Joint Pathology Center
Veterinary Pathology Services

WEDNESDAY SLIDE CONFERENCE 2023-2024

Conference #21 03 April 2024

CASE I:

Signalment:
11-year-old, intact male German Shepherd Dog (*Canis familiaris*)

History:
This patient presented to the Emergency Service for evaluation of a cough of 1-week duration that was unresponsive to Baytril and Torbutrol. His cough became acutely worse, at which time he regurgitated and became dyspneic. Over the next 24 hours the patient became lethargic, weakly ambulatory, and febrile (104^{o}F). Pertinent previous history included chronic intermittent coughing followed by vomiting/regurgitation. Three-view thoracic radiographs showed a bilateral interstitial to alveolar pattern in the cranioventral lung lobes, marked distention of the thoracic esophagus, and a homogenous oblong soft tissue mass in the cranial mediastinum. An inflammatory leukogram was identified on CBC. While hospitalized he became progressively weaker with marked reduction in pelvic limb reflexes, requiring a sling to walk. Euthanasia was elected.

Gross Pathology:
The thoracic cavity contained approximately 2 ml of a non-viscous, translucent, dark red (serosanguinous) fluid. The thoracic esophagus was diffusely distended and was 3.5 cm at its widest dimension. The cranial mediastinum

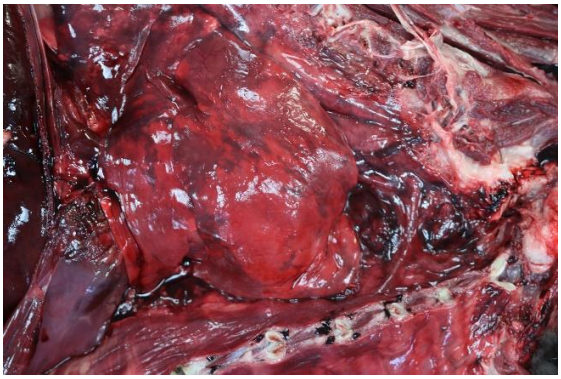

Figure 1-1. Mediastinum, dog. The cranial mediastinum is expanded by a poorly demarcated, 14.4 cm x 3.6 cm x 4.5 cm, multilobulated, firm to friable, tan to dark red mass (*Photo courtesy of:* Schwarzman Animal Medical Center, Department of Anatomic Pathology. www.amcny.org)

was focally expanded by a poorly demarcated, 14.4 cm x 3.6 cm x 4.5 cm, multilobulated, firm to friable, tan to dark red mass. The cut surfaces of this mass comprised anastomosing, firm, tan trabeculae separated by a soft to friable, dark red tissue.

The right middle lung lobe was diffusely red and firm. Sections from the right middle lung lobe sank in 10% buffered formalin. The right cranial, right caudal, accessory, and left cranial lung lobes were diffusely red and contained multifocal to coalescing, mildly to moderately firm regions. Sections from these lung lobes inconsistently floated, floated low, or sank in 10% buffered formalin. The left

caudal lung lobe was diffusely red and rubbery. Sections from the left caudal lung lobe floated in 10% buffered formalin. From the cut surfaces of all lung lobes oozed a moderate amount of non-viscous, translucent, dark red (serosanguinous) fluid.

Laboratory Results:

Parameter	Value	Reference Range
WBC	25.8 K/µL	4.9-17.6 K/µL
Neutrophils	23.33 K/µL	2.94-12.67 K/µL
Bands	258 /µL	0-170 /µL
Lymphocytes	0.52 K/µL	1.06-4.95 K/µL
Monocytes	1.806 K/µL	0.13-1.15 K/µL
Eosinophils	0 K/µL	0.07-1.49 K/µL
Basophils	0 K/µL	0.0-0.1 K/µL
Platelets	345 K/µL	143-448 K/µL

Microscopic Description:

Mediastinal mass cytology: Touch imprints of the cranial mediastinal mass comprise large numbers of streaming, mildly pleocellular spindle cells mixed with small numbers of mast cells, few small to intermediate size lymphocytes, and few macrophages containing variable amounts of hemosiderin on a background of peripheral blood. There are scattered mesothelial cells.

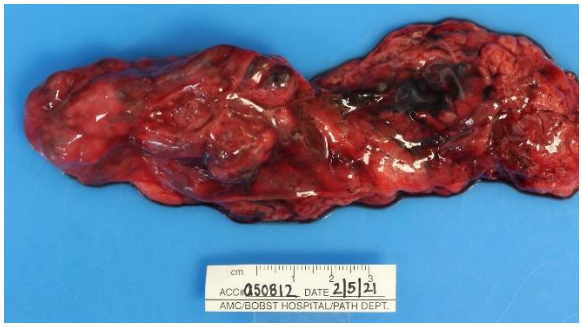

Figure 1-2. Mediastinum, dog. Dissected mediastinal mass. (*Photo courtesy of:* Schwarzman Animal Medical Center, Department of Anatomic Pathology)

Lung cytology: Touch imprints of the right middle lung lobe comprise abundant degenerate and viable neutrophils and macrophages mixed with small numbers of respiratory epithelial cells on a hemodiluted background with abundant extracellular bacteria. Macrophages infrequently contain hemosiderin. Intracellular bacteria are not identified.

Mediastinal mass histology: Expanding the fibrofatty stroma is a poorly demarcated, unencapsulated neoplasm consisting of mildly pleomorphic oval to spindle to polygonal cells arranged in fascicles, short and long streams, small bundles, and solid regions supported by a fine fibrovascular stroma with congested blood vessels and traversed by dense connective tissue trabeculae. Neoplastic cells are oval to spindle to polygonal with indistinct cell borders and small to moderate amounts of eosinophilic cytoplasm with inconsistent cytoplasmic vacuolization. Nuclei are round to oval with evenly distributed, finely stippled chromatin and up to one prominent nucleolus. No mitotic figures are identified in ten HPF (40x x FN22; 2.37mm^2). Scattered within the neoplastic population are a small number of individualized macrophages and small lymphocytes, as well as rare plasma cells. Macrophages infrequently contain intracytoplasmic, deep to golden brown pigment (hemosiderin and lipofuscin). The neoplastic population is multifocally separated by areas of hemorrhage that occasionally form large cavitated regions bordered by neoplastic cells. Rare mineral is present. Hassall's corpuscles and protein-filled perivascular spaces are not identified.

Immunohistochemistry: Approximately 90% of the neoplastic population display moderate to strong, cytoplasmic to membranous immunoreactivity for cytokeratin AE1/3 and are dif-

fusely immunonegative for vimentin. Individualized CD3 immunopositive round cells are scattered throughout the mass and comprise less than 5% of the total cell mass.

Contributor's Morphologic Diagnosis:
Mediastinal mass: Thymoma, epithelial-rich (prominent spindle cell morphology) with multifocal to coalescing, moderate to severe hemorrhages and few intratumoral small lymphocytes.

Contributor's Comment:
Based on the clinical presentation, identification of a cranial mediastinal mass with thoracic radiographs, megaesophagus, and an interstitial to alveolar pulmonary pattern in the cranioventral lung lobes, the working clinical diagnosis was thymoma-associated myasthenia gravis with secondary aspiration pneumonia. Gross exam confirmed a multilobular mass in the cranial mediastinum, megaesophagus, pleural effusion, and aspiration pneumonia. Differentials for a cranial mediastinal mass include neoplasia (i.e., thymic epithelial tumor, lymphoma, thymic germ cell tumor, stromal sarcoma, tumors of ectopic thyroid and parathyroid tissues, chemodectoma, lipoma, schwannoma, metastatic disease), thymic cyst, brachial cyst, abscess, and granuloma.[14,15,17,23,26] In this case, histopathology supported a diagnosis of an epithelial-rich thymoma with prominent spindle morphology.
The distinction between WHO classified type A and type AB thymomas is difficult, as both share a predominance of bland spindle cells and are distinguished by the percentage of immature T cells (few in type A and high numbers in type AB).[9,10] In this case, three representative sections were evaluated as part of the postmortem examination, and all contained a paucity of lymphocytes. These findings would be most consistent with a type A thymoma.

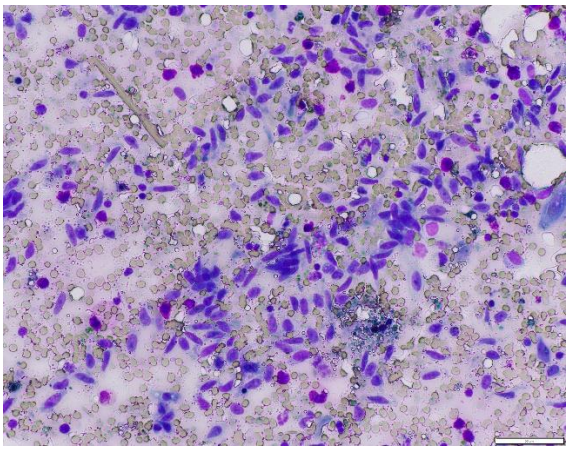

Figure 1-3. Mediastinum, dog. Touch imprints demonstrate numerous pleocellular spindle cells against a background of peripheral blood. (Wright-Giemsa, 400X) (*Photo courtesy of:* Schwarzman Animal Medical Center, Department of Anatomic Pathology)

However, as type AB thymomas can have lymphocyte-poor and lymphocyte-rich regions, it is possible that lymphocyte-rich regions were not represented in the examined sections and thus a type AB thymoma could not be entirely ruled out.[9,10] As euthanasia was elected before confirmatory AChR antibody titers could be submitted, myasthenia gravis could not be confirmed but was suspected.

As a lymphoepithelial organ, the thymus is formed by a combination of epithelial and lymphoid tissues (differentiating and proliferating T lymphocytes).[5,21] In addition, a small population of myoid cells that resemble striated muscle fibers are of unclear origin and thought to play a key role in the development of thymoma-associated myasthenia gravis.[21] During development, the thymic epithelium forms from the 3rd pharyngeal pouch's endoderm that subsequently becomes invaded by blood vessels and connective tissue trabeculae from the adjacent mesoderm and infiltrated by

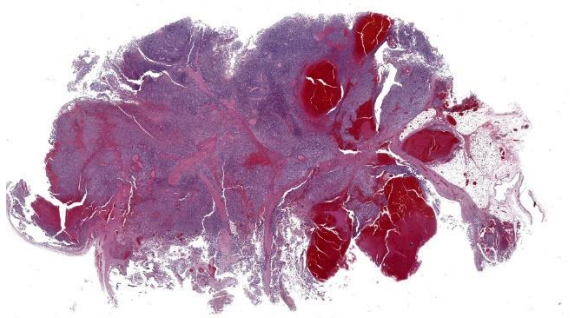

Figure 1-4. Mediastinum, dog. One section of a moderately cellular, cystic neoplasm with multifocal areas of hemorrhage is submitted for examination. (HE, 5X)

bone marrow derived lymphocyte progenitors.[5,8,21] Grossly, the thymus is separated into distinct lobes (paired cervical lobes, an intermediate lobe at the thoracic inlet, and a thoracic lobe), but the arrangement and prominence of each lobe varies among species.[5,21] In both ruminants and pigs, the cervical lobes are large.[5,6,21] Further, in ruminants the thoracic lobe is in the dorsal part of the cranial mediastinum.[5] In horses, the cervical lobes are small with a large thoracic lobe.[5,21] In domestic small animals, the cervical lobes are small (cats) to absent (dogs).[5,21] In both cats and dogs, the thoracic lobe is in the ventral cranial mediastinum and extends to the pericardium where in cats it can mold to the pericardial surface.[5] The thymus is partially separated into lobules by connective tissue septa that extend from the capsule so that each lobule comprises a central medulla and surrounding cortex.[5,21] The cortex is created by epithelial reticular cells and densely packed differentiating precursor T lymphocytes.[5,21] Meanwhile, the medulla contains epithelial reticular cells (relatively larger and more prominent when compared to the cortex), Hassall's corpuscles, interdigitating dendritic cells, and relatively fewer, less densely packed lymphocytes.[5,21] The thymus reaches its maximum size at birth and involutes after sexual maturity, although remnants may be retained through adulthood.[5,21] Differentiation between normal age-associated involution and disease-associated thymic atrophy requires interpretation of thymic features considering the patient's age, clinical presentation, and nutritional state.[5,21]

Thymic disorders include thymic hyperplasia, congenital thymic cysts, inflammatory diseases, hemorrhages and hematomas, neoplasia (i.e., thymic lymphoma and thymic epithelial tumors), and congenital immunodeficiency disorders.[5,21] The most common reaction pattern in the thymus is lymphoid atrophy, which can occur secondary to a multitude of underlying diseases processes including physical and physiologic stresses, cachectic states, toxins (lead, mercury, halogenated aromatic hydrocarbons, mycotoxins (i.e., fumonisin B1 & B2 and aflatoxin)), drugs, radiation, neoplasia, and viral infections.[5,21] True thymitis is rare but can be seen with certain infectious etiologies (i.e., *Neorickettsia helminthoeca* [salmon poisoning], *Neorickettsia elokominicas* [Elokomin fluke fever], epizootic bovine abortion, and porcine circovirus 2).[5,21] Thymic hemorrhage and hematomas are more frequently reported in young dogs, for which reported causes include anticoagulant rodenticides, dissecting aneurysm, trauma, and idiopathic/spontaneous.[5,21]

Tumors primary to the thymus are uncommon and include thymic lymphoma, thymic epithelial tumors, and thymic germ cell tumors.[5,7,9,10,20,21] Thymic epithelial tumors (TET) are uncommon cranial mediastinal neoplasms originating from the thymic epithelium and include thymoma, thymic carcinoma, and thymic neuroendocrine tumors.[1,5-7,14,15,18,20-26] Thymomas have been reported in numerous species, including dogs, cats, small

and large ruminants, horses, pigs, rabbits, ferrets, birds, non-human primates, rodents, a polar bear, and an otter, and are most typically identified in adult to aged animals, without a sex predilection.[2,3,5,6,8,14,15,17,18,20-24,26] They are reportedly more common in goats with a 25% incidence reported in a single closed herd of Saanen dairy goats.[3,5,6,14,15,17,20,21,24,26] Generally, thymomas are not associated with a specific breed predisposition in domestic small animals but medium to large breed dogs are often affected.[15,20] In one retrospective case series, Labrador retrievers and German shepherd dogs were overrepresented.[15,20]

Thymomas can be incidental but, when present, clinical signs are non-specific and include weight loss, vomiting/regurgitation, respiratory distress, dyspnea, cough, lethargy, exercise intolerance, and edema of the ventral head, neck, and forelimbs.[6,15-17,18,20,21,23,24] In cases of cervical thymoma, a visible or palpable subcutaneous mass may be present.[6] Associated imaging findings include a soft tissue opacity in the cranial mediastinum, dorsal displacement of the cardiac silhouette and/or trachea, and/or pleural effusion.[6,15,26] Advanced imaging (CT or MRI) is the most reliable method to assess tumor invasiveness.[6,15,17] Additional clinical signs, gross examination findings, and imaging findings associated with paraneoplastic syndrome(s) may be present.

The most common location of thymoma is the cranial mediastinum, but thymomas have been reported in the neck, thoracic inlet, and rarely in ectopic thymic tissues.[6,8,17,20,21,23] Cytologic features indicative of thymoma include lymphocytes (predominantly small T lymphocytes), thymic epithelial cells, and few mast cells, eosinophils, and/or macrophages.[15,17,26] As the non-neoplastic lymphoid component exfoliates readily, neoplastic epithelial cells

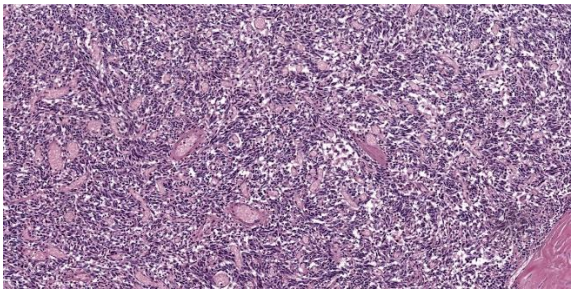

Figure 1-5. Mediastinum, dog. Spindled epithelial cells are arranged in short streams and bundles. There are numerous congested blood vessels. (HE, 128X)

are variably present in cytologic preparations.[15,20,24,26] In this case, the predominant cell population on cytology was mildly pleocellular spindle cells mixed with few mast cells, lymphocytes, and hemosiderin-laden macrophages. Initially, these spindle cells were of unclear etiology (neoplastic versus reactive population), but when evaluated along with the histology, they represented neoplastic epithelial cells displaying a spindle morphology.

The histologic appearance of thymomas is diverse due to the range of neoplastic cell morphologies and degree of benign lymphocyte infiltration.[1,5,6,14,17,20,23] Additional features inconsistently seen in thymomas include loss of normal thymic architecture, loss of corticomedullary junction distinction, protein-filled cysts, necrosis, and hemorrhages.[6,8,20,21,26] Neoplastic thymic epithelial cells can display spindle, oval, and round to polygonal morphologies and exhibit various growth patterns.[3,14,17,20,23,26] Typically, neoplastic cells have ill-defined cell margins and single nuclei with vesicular chromatin and up to one prominent nucleolus.[3,15,17,20,23,26] An uncommon clear cell variant comprised of large round cells with abundant amounts of clear cytoplasm and prominent cell margins has been described.[15,20] In one study, 30.6% of canine

cases were inconclusive on cytology and 66.7% of cases had diagnoses with histology alone.[24] Thus a small percentage of tumors will require immunohistochemistry for final diagnosis. [24, 26]

Thymomas have historically been categorized based on their cellular composition, defined as epithelial-rich, lymphocyte-rich, and mixed/ intermediate types with clear cell, spindle cell, and pigmented variants.[8,14,15,17,20,21,23,24] It is rare for thymic epithelial tumors to consist purely of epithelial cells.[21] Tumors can be further differentiated as to whether they are encapsulated /non-invasive or invasive.[3,15,17,21] Recently, the human WHO classification has been shown to be applicable in canines.[21,24] In people the histologic subtype carries prognostic significance, but prognostic significance between subtypes has not been established in veterinary medicine.[9,10,20,21,24]

The WHO classification is based on epithelium morphology, severity of cellular pleomorphism, and degree of proliferating non-neoplastic lymphocyte; under this scheme, thymomas are classified into A, AB, B1, B2, and B3 type, micronodular, and metaplastic thymomas.[2,7,9,10,21,24,25] Type A thymomas contain bland oval to spindle-shaped neoplastic epithelial cells arranged in streaming, storiform, fascicular, pericytomatous, or pseudorosettes with a paucity of T-lymphocytes.[7,9,10,21,24,25] Micronodular thymomas likely represent a subvariant of type A tumors where spindle shaped neoplastic epithelial cells are separated by large aggregates of lymphocytes.[7,9,10,21] Type A thymomas with atypia exist and represent a separate histologic variant.[7,9,10,21] Type AB thymomas comprise similar oval to spindle shaped neoplastic cells but are mixed with a focal to diffuse abundance of non-neoplastic lymphocytes.[7,9,10,21,24,25] Polygonal epithelial cells can be present in both type A and AB thymomas.[9,10] Type B thymomas contain oval to polygonal neoplastic cells with varying numbers of non-neoplastic lymphocytes, as well as variably present perivascular spaces and Hassall's corpuscles and are further divided into B1, B2, and B3 types.[7,9,10,21,24,25] Type B1 tumors maintain a thymus-like microarchitecture and comprise non-clustered neoplastic cells that lack atypia and are mixed with small numbers of lymphocytes.[7,9,10,21,24,25] In Type B2 tumors individualized or clustered oval polygonal neoplastic cells lack atypia and are mixed with large numbers of lymphocytes.[7,9,10,21,24,25] Lastly, Type B3 thymomas comprise mild to moderately atypical polygonal neoplastic cells arranged in sheets and contain a paucity of small lymphocytes.[7,9,10,21,24,25] Metaplastic thymomas are characterized by islands of polygonal neoplastic cells displaying variable atypia on a background of bland spindle cells.[7,9,10,21,24,25] Thymic carcinomas comprise neoplastic thymic epithelial cells that display overt features of malignancy and an invasive growth pattern.[7,9,10,20,21,24,25]

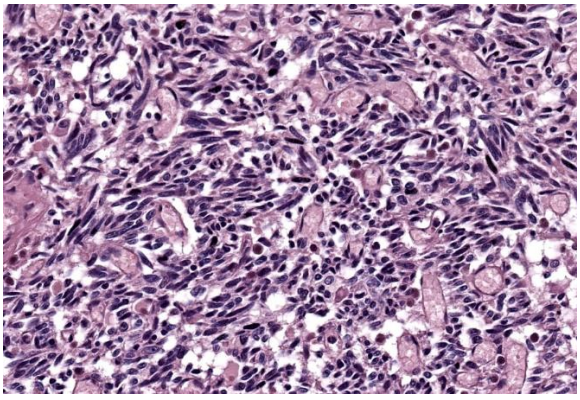

Figure 1-6. Mediastinum, dog. High magnification of neoplastic cells. (HE, 384X)

Molecular profiling in humans shows mirroring of the WHO histological classifications and molecular subgrouping, including A-like and AB-like clusters with frequent GFT2I and HRAS mutations, an intermediate B-like cluster with T-cell signaling profile, and a carcinoma-like cluster with high tumor molecular burden and frequent CDKN2A and TP53 alterations.[7] Further, thymic carcinomas and B2 and B3 thymomas showed a higher frequency of large copy number variation involving entire chromosomes.[7] The GTF2I gene encodes for the TFII-I protein involved in transcription regulation and, in people, GTF2I mutations appear to play a pivotal role in thymic epithelial tumors and may represent a thymoma-specific oncogene.[7,25]

Typically, thymomas have a benign biologic behavior and the vast majority are slow growing and heavily encapsulated.[3,5,6,14,15,17,18,20,21,23,26] However, all thymoma subtypes have the potential to behave aggressively with infrequent local tissue invasion and rare metastasis.[24] When metastases occur, they most commonly occur to the lungs and regional lymph nodes.[17] With surgical excision, most thymomas have a favorable long-term prognosis.[1,6,15,17,21,23,24,26] The results of one case series in goats suggests that anatomic location may be a key factor in influencing survival where the prognosis for cervical thymomas with surgical excision were favorable, while excision of mediastinal thymomas more likely carried a guarded prognosis.[6] In small animals, there is limited information regarding radiation as a treatment modality in thymomas with reports suggesting a high overall response rate (50-75%) with radiation therapy alone; however, complete response is reportedly rare.[15,17,24] In people, the main prognostic factors described with thymomas are tumor size, tumor spread, resection status, histologic subtype, patient characteristics (i.e., age and comorbidities), and response to treatment.[7] Prognostic factors in veterinary medicine vary and can be contradictory between studies, but reported factors associated with increased survival times are surgical excision and absence of megaesophagus; those associated with decreased survival times include presence of a paraneoplastic syndrome, myasthenia gravis (as a single variant), metastases, moderate to marked neoplastic cellular pleomorphism, percentage lymphocyte composition, presence of another concurrent tumor, and higher pathologic stage (based on the Masaoka-Koga Staging Systems).[15,17,20,24,26] The Masaoka-Koga Staging System evaluates tumor encapsulation, invasion location, and metastases.[3,7,24,26] In humans, the Masaoka-Koga Staging System is significantly associated with survival and decreased survival rates are seen with increasing stage regardless of histologic subtype.[7,15,24] In one report in dogs, patients with lower staging (stages I, IIa, and IIb) had significantly longer survival times than those with higher staging (stages III, IVa, and IVb);however, a contradictory study did not find a prognostic significance of between stages in dogs.[15,24]

Paraneoplastic syndromes are complications of malignancy distant from the primary tumor that in some instances can be more harmful than the direct effects of the neoplasm.[4,15,24] These syndromes can aid in tumor identification, as well as function as markers of response to treatment.[4] The high rate of paraneoplastic syndromes associated with thymic disorders is related to the crucial function of the thymus in immune tolerance through differentiation, selection, and maturation of T lymphocytes from progenitors.[1,5,8,11,20,21,24,26] Naïve, CD34+ and CD7+ T lymphocytes are

pluripotent and lack helper, suppressor, and killer functions; these progenitors undergo a sequential pattern of antigen expression as a function of orderly gene rearrangements encoding the T cell receptor (TCR).[21] While T lymphocytes traverse from the cortex to the medulla, they undergo differentiation and selection via both positive and negative selection, after which only a small percentage of progenitor T lymphocytes survive.[5,21] Positive selection occurs in the cortex, where only lymphocytes that recognize MHC molecules, but not self-antigens, are allowed to mature.[5,21] Meanwhile, negative selection occurs at the corticomedullary junction, where lymphocytes that recognize both MHC and self-antigen are removed.[5,21] Thymic "nurse cells" produce IL-1 which drives lymphoproliferation.[21] Once mature, T lymphocytes leave the thymus, enter the bloodstream, and circulate through secondary lymphoid organs.[5,21] Thymomas have been associated with a vast array of paraneoplastic processes with related clinical signs; these include myasthenia gravis, hypercalcemia of malignancy, immune-mediated polymyositis, keratoconjunctivitis sicca (KCS), immune-mediated thrombocytopenia, immune-mediated anemia, granulocytopenia, hypergammaglobulinemia, cardiac myositis with arrhythmias, feline exfoliative dermatitis, erythema multiforme, and T lymphocytosis.[2-4,11,13-15,17-21,23,24,26]

Disruption of central tolerance has been proposed as one of the mechanisms leading to autoimmunity associated with thymoma.[11] Thymic epithelial cells express elevated levels of MHC I and II molecules.[20] Aberrant expression of autoantigens and decreased expression of MHC molecules and autoimmune regulator (AIRE) on neoplastic epithelial cells result in defective thymic selection and survival of autoreactive lymphocytes.[20] Clinical signs associated with paraneoplastic syndromes may resolve after surgical excision of the mass, but sometimes clinical features of paraneoplastic syndromes can develop after mass removal.[2-4,11,13-15,17-21,23,24,26]

Myasthenia gravis and hypercalcemia related to parathyroid hormone-related peptide (PTHrP) are the most frequently reported thymoma-associated paraneoplastic syndromes in dogs with reported prevalence of 10-45% (myasthenia gravis) and 30% (hypercalcemia).[2,6,14,15,20,26] In this case, myasthenia gravis was suspected based on the clinical presentation but was not confirmed with ancillary testing. The syndrome is classically categorized as either acquired or congenital myasthenia gravis; however, recently alternative terminology has been suggested where myasthenia gravis exclusively refers to acquired forms and the term congenital myasthenia syndromes is proposed for congenital forms.[12,18,19,21] Acquired myasthenia gravis can occur with or without thymoma, and is associated with thiourylene medication administration in cats.[12]

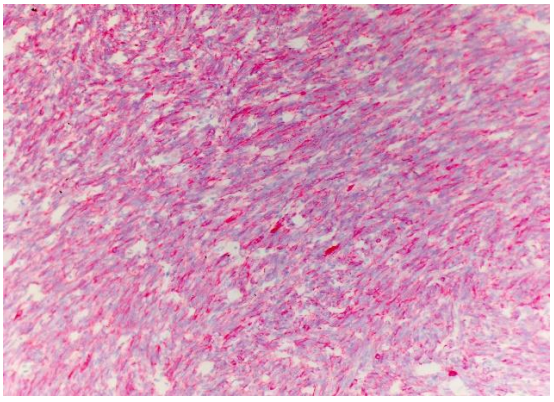

Figure 1-7. Mediastinum, dog. Neoplastic cells demonstrate moderate cytoplasmic immunopositivity for cytokeratin. (anti-AE1/AE3, 400X). *Photo courtesy of:* Schwarzman Animal Medical Center, Department of Anatomic Pathology)

Immune-mediated myasthenia gravis is most commonly due to the production of autoantibodies towards acetylcholine receptors (AChR) leading to destruction of the post-synaptic membrane and reduction of available functional AChR at the neuromuscular junction.[2,5,12-14,18-21] Less frequently, autoantibodies targeted against other muscle specific proteins (muscle specific kinase [MUSK], titan, and ryanodine proteins) have been reported. The pathogenesis of autoantibody production with thymoma is not entirely known but thymic myoid cells and dysregulation of T lymphocyte selection is thought to play a role.[2,13-15,18-21] Differentiating T lymphocytes recognize autoantigens to AChR or other skeletal muscle specific protein subunits and, due to a defective selection process, they survive and in turn stimulate proliferation and differentiation of B lymphocytes with subsequent autoantibody production.[12,18,20,21] At the neuromuscular junction, proposed mechanisms for receptor damage/inactivation include direct damage or formation of cross-linked antibodies leading to receptor internalization.[19]

The clinical manifestations of myasthenia gravis are divided into focal, generalized, or acute fulminating forms.[12,21] Focal manifestations affect an isolated skeletal muscle group outside the appendicular skeletal muscles (i.e., facial, esophageal, pharyngeal, and laryngeal skeletal muscles).[12,21] Generalized manifestations are defined as appendicular skeletal muscle weakness with or without facial, esophageal, pharyngeal, or laryngeal skeletal muscle involvement.[12] In fulminant forms patients present with an acute, rapidly progressive, and severe form of generalized myasthenia gravis that can progress to respiratory collapse requiring intubation and mechanical ventilation.[12]

Clinical signs vary between patients and include exercise intolerance, decreased or absent peripheral reflexes, cervical weakness, muscle weakness, flaccid paralysis, episodic collapse, hypersalivation, dysphonia, dysphagia, and regurgitation.[2,12,13,18-21,26] Initially enough functional AChRs are present to allow for normal transmission, but with sustained muscle activity the number of available receptors decreases, leading to progressive or episodic weakness and collapse.[19] Megaesophagus is a common sequela that predisposes patients to aspiration pneumonia.[5,21,26] Diagnosis of myasthenia gravis requires detection of circulating antibodies to AChR, however a subset of dogs with generalized disease will be seronegative.[12,13,15,18,20] There is a correlation between fluctuations in autoantibodies and clinical course of disease.[18] Clinical responses to administration of an anticholinesterase drug (edrophonium chloride; Tensilon) can be helpful but are not absolutely specific.[12,13,15,19,20] Therefore, ancillary testing results should be evaluated in concert with thoracic imaging and clinical signs.[12] Complete excision may lead to normalization of neuromuscular junction activity and resolution of clinical signs, but signs of myasthenia gravis can also develop after thymectomy.[12,15,18-21]

Contributing Institution:
Schwarzman Animal Medical Center
Department of Anatomic Pathology
www.amcny.org

JPC Diagnosis:
Mediastinal mass: Thymoma, type A.

JPC Comment:
The contributor provides an excellent, thorough overview of thymoma, the various applicable classification schemes, and associated paraneoplastic syndromes; it is an enjoyable,

informative read and a lily that requires no gilding.

This week's conference was moderated by Dr. Amy Durham, Professor of Anatomic Pathology at the University of Pennsylvania School of Veterinary Medicine, who opened discussion of this case by noting that the basics of this neoplasm, including tissue identification and cell of origin, are not particularly obvious without the clinical history and diagnostic results provided by the contributor. Conference participants seemed to agree, as, in addition to thymoma, a wide range of differentials were presented, including various sarcomas and vascular entities. The moderator noted that a well-curated immunohistochemistry plan, including vimentin, cytokeratin, and lymphocyte markers, makes the diagnosis. Vimentin is particularly helpful in this case given the spindle-cell morphology of the epithelial cells, which is uncommon in dogs and can easily cause the neoplasm to be misidentified as mesenchymal without immunohistochemistry.

A particularly astute conference participant noted the presence of multifocal pockets of ciliated epithelium within the thymic parenchyma and suggested that these might represente branchial cysts. The moderator noted that branchial cysts, which are persistent remnants of the embryonic pharyngeal pouches, are commonly encountered in the mediastinal tissues and, while interesting, are typically of no moment.

Conference participants discussed the value of the various classification schemes which, as noted by both the contributor and the moderator, currently have no prognostic value. The moderator noted that she typically prefers to diagnose these simply as thymomas, without subclassification, unless the specifics of a particular case warrant emphasizing the tumor's morphology. Other conference participants shared this approach, but all remained ready to adopt any classification scheme that becomes clinically useful in the future. During discussion of the morphologic diagnosis, however, a resident noted that the WHO classification scheme is exhaustively detailed in the primary veterinary pathology textbooks; therefore, for educational purposes, this delightful and slightly enigmatic neoplasm's WHO Type A classification was retained in the morphologic diagnosis.

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CASE II:

Signalment:
13-year-old, female spayed Yorkshire terrier (*Canis familiaris*)

History:
A 13-year-old Yorkshire terrier presented with a previously debulked oral mass associated with the caudal hard palate and soft palate. After 3 months of palliative treatment, the dog presented with inappetence, weight loss, and increased respiratory effort. Physical examination showed rapid growth of the mass since the most recent checkup, with partial upper airway obstruction. Euthanasia was elected given the rapid clinical decline.

Gross Pathology:
Within the caudal oral cavity and oropharynx, there is an irregularly shaped, 5 x 3 x 1.5 cm, firm, pale tan to dark gray mass extending ventrally from the hard and soft palate. The submandibular lymph nodes are enlarged, firm, and gray on cut section with loss of corticomedullary architecture. There are dozens of, firm, pale tan to gray, round to multinodular masses ranging from 2-10 mm in diameter within the lungs, mediastinum, spleen, liver, and right adrenal gland.

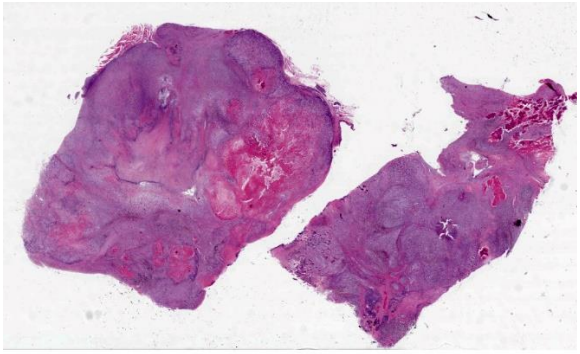

Figure 2-1. Oral mucosa, dog. Two sections of oral mucosa are submitted for examination. Multiple area of bone are visible at subgross magnification in both sections. (HE, 5X)

Microscopic Description:
Oral mucosa: Expanding the lamina propria is an unencapsulated, highly cellular mass composed of a pleomorphic population of neoplastic cells supported by sparse fibrovascular stroma. Neoplastic cells range from polygonal and arranged in sheets or thin cords, to spindle-shaped and arranged in streams and bundles. The neoplastic cells have variably distinct borders, abundant eosinophilic cytoplasm, round to ovoid nuclei, finely stippled chromatin, and 1-2 prominent nucleoli. Anisocytosis is marked and anisokaryosis is moderate. There are 24 mitotic figures in 10 examined 400X fields. Rarely, neoplastic cells contain scant black, fine, granular intracytoplasmic material (melanin). Sporadically throughout the mass, there are numerous round cells with abundant black, granular intracytoplasmic material (melanomacrophages). Within the neoplasm are several foci of homogenous, eosinophilic, extracellular matrix (osteoid) that is frequently associated with individual or thin aggregates of neoplastic cells, and rarely mineralized. Adjacent to and entrapped within the neoplasm are occasional islands of salivary gland tissue and salivary ducts. Centrally within the mass, there are large foci of coagu

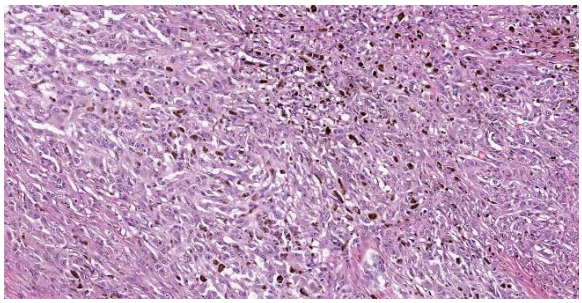

Figure 2-2. Oral mucosa, dog. The neoplasm is composed of pleomorphic cells which range from polygonal to spindle, and approximately 20% of the cells contain cytoplasmic melanin. (HE, 178X)

lation necrosis with loss of differential staining, pyknotic and karyorrhectic debris, and occasional pockets of neutrophils. The overlying mucosa is multifocally ulcerated and replaced by a mat of fibrin and necrotic cellular debris with abundant bacteria of mixed morphology.

Immunohistochemistry for SOX10, PNL2, Melan-A, and S100 was performed. >80% of neoplastic cells exhibit strong, nuclear labeling for SOX10 and cytoplasmic labeling for S100. Approximately 50% of neoplastic cells exhibit cytoplasmic labeling for PNL2 with regional variability throughout the tissue. Only sparse neoplastic cells exhibit strong cytoplasmic labeling for Melan-A (<10%).

The same neoplastic population with variable deposition of osteoid comprises all masses in other examined tissues (metastatic sites).

Contributor's Morphologic Diagnosis:
Oral mucosa: Malignant melanoma, osteogenic.

Contributor's Comment:
Malignant melanoma, composed of neoplastic melanocytes of neural crest ectoderm origin, is one of the most common oral tumors in dogs.[16] Canine oral melanomas have been associated with worse clinical outcomes than those arising at other sites. Most canine oral melanomas have metastasized by time of diagnosis, and the median survival time with treatment is under 3 years.[7,9,16] However, a subset of oral melanocytic tumors in dogs exhibits a less aggressive biological behavior despite being characterized histologically as malignant.[5] Mitotic index, nuclear atypia, and Ki67 positivity are reported as relevant prognostic features.[14] Oral melanomas frequently contain a significant amelanotic component, as in this case, but this does not correlate well with biological behavior.

Melanomas can vary widely in their histologic appearance. Cellular morphology can range from round to polygonal to spindle-shaped, often within the same anaplastic tumor, likely reflecting their neural crest origin drawing from both neural and epithelial roots. Junctional activity (subepithelial proliferation of neoplastic cells) is considered a characteristic feature that should increase suspicion for melanoma, even when pigmentation is sparse or absent.

The presence of osteoid in the stroma of this melanoma is a rare concurrent finding in canine melanomas, with only a few case reports in the literature.[3,4,8,11] While this osteogenic variant of melanoma is rare, it is important to recognize since osteosarcoma and malignant melanoma differ in prognosis and treatment. Human cases of melanoma with stromal osteoid and/or bone are also rare.[12] Heterologous differentiation in human melanoma is well-recognized and characterized, although these patterns are not widely used in veterinary species and prognostic significance has not been established. Such differentiation patterns in

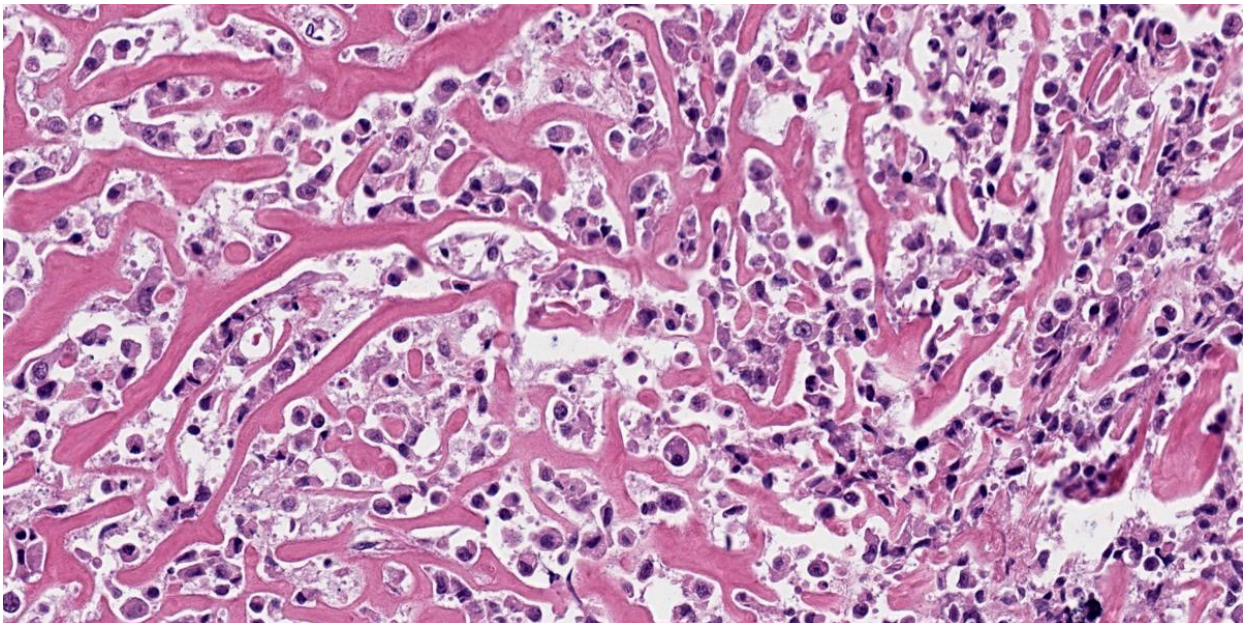

Figure 2-3. Oral mucosa, dog. Neoplastic cells are surrounded by thin anastomosing trabeculae of osteoid. (HE, 381X)

clude osteocartilaginous, fibroblastic, rhabdomyoblastic, Schwannian, and ganglionic.[1] Cases with osteoid and bone formation are classified as "osteosarcomatous" or "osteocartilaginous" which, as implied by the names, frequently also contain cartilage.[2,6,10]

The mechanism for osteoid and bone formation with canine or human melanoma is unresolved, but multiple hypotheses have been proposed.[1] Because some cranial skeletal elements are derived from neural crest, some postulate that metaplasia of pluripotent precursors results in production of osteoid and bone by the tumor cells. Others have speculated that it represents a stromal response to local trauma, although this does not explain production at sites of metastasis. Alternatively, osteoid and bone could be produced by the tumor stroma in response to release of factors by the neoplastic cells. Such factors have not been characterized in cases of osteogenic melanoma, but could include bone morphogenetic proteins, as an example. Of these possible sources, direct production by metaplastic tumor cells is favored in this case since the matrix is directly surrounded by cells that are morphologically consistent with the neoplastic population; however, immunohistochemistry may contradict this interpretation since most of these "osteogenic" cells are negative for our melanocytic markers.

Because anaplastic melanomas can pose a diagnostic challenge, particularly with poor pigmentation and divergent differentiation, immunohistochemical evaluation is a useful tool. Markers for which melanocytic tumors are typically positive include Melan-A, PNL2, S100, SOX10, TRP-1, and TRP-2.[13,15] Overreliance on a single immunohistochemical marker can result in false negatives. This occurred in the present case, as the top clinical differential was a poorly-differentiated sarcoma based on a reported negative result for Melan-A on the initial mass debulk.

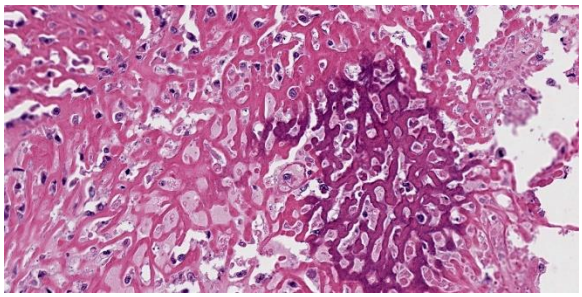

Figure 2-4. Oral mucosa, dog. Multifocally, osteoid is mineralized. (HE, 381X)

Contributing Institution:
The Ohio State University
College of Veterinary Medicine
Department of Veterinary Biosciences
https://vet.osu.edu/biosciences

JPC Diagnosis:
Oral mucosa: Osteogenic melanoma.

JPC Comment:
As noted by the contributor, osteogenic melanoma is rare in both human and veterinary medicine. Due to the paucity of reported cases, it is unclear if cartilaginous or osteogenic differentiation is of prognostic significance; therefore, current prognoses and treatment plans for these neoplasms are the same as for conventional, non-productive melanomas.[4]

Most reported cases of osteogenic melanoma have occured primarily in older, small breed dogs, are typically oral, and arise primarily in the gingiva.[4] As noted above, the biological behavior of these tumors is, in the small number of reported cases, similar to oral melanoma.[4] Features common to reported cases of osteogenic melanoma are the absence of primary bony involvement, the presence of junctional activity, and neoplastic cells with distinct melanin granules or Melan-A and S-100 positive cells adjacent to and/or embedded in tumor osteoid.[4]

While the molecular basis for the osteogenic part of osteogenic melanoma remains unclear, melanomas are known to express "runt-related transcription factor 2," or RUNX2, an osteogenic master regulator typically only expressed in osteoblasts and in mesenchymal stem cells during osteogenic commitment.[2,17] In vitro studies have shown that melanoma cells can be induced to form bone directly by expression of both bone sialoprotein and RUNX2.[4] Increased RUNX2 expression also induces the expression of specific metalloproteinases and collagenases that potentiate matrix degradation and tumor invasion. Because of this, it has been suggested that the presence of neoplastic bone in malignant melanoma suggests that mesenchymal conversion has occurred and that osteogenic melanoma may, therefore, have a greater metastatic potential when compared to its non-osteogenic counterpart.[4]

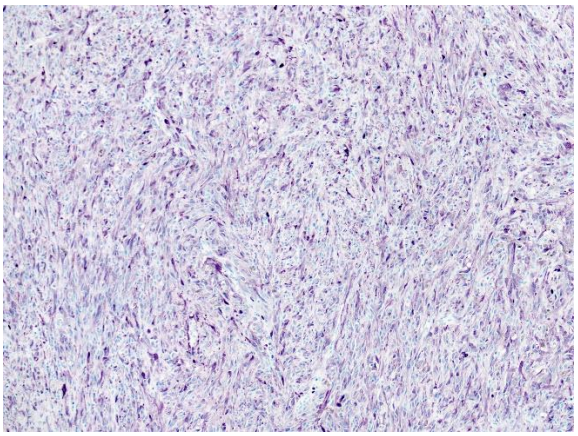

Figure 2-5. Oral mucosa, canine. >80% of neoplastic cells exhibit cytoplasmic labeling for S100. (*Photo courtesy of*: The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences, https://vet.osu.edu/biosciences) (anti-S100, 100X)

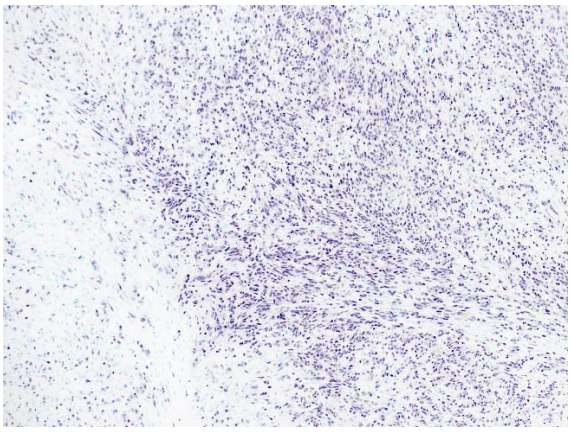

Figure 2-6. Oral mucosa, canine. >80% of neoplastic cells exhibit nuclear labeling for SOX10, with some regional variability. (*Photo courtesy of*: The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences) (anti-SOX10, 100X)

The presence of osteoid, multinucleated cells, and suspected melanin-containing cells initially stymied diagnostic progress among conference participants, and discussion quickly narrowed to the two main differentials of melanoma and osteosarcoma. Further frustrating efforts was the paucity of oral mucosa in the examined section, making it difficult to assess junctional activity, the presence of which would have helped convince a skeptical audience of the melanocytic origin of this neoplasm. Conference participants discussed and reviewed melanocytic immunohistochemical marker PLN2, which was convincingly positive in approximately 30-40% of neoplastic cells. Participants remarked on the quantitative and qualitative variability in staining among the neoplastic cells, causing the moderator to speculate that not all neoplastic cells are perfectly clonal, and this variability in gene expression, coupled with genetic variability induced by the tumor microenvironment could account for the pleomorphic quality often seen with immunohistochemical staining.

Conference participants discussed whether the prominent bone within section was produced by the neoplasm, or part of normal anatomic structures. The moderator noted that the section was not decalcified, which would normally be required for section through the mandible or maxilla, suggesting that all the bone in section was produced by the tumor.

The moderator cautioned participants to maintain an index of suspicion for osteogenic melanoma, particularly in the oral cavity where neoplasms such as ossifying fibroma and osteosarcoma or lesions producing cemento-osseous matrix are more apt to come to mind when presented with spindle cells producing an osteoid-like matrix.

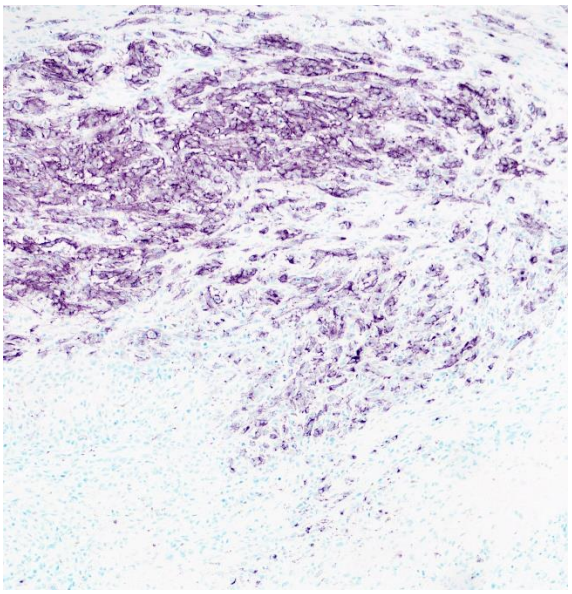

Figure 2-7. Oral mucosa, canine. Approximately 50% of neoplastic cells exhibit cytoplasmic labeling for PNL2 with significant regional variability. (*Photo courtesy of*: The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences) (anti-PNL2, 100X)

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CASE III:

Signalment:

7-year-old, female spayed Bassett hound (*Canis familiaris*)

History:

The patient initially presented to a veterinary clinic with a two-day history of inappetence

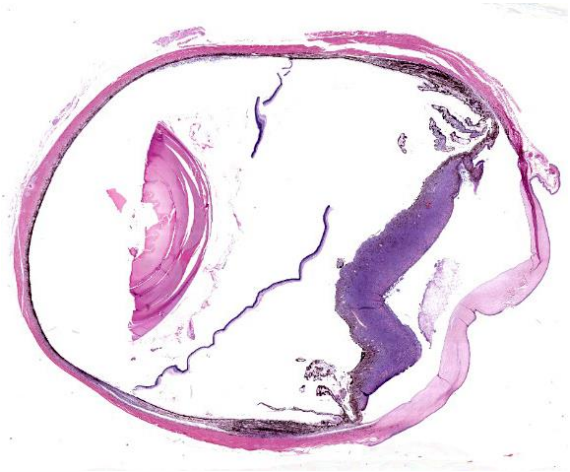

Figure 3-1. Eye, dog. A section of the globe is submitted for examination. At subgross magnification, the uvea is markedly expnded by a neaoplastic round cell tumor. The lens is pushed artifactually into the vitreous in this section. (HE, 5X)

and lethargy. Blood work performed in-house at that time revealed elevated liver enzyme activities. Splenomegaly was detected by ultrasound, and the spleen was consequently removed surgically. Three months later, the dog re-presented with exophthalmos of the left eye. The clinician diagnosed a luxated lens and glaucoma. The eye was enucleated and submitted for histology. The clinical concern was metastasis to the eye from the previously diagnosed splenic mass.

Gross Pathology:

The submitted trimmed globe was 24 x 24 x 27 mm.

Microscopic Description:

Globe: There is severe multifocal infiltration of the uveal tract by unencapsulated sheets of moderately pleomorphic round cells, resulting in thickening of the choroid (to 600 µm) and iris (to 2.5 mm). Neoplastic cells have scant, lightly eosinophilic cytoplasm and irregularly round to elliptical nuclei with 1-2 atypically large nucleoli. The mitotic rate averages 2-4 per HPF. There is moderate anisokaryosis. Similar cells are present in the lumina of choroidal vessels, as well as in ciliary process epithelium and in the anterior chamber. The neoplastic round cells form sheets adherent to the anterior aspect of the iris. They are also present between the sensory and non-sensory retina, admixed with melanin-containing macrophages of presumptive retinal pigmented epithelium (RPE) origin. Modest numbers of small lymphocytes, interpreted as an inflammatory response, are present at the margins of the intra-uveal neoplastic infiltrates. Other changes include retinal detachment with tombstone hypertrophy of the RPE, deep neovascularization of the corneal substantia propria, hemorrhage with fibrin exudation in the anterior chamber, and hemorrhage in the posterior chamber and in the vitreous. There is posterior luxation of the lens, edema of ciliary processes, mild vacuolation of lens fibers at the lenticular bow, cytoplasmic vacuolation with partial loss of corneal endothelium, and partial occlusion of the drainage angle.

Contributor's Morphologic Diagnosis:

Eye, uvea: Lymphoma, metastatic, with secondary retinal detachment, intraocular hemorrhage, uveitis and glaucoma, Basset hound, canine.

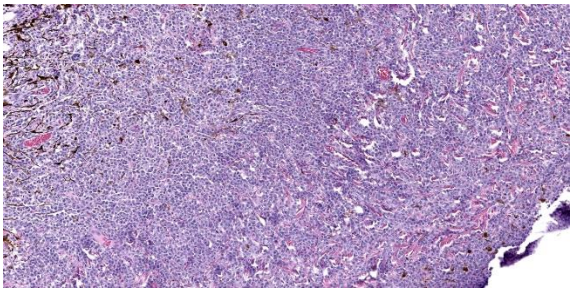

Figure 3-2. Eye, dog. The iris is markedly expanded by sheets of neoplastic lymphocytes. (HE, 102X)

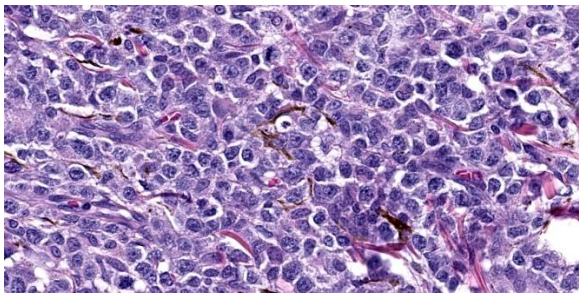

Figure 3-3. Eye, dog. High magnification of neoplastic lymphocytes within the iris.

Contributor's Comment:

The dog was diagnosed three months earlier with intrasplenic T cell lymphoma. This was based on positive staining for CD3 and negative staining for CD79a. A comparable staining pattern was evident in the intraocular mass. The clinician's concern about intraocular metastasis, resulting in lens luxation and glaucoma, was warranted.

Lymphoma is the most common metastatic intraocular tumor of dogs.[1] It may occur in as many as one third of canine lymphosarcoma cases.[8,11] Primary intraocular lymphomas (presumed solitary ocular lymphoma [PSOL], corresponding to the primary intraocular lymphoma [PIOL] of human patients) can also occur. Such tumors are considered rare in dogs.[5,7,11]

The clinical presentation in this case (i.e., glaucoma and lens luxation) is fairly typical for dogs with lymphoma metastatic to the eye. Clinical features may also include uveitis, retinal hemorrhage, retinal detachment, hyphema, and conjunctivitis.[1,5] Most of these features were found histologically in this dog. Ocular signs may represent the initial finding in dogs with generalized lymphoma, prompting owners to take the animal to a clinician. This salmagundi of intraocular changes can mimic other primary intraocular conditions and mislead clinicians, analogous to the masquerade syndrome in people.[2]

Confirmation of metastasis of lymphoma to the eye(s) can be of prognostic value for dogs undergoing standard chemotherapeutic regimens. Such dogs have a lifespan only 60 – 70% as long as those without ocular metastasis. Intraocular metastasis of lymphoma to one or both globes is therefore an ominous development.[1] Neoplastic lymphocytes are thought to localize initially in the anterior ciliary body and/or iris root.[5] When the intraocular lymphoma is at a more advanced stage, as in this case, the anterior uvea tends to be the most severely infiltrated component, followed by the choroid.[1] Median survival time in one study of 100 dogs with intraocular lymphoma was 104 days. There was no statistical difference in survival time between animals with lymphoma of T versus B cell derivation.[5] Survival times in dogs with true primary intraocular lymphoma are longer (MST: 769 days) and some appear to have been cured by surgical enucleation alone.[5] Survival times in dogs undergoing chemotherapy for intraocular lymphoma can be as long as one year, compared to 2-4 weeks for untreated dogs.[5]

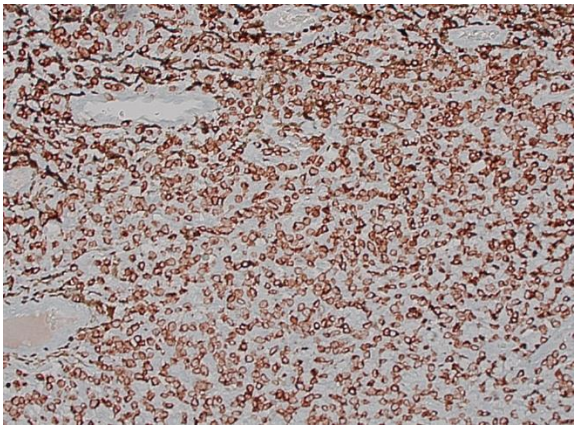

Figure 3-4. Eye, dog. Neoplastic lymphocytes stain strongly immunopositive for CD3 (a T-cell marker). (anti-CD3, 400X)

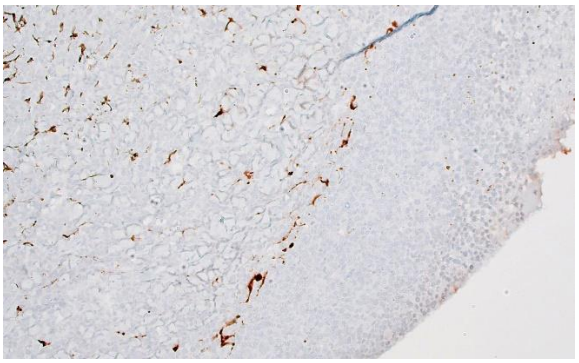

Figure 3-5. Eye, dog. Neoplastic cells are immunonegative for CD20 (a B-cell marker). (anti-CD20, 400X)

There is an interesting disparity between intraocular lymphoma in dogs with multicentric T cell lymphoma relative to human patients. People with generalized T cell lymphoma rarely develop intraocular metastases.[5] By contrast, a voluminous literature now exists about primary intraocular lymphoma in people, generally of B lymphocyte origin, which may also involve brain, spinal cord and/or meninges.[2] These are thought to arise in a multicentric fashion in the CNS and in the retina-vitreous.

In our experience, clinicians prefer to enucleate eyes in which they suspect intraocular neoplasia rather than use cytology first to establish the identity of the tumor. Some current cytology texts provide guidelines for interpretation so that cytology and/or antigen receptor rearrangement (PARR) testing may be used more in the future following anterior chamber paracentesis.[9,10] For understandable reasons, cytology is more commonly used in human ophthalmology for intraocular masses, relative to dogs or cats. It is likely in this case that cytological sampling of anterior chamber could have generated a diagnosis of lymphoma.

Contributing Institution:
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1174 Snowy Range Road
Laramie, WY 82070.
http://www.uwyo.edu/wyovet/

JPC Diagnosis:
Eye, uvea: Lymphoma, intermediate cell, mid-grade.

JPC Comment:
This case provides an excellent, classic example of the ocular manifestation of canine multicentric lymphoma. As the contributor notes, ocular involvement is seen in over one-third of dogs with multicentric lymphoma and is second only to enlarged lymph nodes as the most common clinical finding in canine lymphoma. Due to hematogenous spread, the uveal tract, most commonly the choroid, is affected in the vast majority of cases (97% in one large retrospective study), followed by the retina (46%), the cornea (32%), and the sclera (43%).[5] As in this case, peripheral T-cell lymphoma is the most common histologic subtype encountered in the eye, followed closely by diffuse large B-cell lymphoma (DLBCL).[5]

While lymphoma is the most common metastatic intraocular tumor, it is, of course, not the only one. A recent large retrospective study of 173 canine intraocular tumors found that the median age of diagnosis was 10 years, and the most commonly represented breeds included neoplasia stalwarts Labrador Retrievers and Golden Retrievers as well as mixed breed dogs.[4] In this patient population, the primary multicentric malignancies were lymphoma and histiocytic sarcoma, consistent with previous reports.[4] Intraocular histiocytic sarcoma is associated with a particularly short post-diagnosis survival time lending support to the view that intraocular histiocytic sarcoma is most

commonly manifestation of disseminated, multicentric disease.[4] Excluding the hematopoietic/multicentric tumors, hemangiosarcoma was found to be the most common metastatic intraocular tumor. Metastatic epithelial neoplasms represented in the study included mammary, pancreatic, and urothelial carcinomas.[4]

The moderator began discussion of this case by noting the mild edema and multifocal vascularization of the cornea, which had been overlooked in initial descriptions of the slide. The moderator cautioned participants to examine all tissues present, particularly when, as in this case, the slide is dominated by one major, attenting-grabbing lesion. And while a dramatic lesion, conference participants made relatively short diagnostic work of this neoplasm. Participants briefly discussed the possibility of melanoma, which the moderator noted would, in dogs, typically be nodular rather than the diffusely thickend iris examined in section. The common top differential was lymphoma, confirmed by the diffuse positive cytoplasmic immunoreactivity of neoplastic cells for CD3.

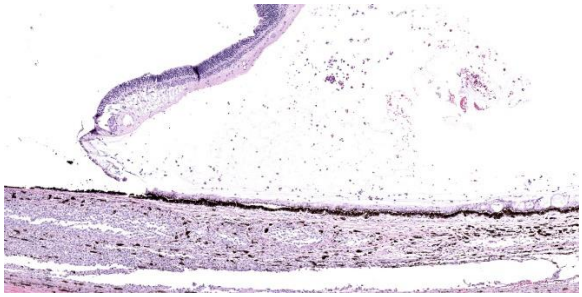

Figure 3-6. Eye, dog. Neoplastic cells expand the choroid beneath the detached, mildly atrophic retina. (HE, 66X).

Conference discussion moved to the clinical diagnosis of glaucoma and the lack of convinving evidence of glaucoma on histologic examination. The moderator noted that degeneration of the retinal ganglion cell layer is a key histologic hallmark of glaucoma that was absent in the examined section. Participants remarked on the tombstoning and hypertrophy of the retinal pigmented epithelium which suggests that the observed retinal detachment is unlikely artifact, though a cause for the detachment was not apparent histologically.

The moderator noted that presence of intraocular lymphoma automatically makes for stage V disease under the WHO lymphoma guidelines. This is a codification of the lymphoma dogma of "if it's in the eye, it's everywhere." The moderator noted that this view is changing, pointing to the presence of presumed solitary ocular lympoma [PSOL] which can often be successfully treated by enucleation.

Discussion ended with a brief survey of unique feline ocular neoplasias, including feline diffuse iris melanoma. The moderator noted that the examined section would be suspicious for this entity if the patient were a cat as the histological appearance is similar. Conference participants also discussed feline posttraumatic ocular sarcoma, an aggressive neoplasm that is locally invasive with metastatic potential. The sarcoma arises from metaplastic lens epithelium and can invade extraorbital tissues, typically via the limbus or the optic nerve.

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CASE IV:

Signalment:
11-year-old, female spayed German shorthaired pointer (*Canis familiaris)*

History:
This patient initially presented for bilious vomiting and a pancreatic mass was subsequently identified on abdominal ultrasound and confirmed with computed tomography (CT). Partial pancreatectomy to excise a 15 mm diameter mass from the right distal limb of the pancreas was performed. The patient presented 6 weeks later for a recheck CT at which time an additional pancreatic mass was identified. Another partial pancreatectomy, this time of the left limb and body of the pancreas, was performed.

Gross Pathology:
A formalin-fixed, 15 x 3 x 3 cm segment of the left limb and body of the pancreas was submitted for histologic evaluation. Near the proximal aspect was a rounded, 4 x 3 x 2.5 cm, light brown mass. The cut section revealed multifocal to coalescing rounded, smooth, firm, light brown, waxy nodular material (up to 1.4 x 1.2 x 1.2 cm diameter) with embedded and semi-firm brown tissue.

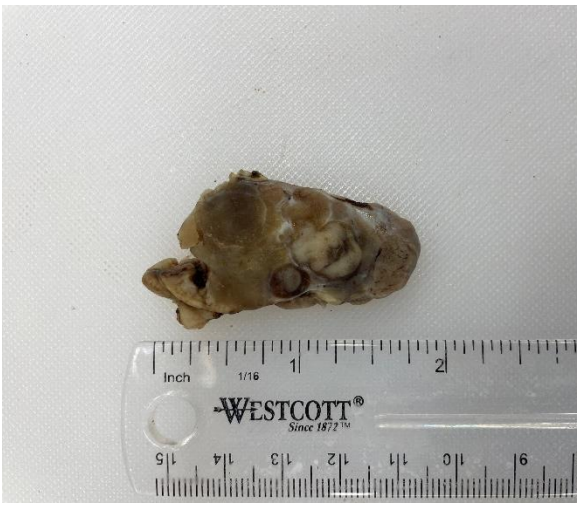

Figure 4-1. Pancreas, dog. A 15 x 3 x 3 cm segment of the left limb and body of the pancreas was submitted for histologic evaluation. (*Photo courtesy of:* Department of Population Health and Pathobiology, North Carolina State University College of Veterinary Medicine, Raleigh, NC 27607 https://php.cvm.ncsu.edu/)

Microscopic Description:
Pancreas: Markedly expanding, replacing, and effacing the pancreatic architecture and compressing resident pancreatic tissue, is a poorly-demarcated multilobular mass of neoplastic polygonal cells and extracellular matrix. Neoplastic cells form irregular acini and ducts and are embedded within and adjacent to an abundant, acellular, eosinophilic, hyalinized matrix that comprises approximately 30-70% of the mass (varied in different sections). Neoplastic epithelial cells have variably distinct cell borders, moderate to abundant brightly eosinophilic granular cytoplasm, and a large vesicular nucleus often with central single prominent nucleolus, but occasionally up to three nucleoli. Anisocytosis and anisokaryosis are moderate with frequent karyomegaly and 14 mitotic figures in 10 hpfs (2.37 mm^2). Multifocally, ducts have lost cellular detail and contain eosinophilic cellular debris. Cellular debris and few macrophages with intracellular pyknotic debris are scattered throughout the neoplasm. Differentiation between overtly neoplastic cells and resident cells is limited in marginal areas where more normal tubules and acini are also in close proximity to the hyalinized matrix.

Contributor's Morphologic Diagnosis:
Pancreas: Exocrine pancreatic adenocarcinoma, hyalinizing type.

Contributor's Comment:
This pancreatic mass is consistent with a hyalinizing type of exocrine pancreatic carcinoma. A recent review of pancreatic carcinomas in the dog reports these tumors are rare with no clear sex-predilection and a potential breed predisposition in Airedale terriers.[1] Clinical signs are typically non-specific and can include vomiting, inappetence, diarrhea, polyuria/polydipsia, and a palpable abdominal mass.[1] The hyalinizing variant has only been reported in dogs, with most occurring in the right limb of the pancreas.[3]

The composition of the hyalinized matrix has not been fully characterized, though application of special and immunohistochemical stains to masses of this type have not been consistent with amyloid A, laminin, collagen, immunoglobulin, or alpha-1 antitrypsin.[3]

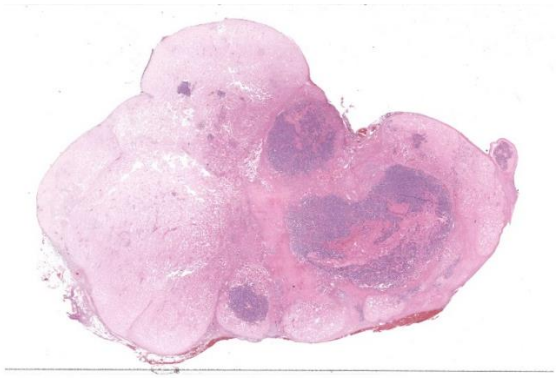

Figure 4-2. Pancreas, dog. A nodule of neoplastic acinar tissue is largely replaced by eosinophilic hyaline material. (HE, 4X)

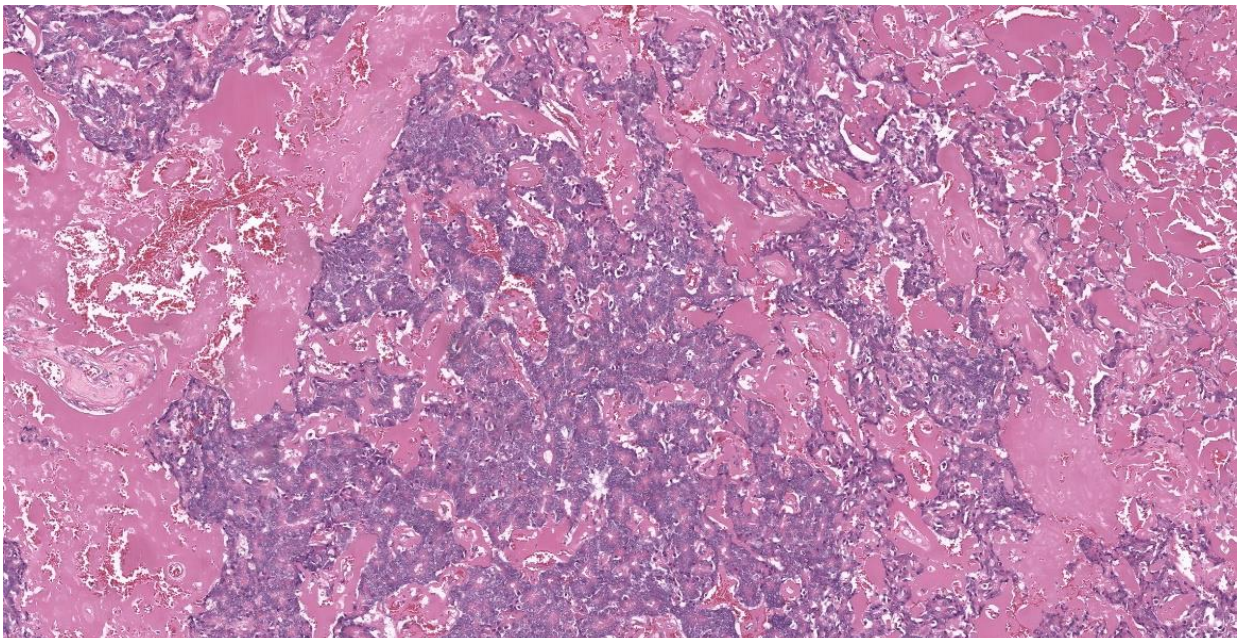

Figure 4-3. Pancreas, dog. Foci of neoplastic acinar tissue are embedded in abundant eosinophilic hyaline material. (HE, 80X)

Types of recognized and reported variants of exocrine pancreatic carcinomas in the dog include acinar, ductal, hyalinizing, and mixed endocrine-exocrine.[1,2] Growth patterns are described including acinar, solid, rosette, clear, mucinous, and tubulopapillary with variable differentiation.[1,4] Acinar is most commonly reported in dogs, as compared to humans, in which exocrine pancreatic carcinomas are more often ductal, though differentiation can overlap within the same tumor.[1] Exocrine pancreatic carcinomas have the potential for metastasis, especially to the liver and local lymph nodes.[3] Evidence of metastasis was not identified in additional tissues submitted for this patient.

As cases are rare, no particular set of prognostic features has been established, though the hyalinizing variant may have better post-diagnosis survival times, especially if the patient survives the acute-post surgical interval.[3] The patient in this case was alive at time of case submission, nearly seven months after initial diagnosis.

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JPC Diagnosis:
Pancreas: Exocrine adenocarcinoma, hyalinizing type.

JPC Comment:
As the contributor notes, pancreatic neoplasia in dogs is rare, and the hyalinzing variant, so beautifully demonstrated by this case, is rarer still. Most canine pancreatic neoplasms are derived from the exocrine epithelium and are usually malignant, with clinical signs appreci

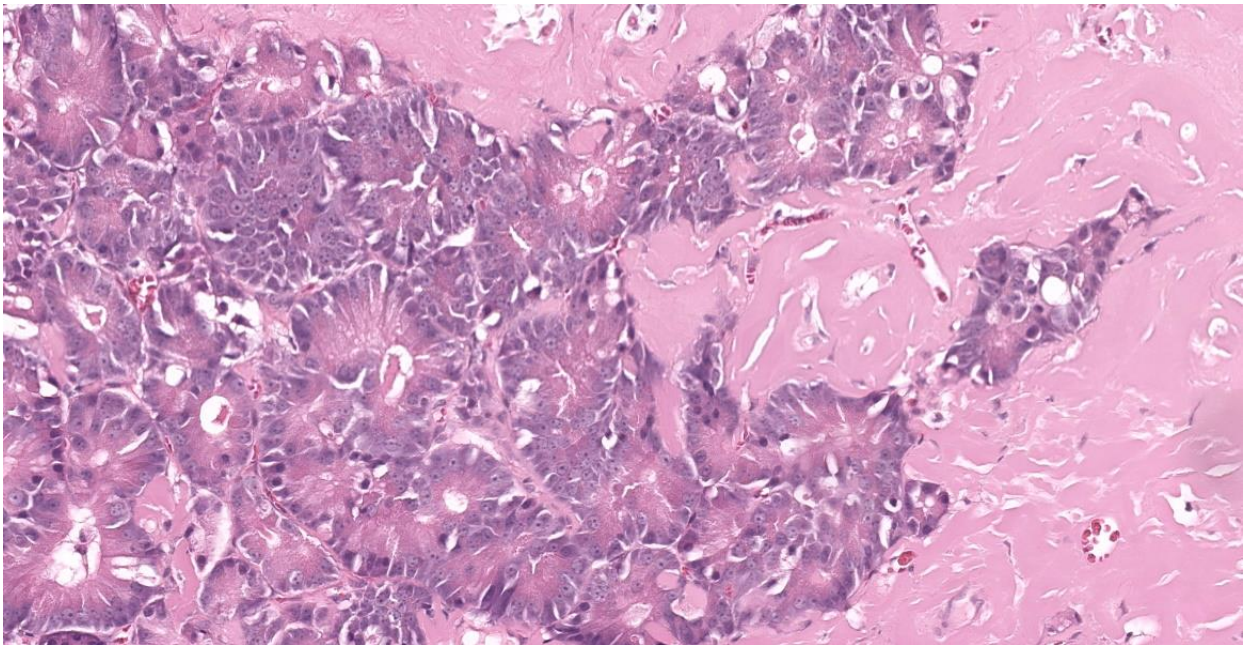

Figure 4-4. Pancreas dog. High magnification of neoplastic acinar cells embedded in hyaline material. (HE, 237X)

ated only after extensive local growth or metastasis to the liver, lymph nodes, or lymph nodes has occurred.[3]

Canine exocrine pancreatic carcinomas come in many varieties, with cuboidal, polygonal, or columnar cells of varying polarities and degrees of granulation forming tubules, acinar structures, or solid sheets of cells on stromal elements raning from delicate and fibrovascular to scirrhous. Histologic subcategories based on morphology are described for canine pancreatic neoplasias, though these subcategories currently have no confirmed prognostic significance or ability to predict biologic behavior.[3]

The hyalinizing variant of exocrine pancreatic carcinoma was first and most extensively described in a case series of six older mid-sized dogs; in most of these patients, the mass was present grossly as a single solitary mass, and most were discovered incidentally.[3] As nicely demonstrated by this case, the feature that distinguishes the hyalinzing variant from a traditional exocrine pancreatic carcinoma is the presence of large contiguous areas of accumulated extracellular hyaline material that has the appearance, though not the staining or immunohistochemical properties, of amyloid.[3] Neoplastic cells typically form tubules and are well-differentiated, with recognizable zymogen granules within the cytoplasm.[3] In the initial case study, these histological features suggested a low-grade malignancy in all six cases, though some features of malignancy were noted, such as mild cellular atypia, frequent mitotic figures, and stromal invasion.[3]

As the contributor notes, based on the few reported cases of hyalinizing exocrine pancreatic adenocarcinoma, it is possible that this variant has a less aggressive course than its conventional counterpart. In contrast to conventional exocrine pancreatic adenocarcinomas, which have often metastasized or in-

vaded at the time of diagnosis, in the initial descriptive case study of 6 dogs, metastatic disease was present in only 1 dog, and this dog lived for 16 months post-diagnosis.[3] Speculating on the possible causes for this relatively indolent behavior, researchers posited that perhaps the unique accumulations of hyalinized matix within the tumor provided mechanical or biochemical impediments to the cell-matrix interactions required for tumor growth, invasion, and metastasis.[3]

Conference participants were struck by the abundant matrix that is the main histologic feature of this neoplasm. This matrix, together with the relatively differentiated neoplastic cells, made for an easy, though unfamiliar diagnosis. Discussion focused predominately on the nature of the matrix itself which, as noted, is of unknown composition. Participants evaluated a Masson trichrome, which stained the matrix an unusual gray-blue which was quite distinct from the vibrant blue staining of normal mature collagen. Evaluation of a Movat pentachrome stain revealed a deep yellow-orange color, most consistent with, but not specific for, collagen. While a tumor of first instance for most conference participants, the moderator noted that these tumors do occasionally appear in her diagnostic workload, including, an as-yet unpublished case in a cat.

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