CONTRIBUTOR NAME*	Jessica Marin
CONTRIBUTOR EMAIL*	jdmarin@ncsu.edu
COAUTHORS	Hiroyuki Mochizuki, Devorah Stowe
COMPANY OR UNIVERSITY	North Carolina State University

*Corresponding author.

SPECIMEN: Blood smear

SIGNALMENT: 1-year-old male castrated domestic shorthair cat

HISTORY AND CLINICAL FINDINGS:

The patient was presented to the NC State Veterinary Hospital for a one week history of intermittent open-mouth breathing and tachypnea. On physical examination, the patient was dyspneic with an increased inspiratory effort, significant stridor, which obscured auscultation of bronchovesicular sounds, and extension of the head and neck with an exaggerated swallow. The remainder of the physical exam was considered unremarkable. Initial diagnostics performed at this time included CBC, serum chemistry, thoracic and cervical radiographs, CT of the head and neck, and upper airway endoscopy. A pathologist review of the patient's blood smear was ultimately performed. Prior to CT imaging, intubation was noted to be difficult due to markedly swollen, pale, and edematous arytenoids and epiglottal folds.

The patient previously presented to NC State Veterinary Hospital about 5 months prior for evaluation of a 3-month history of large to mixed bowel diarrhea and a 1-month history of vomiting. A CBC was not performed at this visit. The patient's GI signs ultimately improved on a select protein diet and tylosin administration.

LABORATORY DATA:

Hemogram Results:

TEST	UNITS	RESULT	REFERENCE INTERVAL
WBC	x 10^3/UL	**23.72 H**	3.77 - 16.73
RBC	x 10^6/UL	8.55	7.11 - 12.02
HGB	G/DL	13.8	11.3 - 17.2
HCT	%	39.4	33 - 51
MCV	FL	46.1	38.3 - 49.9
MCH	PG	16.1	13.1 - 16.8
MCHC	G/DL	34.9	32.2 - 35.7
RDW	%	15.3	13.4 - 16.9
PLATELET	x 10^3/UL	281	198 - 434
MPV	FL	15.4	8.8 - 21.3
PCT	%	0.43	0.28 - 0.85
PCV	%	41	32 - 48
Plasma Protein	G/DL	7.5	6.6 - 8.1
Segmented Neut	x 10^3/UL	**14.47H**	2.773 - 6.975

Lymph	x 10^3/UL	**7.119H**	0.415 - 4.996
Monocyte	x 10^3/UL	**1.186H**	0.068 - 0.78
Eosinophil	x 10^3/UL	**0.949H**	0.118 - 0.879
Absolute Retic	/UL	36000	

Serum Chemistry Results:
The patient had mild elevations in both ALT and AST. The remainder of the results were within reference intervals.

TEST	UNITS	RESULT	REFERENCE INTERVAL
ALT	IU/L	**184 H**	22 – 105
AST	IU/L	**67 H**	12 – 44

Figure 1(A)

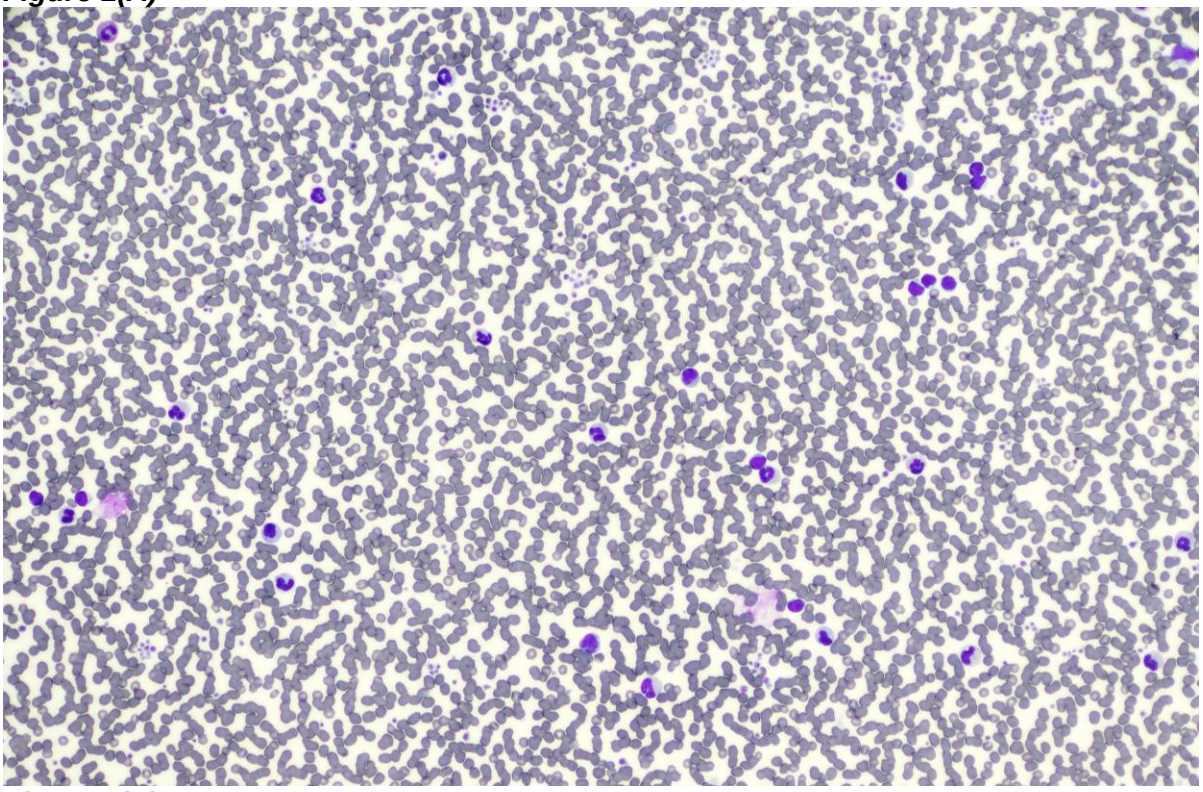

Figure 1(B)

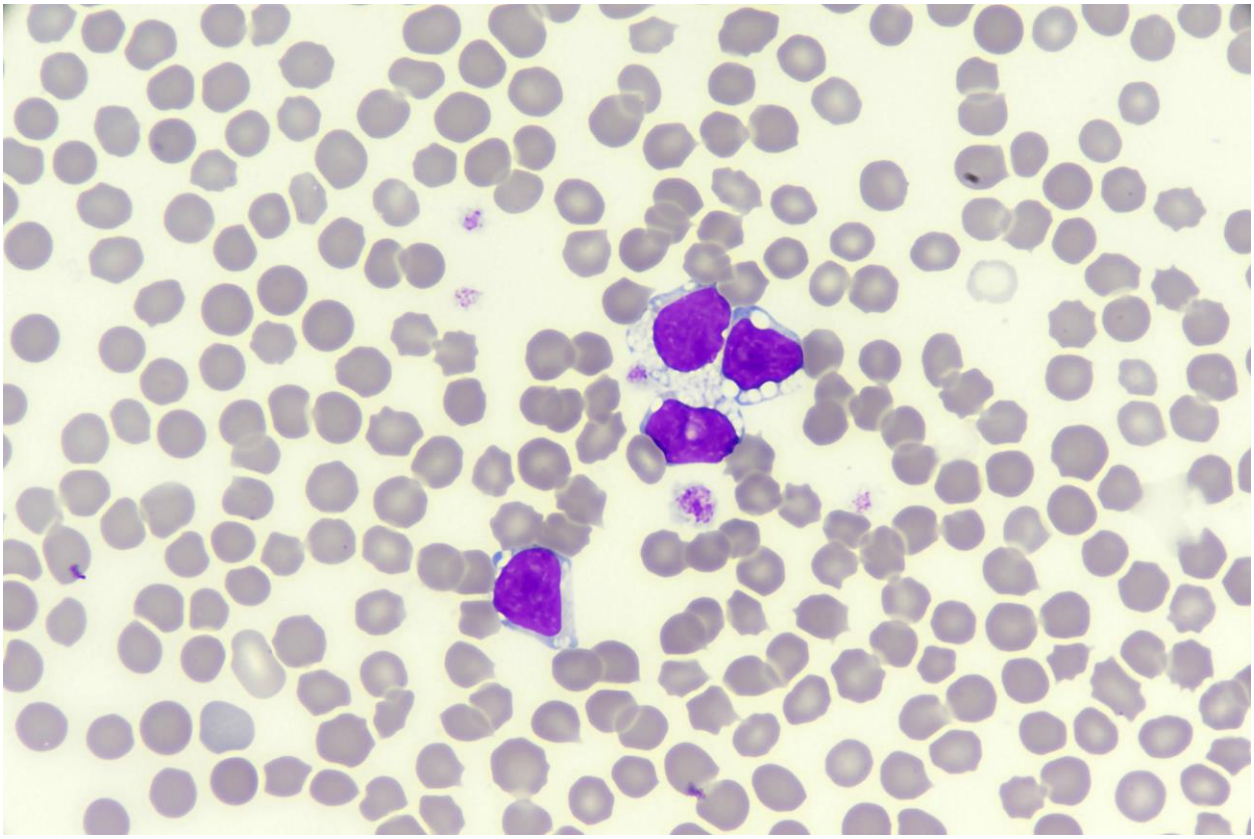

Figure 1(C)

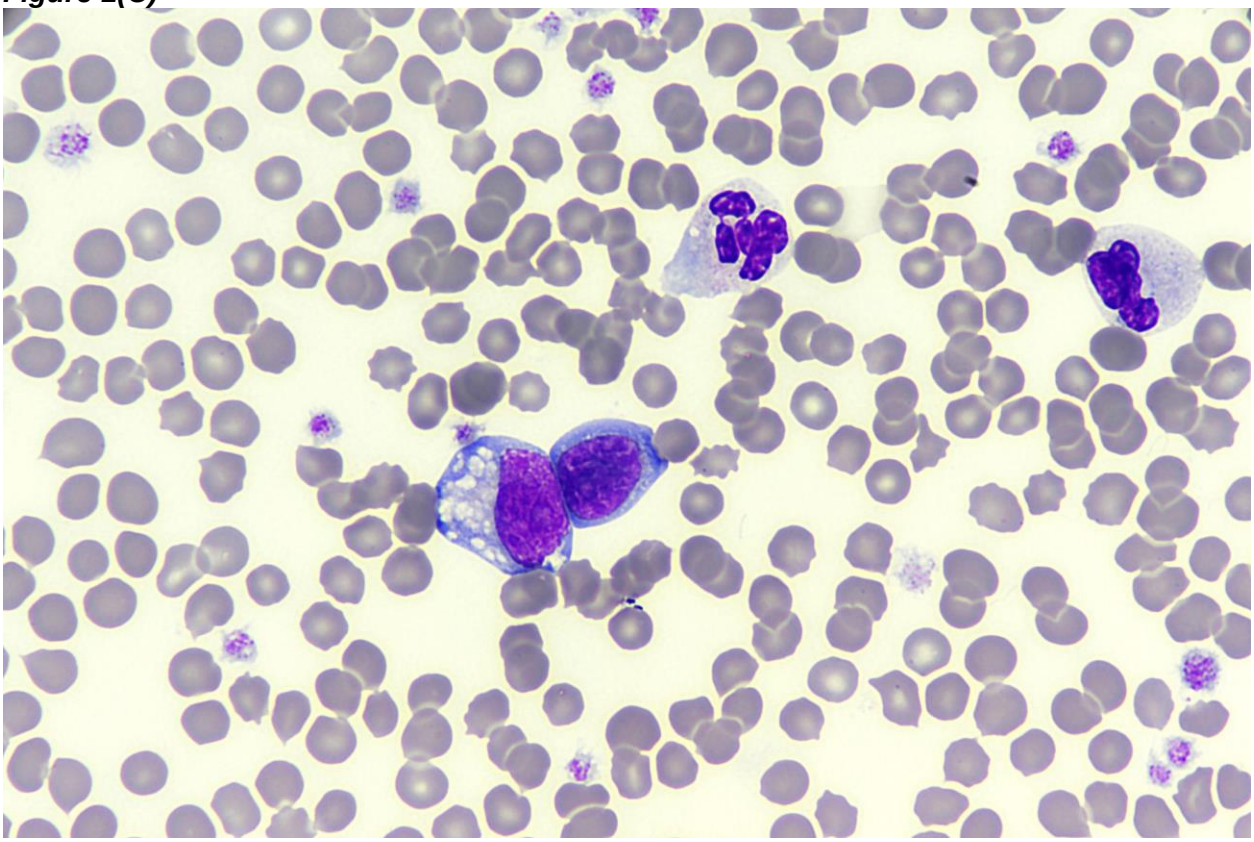

Figure 1 (A-C). Photomicrographs of blood smear from a 1-year-old male castrated domestic shorthaired cat. Wright-Giemsa stain. (A) Direct Smear Preparation ×20 objective. (B-C) Direct Smear Preparation ×100 objective.

ADDITIONAL DIAGNOSTIC TESTS:

Imaging Findings

- Thoracic radiographs: Mild cardiomegaly and aerophagia. No radiographic evidence of lower airway disease.
- CT findings: Swollen peri-arytenoid tissues and moderate regional lymphadenopathy. No other oral, nasal, pharyngeal, or laryngeal abnormalities noted. No polyps, masses or foreign material identified.

Upper Airway Endoscopy:

Proliferative tissue noted at the dorso-caudal aspect of the soft palate.

Pharynx aspirate and cytological interpretation

Marked suppurative inflammation with bacterial infection (predominantly cocci); uniform epithelial cells.

QUESTIONS:

1) What are three differentials for the blood smear findings?

2) Which statement is correct?

    A) Primary lysosomes originate from the Smooth Endoplasmic Reticulum
    B) Mannose-6-phosphate (M6P) present on lysosomal soluble hydrolases are degraded in the cis-Golgi network
    C) Lysosomal proteases play a significant role in regulating macroautophagy
    D) Glycosaminoglycans (GAGs) are typically absent in the extracellular matrix (ECM)

CYTOLOGIC DESCRIPTION: The leukocyte density appears mildly increased with mildly increased segmented neutrophils, monocytes and eosinophils and moderately increased lymphocytes. Most lymphocytes are small to intermediate in size, with round to oval nuclei and clumped chromatin. Moderate numbers of lymphocytes have mildly expanded clear to pale blue cytoplasm, with few clear distinct vacuoles. Occasional neutrophils and monocytes also contain similar vacuoles. The remainder of the CBC findings were confirmed.

CYTOLOGIC INTERPRETATION/DIAGNOSIS: Moderate numbers of lymphocytes (with fewer neutrophils and monocytes) with cytoplasmic vacuoles. Considerations for findings include storage disease, robust immune reaction and less likely lymphoid neoplasia.

ADDITIONAL FINDINGS:

Urine Metabolic Screening (U Penn)

Substrate	Result
Amino Acids	Increased amino aciduria - felinine present
Organic Acids	Hippurate/adipic acid present
Carbohydrates	Negative
Clinistix	Negative
Nitroprusside	Negative for cystine
Ketones	Negative
MPS Spot Test	**Slightly Positive**
MMA Spot Test	Negative

Table 1: MPS spot test was slightly positive, indicating a possible metabolic disorder. Felinine and hippurate/adipic acid is normal in feline urine.

Plasma Lysosomal Enzyme Assay (IDUA, 4S and GUSB) (U Penn)

Enzyme	Disease	Activity: % of Normal Control
α-L-iduronidase	MPS I	139.4
N-acetylgalactosamine-4-sulphatase	**MPS VI**	**29.5**
β-D-glucuronidase	MPS VII	119.9

Table 2: Low plasma concentrations of N-acetylgalactosamine-4-sulphatase (4S) suggests that the patient had mucopolysaccharidosis type VI (MPS VI).

Genetic Testing (U Penn)
Testing for the previously documented *ARSB* gene mutation (MPS VI L476P (c.1427T>C) variant) was negative.

DIAGNOSIS: Mucopolysaccharidosis type VI (MPS VI)

CLINICAL OUTCOME/FOLLOW-UP: Following diagnosis MPS VI, the patient was maintained on oral corticosteroids. The patient's laryngeal soft tissue edema remained largely unchanged, and approximately 5 months later, the patient had acute onset of vomiting and diarrhea which later culminated in bronchopneumonia. Shortly thereafter, the patient went into respiratory and cardiac arrest due to complications associated with severe laryngeal edema resulting in upper airway obstruction.

Gross Post Mortem Exam and Histopathological Findings (NCSU)
Prominent autopsy findings included moderate diffuse laryngeal and caudal pharyngeal edema; moderate multifocal chronic cartilage erosions of the glenohumeral and coxofemoral joints; marked regional acute bronchopneumonia of the lungs; mild chronic mitral valve endocardiosis of the heart; and marked diffuse bone marrow pallor. Histologic examination revealed vacuolized, lightly staining cells (presumed macrophages or lymphocytes) within the spleen, liver, kidney, pharynx, and larynx. Evaluation of the lungs reveals a bronchopneumonia that was most likely due aspiration pneumonia.

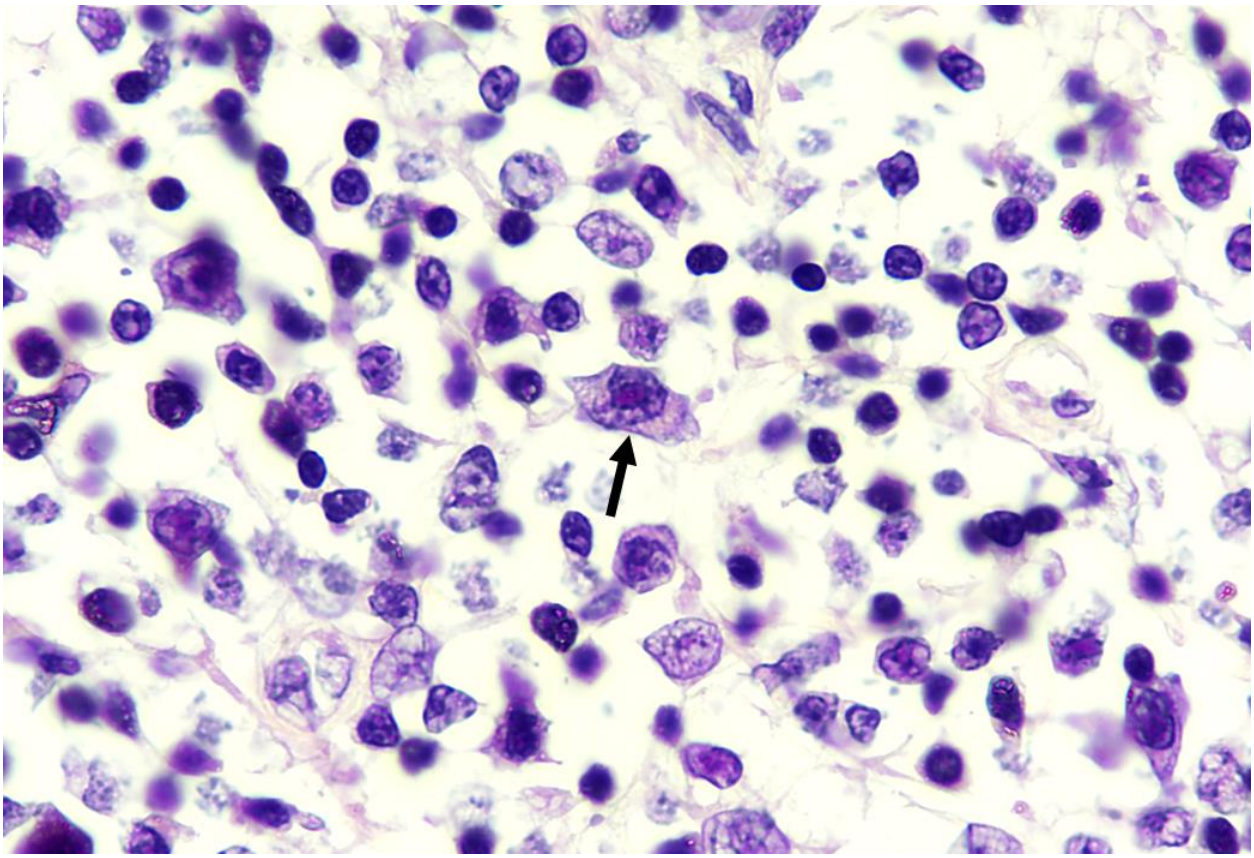

Figure 2. Photomicrograph of a tissue section from the aryepiglottic fold from a 1-year-old male castrated domestic shorthaired cat. H&E stain ×100 objective. Arrow indicates an

example of the described vacuolized, lightly staining cells (presumed macrophages or lymphocytes).

ANSWERS TO QUESTIONS:
1. Storage disease, robust immune reaction, lymphoid neoplasia
2. C

DISCUSSION:

Lysosomal storage diseases encompass a rare group of inherited metabolic disorders that are characterized by abnormal lysosomal function as well as the intralysosmal accumulation of undegraded substrates. Lysosomes are catabolic intracellular organelles that are responsible for the breakdown and recycling of a wide variety of substrates. Lysosomes are also important metabolic sensors that are crucial for metabolism and cell growth.[1] The ability of lysosomes to successfully degrade complex substances is highly dependent on the approximately 40 to 60 different lysosomal acid hydrolases. The majority of lysosomal storage diseases are caused by mutations in genes encoding for one of these diverse groups of proteins. The Mucopolysaccharidoses (MPSs), a subgroup of lysosomal storage disorders, are caused by deficiencies in enzymes involved in the degradation of glycosaminoglycans (GAG).[1] In humans, there are 7 major types of MPS (MPS I, II, III, IV, VI, VII, and XI). With the exception of MPS II, all forms are autosomal recessive.[2] Skeletal, cardiovascular, ocular and neurological abnormalities are common threads amongst the different types of MPS, which is a reflection of the diverse functions of GAGs.

MPS VI, also known as Maroteaux-Lamy Syndrome in the humans, corresponds to a mutation in the *ARSB* gene, leading to a deficiency of N-acetylgalactosamine-4-sulfatase, an enzyme which is necessary for the degradation of dermatan sulfate and chondroitin-4-sulfate (C4S).[2,3] Dermatan sulfate is found in various connective tissues, particularly cartilage and bone.[4] The severity of clinical phenotype and progression of disease varies from patient to patient. In rapidly progressive MPS VI, patients are reported to suffer from impaired growth, coarse facial features, dysostosis multiplex, restriction of joint movement, flexion contractures, impaired vision and/or hearing, nerve entrapment syndromes such as carpal tunnel syndrome and spinal cord compression, organomegaly, umbilical and/or inguinal hernias, reduced pulmonary function, and cardiac valve disease. The central nervous system tends to remain unaffected.[3]

Initial diagnostics for MPS typically involve urine GAG screening, which looks for increases in total urinary GAG. One of the caveats to this test is that patients with more mild forms of the disease typically excrete small amounts of GAGs into the urine, and as such, can go undetected.[8] Enzyme assays are also considered to be a crucial part of the first stage of laboratory diagnostic testing for MPS (and other LSDs). Molecular examination is typically part of the second stage of laboratory diagnostic testing performed in human patients suspected of the disorder, thus allowing for confirmation of enzyme activity assays and determination of mutation in the proband.[9]

MPS VI has been reported in both domestic canines and felines.[5] In felines, there is a predilection for this disease in the Siamese cat. Affected animals are typically purebred or inbred.[4] Several breeding colonies of Siamese cats with MPS VI have subsequently been established to evaluate the efficacy of bone marrow transplantation, enzyme replacement, and gene therapies for future application to human MPS VI patients.[4] As a result of forming these colonies, two mutations in the *ARSB* gene were identified and found to cause different clinical phenotypes (mild and severe diseases) in felines, similar to the genotype to phenotype relationship observed in humans.[6]

In the case presented, while the patient's full lineage is unknown, the patient and a littermate were both adopted from a local animal shelter at approximately 2 months of age. The

littermate had no reported clinical abnormalities and had grown substantially more than the patient. This piece of the history may support an autosomal recessive disorder, although other modes of inheritance certainly can't be excluded in the absence of further molecular genetic testing. The patient's overall clinical picture appears to fit best with a more mild clinical phenotype, which is also corroborated by the "slight" positive urine MPS spot test. The most significant pre-mortem physical abnormality in this case was the persistently edematous laryngeal soft tissues. Interestingly, airway abnormalities in human MPS patients are a relatively frequently reported cause of morbidity and mortality among patients with MPS. Infiltration by stored GAGs oropharyngeal manifestations such as macroglossia, enlargement of palatine and pharyngeal tonsils, and similarly to the patient presented, thickening and redundancy of soft parts of the oropharynx.[7] These changes can lead to progressive obstruction, as illustrated in this case. Cytoplasmic inclusions were noted in neutrophils and monocytes, but were most pronounced and repeatable in the lymphocytes, as previously described.[10] Gross postmortem and histopathological findings including cartilage erosions, mild endocardiosis of the mitral valve and vacuolized, lightly staining cells (presumed macrophages or lymphocytes) within the spleen, liver, kidney, pharynx, and larynx; are consistent with previously reported findings in MPS VI affected cats.[6] This case is a wonderful illustration of how careful examination of routine diagnostics, such as a peripheral blood smear, can allow for an impressive timely diagnosis of a very uncommon disease.

REFERENCES:

1. Parenti G, Andria G, Ballabio A. Lysosomal storage diseases: from pathophysiology to therapy. Annu Rev Med. 2015;66:471-86.
2. Stapleton M, Arunkumar N, Kubaski F, et al. Clinical presentation and diagnosis of mucopolysaccharidoses. Mol Genet Metab. 2018 Sep;125(1-2):4-17.
3. Hendriksz CJ, Giugliani R, Harmatz P, et al. Design, baseline characteristics, and early findings of the MPS VI (mucopolysaccharidosis VI) Clinical Surveillance Program (CSP). J Inherit Metab Dis. 2013 Mar;36(2):373-84.
4. Crawley AC, Muntz FH, Haskins ME, et al. Prevalence of mucopolysaccharidosis type VI mutations in Siamese cats. J Vet Intern Med. 2003 Jul-Aug;17(4):495-8.
5. Gurda BL, Bradbury AM, Vite CH. Canine and Feline Models of Human Genetic Diseases and Their Contributions to Advancing Clinical Therapies. Yale J Biol Med. 2017 Sep 25;90(3):417-431.
6. Crawley AC, Yogalingam G, Muller VJ, Hopwood JJ. Two mutations within a feline mucopolysaccharidosis type VI colony cause three different clinical phenotypes. J Clin Invest. 1998 Jan 1;101(1):109-19.
7. John A, Fagondes S, Schwartz I, et al. Sleep abnormalities in untreated patients with mucopolysaccharidosis type VI. Am J Med Genet A. 2011 Jul;155A(7):1546-51.
8. White K, Medelsohn N, Hale S, et al. The Mild Side of MPS Disorders: Are These Cases Being Missed by Urine Screening and Other Common Diagnostic Methods? Mol Genet Metab. 2012 Feb;105(2):S65.
9. Mokhtariye A, Hagh-Nazari L, Varasteh AR, Keyfi F. Diagnostic methods for Lysosomal Storage Disease. Rep Biochem Mol Biol. 2019 Jan;7(2):119-128.
10. Alroy J, Freden GO, Goyal V, Raghavan SS, Schunk KL. Morphology of leukocytes from cats affected with alpha-mannosidosis and mucopolysaccharidosis VI (MPS VI). Vet Pathol. 1989 Jul;26(4):294-302.