, like the Maltese from this report and, attention should be given to the differential diagnosis with other neuropathies in dogs.

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