MENINGEAL THICKNESS IN A FRENCH BULLDOG

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* Presenter

SIGNALMENT:

Lola, 10 years old, female neutered French Bulldog.

HISTORY

LOLA was presented to the Small Animal Veterinary Teaching Hospital *(Hospital Clinic Veterinari)* of the *Universitat Autònoma de Barcelona* (UAB), Spain, on April 9th 2019 for a sudden onset of clusters seizures. The owners described that seizure duration was less than one minute characterized by tonic-clonic movements of all limbs with total impairment of consciousness. Autonomic signs such as sialorrhea and behavioral changes were noted before the episode.

CLINICAL FINDINGS

On physical examination, Lola was panting, had congestive mucous membranes and a body temperature of 39ºC. The neurologic examination revealed circling to the left, delayed postural reactions in all limbs and absent menace response bilaterally. The neurological examination was consistent with a prosencephalic lesion. Main differential diagnoses included neoplasia, vascular and inflammatory/infectious diseases.

DIAGNOSTIC TESTS

Lola was hospitalized in the intensive care unit (ICU) for emergency treatment and monitoring of seizures, as well as, a complete diagnostic workup. An intravenous (IV) catheter was placed to administer IV fluid therapy with Ringer's Lactate (2 mL/kg/hour) and a single IV dose of diazepam (1mg/kg). Phenobarbital therapy was also started at 4mg/kg/IV twice a day in order to control seizures.
A complete blood cell count with a blood smear and biochemistry profile were performed and the results are listed in tables 1 and 2, respectively. The biochemistry results were within normal limits.

Thoracic radiographs, abdominal ultrasound and magnetic resonance imaging of the brain were performed. Thoracic radiographs did not show any significant pulmonary changes and abdominal ultrasound was unremarkable.
Magnetic resonance of the brain revealed diffuse thickening of the meninges of the left hemisphere, with extension to the *falx cerebri*. The lesion was hypointense on T2w and FLAIR (Figure 1), isointense on T1w, and showed marked diffuse and homogeneous contrast enhancement (Figure 2). Severe mass effect causing midline shift to the right and distortion of the left lateral ventricle were also observed.

There was also an intramedullary lesion over C2 that was poorly marginated and located in the central area of the spinal cord occupying 40% of its width. This lesion was hyperintense on T2w and hypointense on T1w, and did not enhance after contrast administration.

A neoplasm (such as lymphoma, granular cell tumor, histiocytic sarcoma, less likely meningioma) or idiopathic hypertrophic pachymeningitis were the main differentials for the lesion observed in the brain MR images. The presence of an intramedullary lesion over C2 was consistent with syringohydromyelia probably secondary to the brain lesion.

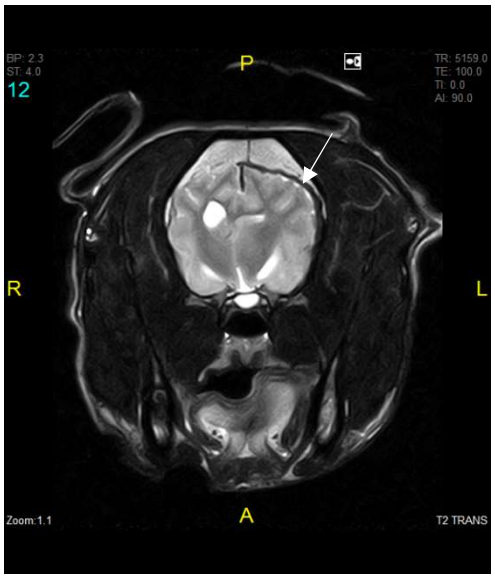

Figure 1. Transverse T2-W. Diffuse extra-axial lesion in the left cerebral hemisphere. Note the hypointense signal of the lesion and the moderate mass effect (arrow).

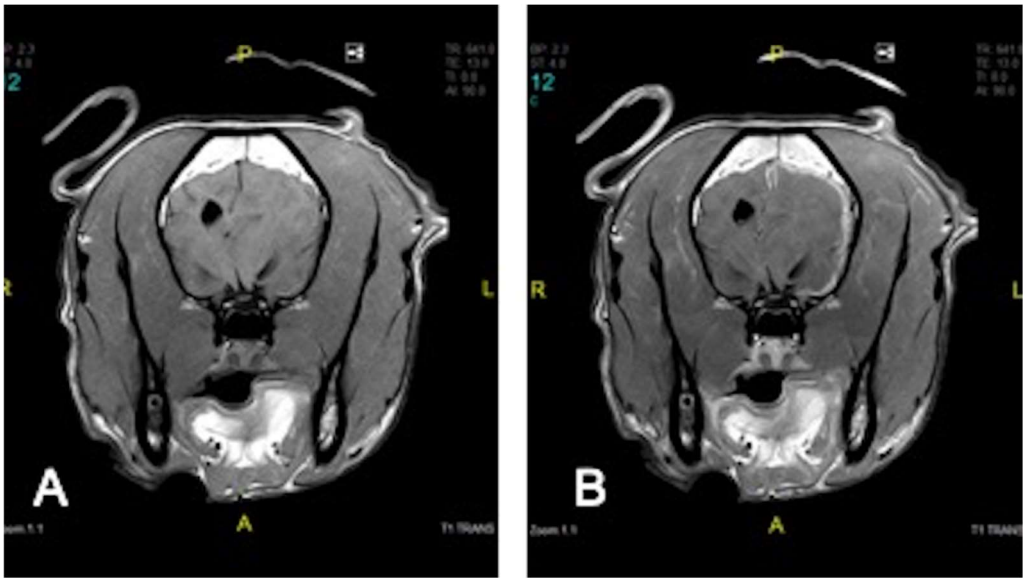

Figure 2. (A) Transverse T1-w and (B) T1-w after contrast administration images. The lesion is isointense to the brain parenchyma and shows marked and homogeneous contrast enhancement.

Cerebrospinal Fluid (CSF) analysis showed albumin-cytological dissociation (Table 3). A craniotomy plus durectomy were performed to biopsy the lesion and to relieve mass effect. The brain meninges appeared thickened and had a highly irregular surface. A piece of the affected meninges was biopsied and submitted for histopathologic examination. Before immersing the sample in formalin, multiple cytological imprints were performed and analyzed.

Table 1. Hematological results

Parameters (units)	Result	Reference interval
RBCs ($x10^6/\mu L$)	6.46	5.5-8-5
Hematocrit (%)	43	37-55
Hemoglobin (g/dL)	16	12-18
MCV (fl)	72.3	62-77
MCHC(g/dL)	36.1	33-37
Reticulocytes ($x10^3$cells/μL)	34.238	0-60.000
WBC ($x10^3$cells/μL)	8.190	6.000-17.000
Neutrophils (cells/μL)	5.897	3.000-11.500
Band cells (cells/μL)	0	0-300
Lymphocytes (cells/μL)	1.638	1.000-4.800
Monocytes (cells/μL)	328	150-1.350
Eosinophils (cells/μL)	328	100-1.500
Basophils (cells/μL)	0	0-200
Platelets ($x10^3$cells/μL)	80	200-500

RBC: red blood cells, **MCV**: median corpuscular volume, **MCHC**: Mean Corpuscular hemoglobin concentration and **WBC**: white blood cells.

∗ **Advia 120.**

Table 2. Biochemistry results

Parameters (units)	Result	Reference interval
Creatinine (mg/dL)	1.02	0.5-1.5
Urea (mg/dL)	34.6	21.4-59.9
Total cholesterol (mg/dL)	244.3	135-270
Glucose (mg/dL)	93.7	65-118
Total protein (g/dL)	6.61	5.6-7.5
ALKP (UI/L)	23.64	20-156
ALT (GPT) (UI/L)	32.5	21-102
Calcium (mg/dL)	10	9-11.3
Potassium (mmol/L)	4.16	4.37-5.35
Phosphorus (mg/dL)	3.96	2.6-6.2

ALKP: Alkaline Phosphatase and **ALT**: Alanine Aminotransferase.
∗Beckman Coulter AU480

Table 3. Cerebrospinal fluid results

Parameters (units)	Result	Reference interval
Cellularity	2/µL	<5 /µL
WBC∗	2/µL	<5/µL
RBC∗	1/µL	0/µL
Protein concentration	49.5 mg/dL	<25-30 mg/dL

WBC: White blood cell; **RBC:** Red blood cell
∗ Sysmex XN 1500
The reference values are extracted from: Rose E. Raskin and Denny J. Meyer. (2010). Canine and Feline cytology. A color atlas and interpretation guide. Second edition. Saunders Elsevier. St Louis Missouri; 325-347.

CYTOLOGICAL DESCRIPTION

The pachymeninx imprints showed adequate nucleated and good cell morphology. The background was clear, slightly pinkish and granular, with a moderate number of erythrocytes, occasional platelets and platelet clumps, and a moderate number of naked nuclei and lysed cells. A population of round to polygonal-large cells that exfoliated individually were seen. In some areas, these cells were in non-cohesive groups that occasionally surrounded vessels and a small amount of pink, amorphous extracellular matrix (Figure 3). Cells presented a round nuclei located paracentrally, with reticular chromatin and occasionally one or multiple evident nucleoli. The nucleus to cytoplasm ratio was low. There was abundant cytoplasm that contained multiple pink granules with different shape and size. Some of the cells also contained a variable amount of clear round vacuoles. Moderate anisocytosis and anisokaryosis were noted. Binucleated cells and multinucleated cells were occasionally encountered (Figures 4 and 5). A moderate number of plasma cells and small lymphocytes, occasional macrophages and rare non-degenerate neutrophils was also detected. Some of the macrophages had a small amount of blue granules that were suggestive of hemosiderin. Scant mesenchymal meningeal cells arranged in loss aggregates were also seen. These cells had oval nuclei with stippled chromatin. The nucleus to cytoplasm ratio was moderate. The cells had a moderate amount of clear blue cytoplasm with poorly defined margins. Mild anisocytosis and anisokaryosis were noted in the cells. No microorganisms were seen.

CYTOLOGICAL INTERPRETATION

- Highly suggestive of Granular Cell Tumor
- Moderate lymphoplasmocytic inflammation with a macrophagic component
- Evidence of previous hemorrhage

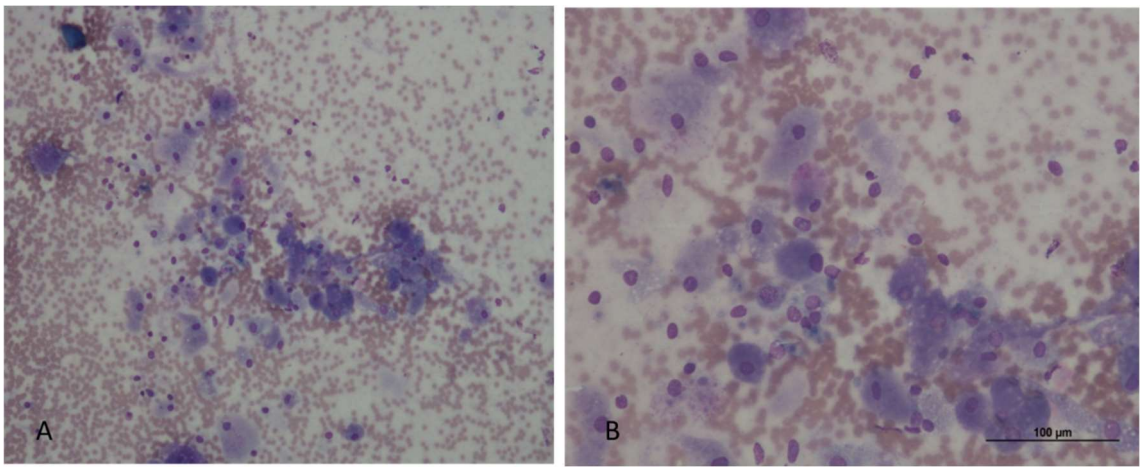

Figure 3. (A) Imprints from the meningeal lesion, 100x (May-Grünwald-Giemsa stain). At low magnification, the disorganized groups of large, round to polygonal cells are frequently seen. (B) Imprints from the meningeal lesion, 200x (May-Grünwald-Giemsa Stain). Note the large size of cells.

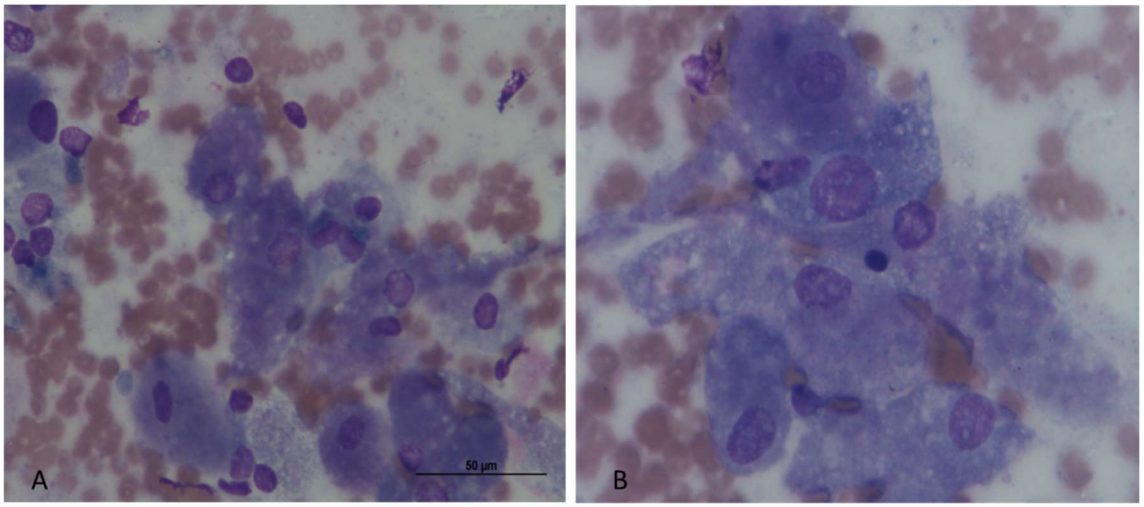

Figure 4. (A) Imprints from the meningeal lesion, 400x (May-Grünwald-Giemsa Stain). Cells have a low nuclear to cytoplasm ratio. The cytoplasm contains multiple pink, different sized granules and a variable number of clear, round vacuoles. Note the mild anisocytosis and anisokaryosis. (B) Imprint from the meningeal lesion, 600x (May-Grünwald-Giemsa Stain). Note the oval nuclei with stippled chromatin and sporadically evident nucleoli.

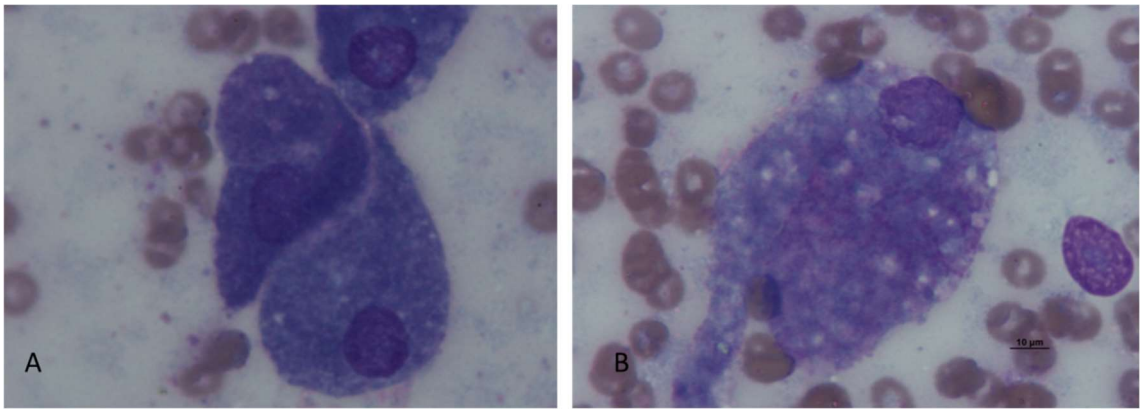

Figure 5. (A and B) Imprints from the meningeal lesion, 1000x (May-Grünwald-Giemsa Stain). Note intracytoplasmatic vacuoles and pink granulation.

HISTOLOGICAL DESCRIPTION

Macroscopically, dura mater was thick, firm and had a rough and irregular surface. The inner face of dura mater presented a whitish material associated with bloody areas. Adjacent cranial bone fragment was apparently normal.

Microscopically, the pachymeninx appeared very thickened due to the accumulation of dense fascicles of collagen fibers, with multiple basophilic areas showing features of chondroid metaplasia. The deepest part of pachymeninx, was disorganized, infiltrated and destroyed by the presence of high number of rounded, large cells (Figure 6). Round and eccentric nuclei and abundant cytoplasm containing multiple small and pale granules were noted. No mitoses were observed. The cells were arranged individually or in small groups infiltrating the meninx (Figure 7), but without reaching the bone.

Immunohistochemistry revealed neoplastic cells with strong reaction against S100 protein (Figure 8) and ubiquitin (Figure 9) markers. Few cells showed mild cytoplasmic reaction against vimentin (Figure 10). Cells did not react against CD18- histiocytic marker (Figure 11).

HISTOLOGICAL AND IMMUNOHISTOCHEMISTRY INTERPRETATION

- Granular cell tumor affecting the meninges
- Chondroid hyperplasia and metaplasia of the dura mater.

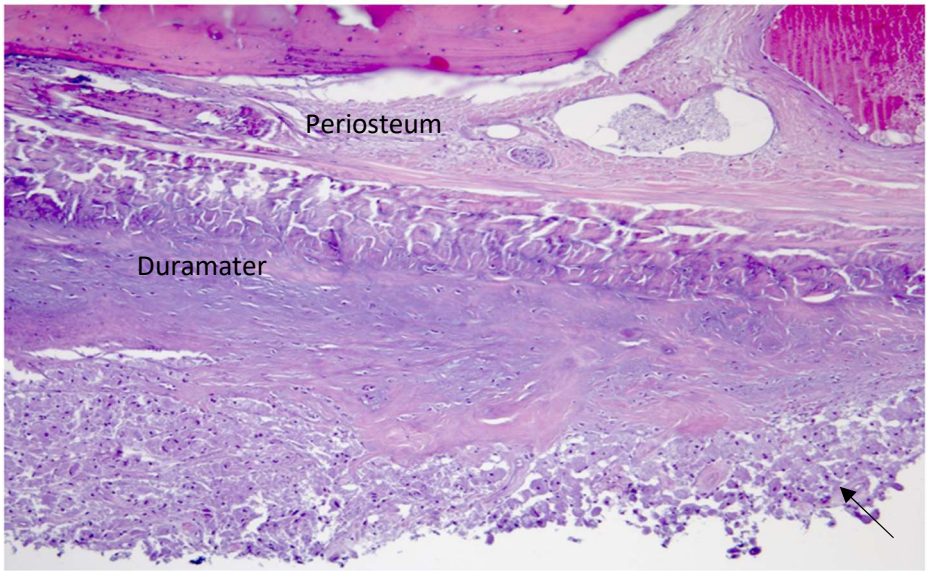

Figure 6. Hematoxylin eosin stain. The granular cell population (arrow) is growing and infiltrating the inner part of the dura mater.

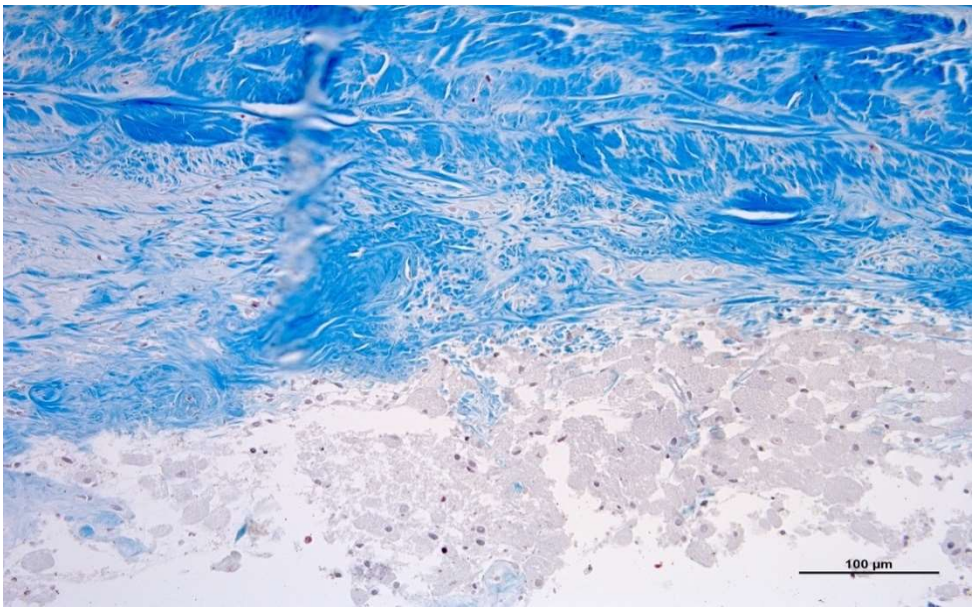

Figure 7. Masson trichrome stain. The collagen fibers and the bone stain positive while the granular cell population shows no cytoplasmic staining. Note how the neoplastic cells are invading and fragmenting collagen fibers of the dura mater.

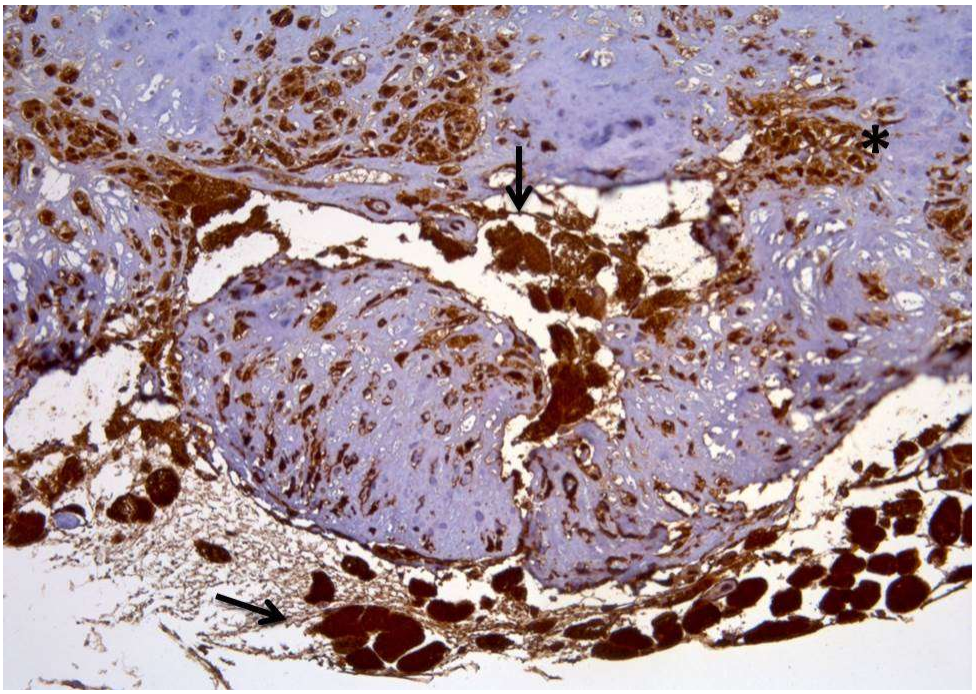

Figure 8. S-100 Immunohistochemistry. Granular cells are strongly immunostained by S-100 protein antibody (arrow). Chondrocytes (*) in the metaplastic areas of dura mater also are immunopositives but show lower intensity.

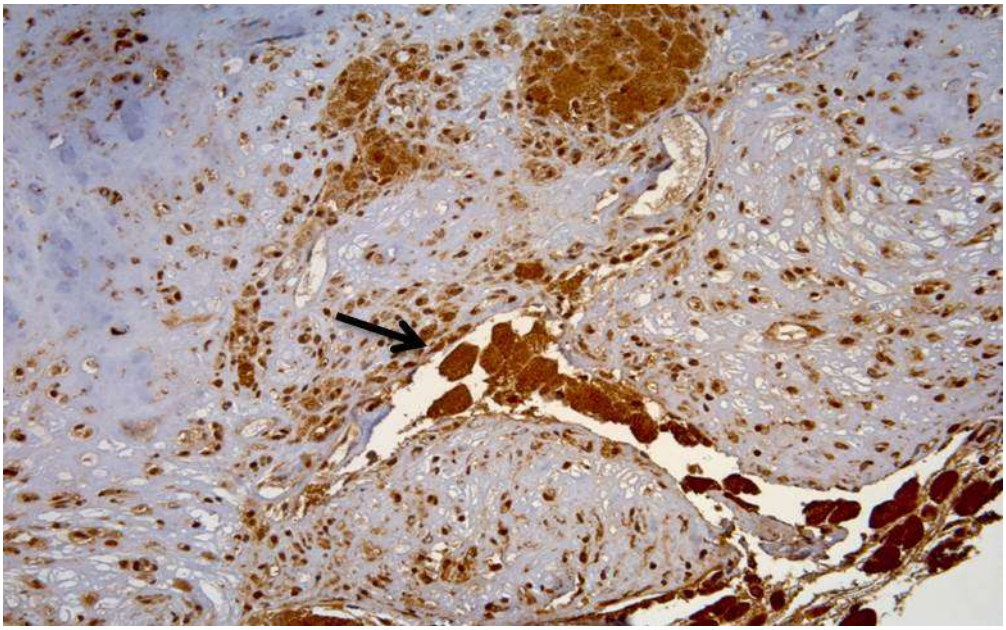

Figure 9. Ubiquitin immunohistochemistry. Infiltrating granular cells show strongly immunopositive against Ubiquitin antibody. Neoplastic cells grouped inside the dura mater (arrows) are also immunopositives.

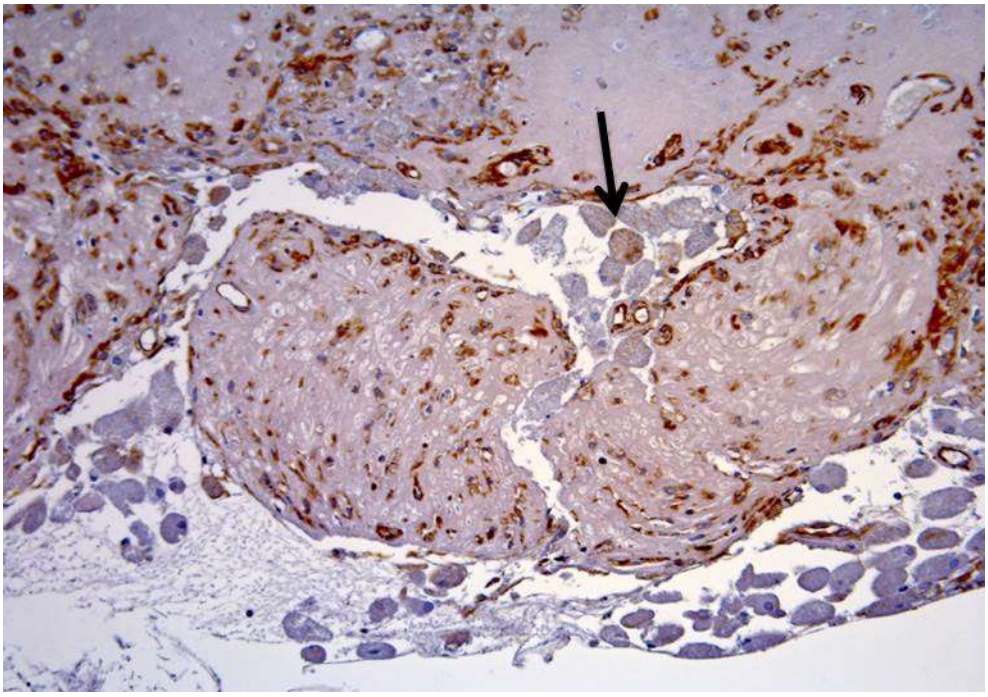

Figure 10. Vimentin immunohistochemistry. Chondrocytes and fibroblasts stain positive against vimentin while only a low number of neoplastic cells show a mild cytoplasmic immunopositivity (arrow).

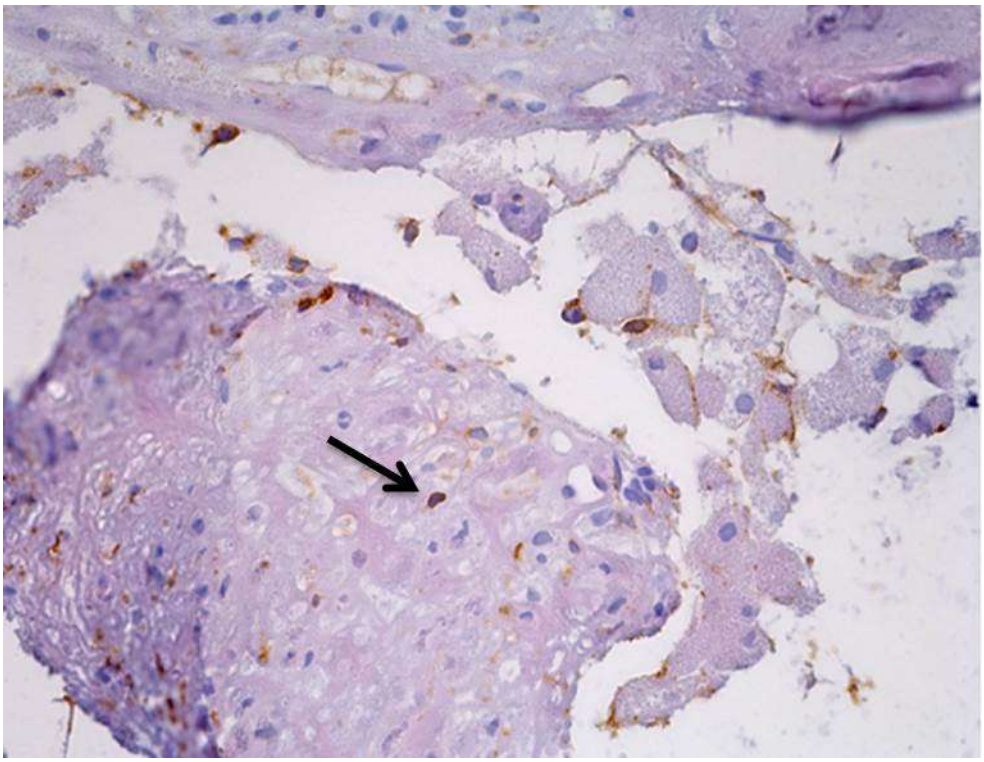

Figure 11. CD18 Immunohistochemistry. No immunopositivity was observed in the granular cell population against this histiocytic marker. Some histiocytes admixed with the neoplastic population where detected (arrow).

FOLLOW-UP

During hospitalization and after surgery Lola did not had any other seizure. Initially, the dog was depressed and slightly ataxic but had a favorable clinical progression and she was discharged four days later with an initial treatment of cephalexin, omeprazole, phenobarbital and prednisone. Ten days after the craniotomy the dog was completely recovered, had a normal neurological exam and the owners reported that she had not had suffered more seizures. The antibiotic treatment was stopped, the corticosteroid dose was reduced and a treatment with a tyrosine kinase inhibitor (TKI) Toceranib, was initiated *(Palladia®)*. One-month post craniotomy the dog was still stable.

DISCUSSION

Granular Cell Tumors (GCT) are considered rare neoplasm both in human beings and in domestic animals (1). GCT involving the central nervous system in dogs are even less frequent and they have been described in the brain and in the spinal cord (3).
This tumor type is the most common central nervous system (CNS) neoplasm reported in rats and has been rarely described in human, dogs, cats and ferrets (4). In humans, ultrastructural studies suggest that the granular cells arise from transformed astrocytes and these tumors appear to be a mixture of astrocytes and granular cells (5,8). In veterinary medicine, the histogenesis of this tumor remains uncertain and a multiple embryologic origin of the cells is suspected (1,3,4). Morphological and ultrastructural studies performed in rats support a meningeal origin and consider these neoplasms a variant of meningeal meningioma (1,5).

Granular cell tumor is a descriptive term for an histogenetically heterogeneous group of tumors (8). The granularity that characterize these neoplasms is thought to be the result of lysosomal accumulation, reflecting metabolic derangements of the cell of origin (2,6). On cytology and histopathology, they are characterized by the presence of large, rounded cells with an eccentric nuclei and multiple eosinophilic granules. In the previous reported cases in dogs with meningiomas that have a granular cell component, the majority of the large polygonal cells contained variable amounts of cytoplasmic PAS-positive granules. Immunohistochemically the cells stain against ubiquitin, and they are partially positive for S-100, α-1-antichymotrypsin, α-1-antitrypsin and vimentin (2,4,6, 9). It is important to note that although these cells can have a macrophage/histiocytic appearance they do not express leukocyte markers (8).
Cytological and histological characteristics of this case were consistent with GCT cells. To confirm diagnosis, an immunohistochemical including S-100, ubiquitin, vimentin and CD18 panel was performed. Cells had a strong immunopositivity for ubiquitin and S100 protein partial positivity to S100 protein; a mild immunoreaction was observed in some cells against vimentin and all were negative for CD18 and for cytokeratin. A PAS stain was also performed and neoplastic cells resulted positive as previously described (2).

Although there is not a unique distinctive characteristic to identify these tumors on MRI, there are several patterns shared. Normally GCT are hyperintense on T1w, iso- to

hyperintense on T2w, have a plaque like distribution and have a moderate to severe degree of peritumoral edema and mass effect (1). In the case presented the lesion did not show the most typical pattern because it was isointense on Tw images. However, it was hypointense on T2w and FLAIR images and caused a severe mass effect. Regarding therapy, the dog was started with TKI. TKI have been shown *in vitro* efficacy against astrocytoma cells (10).

In conclusion, this report describes a GCT affecting the pachymeninx of the left forebrain in a French bulldog. GCT is a rare neoplasia in dogs, CSF analysis do not show the typical granular cells and a cytological imprint of the tumor or a histopathological study of the mass are needed for a definitive diagnosis.
To the author's knowledge, this is the first report evaluating the response and outcome of this neoplasia in a dog.

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