Diagnostic Exercise

From The Davis-Thompson Foundation*

Case #**242**; Month: **August**; Year:**2024**

Answer sheet

Title: Pulmonary mineralization associated with Cushing's disease in two Shih-Tzu dogs.

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History:
Case A is a 10-year-old, spayed female Shih-Tzu dog, with a history of pulmonary hypertension, mild mitral and tricuspid regurgitation, seizures/syncopal episodes, Immune mediated hemolytic anemia – immune mediated thrombocytopenia (IMHA-IMTP), and chronic steroid use (prednisone, discontinued 1 year prior to euthanasia) for the treatment of IMHA-IMTP and immunosuppressants (azathioprine). Blood chemistry and hemogram of this animal revealed increased ALP, neutrophilia and monocytosis. Vetmedin was used for treatment of Mitral Valve Disease (MVD).
Case B is a 7-year-old, neutered male Shih-Tzu dog with a clinical diagnosis of Cushing disease and early-stage mitral valve disease. During months prior to death, has experienced episodes of shaking, weakness, unsteadiness, and inconsistent vomiting. Patient was on Trilostane and Vetmedin for MVD. Similar as in case A, Blood chemistry and hemogram of this animal revealed increased ALP, neutrophilia and monocytosis. The animal was euthanized and submitted for necropsy.

Necropsy findings:
Case A: The most significant findings were restricted to the lung, which were pink with a soft spongy consistency. The liver was moderately enlarged with round margins (Figures 1 and 2).
Case B: The lungs were pink with a texture reminiscent of a semi-firm sponge. Other findings included numerous nodular masses in booth adrenal glands, bilateral endocardiosis, multifocal areas of dermal mineralization and a friable red/gray mass in the pituitary gland (Figures 3 and 4).

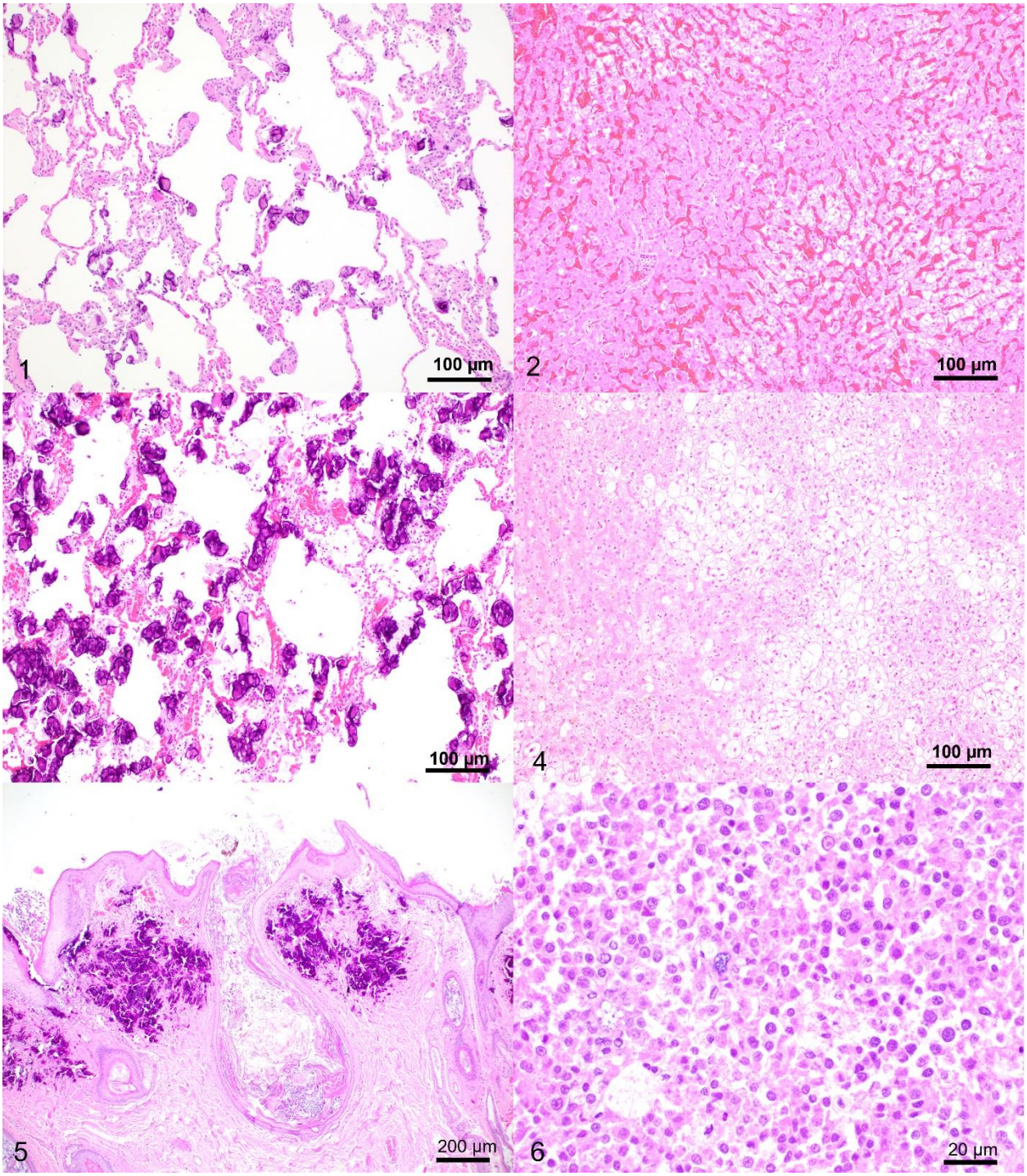

Figure 1 and 2: Dog A: lung and liver, H&E 10X. Figure 3 and 4: Dog B: Lung and liver, H&E 10X. Figure 5 and 6: Dog B: Skin and pituitary mass, H&E 10X.

Histologic description:
Figure 1 and 3: Lung: Multifocally in the alveolar interstitium, there are numerous deposits of a basophilic, irregular material (interpreted as mineral). In addition, alveolar hyperinflation/emphysema is noted. In slide 3, there is mild neutrophilic inflammation in alveoli, plus focal hemorrhages.

Figure 2 and 4: In the liver, there are numerous clusters of hepatocytes displaying vacuolar degeneration, likely due to the deposit of glycogen (interpreted as corticoid-induced hepatopathy).

Figure 5: Skin: In the superficial dermis, there is a band of collagen mineralization, characterized by the deposit of a basophilic irregular material. In the center, a hair follicle is dilated with abundant keratin.

Figure 6: Pituitary mass: there is a dense cellular mass, composed of sheets and lobules of polygonal cells with moderate amounts of basophilic cytoplasm, conspicuous cell margins and centrally located nucleus. Occasional megakaryosis is noted, and one or two mitoses are identified in 2.37 mm2.

Morphologic diagnosis:

Case A:
Lung: Pulmonary interstitial mineralization, multifocal, moderate.
Liver: Hepatic glycogen like vacuolar degeneration, multifocal to coalescing.

Case B:
Lung: Pulmonary interstitial mineralization, multifocal to coalescing, moderate to severe, with mild neutrophilic alveolitis.
Liver: Hepatic glycogen-like vacuolar degeneration, multifocal to coalescing.
Skin: Dermal mineralization (Calcinosis cutis), with follicular atrophy.
Pituitary mass: Pituitary adenoma, basophilic cells.

Etiology: Hyperadrenocorticism. Case A: Iatrogenic. Case B: Pituitary adenoma.

Name the disease: Cushing's disease.

Comments:

Mineralization of tissues, with deposits of calcium, phosphorus and other minerals may occur in two different scenarios: Dystrophic and metastatic mineralization. Dystrophic mineralization is the deposit of calcium, phosphorus and other minerals in tissues with areas of damage or injury, in absence of systemic mineral imbalance. Metastatic mineralization is associated with systemic mineral imbalance, such as in cases of renal failure, vitamin D toxicosis, Increased parathormone (PTH) and PTH-related protein, alkalosis, neoplasms, granulomatous diseases, osteolytic diseases, or sometimes may be idiopathic (8).

In the lung, mineralization of pulmonary interstitium is a feature or uremic pneumonopathy in dogs with renal failure, but may also result from hypercalcemia, hyperphosphatemia, Vitamin D toxicosis, alkalosis and hyperadrenocorticism (3).

On necropsy, the lung is often firm and gritty upon palpation (5).

Histologically, there are variable amounts of basophilic material deposited in smooth muscle and connective tissue fibers in alveolar septa, pulmonary veins and the walls of bronchioles (3).

Kidney failure leads to the buildup of phosphates in the body, triggering a secondary condition of overactive parathyroid glands and high calcium levels. Calcium is deposited in the stomach lining, kidneys and lungs. Another sequel of renal failure is uremia. The magnitude of uremic lesions can either be a result of endothelial damage and cell death, resulting in vasculitis, which triggers the formation of emboli leading to infarction in various tissues, or the development of lesions in the oral cavity and stomach due to the corrosive impact on the epithelial cells (9).

Excessive intake of vitamin D, either through calcinogenic plants like *Tristen flavescens* in herbivores or ingestion of cholecalciferol-containing rodenticides by dogs and cats, causes severe mineralization of soft tissues. This often affects major vessels, the lungs, and the heart, with noticeable mineral deposits in the endocardium (6).

Metabolic alkalosis is characterized by elevated levels of serum bicarbonate or an increase in base excess, which is typically determined through blood gas analysis. Elevated base excess may stem from a primary metabolic alkalosis, where the blood pH is higher than normal, or it may indicate a compensatory reaction to respiratory acidosis, characterized by increased PCO_2 and decreased pH relative to normal levels. Additionally, metabolic alkalosis can coincide with a primary respiratory acid-base disorder, resulting in a mixed acid-base abnormality (4). In the author's experience, cases of pulmonary mineralization associated with metabolic alkalosis are extremely rare.

Hyperadrenocorticism has also been associated with pulmonary mineralization (3). Although the pathogenesis is still unknown, a variety of mechanisms have been suggested. In dogs with hyperadrenocorticism, mineralization is likely a result of cortisol’s protein catabolic effects. The increased levels of cortisol can lead to enhanced breakdown of proteins, a process known as protein catabolism. This protein degradation can cause calcium and phosphorus to deposit in the extracellular framework of the damaged proteins. These deposits occur despite normal levels of calcium and phosphorus in the serum, indicating that the mineralization is not due to abnormal serum concentrations but rather the local environment created by the degraded proteins. Consequently, the abnormal protein structures serve as a nidus for mineral deposition, further complicating the condition This phenomenon underscores the complex interplay between hormonal imbalances and tissue pathology in hyperadrenocorticism (2).

Another proposed mechanism involves the elevation of corticosteroid-induced alkaline phosphatase isoenzyme, which is commonly seen in these animals. This elevation can occur by way of a pituitary adenoma (similar as in case B), leading to increases in ACTH, or via iatrogenic glucocorticoid use (as described in case A). The

increased levels of corticosteroids can lead to vasoconstriction and hypertension due to regulation of water, sodium, and other electrolytes (2).

Corticosteroid use has been linked to the potentiation of the vasoconstrictive effects of catecholamines, most notably, norepinephrine (10).In both cases described above, stress leukograms were noted on hematology (a common finding in Cushing's disease) and can be correlated with elevated levels of catecholamines leading to vasoconstriction and hypertension in the lungs. In both cases reported here, pulmonary hypertension was diagnosed via echocardiogram. In the presence of pulmonary hypertension, it can be speculated that there was diffusion impairment across the vascular endothelium, leading to a decrease in circulation and excretion of vascular constituents such as mineral (calcium and phosphorus) (7). The summation of this process ultimately may have led to mineral accumulation in the pulmonary interstitium.

Pulmonary Mineralization is a well described condition with various causes, such as renal failure, Alkalosis and Vitamin D intoxication in dogs. Cushing's disease must be considered as a differential diagnosis, and should be correlated with gross, radiographic and clinicopathologic findings to ultimately confirm your diagnosis of hyperadrenocorticism (1).

References:

1 Berry CR, at al. Frequency of Pulmonary Mineralization and Hypoxemia in 21 dogs with Pituitary-Depedent Hyperadrenocorticism. *J Vet Intern Med* 2000;14:151-156.
2. Blois SL, et al. Diagnosis and outcome of a dog with iatrogenic hyperadrenocorticism and secondary pulmonary mineralization. *Can Vet J* 2009;4: 397-400.
3. Caswell J, Williams K. In: Maxie MG, ed. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Vol. 2. 6th ed. St. Louis, MO: Elsevier, 2016:494.
4. Ha YS, et al. Incidence, Nature, and Etiology of Metabolic Alkalosis in Dogs and Cats. *Vet Int Med* 2013; 27:847-853.
5. King JM, et al. Mansbridge B, ed. The Necropsy Book. A guide for veterinary students, residents, clinicians, pathologists and biological researchers. 7th ed. Charles Louis Davis DVM foundation, 2013.
6. Lopez A, Martinson SA. Respiratory system, thoracic cavity, mediastinum and pleurae. In: Zachary JF, ed. Pathological Basis of Veterinary Disease. 7th ed. MO: Elsevier, 2016: 547-642.
7. Masters AK, et al. Effects of short-term anti-inflammatory glucocorticoid treatment on clinicopathologic, echocardiography, and hemodynamic variables in systemically healthy dogs. *Am J Vet Res* 2018;79:411-423.
8. Miller MA, Lyle T, Zachary JF. Mechanisms and morphology of cellular injury, adaptation and death. In: Zachary JF, ed. Pathological Basis of Veterinary Disease. 7th ed. MO: Elsevier, 2016: 16-73.
9. Sula MJ, Lane LV. The Urinary system. In: In: Zachary JF, ed. Pathological Basis of Veterinary Disease. 7th ed. MO: Elsevier, 2016: 699-766.
10. Ulian ME. The role of corticosteroids in the regulation of vascular tone. *Card Res* 1999; 41:1:55-64.

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