Diagnostic Exercise

From The Davis-Thompson Foundation*

Case #: 105 Month: September Year: 2018

Answer Sheet

Title: *Canine, Brain mass (cerebrum), Anaplastic Ependymoma (Grade III)*

Contributors: Matthew Sheley, DVM Anatomic Pathology Resident III, Kevin Woolard, DVM, PhD, DACVP, School of Veterinary Medicine, University of California, Davis, William R. Pritchard Veterinary Medical Teaching Hospital, Davis, CA.

Diagnosis: Brain: anaplastic (grade III) ependymoma.

Microscopic Findings: Multiple sections through a multilobular, poorly demarcated, heterogeneous, moderately cellular mass are examined. Lobules of the mass are composed of various epithelioid profiles embedded in a thin, dense, fibrovascular meshwork. Groups of epithelioid neoplastic cells form irregularly shaped nests, thin chords, and occasional tubular structures. The nests contain dense polygonal to columnar cells with distinct cellular borders, small to moderate amount of eosinophilic, fibrillar to finely vacuolated cytoplasm, and a large round to ovoid nucleus with stippled to vesiculate chromatin and 2-4 small nucleoli. Anisocytosis and anisokaryosis are moderate to marked, 35 mitotic figures are counted in 10 high-power (400x) fields, and mitoses are occasionally bizarre. Within nests, cells occasionally form tubules with more differentiated columnar cells that rarely exhibit mitotic figures. These tubules are often filled by basophilic, wispy, myxomatous material. Neoplastic cells rarely palisade around a central small clear space forming vague pseudorosettes. The neoplastic cell population often forms thin chords in which the cells become thin, spindloid to columnar, and palisade. Nuclei of these cells also become thin and elongate, spanning across most of the cytoplasm (tanycytic nuclei). Occasionally neoplastic cells transition into short streams of smaller spindloid cells with scant cytoplasm and small, ovoid, hyperchromatic nuclei (primitive appearance). Approximately 25% of the neoplasm is multifocally necrotic, characterized by small irregular pools of karyorrhectic nuclear debris and light eosinophilic cytoplasmic debris. There is moderate multifocal acute hemorrhage in all sections. Streams of thickened, somewhat disorganized fibrous tissue commonly surround nests of neoplastic cells (interpreted as a desmoplastic response). Multifocally, groups of neoplastic cells are disrupted and overlain by streams of nuclear chromatin (likely crush artifact).

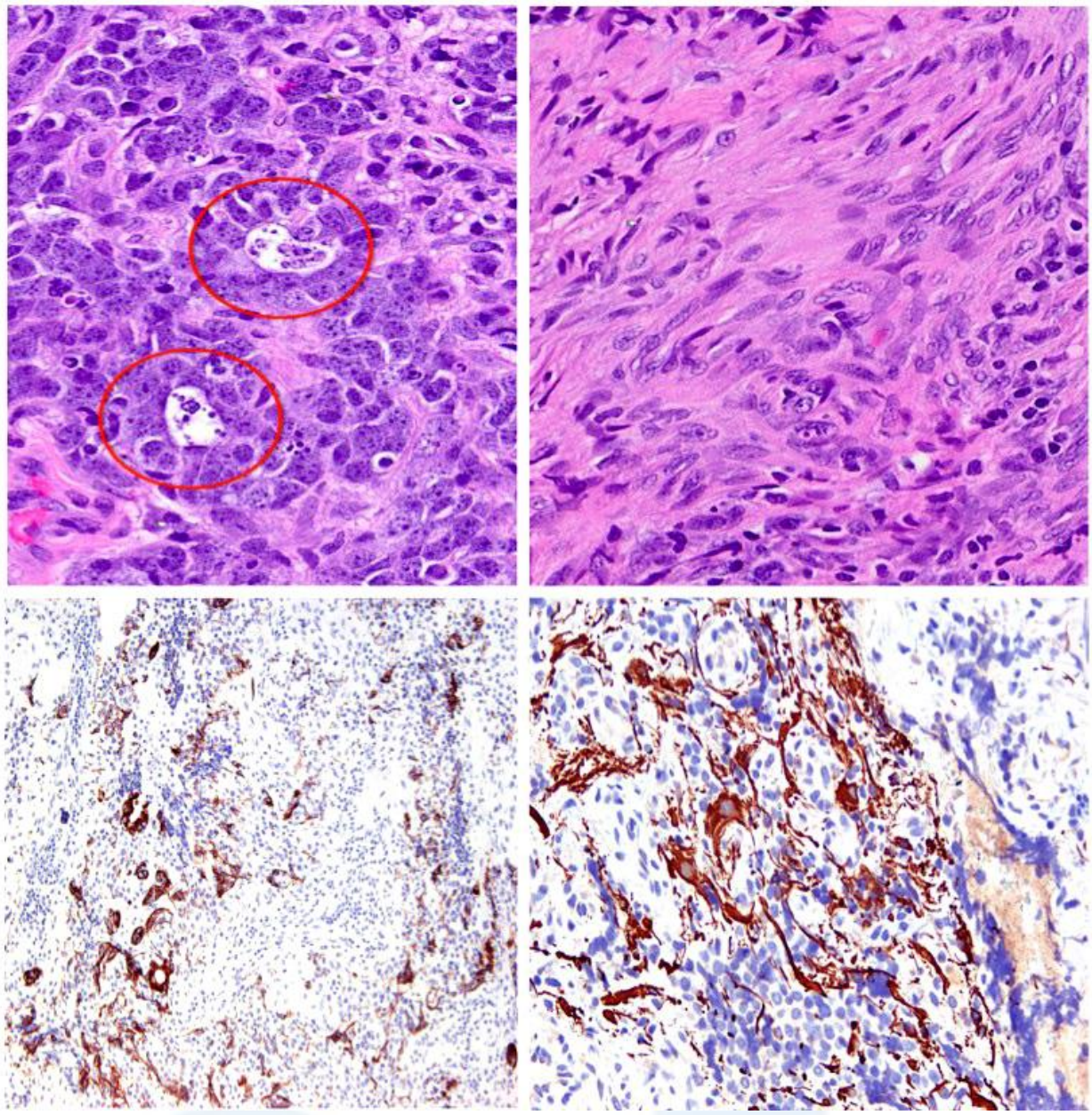

Figure legend: A majority of the tumor cells are epithelioid, occasionally arranged into crude rosette and tubule formations (encircled, upper left). In other regions, the tumor cells are more primitive (undifferentiated) and sometimes elongated with an almost mesenchymal phenotype (upper right photo). Immunohistochemistry (bottom row (left, pan-cytokeratin; right, GFAP): Approximately 30-40% of the nests of neoplastic cells exhibited variable cytoplasmic immunoreactivity to anti-pancytokeratin antibody. Approximately 60% of nests of neoplastic cells exhibited cytoplasmic anti-GFAP antibody immunoreactivity.

Discussion: The MRI of this neoplasm depicted a mass that expanded and distorted the olfactory and frontal lobes, and appeared to extend both rostrally to the ethmoid bone and caudally to the anterior lateral ventricle. Based on this location and the cytomorphology of the

neoplastic population, differentials included metastatic carcinoma, invasive nasal carcinoma, and glioma, including anaplastic ependymoma. A majority of this tumor was composed of epithelioid cells, which were occasionally arranged into crude rosette and tubule formations. A much smaller percentage of the cell population exhibited more classic features of ependymal cells: small primitive cells with scant cytoplasm loosely arranged into pseudorosettes. Additionally, tanacytic nuclei were occasionally present, which are most commonly found in anaplastic ependymomas and (rarely) glioblastoma mutiforme (GBM). This tumor did not express other features of a GBM.

Cytology was performed during surgical removal of the mass and revealed rafts of cohesive cells with variable amounts of eosinophilic cytoplasm. Some cells had larger nuclei with moderate amount of cytoplasm while others had small ovoid nuclei with scant streaming cytoplasm. Occasional cytoplasmic processes were identified and cells rarely vaguely palisaded. These findings are consistent with, but do not confirm, an ependymoma as other tumors share these features.

Immunohistochemical assays further supported a diagnosis of anaplastic ependymoma. Approximately 60% of nests of neoplastic cells exhibited cytoplasmic anti-GFAP antibody immunoreactivity; approximately 30-40% of the nests of neoplastic cells exhibited variable cytoplasmic immunoreactivity to anti-pancytokeratin antibody; and approximately 10% of nests of neoplastic cells exhibited variable cytoplasmic anti-Neurofilament 200 kD (NF200) antibody immunoreactivity, all of which are consistent with an anaplastic ependymoma. An invasive or metastatic carcinoma would not react with GFAP or NF200. Lastly, neoplastic cells were grown in culture and formed non-adherent neurospheres, similar to neural stem cells, further supporting an ependymoma over a carcinoma. Tissue obtained during surgery was also placed into serum-free culture, producing non-adherent neurosphere-like structures that strongly co-expressed GFAP and nestin, consistent with an ependymal derived cell population.

Ependymomas rarely occur in animals. There are only a handful of reports of canine ependymomas, and a few have been diagnosed in cattle, horses, cats, and rats. Similarly, they are rare in humans and WHO classification, even in humans, is not clearly established. However, high mitotic rate, marked pleomorphism, and abundant necrosis, all of which were present in the current case, garner a grade III diagnosis in humans and did in the current case.

Ependymomas are challenging to accurately diagnose as they lack a definitive immunohistochemical marker. In humans, the presence of epithelial membrane antigen (EMA) is often used as a marker, but it is non-specific as it is also expressed in some glial tumors, including GBM. Additionally, antibody clones used in human tumors do not appear to be cross-reactive to canine samples. The presence of cytokeratin immunoreactivity is controversial in ependymomas, as the percentage reported varies widely in the human literature, confounded by

potential cross-reactivity between other intermediate filaments, including GFAP. Thus, a diagnosis of ependymoma requires careful consideration of other possible neoplastic entities, and optimally centers on ultrastructural features for a definitive classification.

References and Recommended literature:

1. Burger PC, Scheithauer BW, DeMasters BK (2012): Diagnostic pathology: neuropathology; second edition. Amirsys. 107 – 111.
2. Carrigan MJ, Higgins RJ, Carison GP, Naydan DK (1996):Equine papillary ependymoma. Veterinary Pathology, 33, 77–80.
3. McKay JS, Targett MP, Jeffery ND (1999): Histological characterisation of an ependymoma in the fourth ventricle of a cat. Journal of Comparative Pathology, 120, 105–113.
4. Michimae Y, Morita T, Sunagawa Y, Sawada M, Okamoto Y, Shimada A (2004): Anaplastic ependymoma in the cervical spinal cord of a Maltese dog. The Journal of Veterinary Medical Science, 66, 1155–1158.
5. Rittinghausen JK, Deerberg F (1989): Spontaneous tumours of the spinal cord in laboratory rats. Journal of Comparative Pathology, 100, 209–215.
6. Sfacteria A, Macrì F, Perillo L, Rapisarda G, Lanteri G, Mazzullo G (2010): Cytologic and histologic features of spinal cord ependymoma in a young dog: a case report. Veterinarni Medicina. 1;55(1):35-8.
7. Simpson DJ, Hunt GB, Tisdall PLC, Govendir M, Zaki S, France MP, Malik R (1999): Surgical removal of an ependymoma from the third ventricle of a cat. Australian Veterinary Journal, 77, 645–648.
8. Vural SA, Besalti O, Ilhan F, Ozak A, Haligur M (2005): Ventricular ependymoma in a German Shepherd dog. The Veterinary Journal, 172, 185–187.

*The Diagnostic Exercises are an initiative of the **Latin Comparative Pathology Group (LCPG)**, the Latin American subdivision of The Davis-Thompson Foundation. These exercises are contributed by members and non-members from any country of residence. Consider submitting an exercise! A final document containing this material with answers and a brief discussion will be posted on the CL Davis website (http://www.cldavis.org/diagnostic_exercises.html).

Associate Editor for this Diagnostic Exercise: Patricia Pesavento
Editor-in-chief: Vinicius Carreira