32401
C.L Davis
Foundation

WEDNESDAY SLIDE CONFERENCE 2014-2015
CASE SUBMISSION FORM

File Code: _____(JPC use)

Identification:

a. Surgical biopsy No Necropsy Yes Tissues from multiple animals? No

b. Label on Histoslides: 14L-0353 AFIP

c. Label on Gross/EM Photographs:

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Signalment: (please give: age, gender, breed, scientific name, and species):
Age: 3 years 6 month old
Gender: Male
Breed: Great Dane
Scientific name: *Canis lupus familiaris*
Species: Canine

History: This animal presented with a 3 week history of altered mentation, inappetence, and weight loss along with head pressing and vacant episodes. Also, this case had a 1 week history of bilaterally symmetrical repetitive myoclonus in the flexor muscles of the thoracic limbs when at rest, approximately one every 1-2 seconds, which was abolished by standing, walking and sleep. Cranial nerve examination was unremarkable apart from incomplete pupillary light reflex. The lesion was localised to the forebrain (especially thalamus/basal nuclei). MRI was performed and revealed a large extra axial

mass ventral to and effacing / compressing the thalamus and dorsal to the pituitary gland. Differential diagnoses were the following: germ cell tumor, meningioma, pituitary adenocarcinoma.

Gross Pathology: In the brain there was a poorly demarcated, irregularly shaped approximately 1cm by 1cm by 1cm mass which was similar colour and texture to the adjacent brain parenchyma resulting in indistinct margins which somewhat impaired accurate assessment of the full size and shape of the mass (see MRI findings below). The mass expanded ventrally to the right side of the thalamus causing the third ventricle to deviate laterally.
Apart from mild pulmonary oedema and mild enlargement of the liver and spleen, no other abnormalities were detected grossly at the necropsy.

Laboratory Results (clinical pathology, microbiology, PCR, ELISA, etc.):
Haematology: no significant findings;
Serum biochemistry: mild increases in urea, creatinine and cholesterol, otherwise unremarkable;
CK: mild elevation, unlikely to be of significance;
T4: below detection limit of the assay (hypothyroidism or sick euthyroid syndrome);
TSH: normal;
Basal cortisol: subnormal, in keeping with recent steroid administration, but measurable, so this in combination with the lack of response to steroids made hypoadrenocorticism extremely unlikely;
CT of chest and abdomen: was performed prior to intracranial imaging to evaluate for thoracic or abdominal neoplasia, the only significant findings were evidence of cervical spondylomyelopathy (Wobbler) with moderate spinal cord compression and thyroid glands were mildly hypoattenuating with subjectively normal size (this may be a normal variant or represent hypothyroidism).
MRI: revealed a well-defined, large (1.7 x 2.3 x 2.2cm) extra-axial mass at the level of the sella turcica extending symmetrically dorsally and dorso-laterally having the shape of a tree. The mass is homogeneous and mildly T2w hyperintense to the white matter. It is T1w isointense to the brain parenchyma and mildly hyperintense in the FLAIR sequences. It is moderately enhancing after contrast administration, with mildly more enhancement peripherally. In the dorsal aspect of the mass there is a poorly defined, small, wedge-shaped T2w heterogeneous hypointesity which is hyperintense in T1sequences and heterogeneously isointense to the mass in FLAIR images. There is a mildly T2w hyperintense area surrounding the mass, which is hyperintense on the FLAIR images. The lateral ventricles are moderately enlarged and the third ventricle and the thalamus are moderately displaced and compressed dorsally due to the mass effect created by the mass. The brainstem and the cerebellum is compressed caudally, the cerebellum has a mildly straightened cranial surface. Radiological diagnosis: large extra-axial supracellar mass with mild peri-lesional oedema (Ddx: pituitary carcinoma, germ cell tumour, meningioma); moderate ventriculomegaly.

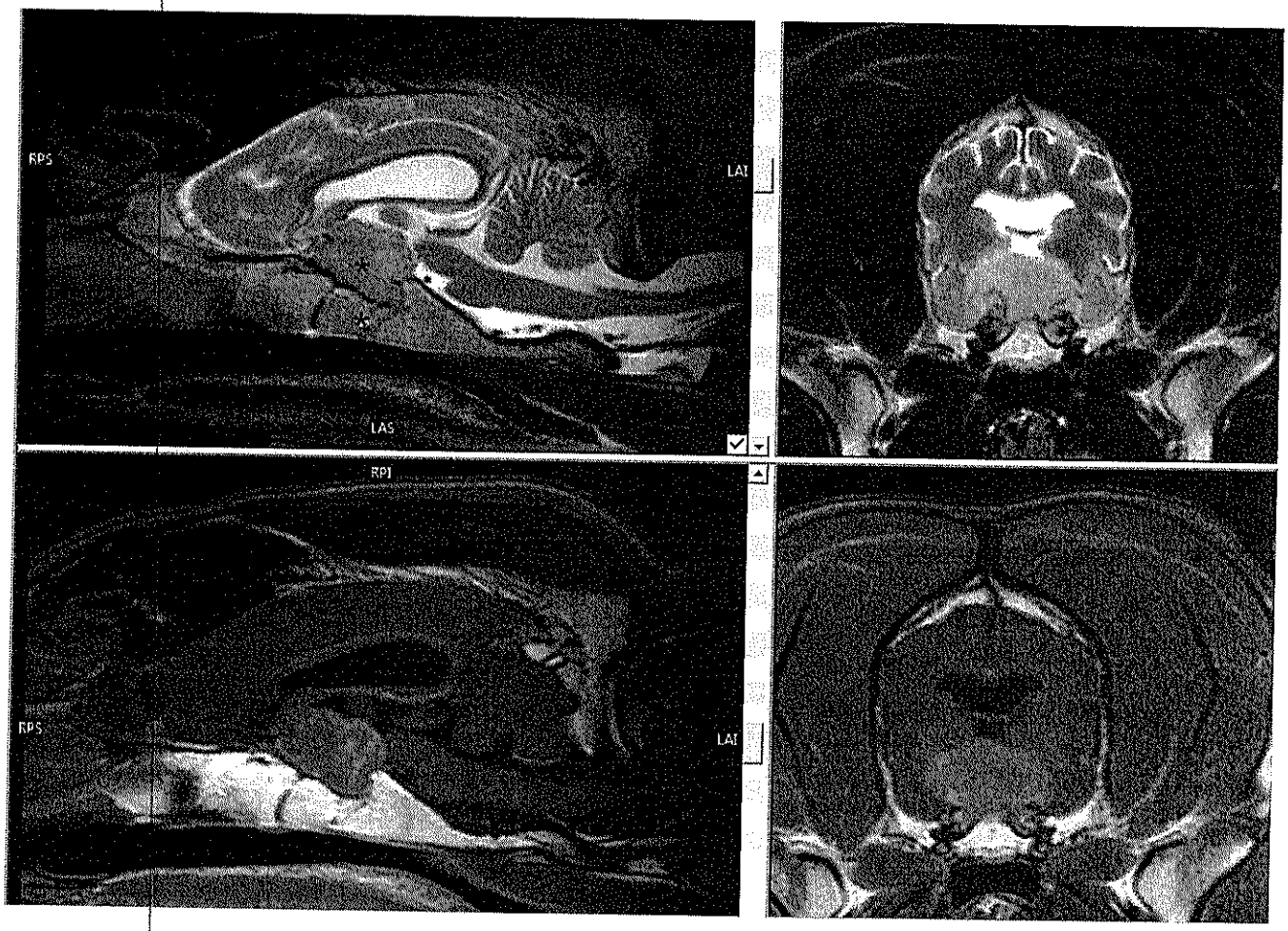

Figure: T2W (top) and T1-post contrast (bottom) MRI images of sagittal (left) and transverse (right) planes through the supracellar mass (*).

Immunohistochemistry was performed with the following results:

- GFAP: neoplastic cells were negative;
- Vimentin: The areas of the neoplasm exhibiting spindloid morphology exhibit strong positive staining;
- CD18: Neoplastic cells are negative;
- Pancytokeratin: The areas of the neoplasm exhibiting epithelioid morphology exhibit strong positive staining. The main population of large round cells often exhibit small aggregates of positively staining cytoplasmic material;
- C-Kit: Neoplastic cells are diffusely positive.

Histopathologic Description:

Located midline, within and effacing the thalamus, and also infiltrating in to the surrounding brain parenchyma there is a well demarcated non-encapsulated neoplastic mass. Within the neoplasm there is a heterogeneous population of neoplastic cells with the main population being sheets of pleomorphic round cells. Neoplastic cells exhibit mild anisocytosis, varying in size from 15 micrometres to 30 micrometres in

diameter and have moderate amount of finely granular, occasionally vacuolated eosinophilic cytoplasm with mostly distinct cell borders. The nuclei of the cells are round, peri-central to centrally positioned, approximately 10 micrometres to 25 micrometres in diameter with coarsely clumped chromatin and 1 to 2 round, deeply basophilic nucleoli. Stroma is scant around these neoplastic cells and mitotic rate is high and variable, averaging between 3 and 6 per high power field (higher in some slides). Mitoses occasionally are abnormal in appearance.
Another smaller population of neoplastic cells are scattered multifocally throughout the main neoplastic population in acini and clusters (variable, depending on slide). When acini have formed there is a scant quantity of homogenous amphophilic material within. These cells are polygonal, approximately 10 micrometres to 15 micrometres, with indistinct cell borders. The nuclei are mostly centrally placed, basophilic with coarsely clumped chromatin and a single nucleolus. There is a moderate amount of cytoplasm and it is homogenously eosinophilic. There are only scant quantities of stroma and mitotic rate is moderate in this area with 2 to 3 mitosis per high power field. (Poorly differentiated epithelial cells).
A third population of neoplastic cells are arranged in streams that dissect through the main neoplastic cell line. These cells are spindloid approximately 15 micrometres to 20 micrometres by 5 micrometres to 10 micrometres with indistinct cell borders. There are moderate quantities of homogenous eosinophilic cytoplasm and a scant quantity of stroma. The nuclei are elongated to oval and approximately 10 micrometres to 15 micrometres by 4 micrometres to 7 micrometres with stippled palely basophilic chromatin with 1, occasionally 2 nucleoli.

Within the neoplasm there are also multifocal to coalescing, moderate, clusters of lymphocytes and plasma cells (lymphoplasmocytic inflammation). Also there are multifocal, variably sized areas of eosinophilic cells devoid of nuclei (malacia, depends on slide).
There is diffuse congestion of vessels with in the neoplasm and surrounding brain parenchyma.
Dorsal to the mass, in the grey matter, there are small multifocal intercellular non-staining areas (oedema, depends on slide).
Within the parenchyma of the brain there are multifocal, variably sized areas (depending on slide) of rarefaction.

Contributor's Morphologic Diagnosis: Thalamic germinoma.

Contributor's Comment: Thalamic/Suprasellar germinomas are rarely reported in veterinary medicine although are more common within the human population. There are also a few reported cases of primary germ cell tumours in the canine CNS[1,3,4,8,15]. They are found in young to middle-aged dogs and cats and young to adolescent humans. Germinomas are the most common CNS germ cell tumour accounting for two thirds of this neoplastic derivative in man[9].

The histopathology of germ cell tumours varies depending on the type. Cordy was the first to accurately describe the histological features of and intracranial germinoma in a dog in 1984[1]. Germinomas are histologically similar to seminomas or dysgerminomas with varying degrees of other cell lines including epithelioid and mesenchymal aspects. They also are reported as having an lymphocytic inflammatory element [15]. The histological features of the case presented are consistent with previous descriptions. The main neoplastic population is a poorly differentiated round to polygonal cell, reminiscent of testicular seminoma, and the multifocal lymphocytic infiltrates present in this case are also a common feature, and typical of testicular seminomas. There are areas of both epithelial and mesenchymal differentiation of these primitive cells which are observed morphologically on haematoxylin and eosin stained sections, and are confirmed immunohistochemically by positive staining with pancytokeratain and vimentin respectively.

The germinoma is a line of germ cell tumours which are more commonly found within the gonads. They are thought to arise in the brain when embryonic cells migrate from the yolk sack to the gonads during development and may inadvertently arrive in the CNS where they can become neoplastic with time [15]. Due to this method of accidental migration to the CNS, almost all germinomas are in a midline location[6].
There are several different types of germ cell tumors: germinoma, seminoma, embryonal carcinoma, mature/immature teratoma, choriocarcinoma and endodermal sinus tumour. These may be grouped in to germinomas and non-germinomas.

GERMINOMATOUS	NON GERMINOMATOUS
Germinoma (brain)	Embryonal carcinoma
Dysgeminoma (ovary)	Teratoma
Seminoma (testis)	Yolk sac tumour/endodermal sinus tumour
	Choriocarcinoma

There are also reported cases of metastatic spread of seminoma and dysgeminomas to the CNS[2,5]. This is considered a rarer event than a primary tumour, partly due to the fact that rate of metastasis is low in these tumours and are more likely to be found in abdominal and thoracic sites[2,5]. In such cases there were grossly apparent primary tumours in the gonads which was not the case in this animal.
Clinical signs associated with intracranial germ cell tumours are variable neurological signs and depend on the area of tumour infiltration but often include sudden onset blindness, depression, ataxia, proprioception deficits, head pressing and even diabetes insipidus [3,8,15].

According to early descriptions the diagnosis of an intracranial germ cell tumour requires three criteria be fulfilled [15]:

1. Location- midline and suprasellar
2. Multiple distinct cell types within one tumour- seminoma or dysgerminoma and secretory glandular and squamous elements (teratomatous differentiation)
3. AFP (α-fetoprotein) positive staining

Immunohistochemistry is required to confirm the presence of a germinoma and recently they have also been found to be C-Kit positive in the cytoplasm of neoplastic germinoma cells in humans[7,9,11-14]. As anti-AFP antibody was unavailable in this institution C-kit was used to in this case, and all of the neoplastic cells were strongly positive. To the best of our knowledge, this is the first reported case of using immunostaining against C-kit to identify germinoma cells in a canine intracranial germinoma, although C-kit positivity in these tumours is mentioned in Veterinary Neuropathology Essentials of Theory and Practice[16]. Recent research in human medicine has found OCT4 and UTF1 are also expressed in CNS germ cell tumours[9,12] and more research is required to consider whether these may become appropriate immunohistochemical markers in veterinary species.
Treatment in human medicine is mostly radiotherapy and chemotherapy[10] but there are no reports of successful treatment in dogs with most dogs being euthanised.

Contributing Institution (please include the contributor's departmental or institutional Web site address): http://www.liv.ac.uk/vetpathology/

Acknowledgments
University of Liverpool Veterinary Neurology department must be mention for submitting the animal for post mortem examination and for providing a detailed clinical history and MRI images.

References (please submit one (1) separate electronic PDF copy of **each** reference (Jubb, Kennedy, Palmer; McGavin texts excluded) and ensure these are cited in the text of your "Contributor's Comment" section above, using a numbering format; use the reference style as required by the journal *Veterinary Pathology*):

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