Diagnostic Exercise
From The Davis-Thompson Foundation*

Answer Sheet

Case: **221**; Month: **September**; Year: **2023**

Title: Primary Hepatic Neuroendocrine Tumor (PHNET) in a Dog (*Canis familiaris*)

Contributors: Cassandra M. Powers[1], DVM; Ebony T. Gilbreath[1], DVM, PhD, Dipl. ACVP; Pamela Y. Martin[2], DVM; Athema L. Etzioni[1], DVM, MS, Dipl. ACVP; Ida L. Phillips[1], DVM, Ph.D. Dipl ACVP; Thomas C. Graham[1], DVM, PhD
power143@purdue.edu
1.Department of Pathobiology and 2. Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Tuskegee University, Tuskegee, AL 36088

Clinical History: A 3-year-old female spayed Mixed Breed canine (*Canis familiaris*) presented to Tuskegee University College of Veterinary Medicine's Small Animal Hospital with a history of marked anorexia for a 3-week period and a slowly expanding abdomen. On clinical presentation and physical examination, an abdominal mass was palpated, and ascites was suspected after abdominal ballottement. Diagnostic testing included a complete blood count (CBC), serum biochemical profile (CHEM), and diagnostic imaging. Bloodwork revealed an increase in hepatic enzyme activity. Diagnostic imaging included survey radiographs and ultrasound of the thorax and abdomen. The radiographs revealed an additional multinodular mediastinal mass, and the ultrasound showed multiple masses within the liver, lungs, and spleen. Due to continued ill thrift and a poor prognosis, the owners elected for humane euthanasia. The carcass was submitted for a postmortem evaluation.

Necropsy Findings: During a complete necropsy, multiple masses were identified within the mediastinum, liver, adrenal glands, spleen, and kidneys (Fig. 1). The multinodular mediastinal mass measured 9 cm x 5.5 cm x 7 cm. The mass bulged on cut surface and was mottled tan to yellow to red (Fig. 2). Within the abdominal cavity there was about 0.75 L of serosanguinous fluid. There were multifocal to coalescing, firm to soft masses throughout all lobes of the liver ranging in size from 9 cm x 8 cm x 5 cm to 1 cm x 0.5 cm x 0.5 cm (Fig. 3). Both adrenal glands were completely replaced by a solitary mass that measured 9 cm x 5 cm x 5 cm. The adrenal mass on cut surface contained hard, white to red caseous material with a distinct vascular network (Fig. 4A-C). Bilaterally, the kidneys contained multifocal, round, white to red, soft to firm masses that invaded into both the cortex and medulla of the renal parenchyma and ranged in size from 0.3 cm to 0.7 cm in diameter (Fig. 4D). The spleen had multifocal, round, tan, masses throughout the parenchyma that measured approximately 1 cm x 2 cm x 0.4 cm (Fig. 5). There was diffuse enlargement of all palpable lymph nodes within both the thoracic and abdominal cavities. No other major macroscopic changes were identified.

Histopathologic Findings: Arising from the hepatic parenchyma was a well demarcated and non-encapsulated densely cellular mass composed of variably round to epithelioid cells arranged in sheets and nests with a moderate fibrous stroma (Fig. 6). The cells had variably distinct borders and lightly amphophilic cytoplasm. The nuclei were round to oval with 1-3 condensed nucleoli. There were 37 mitotic figures observed in ten 400x hpf. There was moderate to marked anisocytosis and anisokaryosis. All other identified masses in the mediastinal space, adrenals, kidneys, spleen, and lymph nodes displayed similar histological changes.

Multiple immunohistochemical stains were used on the liver slides to identify the origin of the neoplastic cells. Results of immunohistochemistry performed on liver mass are summarized in Table 1. Lymphoma, a primary thymic tumor, a primary adrenal tumor, and a primary hepatic tumor were all considered. Neoplastic cells lacked expression of PAX5 and CD3 ruling out lymphoma. E-cadherin and pancytokeratin were used to help rule out epithelial tumors, including a thymic tumor. A panel of stains that included Melan A, Inhibin, Met-enkephalin, and Endorphin were all negative. The results of this panel helped rule out a primary adrenal tumor. An epithelial hepatic tumor was also suspected, but the negative pancytokeratin ruled out cells of epithelial origin, thus a hepatocellular carcinoma and a cholangiocarcinoma were not likely. Finally, a neuroendocrine tumor of hepatic origin was suspected and synaptophysin, neuron specific enolase (NSE), and chromogranin A markers were performed. The NSE and chromogranin A were both negative, but the synaptophysin stain showed strong cytoplasmic positive staining (Fig. 6). Since synaptophysin is a reliable marker for neuroendocrine tumors and the previous IHC results ruled out a primary tumor of mediastinal/thymic or adrenal origin, this metastatic tumor was determined to be of hepatic origin with a neuroendocrine behavior.

Gross Images:

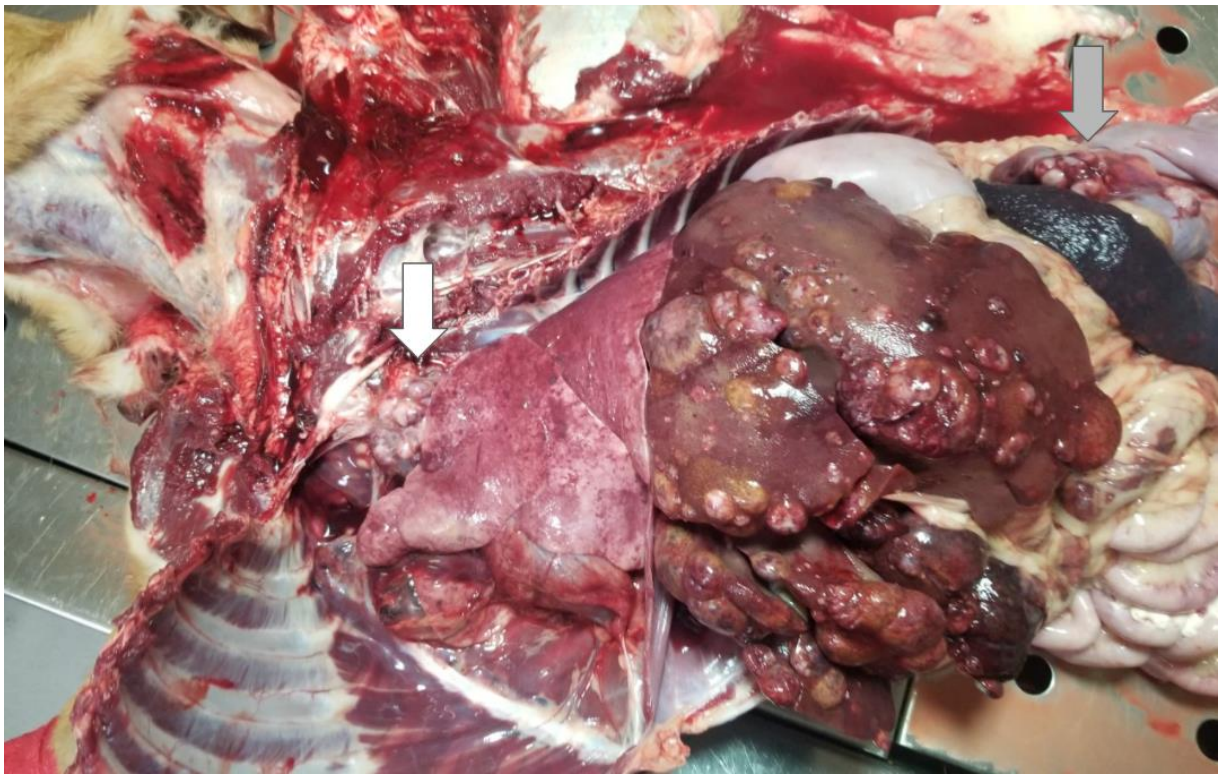

Figure 1: Whole body. Both the thoracic and abdominal cavities are infiltrated by round, infiltrative masses within the mediastinum (white arrow), liver, adrenal glands (gray arrow), kidneys, and spleen.

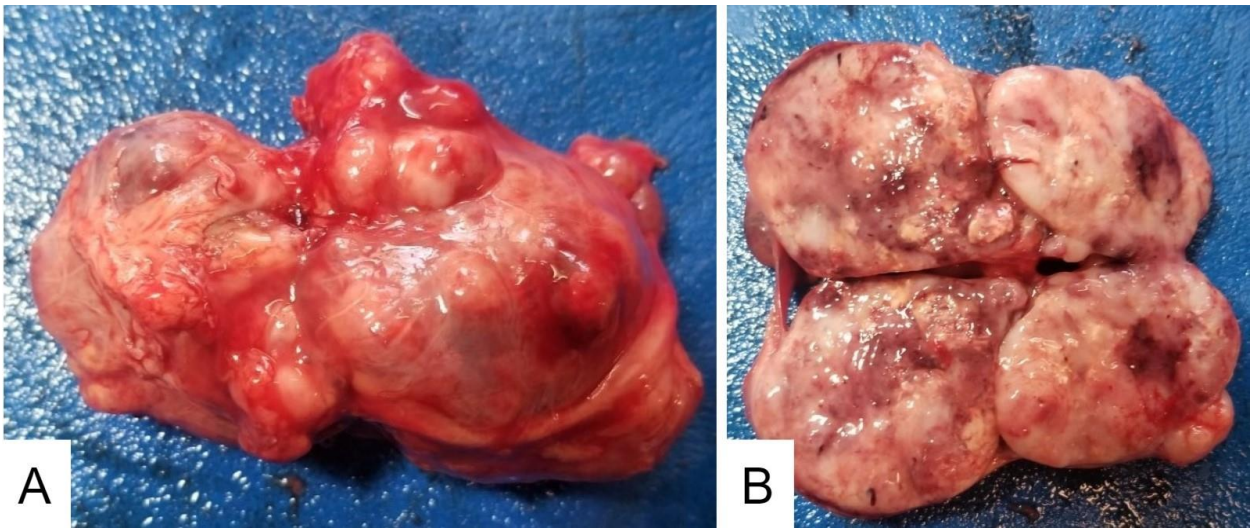

Figure 2: Mediastinal mass. **A** — Within the mediastinal space there was a multinodular mass that measured 9 cm x 5.5 cm x 7 cm. **B** — On cut section the mass bulged out and was a mixture of tan to yellow to red

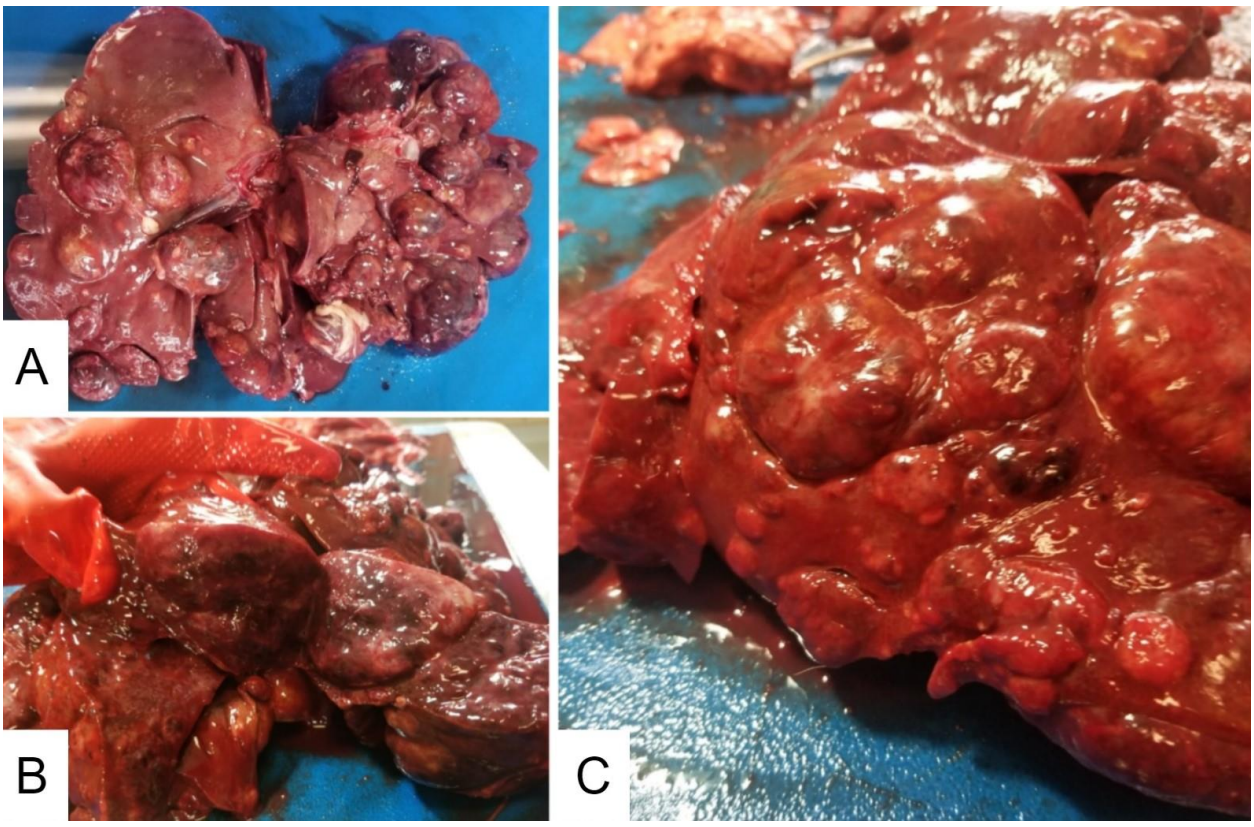

Figure 3: Liver masses. **A** — There were multifocal to coalescing, firm to soft masses infiltrating into the parenchyma of all lobes of the liver ranging in size from 9 cm x 8 cm x 5 cm to 1 cm x 0.5 cm x 0.5 cm. **B** — On cut section the masses bulged and had a dark red to white appearance. **C** — Occasionally, the masses had an umbilicated appearance

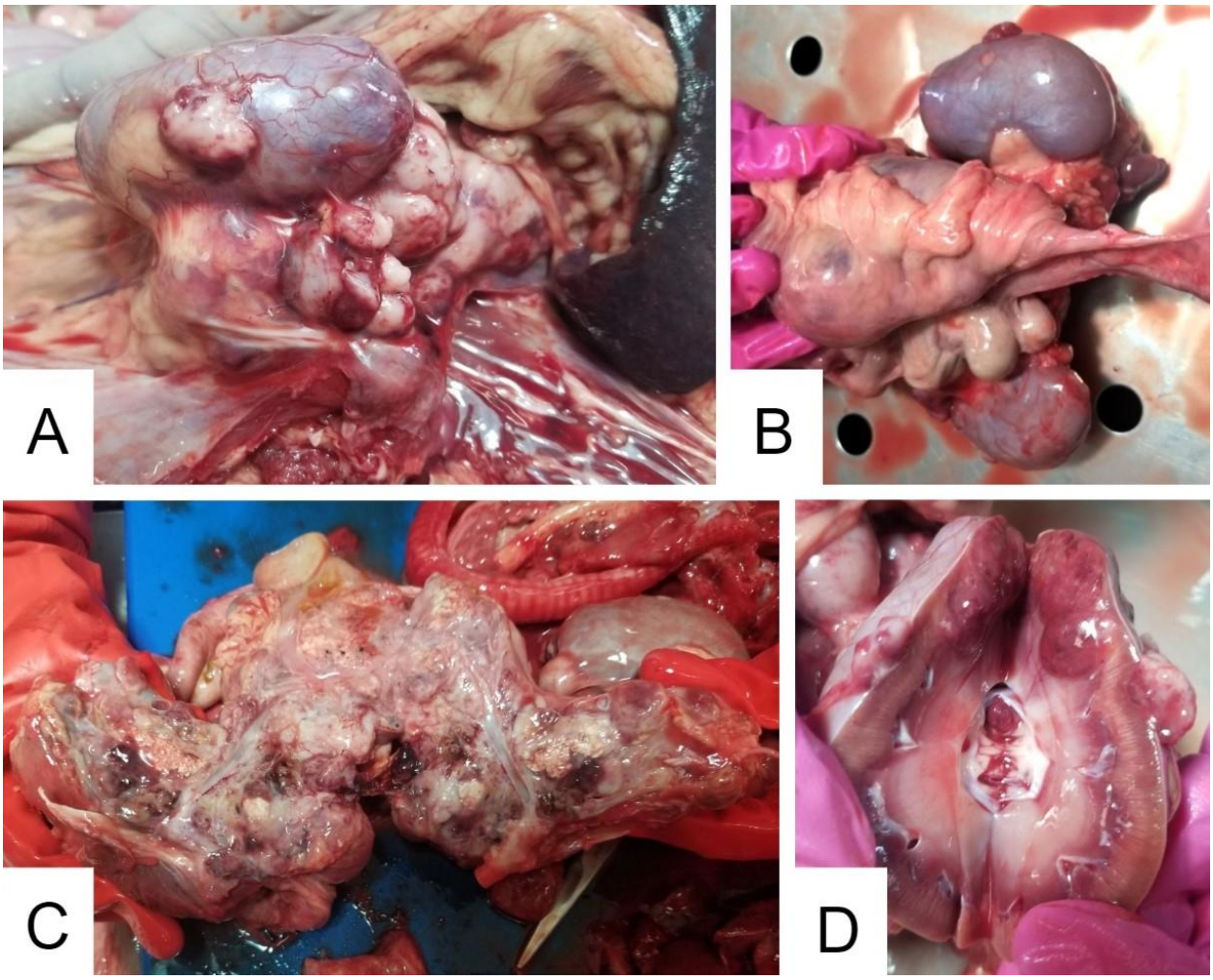

Figure 4: Adrenal masses and kidney masses. **A** — Completely effacing and replacing the adrenal glands is a multinodular mass white, firm mass. **B** — Both of the adrenal glands were completely effaced by a solitary mass that measured 9 cm x 5 cm x 5cm. **C** — On cut section the mass contained hard, caseous material and appeared white to red with a distinct vascular network. **D** — Bilaterally, the kidneys contained multifocal white to red, soft to firm masses that invaded into the renal parenchyma and ranged in size from 0.3 cm to 0.7 cm in diameter.

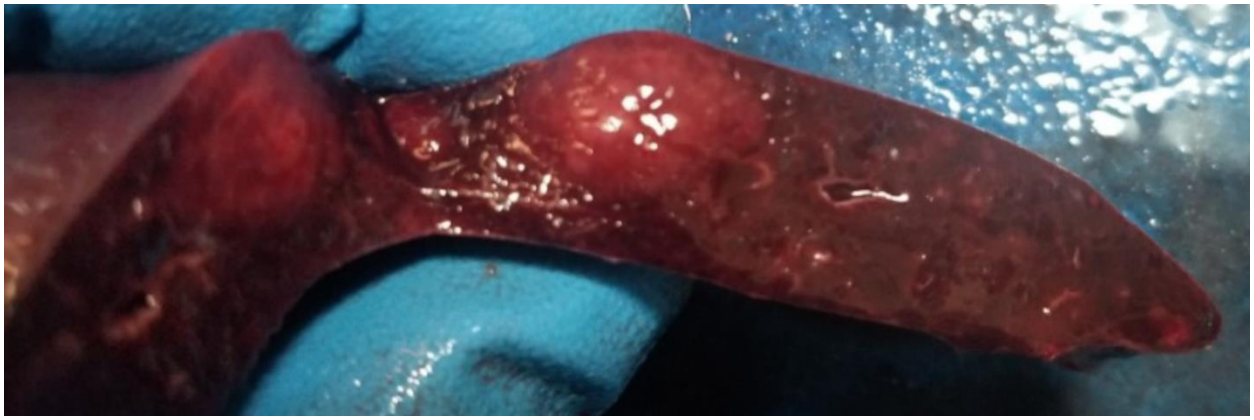

Figure 5: Splenic mass. The spleen had multifocal, tan, masses throughout the splenic parenchyma that measured approximately 1cm x 2cm x 0.4cm.

Microscopic Images:

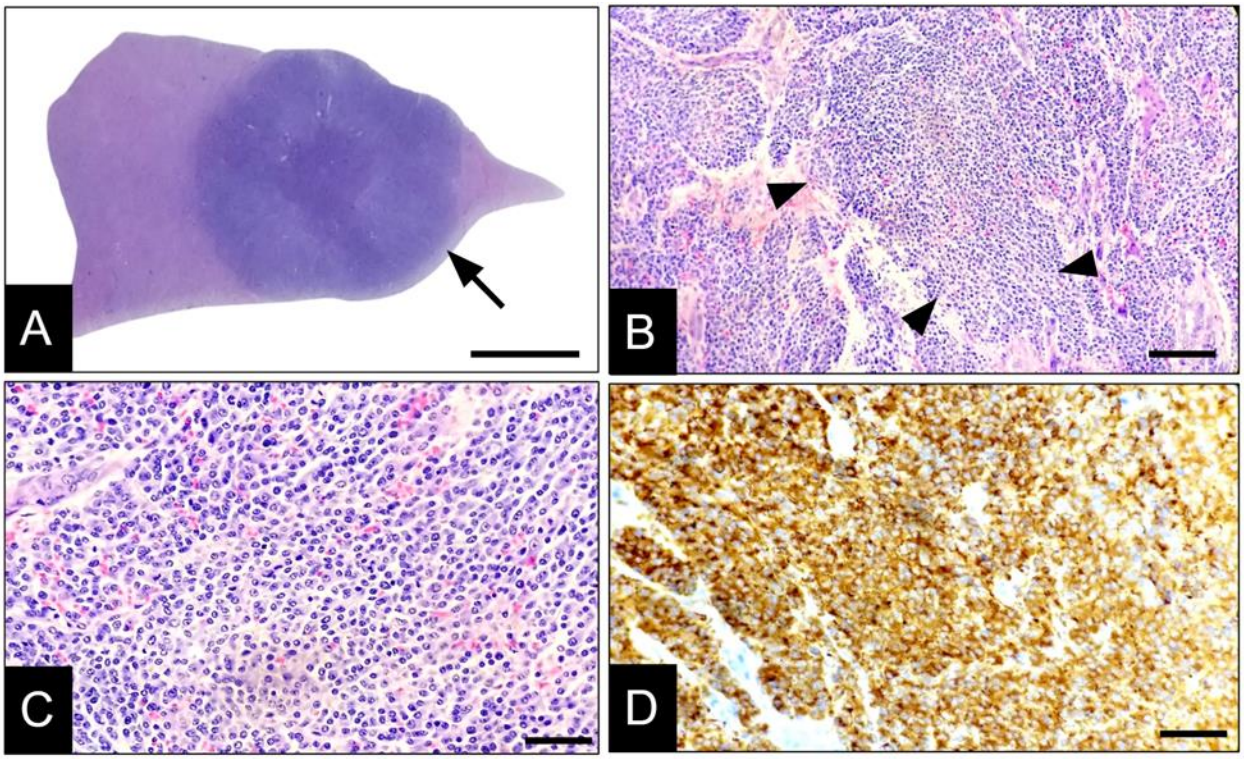

Figure 6: Liver mass. **A** — Arising from the hepatic parenchyma is a well demarcated and non-encapsulated densely cellular and infiltrative mass (arrow). H&E stain; 0x; bar = 1 mm. **B** — The mass is composed of variably round to epithelioid cells arranged in sheets and nests (outlined by arrowheads) supported on a moderate amount of fibrovascular stroma. H&E stain; 20x; bar = 100 um. **C** — The cells have variably distinct cell borders and lightly amphophilic cytoplasm. The nuclei are round to oval with 1-3 condensed nucleoli. There are 37 mitotic figures observed in ten 400x hpf. There is moderate to marked anisocytosis and anisokaryosis. H&E stain; 40x; bar = 50 um. **D** — Synaptophysin stain showed strong cytoplasmic expression within neoplastic cells. H&E stain; 40x; bar = 50 um.

Table 1: Results of immunohistochemistry performed on liver mass

Immunohistochemistry	Results	Interpretation
PAX5	-	Rules out a tumor of B-cell origin (lymphoma)
CD3	-	Rules out a tumor of T-cell origin (lymphoma)
E-cadherin	-	Rules out an epithelial tumor (specifically used to rule out a tumor of thymic origin)
Pancytokeratin	-	Rules out an epithelial tumor (specifically used to rule out a tumor of thymic origin)
Melan A	-	Rules out a tumor of adrenal origin (used in conjunction with other markers)
Inhibin	-	Rules out a tumor of adrenal origin (used in conjunction with other markers)
Met-enkephalin	-	Rules out a tumor of adrenal origin (used in conjunction with other markers)
Endorphin	-	Rules out a tumor of adrenal origin (used in conjunction with other markers)
NSE	-	Used in conjunction with chromogranin A and synaptophysin to diagnose NE tumors
Chromogranin A	-	Used in conjunction with NSE and synaptophysin to diagnose NE tumors
Synaptophysin	+	Confirms a tumor of neuroendocrine origin

NSE, Neuron-specific enolase
NE, Neuroendocrine

Morphologic diagnosis: Primary hepatic neuroendocrine carcinoma with multiorgan metastasis in a 3-year-old mixed breed dog

Comments:
This case proved to be highly misleading on initial presentation. On gross examination and primary histopathology, a mediastinal lymphoma was tentatively diagnosed based on the location and sheet-like distribution of the cells on the initial sections. However, immunohistochemistry for PAX 5 and CD3 were both negative. The locations of the masses, the severity of the disease, and the involvement of both the thoracic and abdominal body cavities led to confusion on which mass should be considered primary. As such, multiple immunohistochemical stains were implemented to help identify the tumor origin. PAX5, CD3, E-cadherin, pancytokeratin, and an adrenal panel of IHCs (Melan A, Inhibin, Met-enkephalin, Endorphin) were all identified as negative. The NSE and chromogranin A stains were both negative, but the synaptophysin stain showed strong cytoplasmic positive staining. Finally, a neuroendocrine tumor of hepatic origin was diagnosed.

A neuroendocrine tumor is a tumor that arises from specialized cells that have traits of both nerve cells and hormone producing cells. They release hormones into the blood in response to signals from the nervous system. These are often invasive carcinomas with diffuse infiltration. In the past "carcinoid" was used to denote this metastatic tumor of neuroendocrine origin. In practice the term "carcinoma" is more commonly used to denote a diagnosis of malignancy for better understanding by clients.

Primary neuroendocrine tumors are a rare diagnosis in many species. It is found primarily in the intestine, liver, gallbladder, lung, esophagus, skin, and nasal cavity. Hepatic neuroendocrine tumors have been reported in rare instances in various species, including cats (Kita et. al 2014), dogs, cows, a baboon (Aloisio et. al 2009), sika deer (Shibata et. al 2020), an African pygmy hedgehog (Lowden et. al 2016), flamingos, a Japanese macaque (Hirata et. al 2019), and bearded dragons (Lyons et. al 2010). Clinical signs in patients with this tumor can include anorexia, weight loss, vomiting, and hepatomegaly. Metastases are commonly found in regional lymph nodes, the peritoneum, and other sites, exhibiting a highly invasive and aggressive nature. To the authors' knowledge, metastasis to the thymus and mediastinal area has not been reported in the dog.

Diagnosis of a primary hepatic neuroendocrine tumor (PHNET) can only be confirmed via immunohistochemical staining. These tumors have been shown to stain positively for at least one of the major neuroendocrine markers. These include synaptophysin, Neuron-specific enolase (NSE), Chromogranin A, and more recently, PGP9.5 (Ichimata et. al 2021, Patnaik et. al 2005). Churukian-Schenk and Grimelius silver stains are also used to highlight the argyrophilic cytoplasmic granules found in the cytoplasm of these tumors. Argyrophil and argentaffin histochemical stains (Grimelius, Pascal, Fontana, etc.) have been used often to confirm the diagnosis of carcinoid tumors. These silver stains demonstrate the presence of black granules in the cytoplasm of these tumor cells (Michishita et. al 2017). These stains were not implemented in this case.

Some neuroendocrine carcinomas appear to display an active endocrine function. In humans, the most commonly reported positively staining peptides in hepatic neuroendocrine tumors are chromogranin-A, chromogranin-B, NSE, synaptophysin, serotonin, gastrin, and insulin (Patnaik et. al 2005). A 2014 feline case of neuroendocrine carcinoma was able to show positive immunohistochemical staining for gastrin (Kita et. al 2014). A 2005 Patnaik et al. paper looked at ten dogs with hepatic neuroendocrine tumors and found that three neoplasms stained positively for serotonin, one stained positively for insulin, and most of them expressed more than two peptides or amines (Patnaik et. al 2005). Metastasis from other organs was excluded based on surgical records and histopathologic samples.

There is no standard of care for treatment of patients with these neoplasms. These tumors are often invasive with diffuse infiltration and limited ability for complete excision. Two recent studies have explored different treatments for this tumor. A 2019 study treated a dog with doxorubicin and metronomic cyclophosphamide. The patient lived approximately 15.5 months from initial presentation. However, it could not be confirmed whether the chemotherapy regimen increased this patient's overall survival time. Ichimata et al. in 2021 reported a case of a French bulldog with a hepatic tumor and gastrointestinal signs that was treated with single-agent toceranib phosphate (TOC). The animal survived 25.1 months after the initial presentation. The authors report this case to be the first report of long-term survival in a dog with PHNET treated with TOC.2

References

1. Aloisio F, Dick EJ Jr, Hubbard GB. Primary hepatic neuroendocrine carcinoma in a baboon (Papio sp.). J Med Primatol. 2009;38(1):23-26. doi:10.1111/j.1600-0684.2008.00304.x
2. Ichimata M, Nishiyama S, Matsuyama F, et al. Long-term survival in a dog with primary hepatic neuroendocrine tumor treated with toceranib phosphate. J Vet Med Sci. 2021;83(10):1554-1558. doi:10.1292/jvms.21-0254
3. Hirata A, Miyamoto Y, Kaneko A, et al. Hepatic neuroendocrine carcinoma in a Japanese macaque (Macaca fuscata). J Med Primatol. 2019;48(2):137-140. doi:10.1111/jmp.12394
4. Kita C, Yamagami T, Kinouchi S, Nakano M, Nao NAGATA, Suzuki H, Ohtake Y, Miyoshi T, Irie M, Uchida K. A Feline Case of Hepatic Neuroendocrine Carcinoma with Gastrin Immunoreactivity. J of Vet Med Sci. 2014;76(6): 887-890. doi:10.1292/jvms.13-0581
5. Lowden LR, Davies JL. Malignant Neuroendocrine Tumour (Carcinoid) of the Spleen in an African Pygmy Hedgehog (Atelerix albiventris). J Comp Pathol. 2016;155(1):88-91. doi:10.1016/j.jcpa.2016.04.003
6. Lyons JA, Newman SJ, Greenacre CB, Dunlap J. A gastric neuroendocrine carcinoma expressing somatostatin in a bearded dragon (Pogona vitticeps). J Vet Diagn Invest. 2010;22(2):316-320. doi:10.1177/104063871002200230
7. Michishita M, Takagi M, Kishimoto TE, et al. Pancreatic neuroendocrine carcinoma with exocrine differentiation in a young cat. J Vet Diagn Invest. 2017:29(3):325-330.
8. Patnaik AK, Newman SJ, Scase T, et al. Canine hepatic neuroendocrine carcinoma: an immunohistochemical and electron microscopic study. Vet Pathol. 2005;42(2):140-146. doi:10.1354/vp.42-2-140

9. Shibata R, Machida Y, Hatakeyama H, et al. Hepatic neuroendocrine carcinoma with metastases to the lymph nodes in a sika deer (Cervus nippon yakushimae). J Vet Med Sci. 2020;82(2):193-196. doi:10.1292/jvms.19-0472
10. Zhao ZM, Wang, J, Ugwuowo UC. et al. Primary hepatic neuroendocrine carcinoma: report of two cases and literature review. BMC Clin Pathol. 2018; 18:3. https://doi.org/10.1186/s12907-018-0070-7

*The Diagnostic Exercises are an initiative of the Latin Comparative Pathology Group (LCPG), the Latin American subdivision of The Davis-Thompson Foundation (DTF). 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 DTF website (https://davisthompsonfoundation.org/diagnostic-exercise/).

Associate Editor for this Diagnostic Exercise: Saulo Pavarini
Editor-in-chief: Claudio Barros