Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2024-2025

Conference #10 — 23 October 2024

CASE I:

Signalment:
Mature ewe, *Ovis aries*, ovine.

History:
The cadaver and viscera of a mature ewe underwent routine meat inspection by an abattoir veterinarian who noted an emaciated cadaver, abnormal kidneys and liver. The liver was donated to the Department of Veterinary Medicine, University of Cambridge, for veterinary public health teaching and was passed to the anatomic pathology service for further investigation.

Figure 1-1. Liver, sheep. The liver is expanded by multifocal to coalescing, well-demarcated, cream nodules that are unencapsulated and infiltrative. (*Photo courtesy of:* The Queen's Veterinary School Hospital, University of Cambridge. https://www.vet.cam.ac.uk)

Gross Pathology:
The liver is expanded by multifocal to coalescing, well-demarcated, cream nodules that are unencapsulated and infiltrative. The nodules are up to 25 mm diameter.

Microscopic Description:
Liver: Apparent sub-grossly within the hepatic parenchyma forming multifocal to coalescing nodular masses is an unencapsulated moderately densely cellular neoplasm that is infiltrative, with small neoplastic nodules infiltrating the surrounding hepatic parenchyma. The neoplastic cells are arranged in sheets supported by a moderately fine collagenous stroma with blood vessels. The neoplastic cells are polygonal and moderately sized, with clearly demarcated cellular boundaries and a moderate amount of cytoplasm that is clear, or exhibits either globular eosinophilic deposits, or fine fibrillar eosinophilic strands, that are PAS-positive. The nuclei are round and generally centrally placed within the cells, with lightly stippled chromatin and frequently a single prominent basophilic nucleolus. There is one mitosis per ten high power fields (per 2.37 mm^2). There is a moderate degree of anisocytosis and anisokaryosis. There are small foci of necrosis and haemorrhage, comprising less than 5% of the total area of neoplasm in the section examined, and scattered rare neoplastic cells exhibit apoptosis, characterised by shrunken brightly eosinophilic cytoplasm and nuclear pyknosis.

The hepatic parenchyma immediately adjacent to the neoplastic nodules is multifocally

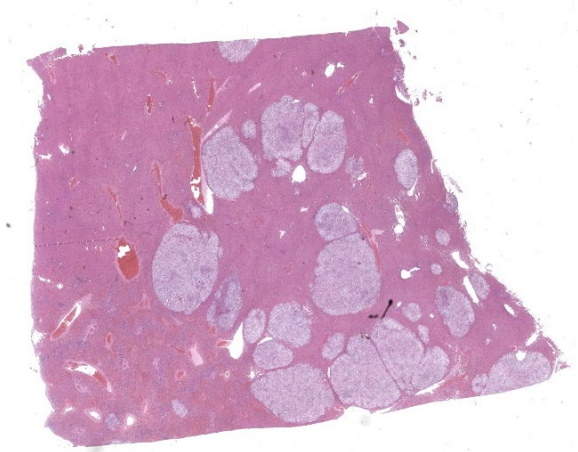

Figure 1-2. Liver, sheep. A multinodular neoplasm is present within the submitted section of liver. (HE, 4X)

compressed. Small numbers of lymphocytes and rarer plasma cells are present multifocally within portal areas, and portal areas also exhibit mild fibrosis multifocally. Deposits of clumped golden-brown granular pigment (haemosiderin) are present multifocally in hepatic Kupffer cells.

Bile duct epithelium exhibits strong positive cytoplasmic immunohistochemical reactivity to cytokeratin 19 (positive internal control tissue). The neoplastic cells do not exhibit positive staining with cytokeratin 19.

Contributor's Morphologic Diagnosis:
Liver: Hepatocellular carcinoma, clear cell variant.

Contributor's Comment:
In this case, a diagnosis of the clear cell variant of hepatocellular carcinoma was made on the basis of the histologic cellular arrangement and morphology. The diagnosis was supported by the macroscopic appearance of the lesion as unencapsulated and infiltrative nodular masses.

Hepatocellular carcinomas have a widely variable histologic appearance due to different cellular arrangements, likely reflecting the degree of differentiation of the neoplastic hepatocytes.[6] Four major histologic variants are described, namely trabecular, pseudoglandular, scirrhous, and solid. As the name suggests, in trabecular hepatocellular carcinomas, the predominant cellular arrangement has a resemblance to normal hepatic trabeculae, although the plates of cells may vary considerably in width, with trabeculae frequently composed of 5-10 cells thickness of neoplastic hepatocytes. In other foci the trabeculae may be quite thin, and this level of variability is a feature that may be helpful in the distinction between well-differentiated trabecular hepatocellular carcinoma and hepatocellular adenoma. In the latter, the trabeculae would be anticipated to be of uniform thickness.[6] In neoplasms exhibiting the pseudoglandular pattern, the neoplastic hepatocytes form rudimentary acini, whereas in the scirrhous form there is dense connective tissue, and aggregates of neoplastic hepatocytes are infiltrated by ductular epithelium.[6] As the name suggests, solid hepatocellular carcinomas are formed by solid sheets of neoplastic hepatocytes. In some variants of this sub-type, the neoplastic hepatocytes exhibit prominent cytoplasmic vacuolation and are described as clear cell hepatocellular carcinomas, as in this case.[6]

Clear cell hepatocellular carcinomas, or hepatocellular carcinomas that exhibit a partial clear cell pattern, have been described in a number of species including dogs,[14] captive fennec foxes,[13] and sheep.[1] It has previously been suggested that the clear cell pattern may be associated with cytoplasmic PAS-positivity and glycogen deposition[1] and PAS-positive cytoplasmic deposits are observed in the case described here.

In cases of hepatocellular carcinoma requiring immunohistochemical diagnostic confirmation, Hep Par-1 can be used to positively identify normal and neoplastic hepatocytes.[6,16] In human medicine, arginase-1 has

been suggested to be a more sensitive and specific marker of hepatocellular differentiation than Hep Par-1 and immunohistochemical staining with arginase-1 may therefore also be of diagnostic utility in veterinary pathology.[4] Normal and neoplastic biliary epithelial cells express cytokeratin 7 and 19, and epithelial membrane antigen. These immunohistochemical staining characteristics can be utilised to distinguish hepatocellular carcinoma from cholangiocellular neoplasms in many cases although some poorly differentiated canine hepatocellular carcinomas may downregulate Hep Par-1 expression and exhibit some cytokeratin 19 immunoreactivity.[6] In our case, cytokeratin 19 was utilised for additional educational confirmation that the neoplastic cells were not of biliary origin.

Fallen stock collection centres, abattoirs and meat packing plants all provide the opportunity to study the prevalence of neoplasms arising in sheep.[1,2,12] Hepatocellular carcinomas appear to be more frequent than cholangiocellular neoplasms and occur in lambs as well as adult animals.[1,2] Extramedullary haematopoiesis has been described as a feature of hepatic tumors in lambs aged less than six months.[1,2] Although considered to be rare in domestic species, hepatoblastomas, believed to arise from hepatic stem/progenitor cells, have also been described in lambs.[5,6] As in other species, the ovine liver is also a site for tumor metastases, lymphoma, and mast cell neoplasms.[8,10,11]

Metastasis has previously been reported in cases of ovine hepatocellular carcinoma.[1] Due to the nature of the abattoir case material described here, a full post mortem examination was not conducted and the presence or absence of metastatic spread to the draining lymph nodes or other viscera could not be determined. The renal lesions were not examined microscopically but the kidneys did not display macroscopic evidence of neoplastic infiltration.

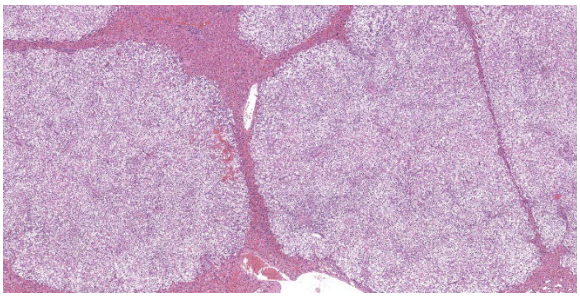

Figure 1-3. Liver sheep. Neoplastic hepatocytes have lost normal sinusoidal architecture and are arranged in sheets. (HE, 44X)

Contributing Institution:
Department of Veterinary Medicine, The Queen's Veterinary School Hospital, University of Cambridge.

Cambridge CB3 0ES, UK.

https://www.vet.cam.ac.uk

JPC Diagnosis:
Liver: Hepatocellular carcinoma, clear cell variant

JPC Comment:
This week's moderator was Dr. Rebecca Smedley of Michigan State University who led participants through a neoplasia-centric conference. This first case was recently published in the *Journal of Comparative Pathology*[9] and we were glad to explore it further.

From subgross, the contrast between neoplastic hepatocytes laden with glycogen (confirmed via PAS) and normal hepatocytes is stark. Several conference participants also noted hyaline inclusions within the cytoplasm of clear cell hepatocytes that were reminiscent of Mallory bodies.[3,7] Mallory bodies (syn. Mallory-Denk Bodies) have been previously noted within hepatocytes of humans (and in some lab animal models) and are composed of a mix of misfolded proteins such as keratins 8 and 18, ubiquitin, and heat shock proteins.[3,7] Associated conditions include alcohol-related steatohepatitis (fatty

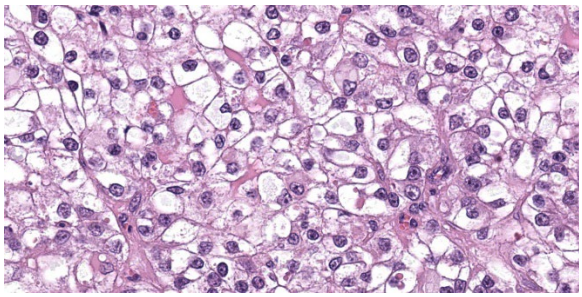

Figure 1-4. Liver sheep. High magnification of neoplastic hepatocytes with cleared eosinophilic cytoplasm. (HE, 627X)

liver disease), copper hepatopathy, and hepatic neoplasms. In human cases of hepatocellular carcinoma, Mallory-Denk bodies are noted in 20-30% of cases, though these should be distinguished from hyaline bodies which are histologically similar, but lack keratin.[7] IHC for P62 and other protein constituents (keratin) can aid in determination. Both elements represent hepatocellular dysfunction and aberrant protein folding.

Given the age (and of course, species) of this animal, we performed a reticulin and copper (rhodanine) stain, which were both negative. Copper-associated hepatopathy may be an inciting cause of HCC in the dog. Additionally, we tried a CK19 and CK7 to characterize hepatocyte and biliary changes as poorly differentiated neoplastic hepatocytes may gain CK19 expression and lose CK7 expression (and may be portend a worsened prognosis), but the stains did not show abnormalities along these lines. .

Finally, Dr. Smedley discussed the potential utility of glypican-3 with the group. As Glypican-3 labels neoplastic hepatocytes but not normal or hyperplastic foci,[15] it would be an invaluable tool in sorting through edge cases where suspect neoplasms also contain rare portal tracts which are typically a distinguishing feature of hepatic hyperplasia. In conjunction with P-glycoprotein or arginase-1 where expression is decreased in neoplastic cells compared with normal hepatocytes, glypican-3 may prove a useful diagnostic tool.

References:

1. Anderson LJ, Sandison AT. Tumors of the liver in cattle, sheep and pigs. *Cancer* 1968;21:289-301.
2. Bundza A, Greig AS, Dukes TW. Primary Hepatocellular Tumors in Animals Killed at Meat Packing Plants: Report of 11 cases. *Can Vet J* 1984;25:82-85.
3. Chelliah, Adeline R, Radhi, Jasim M., Hepatocellular Carcinoma with Prominent Intracytoplasmic Inclusions: A Report of Two Cases, Case Reports in Hepatology, 2016, 2032714.
4. Choi WT, Kakar S. Immunohistochemistry in the Diagnosis of Hepatocellular Carcinoma. *Gastroenterol Clin North Am* 2017;46:311-325.
5. Cotchin E. Spontaneous tumours in young animals. *Proc R Soc Med* 1975;68:653-655.
6. Cullen JM. Tumors of the liver and gallbladder. In: Meuten DM, ed. *Tumors in domestic animals*. 5th ed. John Wiley & Sons, Inc.; 2017.
7. Denk H, Abuja, P.M. & Zatloukal, K. Mallory-Denk bodies and hepatocellular senescence: a causal relationship?. *Virchows Arch* 484, 637–644 (2024).
8. Doige CE. Omasal squamous cell carcinoma in a ewe. *Can J Comp Med* 1983;47:382-384.
9. Hughes K, Radakovic M, Gorman F, Malinowska B, Cullen JM. Immunohistochemical characterization of clear cell variant of hepatocellular carcinoma in a sheep. J Comp Pathol. 2023 Jul;204:47-50.
10. Johnstone AC. Two cases of hepatic mastocytoma in sheep. *Vet Pathol* 1972;9:159-163.
11. Johnstone AC, Manktelow BW. The pathology of spontaneously occurring malignant lymphoma in sheep. *Vet Pathol*

1978;15:301-312.
12. Lovatt FM, Strugnell BW. An observational study involving ewe postmortem examination at a fallen stock collection centre to inform flock health interventions. *Vet Rec* 2013;172:504.
13. Monahan CF, Garner MM, Kiupel M. Hepatocellular Neoplasms in Captive Fennec Foxes (*Vulpes zerda*). *J Zoo Wildl Med* 2018;49:996-1001.
14. Patnaik AK, Hurvitz AI, Lieberman PH, Johnson GF. Canine hepatocellular carcinoma. *Vet Pathol* 1981;18:427-438.
15. Shih TC, Wang L, Wang HC, Wan YY. Glypican-3: A molecular marker for the detection and treatment of hepatocellular carcinoma☆. Liver Res. 2020 Dec;4(4):168-172.
16. Zhang W, Wang Q, Jiang YX, et al. Simultaneous double primary clear cell carcinomas of liver and kidney: a case report and review of literature. *Int J Clin Exp Pathol* 2015;8:995-999.

CASE II:

Signalment:
11 year old, male, Australian shepherd dog, *Canis lupus familiaris*

History:
The dog's owner noted asymmetry of the testes with enlargement of the left testis within a short period of time. After clinical examination, neoplasia of one testis was suspected, and the left testis was removed, and submitted for histopathologic examination. Signs of hormonal imbalance or feminization as well as other clinical symptoms were not observed.

Gross Pathology:
Testis with epididymis (5 x 4 x 3.5 cm) and funiculus spermaticus, testis consisting of approximately 90% neoplastic tissue with a small margin of normal tissue, centrally cystic, multiple confluent whitish and yellowish-brown nodules.

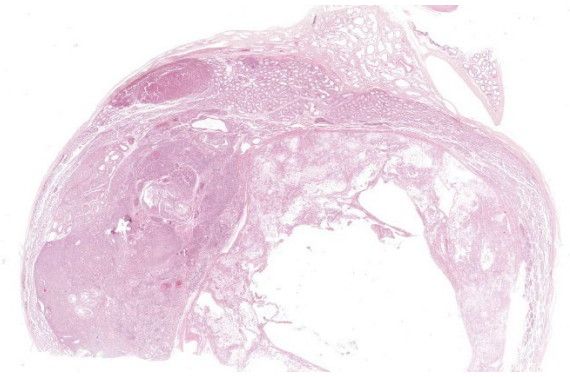

Figure 2-1. Testis, dog. One section of a testis with multiple neoplasms is submitted for examination. (HE, 5X).

Microscopic Description:
Testis: Infiltrating and compressing the testicular parenchyma and extending to one cut edge is a 2 x 1.5 cm large neoplastic proliferation composed of different cells and components. The first cell population consists of polygonal cells with often distinct cell borders, a moderate amount of eosinophilic, granular, or vacuolated cytoplasm (ranging from finely vacuolated to large vacuoles with compression of cytoplasm), and a round nucleus with finely stippled chromatin and an often prominent magenta nucleolus (interstitial cells). There is moderate anisocytosis and anisokaryosis. The mitotic rate is less than 1 per 2.37 mm^2 (10 HPF). These cells form both a 0.5 x 0.3 cm, compact, densely cellular, well-demarcated, partly encapsulated nodule composed of densely packed cords and nest of cells in a fine fibrovascular stroma and a 2 x 1.5 cm moderately cellular, well-demarcated, encapsulated nodule composed of packets and nests of cells with large vacuoles and a central clear space. The second cell population consists of large round cells with distinct cell borders, often a large amount of eosinophilic granular cytoplasm, and large, round, vesicular nuclei with coarse chromatin and 1-2 prominent, magenta nucleoli (germ cells).

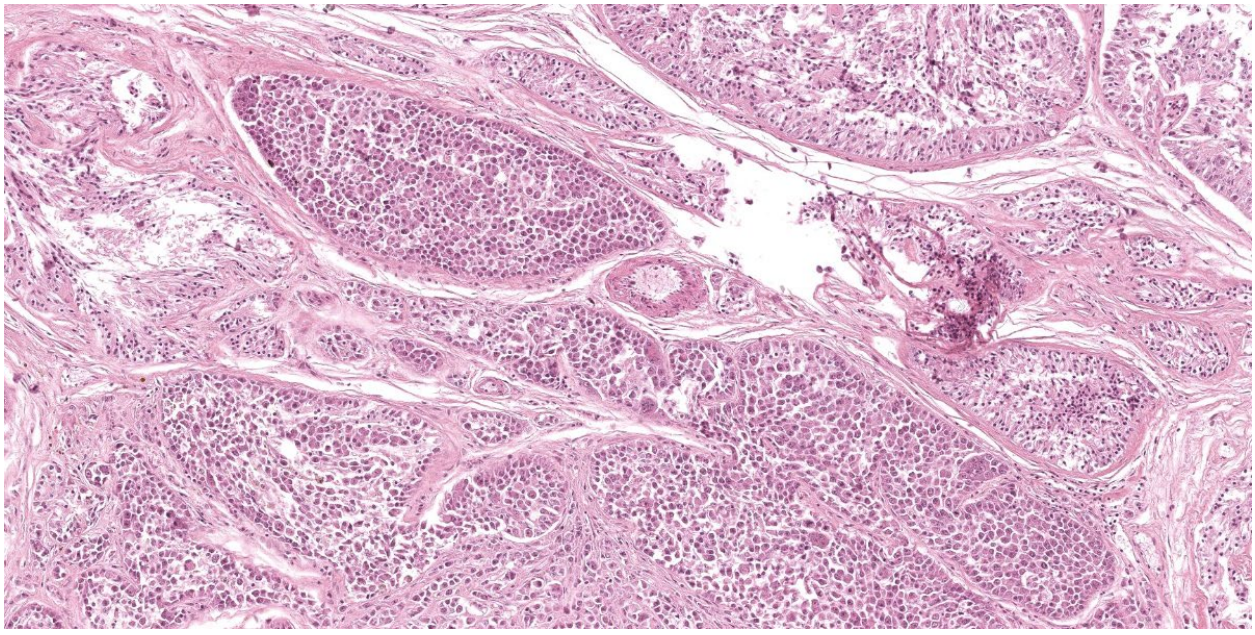

Figure 2-2. Testis, dog. An intratubular neoplasm is present with a mixture of columnar and polygonal cells. (HE, 107X)

There is moderate anisocytosis and anioskaryosis with frequent multinucleated cells, occasionally forming giant cells with up to 8 nuclei. Some macronuclei are visible. The mitotic rate averages 1-3 mitoses per 0.237 mm^2 (1 HPF). These cells are either infiltrate the lumina of the seminiferous tubules, replacing Sertoli and spermatogenic cells, or are intermingled with the third cell population within a 1.5 x 1 cm, moderately cellular, well-demarcated, partly encapsulated, partly infiltrative nodule composed of large nests, packets, and tubules of neoplastic cells, separated and surrounded by bands of fibrous connective tissue. The cells of the second population can sometimes be found within thin walled vessels (vascular invasion). The third population of neoplastic cells is polygonal to columnar with indistinct cell borders , a moderate amount of eosinophilic granular cytoplasm and an oval nucleus with coarsely stippled chromatin and a magenta nucleolus. Palisading of the cells perpendicular to the basement membrane of the seminiferous tubules is frequently noted. The population exhibits mild to moderate anisocytosis and anisokaryosis. The number of mitoses is less than 1 per 2.37 mm^2 (10 HPF). Interspersed within the neoplastic cells are few lymphocytes and plasma cells as well as aggregates of hemosiderin-laden macrophages.

A few seminiferous tubules show remnants of spermatogenesis. Epididymis and Ductus deferens contains single macrophages and no spermatids.

Contributor's Morphologic Diagnosis:
Testis:

1. Interstitial cell tumor in two localizations
2. Seminoma, intratubular multiple and diffuse
3. Mixed germ cell sex-cord stromal tumor (seminoma and Sertoli cell tumor)
4. Atrophy of residual seminiferous tubules

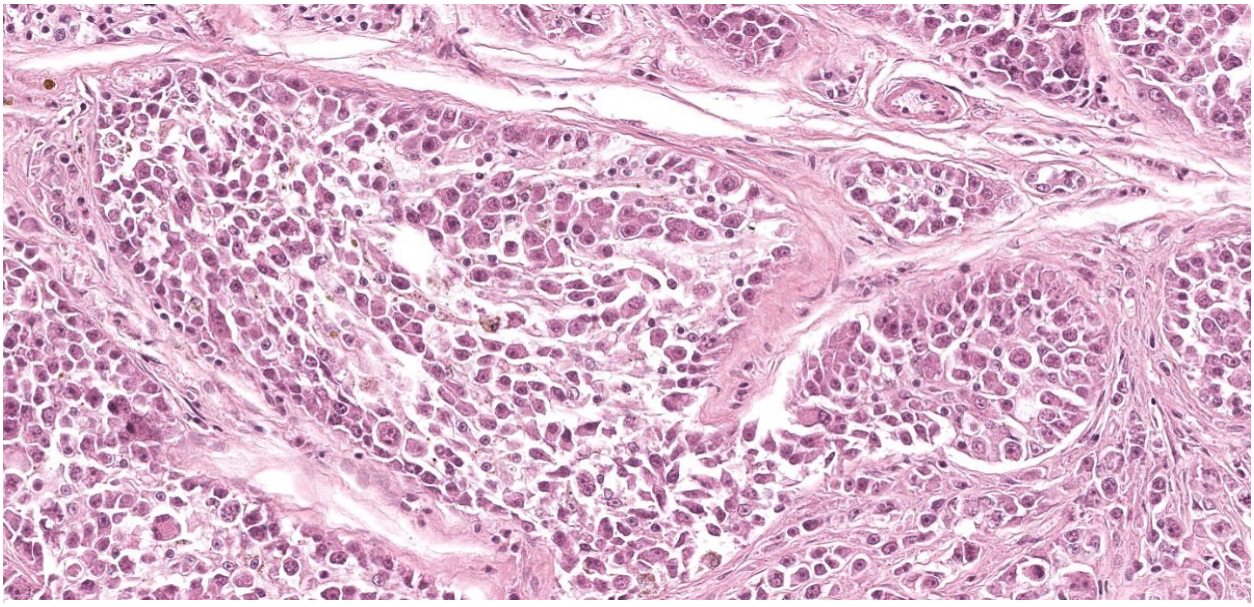

Figure 2-3. Testis, dog. Columnar cells palisade along the basement membrane, while polygonal cells fill the lumen. Nuclear and cytoplasmic features are similar in both populations. (HE, 288X)

Contributor's Comment:

In intact older male dogs, testicular tumors are one of the most common tumors. The cause for that is unknown. Seminoma, Sertoli cell tumors and Leydig cell tumors are found regularly.[1-3] Less common are mixed germ cell sex-cord stromal tumors of the testis,[5] embryonal carcinoma, or teratomas. Adenomas or adenocarcinomas of the rete testis are very rare. Secondary testicular tumors (metastases) are extremely rare.

In dogs, cryptorchism is an important predisposing factor for primary testicular tumors. Several types of neoplasms can be found in one testis,[1,2] so careful examination and lamellation of the testes is obligatory in preparation for histopathology.

The biologic behavior of testicular neoplasms in dogs is often benign, and metastases are rare, with a higher risk in seminomas and Sertoli cell tumors.[3] Leydig cell tumors are considered benign in dogs. Recently, however, two dogs with metastatic Leydig cell tumors have been described. Both cases were found to have a high mitotic count and high Ki-67 index.[4] Unfortunately, there are no other obvious cytologic or histopathologic signs of malignancy in testicular neoplasms in dogs. In malignant cases, distant metastases may be suspected in regional lymph nodes or along the spermatic cord.[1,2]

In the case presented, all three cell types of the common tumor types of canine testicular neoplasms are found, and the examiner must describe the characteristics of the tumor cells and the additional findings in the remaining non-neoplastic tissue. Testicular neoplasms in general and this case in particular are thus very good practice cases for veterinary pathology residents.

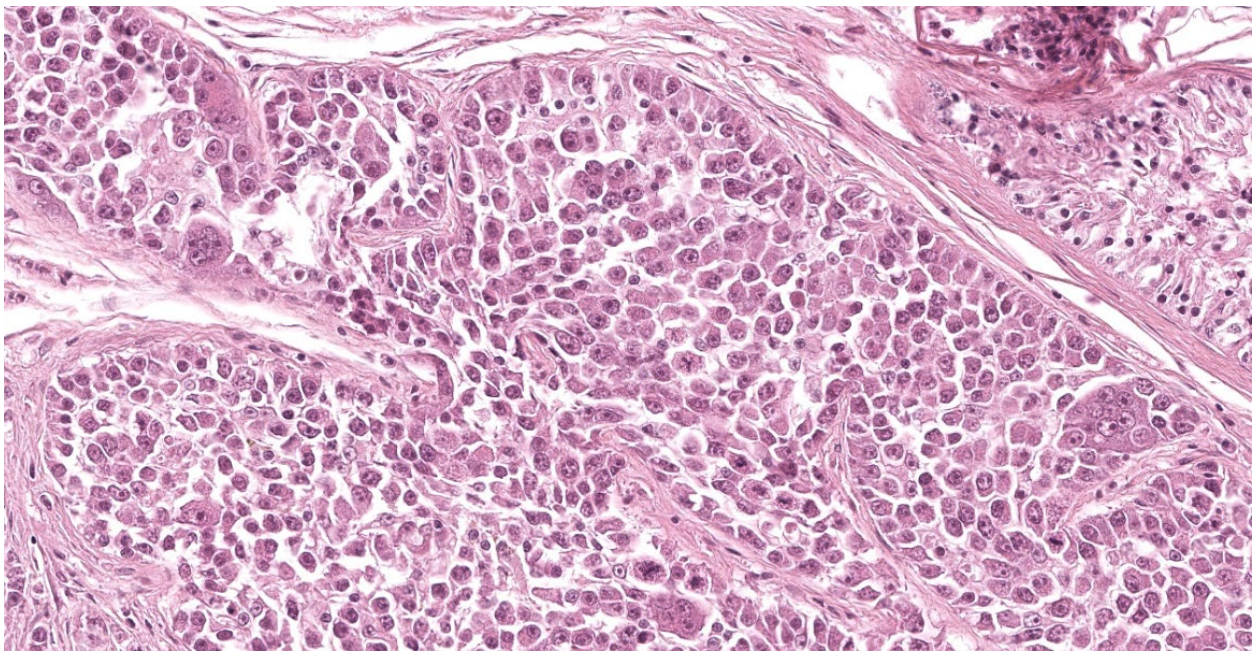

Figure 2-4. Testis, dog. Multinucleated forms are present in both the columnar and polygonal populations. (HE, 286)

Contributing Institution:
Institut fuer Veterinaer-Pathologie, Justus-Liebig-Universitaet Giessen

Frankfurter Str. 96, 35392 Giessen, Germany

http://www.uni-giessen.de/cms/fbz/fb10/institute_klinikum/institute/pathologie

JPC Diagnosis:

1. Testis: Mixed germ cell sex-cord stromal tumor
2. Testis, seminiferous tubules: Atrophy, diffuse, moderate, with aspermatogenesis

JPC Comment:

Case 2 presents an interesting diagnostic challenge in that there are 4 potential separate neoplasms in this neoplasm. Our morphologic diagnoses differed from that of the contributor only academically, in that we were unable to fully resolve what was an individual neoplasm versus a component of the larger mixed germ cell sex-cord stromal tumor. Herein. we simply covered all tumor entities under a single banner of mixed germ cell sex-cord stromal tumor. In addition, and with less spirited discussion, conference participants described degenerative changes to the remaining seminiferous tubules, degeneration of spermatids, and lack of sperm within the epididymis as we.. .

We performed a full workup for this case in conjunction with Dr. Smedley. Within this section of testis, there were 3 separate, distinct morphologies that participants described. Foremost, there was a seminoma-distinct portion that had intratubular round to polygonal cells with radiating chromatin and occasional multinucleation. Second, there was an interstitial (Leydig) cell-distinct portion that was unencapsulated with markedly eosinophilic polygonal cells with vacuolated cytoplasm. Finally, there was an encapsulated, expansile portion that had polygonal cells forming fibrous trabeculae

with palisading that represented an overlap of mrophologies of both Sertoli (sustentacular cells) and seminoma.

Conference participants discussed the nomenclature for this case carefully. Seminomas are a testicular germ cell tumor. Conversely, sex-cord stromal tumors represent neoplasms that exhibit features of either (or both) Sertoli cells and Leydig cells. In cases where seminoma (germ cell) and one or more stromal tumor features are overlapping, a diagnosis of mixed germ cell sex-cord stromal tumor is fitting. In some cases, it may be appropriate to diagnosis multiple discrete tumors within one testis when they are clearly distinct. Conference participants opined that the interstitial cell tumor-distinct population was smaller, separated by normal seminiferous tubules, and was morphologically the most distinct. It is possible that this was a separate tumor that arose earlier that was overshadowed by the emergence of the rapidly growing mixed tumor the contributor describes clinically.

Dr. Smedley emphasized that HE diagnoses alone could be misleading for approximately 20% of canine testicular tumors and that IHC was helpful for resolving exact cell type. We performed IHCs for CD117 (C-kit), desmin,

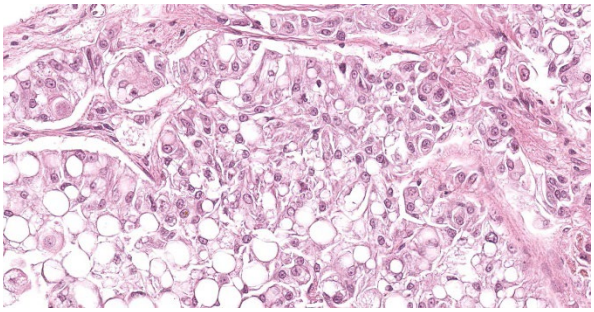

Figure 2-5. Testis, dog. Regionally, neoplastic cells often contain large intracytoplasmic lipid vacuoles. (HE, 381X)

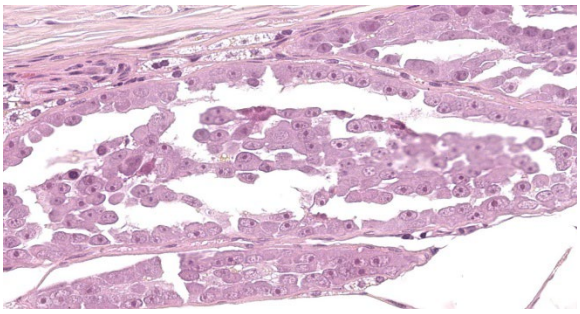

Figure 2-6. Testis dog. A conventional seminoma is present in this section. (HE, 580X)

vimentin, inhibin, LH, Melan-A, and NSE to characterize these neoplasms. Desmin outlined individual seminiferous tubules nicely while interstitial cell tumors were strongly and diffusely reactive for inhibin. GATA-4 is a transcription factor expressed in sex-cord cells, but not germ cells.[6] Dr. Smedley performed this IHC in her laboratory and noted moderate nuclear immunoreactivity within Sertoli cells and select Leydig cells. These patterns confirmed our H&E expectations of this case.

References:

1. Agnew DW, MacLachlan NJ. Tumors of the genital system. In: Meuten DJ, ed. *Tumors in domestic animals*. 5th ed. Ames, IA: Wiley Blackwell; 2017:706-713.
2. Foster RA. Male genital system. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. Philadelphia, PA: Elsevier; 2016; vol. 3:492-497.
3. Kennedy PC, Cullen JM, Edwards JF, Goldschmidt MH, Larsen S, Munson L, Nielsen S. *Histological classification of tumors the genital system of domestic animals*. WHO, Washington, DC: Armed Forces Institute of Pathology; 1998,second series, vol. 4.
4. Kudo T, Kamiie J, Aihara N, Doi M, Sumi A, Omachi T, Shirota K. Malignant

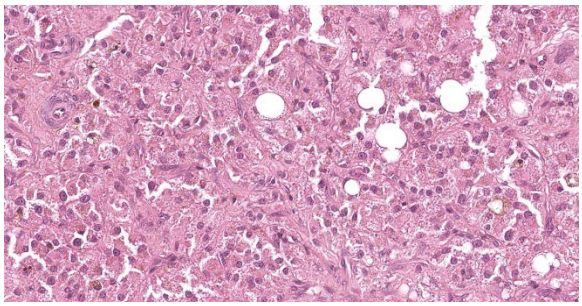

Figure 2-7. Testis, dog. A conventional interstitial cell tumor is present in this section as well. (HE, 363X)

Leydig cell tumor in dogs: two cases and a review of the literature. *J Vet Diagn Invest*. 2019;31(4):557-561.

5. Patnaik AK, Mostofi FK. A clinicopathologic, histologic, and immunohistochemical study of mixed germ cell-stromal tumors of the testis in 16 dogs. *Vet Pathol*. 1993;30:287-295.
6. Ramos-Vara JA, Miller MA. Immunohistochemical evaluation of GATA-4 in canine testicular tumors. *Vet Pathol*. 2009 Sep;46(5):893-6.
7. Reineking W, Seehusen F, Lehmbecker A, Wohlsein P. Predominance of granular cell tumours among testicular tumours of rabbits (Oryctolagus cuniculi dom.). *J Comp Pathol*. 2019;173:24-29.

CASE III:

Signalment:
12-year-old, intact female, dog (*Canis lupus familiaris*)

History:
The dog was submitted to a local veterinary clinic for computed tomography examination. Imaging revealed a tumor of the right adrenal gland measuring 2.5 x 2 cm adjacent to the caudal caval vein. There was no evidence for an intracaval infiltration or a thrombosis of the caval vein but infiltration of the phrenicoabdominal vein was detected. Both ovaries showed multiple cysts. The pre-surgical blood work showed a mildly decreased amount of lymphocytes (0.82, range: 1.05 – 5.1 M/l). Low-dose dexamethasone suppression test revealed inadequately suppressed cortisol secretion with plasma cortisol concentrations of 3.5 µg/dl after three hours, and 3.7 µg/dl after 8 hours of dexamethasone administration, respectively. During surgery, the right adrenal gland including the phrenicoabdominal vein and both ovaries were removed and submitted for histological examination.

Laboratory Results:
The adrenal sample was immunostained using the ABC method with commercially available antibodies specific for chromogranin A, synaptophysin and Melan A. The larger of the two proliferations stained positive for Melan A, the smaller one stained positive for chromogranin A and synaptophysin. The intravascularly detected cellular aggregate also stained positive for Melan A.

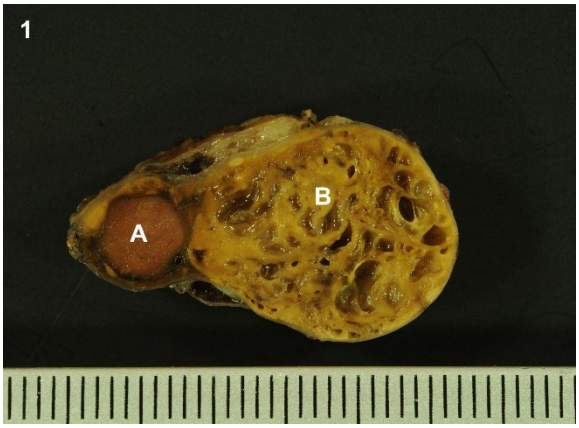

Figure 3-1. Adrenal gland, dog. Two discrete neoplasms are present within the adrenal gland. (*Photo courtesy of:* University of Veterinary Medicine, Hannover , http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

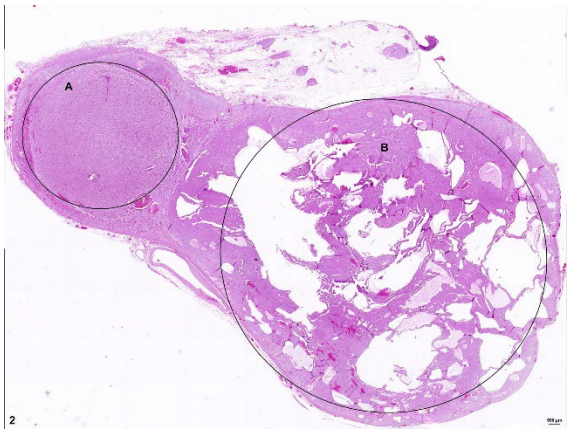

Figure 3-2. Adrenal gland, dog. Both nodules are clearly circumscribed, expansile and separated from each other by normal adrenal tissue (HE, 4X) (*Photo courtesy of:* University of Veterinary Medicine, Hannover , http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

Microscopic Description:

Histologically, two separate neoplastic proliferations are found in the adrenal gland. Both nodules are clearly circumscribed, expansile and separated from each other by normal adrenal tissue (Fig. 2). The larger, more cortically located proliferation measures approximately 1.4 cm in diameter. The nodular, moderately cell-rich, incompletely encapsulated, focally infiltrative mass is characterized by a predominantly solid growth pattern consisting of trabeculae of variable width, partly separated by optically empty or blood-filled spaces (dilated sinusoids). Polygonal, neoplastic cells measure approximately 15 µm in diameter (Fig. 3). The cells show distinct cellular borders and contain a moderate amount of a homogenous eosinophilic, partly highly vacuolated cytoplasm. The centrally located round nucleus displays a moderate amount of heterochromatin and one basophilic nucleolus. There is mild anisocytosis and -karyosis. Mitotic figures are not evident. The cells are accompanied by a scant fibrovascular stroma. Focally, a large aggregate of these tumor cells is present in a blood vessel (*angiosis carcinomatosa*) (Fig. 4).

The smaller nodule, located within the medullary area of adrenal gland measures approximately 0.5 cm in diameter. The well demarcated encapsulated neoplastic mass shows predominantly expansile growth of small polygonal cells measuring approximately 10-12 µm in diameter. Cells are arranged in small lobules and packages separated by thin, fibrous septa (neuroendocrine packaging; Fig. 5). Neoplastic cells are characterized by distinct cell borders, a moderate amount of fine granular, brightly eosinophilic cytoplasm, and a slightly eccentrically located, round, moderately heterochromatin-rich nucleus with up to one distinct nucleolus. There is a moderate anisocytosis and –karyosis. Multifocally, cells measuring up to 30 µm in size with karyomegaly are present. Up to 2 mitotic figures are found per high power field (400x).

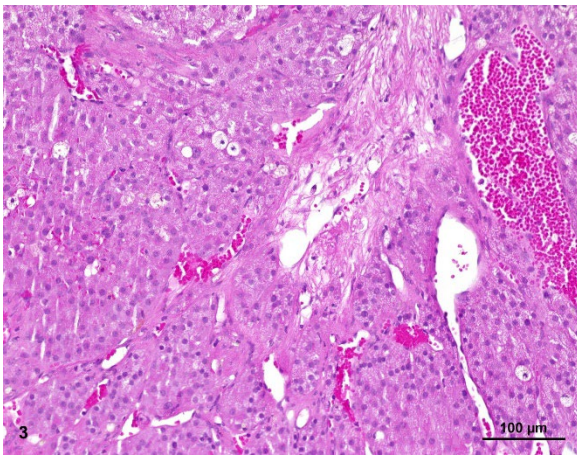

Figure 3-3. Adrenal gland, dog. The larger cortical nodule is composed of trabeculae of heavily vacuolated polygonal adrenocortical cells. (HE, 200X) (*Photo courtesy of:* University of Veterinary Medicine, Hannover , http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

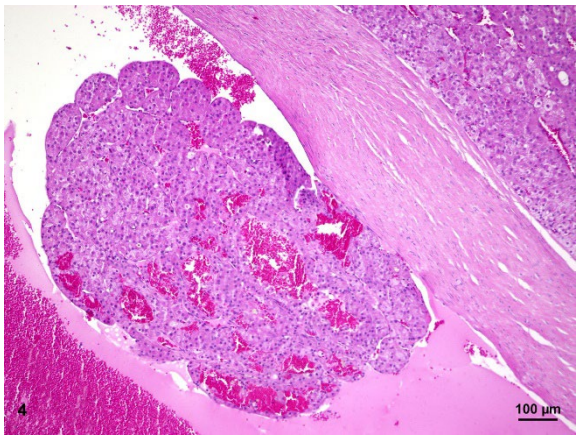

Figure 3-4. Adrenal gland, dog. A cluster of neoplastic cells from the larger nodules is present within a vessel. (HE, 400X) (*Photo courtesy of:* University of Veterinary Medicine, Hannover , http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

Contributor's Morphologic Diagnosis:

Adrenal gland: adrenocortical carcinoma with *angiosis carcinomatosa* and pheochromocytoma, canine.

Contributor's Comment:

The submitted case represents an unusual double lesion of the adrenal gland consisting of concurrent emergence of an adrenocortical carcinoma and a pheochromocytoma. Interestingly, when the dog was first presented to the clinicians, the animal did not show any obvious clinical signs of an altered adrenal function indicating a functional cortical or medullary neoplasia (excessive cortisol and/or catecholamine secretion).

Adrenocortical carcinomas occur in cattle, cats and elderly dogs and are also infrequently described in other species.[12,13] They tend to be larger than adenomas and bilateral occurrence is observed more frequently.[10,12,13] Affecting the zona fasciculata as well as the zona reticularis,[2] they often exhibit an infiltrative growth pattern into the adjacent tissue including the caudal caval vein, where large tumor-cell aggregations can form.[5,12,13]

Adrenocortical tumors can be found in 10-20% of dogs with Cushing syndrome,[6] and severe atrophy of the contralateral adrenal gland occurs in functional neoplasms due to a negative endocrine feedback loop.[12,13] It is estimated that most of the occurring adrenocortical tumors in dogs (up to 80%) are adrenocortical carcinomas.[4] Common sites for metastases are liver and lung.[12,13] The functional variants (endocrinologically active) of adrenocortical tumors usually secrete a massive amount of cortisol causing hyperadrenocorticism.[2,6,11] Clinical signs associated with functional adrenocortical tumors are polydipsia and polyuria, pendulous abdomen, polyphagia, muscle weakness, hypokalemia, hypertension, atrophic dermatosis with thin skin and hair loss as well as bilateral, symmetrical hypotrichosis or alopecia, hyperpigmentation scaling, comedones, and calcinosis cutis.[4,9,10,11,14]

Neoplastic cells of adrenocortical tumors typically show a specific immunoreactivity with antibodies targeting Melan-A, α-inhibin, vimentin, neuron specific enolase. Expression of synaptophysin may be variable.[5,12,13] The larger proliferation in the presented case as well as the intravascularly located neoplastic cells stained immunopositive for Melan-A which proves the presence of an adrenal cortical carcinoma.

Pheochromocytomas represent the most common tumor of the adrenal medulla and mostly occur in older dogs with no gender or breed predisposition. They can also be found in cattle and horses,[12,13] and are described in humans too.[1,3,8] They represent catecholamine-secreting tumors of the neuroectoderm-derived, chromaffin cells of the adrenal medulla or sympathetic ganglia, also termed sympathetic paragangliomas. They are composed of epinephrine-secreting

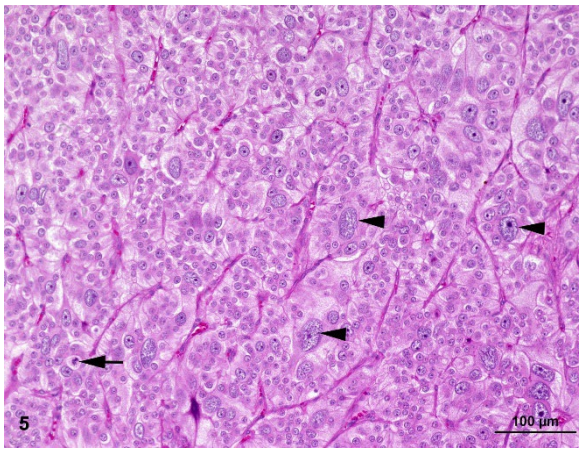

Figure 3-5. Adrenal gland, dog. There is nuclear gigantism (arrows) and occasional mitoses within the second, smaller, medullary neoplasm. (HE, 400X) (*Photo courtesy of:* University of Veterinary Medicine, Hannover, http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

cells, norepinephrine-secreting cells or both and occur mostly unilaterally and vary greatly in size.[1,3,7,12,15] These tumors often go along with clinical signs such as tachycardia, edema, panting, cardiac hypertrophy, hypertension, anorexia, lethargy, ascites, polyuria and polydipsia as well as diarrhea and vomiting resulting from excessive secretion of catecholamine.[3,7,12,13,15] In severe cases, the term 'pheochromocytoma crisis' is used to describe a serious complication characterized by obtundation, shock, disseminated intravascular coagulation, seizures, rhabdomyolysis and acute renal failure.[1] Malignancy of these tumors is assumed when invasion of the adrenal capsule and/or the caudal vena cava and the aorta or the surrounding tissue with potential metastases in distant organs is present.[1,5,12,13,15] Common sites for metastasis are adjacent lymph nodes, kidney, liver, spleen, lung and bone.[1,3,15] The pheochromocytoma of the presented case does not clearly show capsular invasion and/or an infiltrative growth pattern. However, especially the increased mitotic rate as well as the marked amount of pleomorphic cells can be interpreted as indications of a possible precancerous transformation.

During necropsy, a pheochromocytoma may present itself as a large mass occupying the entire adrenal gland, which is partly surrounded by a thin, compressed rim of adrenal cortex, sometimes forming small nodules.[15] Small pheochromocytomas remain confined to the adrenal medulla and are well encapsulated. The tissue shows light brown to yellow-red coloring emerging from hemorrhage and necrosis.

Immunohistological expression of chromogranin A and synaptophysin can be used to confirm the diagnosis.[1,5,12,13] In this case, the neoplasm stained immunopositive for chromogranin A and synaptophysin which confirms a pheochromocytoma in this case.

Contributing Institution:
Department of Pathology, University of Veterinary Medicine, Hannover, Buenteweg 17, 30559 Hannover, Germany.

http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/

JPC Diagnosis:

1. Adrenal gland, cortex: Adrenocortical adenoma
2. Adrenal gland, medulla: Pheochromocytoma.

JPC Comment:
This third case is another 'two-fer' case that the contributor has skillfully presented.

We agree with the contributor that the smaller neoplasm best represents a pheochromocytoma, although we differed from the contributor on the malignancy of the larger adrenocortical neoplasm Conference participants were somewhat split, with the lack of

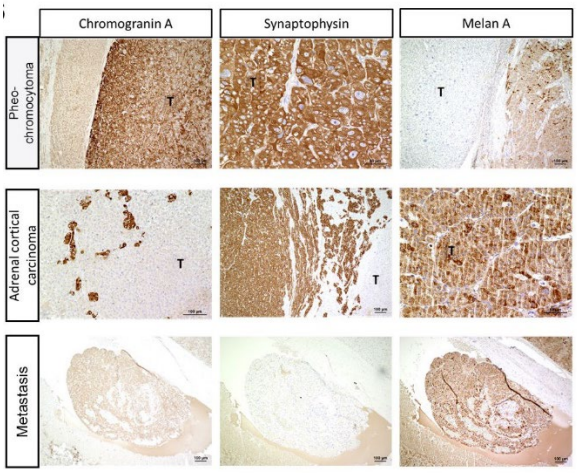

Figure 3-6. Adrenal gland, dog. Immunostaining of the two adrenal neoplasms and the intravascular cluster of neoplastic cells. (*Photo courtesy of:* University of Veterinary Medicine, Hannover , http://www.tiho-hannover.de/kliniken-institute/institute/institut-fuer-pathologie/)

nuclear atypia and well-demarcated nature of this neoplasm as helpful features supportive of an adenoma interpretation. Conference discussion also focused on whether lymphovascular invasion was present. We carefully reviewed the contributor's supplied image and the accompanying feature of the case slide of a large cluster of adrenocortical cells within the lumen of a blood vessel. In line with Meuten et al,[9] we felt that this was more likely to be pseudo-vascular invasion due to tissue sectioning and/or processing.

We performed a number of IHCs for this case to support these diagnoses with one surprise. Chromogranin and synaptophysin are both neuroendocrine markers that label pheochromocytomas, but are not specific *per se* as they do label other neuroendocrine and ganglion cell tumors. Dr Smedley graciously ran a met-enkephalin IHC at MSU for this case as both normal and neoplastic chromaffin cells of the adrenal medulla should react strongly. In this case, both chromogranin and synaptophysin were strongly and diffusely immunoreactive within this smaller neoplasm as well as highlighting the remnant portion of the adrenal medulla. Curiously, met-enkephalin labeled the adrenal medulla, but *not* the neoplasm, an odd finding which was new to Dr. Smedley (we do not have this immunomarker available to us at the JPC). . Although we report this neoplasm as being consistent with a pheochromocytoma, we cannot exclude the remote possibility of a metastasis of a neuroendocrine tumor to the adrenal gland. Curiously, the nuclear features of the pheochromocytooma displayed greater atypia and mitotic rate than is typical for pheochromocytomas.

References:

1. Barthez PY, Marks SL, Woo J, et al. Pheochromocytoma in dogs: 61 cases (1984-1995). *J Vet Intern Med*. 1997;11:272–278.
2. Bielinska M, Parviainen H, Kiiveri S, et al. Review paper: origin and molecular pathology of adrenocortical neoplasms. *Vet Pathol*. 2009;46:194–210.
3. Gilson SD, Withrow SJ, Wheeler SL, et al. Pheochromocytoma in 50 dogs. *J Vet Intern Med.* 1994;8: 228–232.
4. Goiska-Zygner O, Lechowski R, Wojciech Z. Functioning unilateral adrenocortical carcinoma in a dog. *Can Vet J*. 2012;53:623–625.
5. Kiupel M. Histological Classification of Tumors of the Endocrine System of Domestic Animals, vol. 12. Washington, DC: Armed Forces Institute of Pathology; 2008:44–47.
6. Labelle P, Kyles AE, Farver TB, et al. Indicators of malignancy of canine adrenocortical tumors: histopathology and proliferation index. *Vet Pathol.* 2004;41:490–497.
7. Machida T, Machida N. Invasion of pheochromocytoma from the caudal vena cava to the right ventricular cavity in a dog. Case Rep Vet Med. 2020; Feb

11;2020:5382687.doi: 10.1155/2020/5382687.
8. Mauldin EA, Peters-Kennedy J. Integumentary system In: Maxie G, ed. *Jubb, Kennedy and Palmer`s Pathology of Domestic Animals*. 6th ed. Vol. 1. St. Louis: Elsevier, 2016:509–736.
9. Meuten DJ, Moore FM, Donovan TA et al. International Guidelines for Veterinary Tumor Pathology: A Call to Action. Vet Pathol. 2021 Sep;58(5):766-794.
10. Nabeta R, Osada H, Ogawa M, et al. Clinical and pathological features and outcome of bilateral incidental adrenocortical carcinomas in a dog. *J Vet Med Sci.* 2017;79: 1489–1493.
11. Reusch CE, Feldman EC. Canine hyperadrenocorticism due to adrenocortical neoplasia. Pretreatment evaluation of 41 dogs. *J Vet Intern Med.* 1991;5: 3–10.
12. Rosol T, Gröne A. Endocrine Glands. In: Maxie G, ed. *Jubb, Kennedy and Palmer`s Pathology of Domestic Animals*. 6th ed. Vol. 3. St. Louis: Elsevier, 2016:326–428.
13. Rosol TJ, Meuten. Tumors of the endocrine glands. In: Meuten DJ, ed., Tumors in Domestic Animals: 6th ed., Iowa: Wiley & Sons; 2016:766–833.
14. Taylor J, Lee M, Nicholson M, et al. Functional ectopic adrenal carcinoma. *Can Vet J.* 2014; 55:845–848.
15. Zini E, Nolli S, Ferri F, et al. Pheochromocytoma in dogs undergoing adrenalectomy. *Vet Pathol.* 2019;56:358–368.

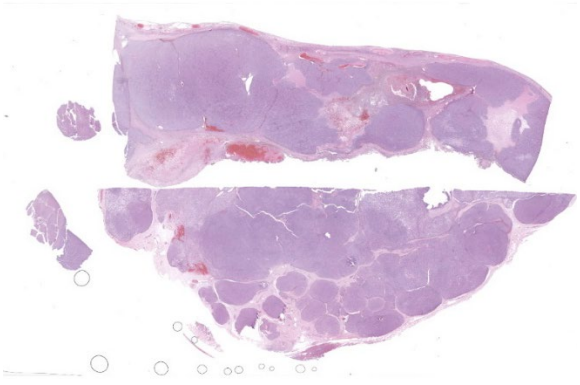

Figure 4-1. Spleen, dog. Two sections of spleen are submitted for examination, and both are effaced by a nodular neoplasm. (HE, 5X)

CASE IV:

Signalment:
3-year-old female canine, Pit Bull mix

History:
Prior to an ovariohysterectomy, the patient was reported to have a 4-month history of unexplained weight loss. Preoperative examination revealed a large intra-abdominal mass suspected to be associated with the spleen or liver and a subcutaneous perivulvar mass. An enlarged, cavitated spleen, a small mass noted on liver, and perivulvar subcutaneous mass were submitted for histopathology.

Gross Pathology:
Approximately 80% of splenic parenchyma was severely enlarged with multiple white to tan, irregular, soft coalescing nodules and necrotic cavitated areas. The liver had a focal, less than 0.5cm in diameter, tan irregular nodule. The perivulvar subcutis mass was tubular, tan, and soft and approximately 4-5cm in diameter.

Laboratory Results:
Cytology identified a spindloid neoplastic population with microvesiculated cytoplasm

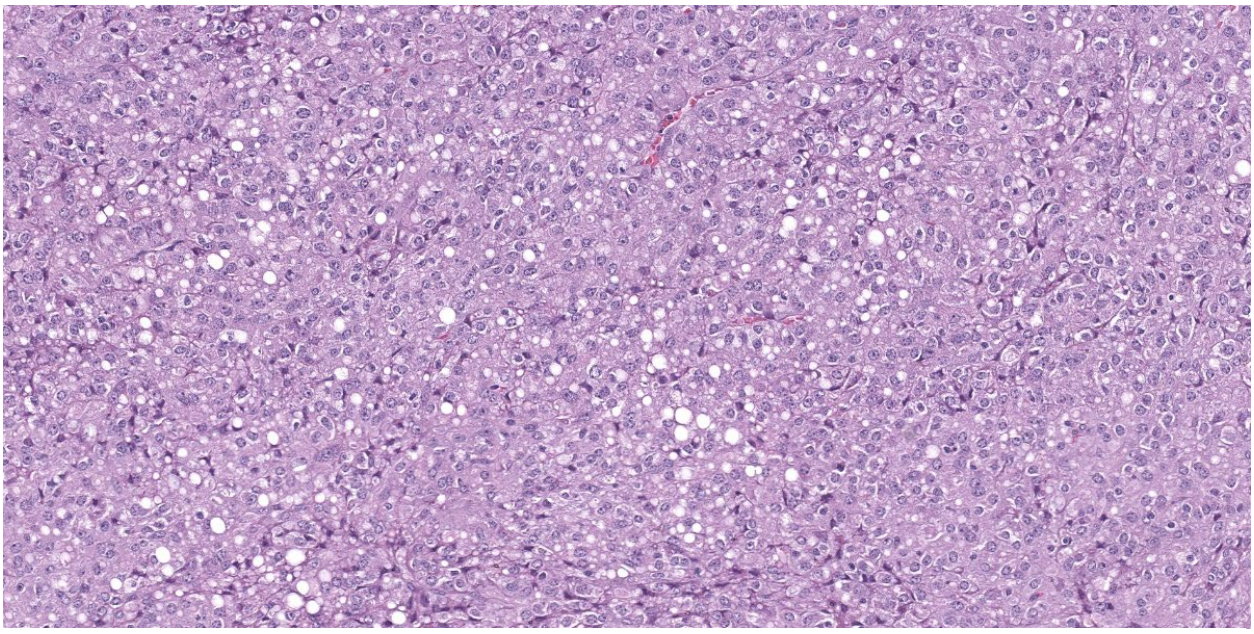

Figure 4-2. Spleen, dog. The neoplasm is composed of sheets of vacuolated spindled to polygonal cells. (HE, 259X)

in both the perivulvar subcutaneous mass and rare to occasionally in the splenic mass. These microvesiculated neoplastic cells were reminiscent of liposarcoma, but an epithelioid neoplasm could not be excluded.

Microscopic Description:
Splenic mass: Splenic parenchyma is expanded and replaced by multifocal to coalescing, poorly demarcated nodules of a moderately cellular neoplasm composed of pleomorphic cells arranged in nests and bundles separated by fine to moderate fibrovascular stroma. Neoplastic cells have indistinct cellular borders, moderate amounts of amphophilic cytoplasm that frequently contain variably sized clear discrete vacuoles (lipid), with one round to ovoid nuclei, with coarse chromatin and 1-3 discrete nucleoli. There is moderate anisocytosis and anisokaryosis with 5 mitotic figures in 10 high power fields ($2.37mm^2$). There are multifocal areas of necrosis with fibrin and hemorrhage, multinucleated giant cells, macrophages with intracytoplasmic dark brown pigment or yellow granular pigment (heme). Remaining spleen is congested with increased plasma cells.

Hepatic mass, incisional biopsy. Replacing approximately 50% of section is an unencapsulated, moderately well demarcated, moderately cellular neoplasm of similar neoplastic cells as described in spleen. Sometimes, there are intracytoplasmic pink globules within cytoplasm of neoplastic cells.

Perivulvar subcutaneous mass: Mass is composed of multiple coalescing nodules of previously described neoplastic cells in spleen separated by moderate amounts of mature collagen interrupted by macrophages laden with brown globular pigment (hemosiderin), lymphocytes, plasma cells, and mineral. There is electrocautery artifact along periphery.

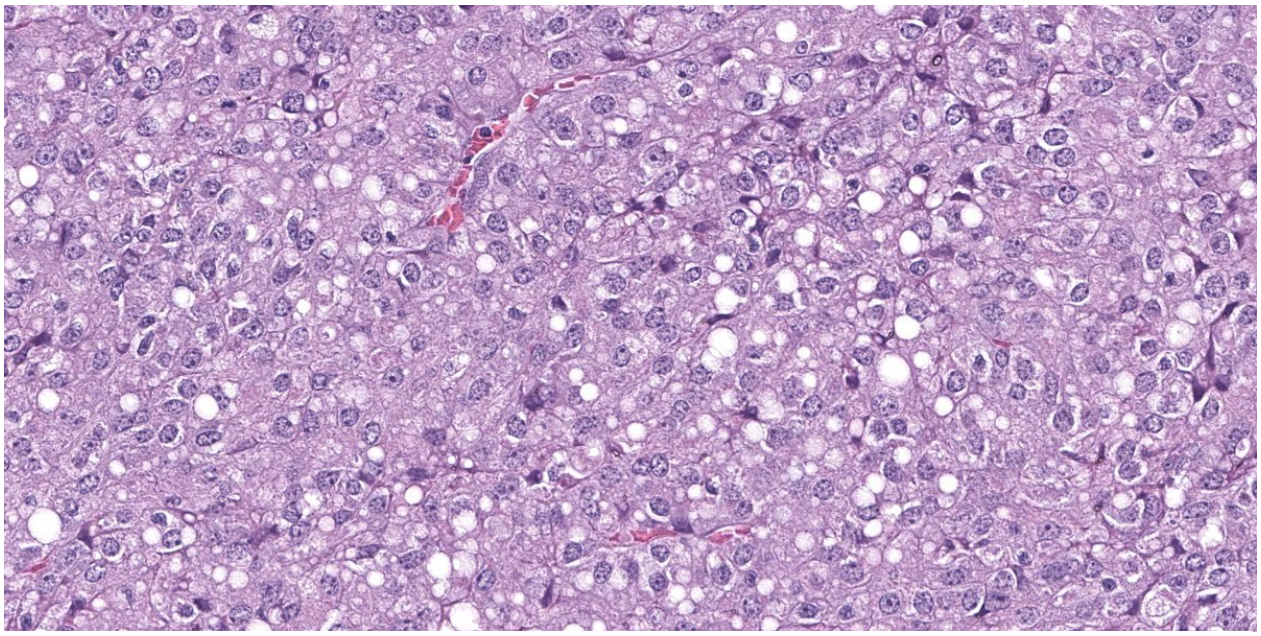

Figure 4-3. Spleen, dog. High magnification of neoplastic cells. Neoplastic cells contain discrete cytoplasmic vacuoles which often coalesce into a single vacuole. (HE, 381X)

Immunohistochemistry/Special Stains:
Hepatic, perivular, and splenic masses are examined and have the same results as below.

1. Cytokeratin: Neoplastic cells do not stain positively.
2. Vimentin: Neoplastic cells have positive cytoplasmic staining.
3. Melan-A: Neoplastic cells exhibit weak cytoplasmic staining which are occasionally stained strongly in cytoplasm. No membranous staining is seen in the neoplastic cells.
4. PNL-2: Approximately 10% of neoplastic cells exhibit cytoplasmic, occasionally membranous staining.
5. CD18: Neoplastic cells do not stain positively.
6. S-100: Neoplastic cells do not stain positively.
7. Synaptophysin: Neoplastic cells do not stain positively.
8. Chromogranin A: The cytoplasm of neoplastic cells diffusely are stained weakly which are considered negative.
9. Oil Red O: Neoplastic cells and vacuoles have positive intracytoplasmic staining.

Contributor's Morphologic Diagnosis:
Spleen: liposarcoma with metastasis to perivulvar subcutis and liver

Contributor's Comment:
Liposarcoma is an uncommon mesenchymal tumor originating from lipoblasts and lipocytes. More commonly, liposarcomas are a subtype of soft tissue sarcomas associated with the skin and subcutaneous tissues. Typically, locally invasive with low metastatic rates, liposarcomas in humans are classified by the World Health Organization into four histological subtypes: well differentiated liposarcoma/atypical lipomatous tumor, dedifferentiated liposarcoma, myxoid/round

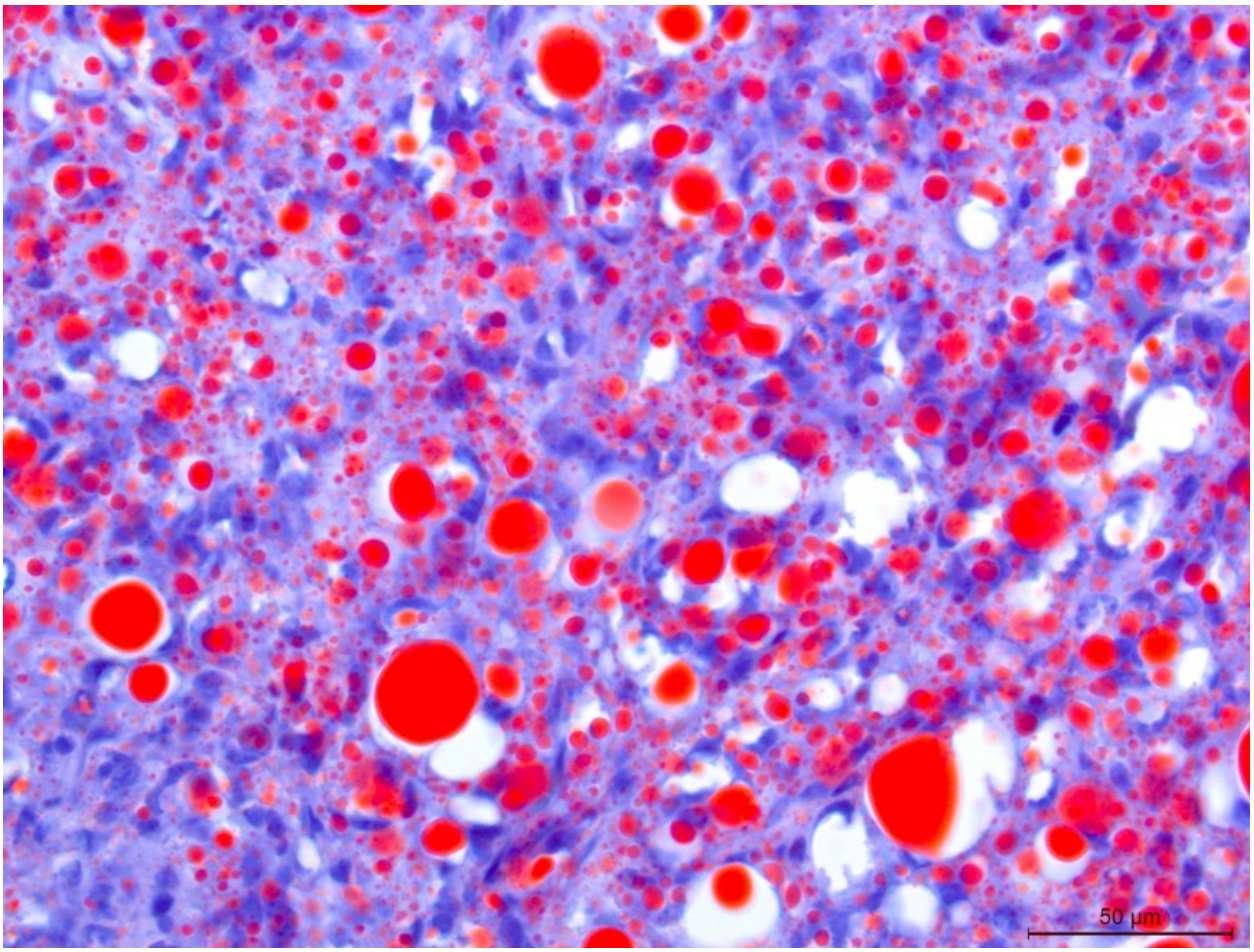

Figure 4-4. Spleen, dog. Cytoplasmic vacuoles stain strongly positive for Oil Red O.(Oil red 0, 400X) (Photo courtesy of: Midwestern University, Glendale, AZ https://clinics.midwestern.edu/animal-health-institute/diagnostic-pathology-center)

cell liposarcoma, and pleomorphic liposarcoma.[1] In domestic animals, there are three subtypes of liposarcomas: well-differentiated, pleomorphic, and myxoid.[4] In well differentiated liposarcomas, the majority of neoplastic cells have intracytoplasmic clear vacuoles.[4] Dedifferentiated liposarcomas arise in association with well differentiated liposarcomas and can have morphologic overlap with pleomorphic liposarcomas that may be multinucleated and irregular with a few to rare neoplastic cells containing intracytoplasmic lipid vacuoles. [4] Myxoid liposarcomas are distinguished by an abundant myxomatous matrix.[4]

Visceral liposarcomas associated with the spleen are a subtype of non-angiomatous non-lymphomatous mesenchymal neoplasm with higher metastatic potential.[3] Non-angiomatous non-lymphomatous mesenchymal neoplasms constitute 23-34% of primary splenic neoplasms and include fibrosarcoma, leiomyosarcoma, undifferentiated sarcoma, and liposarcoma.[2] A study analyzing the outcome of 32 cases of splenic stromal sarcomas in dogs, identified that a mitotic count exceeding 9 mitoses per 10 high power fields ($2.37mm^2$) to be a significant predictor of metastasis.[2] While liver metastases have been reported with splenic liposarcoma, metastases

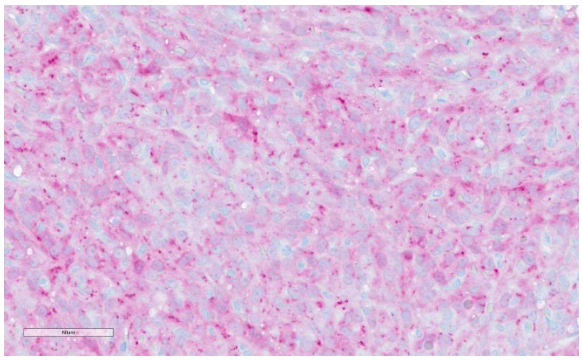

Figure 4-5. Spleen, dog. Neoplastic cells regionally demonstrate strong cytoplasmic immunopositivity for Melan A. (Melan A, 400X)

to subcutis have not been reported.[5]

In this case, given the similarity between neoplastic cells in the liver, subcutis, spleen, and the significantly larger neoplastic mass within spleen, it is favored that the splenic liposarcoma metastasized to both the liver and subcutis. Alternatively, it is possible the subcutaneous liposarcoma and spleen occurred independently. Metastases of sarcomas to subcutaneous tissues is extremely rare in all species. A retrospective study performed in human medicine found less than 0.25% of patients with sarcomas had cutaneous metastases.[10] Leiomyosarcomas were documented to be the most common mesenchymal tumor to metastasize to the skin.[10]

Generally, splenic sarcomas require additional immunohistochemistry or special stains for a definitive diagnosis. Specifically, particularly with a pleomorphic subtype of liposarcoma, Oil Red O is an invaluable stain for lipid within neoplastic cells.[9] Oil Red O is typically performed on frozen tissue sections, though there is a protocol for staining formalin fixed sections. Another lipid stain performed on frozen tissue is Sudan black.[9] Other immunohistochemical stains that may aid in favor of liposarcoma include S-100 and perilipin.[8] In human liposarcomas, immunohistochemical markers include MDM2 (murine double minute 2) and CDK4 (cyclin dependent kinase 4) that have also been used in subtype classification.[5]

Initially, this case was tentatively diagnosed as a melanoma given the positive immunoreactivity to Melan-A. However, given the predominantly negative immunoreactivity to PNL2, this diagnosis was seemingly refuted. In general, PNL2 is more sensitive than Melan-A with less cross reactivity to nonmelanocytic neoplasms.[6]

Contributing Institution:
https://clinics.midwestern.edu/animal-health-institute/diagnostic-pathology-center

JPC Diagnosis:
Spleen (per contributor) and liver: Malignant neoplasm

JPC Comment:
The final case for this conference is a story *within* a story! When we first received this case, we considered the contributor's diagnosis of liposarcoma based on both the gross and H&E features which were certainly suggestive. Although we ultimately reached a different final diagnosis in conference, the case was chosen as liposarcoma is not a common WSC submission.

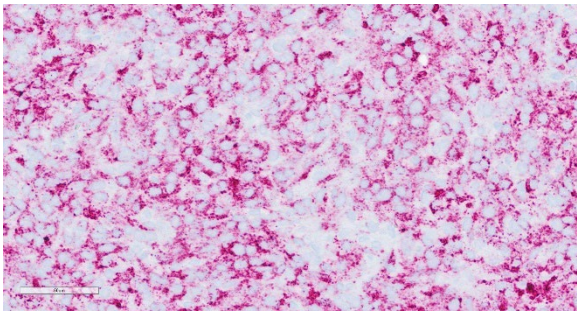

Figure 4-6. Spleen, dog. Neoplastic cells regionally demonstrate strong cytoplasmic immunopositivity for PNL2. (PNL2, 400X)

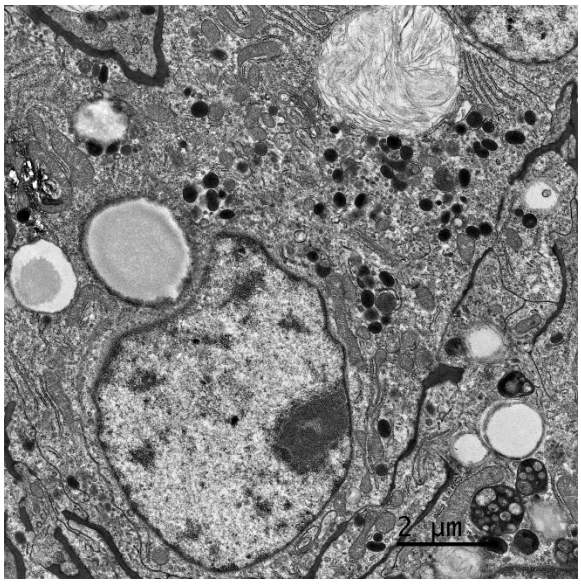

Figure 4-7. Spleen, dog. Ultrastructurally, neoplastic cells contain lipid droplets and clustered melanosomes in their cytoplasm. (Photo courtesy of: Midwestern University, Glendale, AZ https://clinics.midwestern.edu/animal-health-institute/diagnostic-pathology-center)

In prepping this case for conference, we noted that neoplastic cells were reactive for both Melan-A and PNL2 in our lab which did not fit with a liposarcoma. By chance, the contributor also consulted with Dr. Smedley on this case who repeated a melanocytic cocktail (Melan-A, PNL2, TRP-1, TRP-2) and performed a SOX10. The cocktail did label a proportion of cells (and we were cautioned by Dr. Smedley that these particular stains usually only stain a portion of the neoplastic cells. The SOX-10 was negative (which is unusual for melanomas, but the positive cells on the melanoma cocktail was de facie evidence that the tumor was indeed a melanoma.) As a result of these finding the contributor submitted this case to a third institution for transmission electronic microscopy where rare melanosomes within neoplastic cells. Given that Dr. Smedley, a renowned authority of canine melanoma) was on the WSC schedule this year, we knew that we couldn't pass up an opportunity to revisit this case and the superficially conflicting results.

Dr. Smedley focused on differentiating this case from a poorly differentiated sarcoma. Oil Red O in this case highlighted lipid-rich portions of the neoplasm. Lipid within neoplastic cells was also noted on EM. While seemingly an odd finding, the "balloon cell" subtype of melanoma may contain lipid, glycogen, resulting in the cleared swollen cytoplasm that gives this tumor its name. . Lastly, conference participants also considered sebaceous carcinoma as another rule out for lipid-rich tumors.

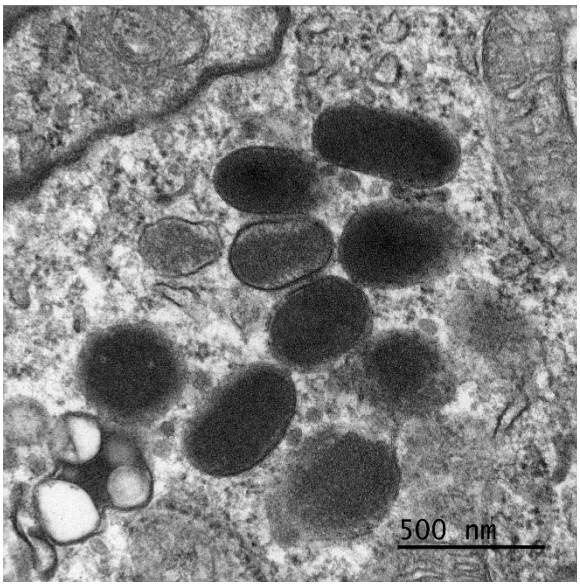

Figure 4-8. Spleen, dog. Higher magnification of melanosomes within the cytoplasm. (Photo courtesy of: Midwestern University, Glendale, AZ https://clinics.midwestern.edu/animal-health-institute/diagnostic-pathology-center)

References:

1. Amer KM, Congiusta DV, Thomson JE et al. Epidemiology and survival of liposarcoma and its subtypes: A dual database analysis. *J Clin Orthop Trauma*. 2020 Jul;11(Suppl 4):S479-S484.
2. Ferrari R, Marconato L, Boracchi P et al. Splenic stromal sarcomas in dogs: Outcome and clinicopathological prognostic factors in 32 cases. *Vet Comp Oncol*. 2024 Mar;22(1):12-21.
3. Gower KL, Liptak JM, Culp WT, Bravo L, Powers B, Withrow SJ. Splenic liposarcoma in dogs: 13 cases (2002-2012). *J Am Vet Med Assoc*. 2015 Dec 15;247(12):1404-7.
4. Meuten DJ, Hendrick M. Mesenchymal Tumors of the Skin and Soft Tissues. In: Meuten DJ, ed: *Tumors in Domestic Animals.* 5th edition. Ames, IO: John Wiley & Sons, Inc. 2017; 640.
5. Nishikawa G, Minamiguchi S, Hata H et al. Dedifferentiated liposarcoma involving the spleen and splenic hilum: a report of a case with a rare growth pattern. *Int Surg*. 2015 Jan;100(1):128-32.
6. Ramos-Vara JA, Miller MA. Immunohistochemical identification of canine melanocytic neoplasms with antibodies to melanocytic antigen PNL2 and tyrosinase: comparison with Melan A. *Vet Pathol*. 2011 Mar;48(2):443-50.
7. Saik JE, Diters RW, Wortman JA. Metastasis of a well-differentiated liposarcoma in a dog and a note on nomenclature of fatty tumours. *J Comp Pathol*. 1987 May;97(3):369-73.
8. Straub BK, Witzel HR, Pawella LM et al. Perilipin 1 Expression Differentiates Liposarcoma from Other Types of Soft Tissue Sarcoma. *Am J Pathol*. 2019 Aug;189(8):1547-1558.
9. Tracy RE, Walia P. A method to fix lipids for staining fat embolism in paraffin sections. *Histopathology*. 2002 Jul;41(1):75-9.
10. Wang WL, Bones-Valentin RA, Prieto VG et al. Sarcoma metastases to the skin. *Cancer*. 2012;118: 2900-2904.