Findings from a Hedgehog

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SIGNALMENT:

3-year-old African pigmy hedgehog

HISTORY AND CLINICAL FINDINGS:

History of anorexia, weakness, weight loss, behavioral change, ataxia, and cervical and abdominal masses. Skin mites were observed.

LABORATORY DATA:

Table 1

CBC	3/8 Pre-treatment	3/12 Post-treatment	Reference interval*	Units
Hct	36.7	31.9	36 +/- 7 (22-64)	%
Hgb	13.7	11.1	12 +/- 2.8 (7-21.1)	g/dl
Rbc	4.3	3.67	6+/- 2 (3-16)	$x10^6$/µl
MCV	85.3	87	67 +/- 9 (41-94)	fl
MCHC	37.3	34.9	34 +/- 5 (17-48)	g/dl
Ptl	137	142	226 +/- 108 (60-347)	x103/µl
MPV	11.6	10	n/a	fl
WBC	85.4	2.0	11 +/- 6 (3-43)	x103/µl
Seg	(30%) 25.6	(11%) 0.220	5.1 +/- 5.2 (0.6-37.4)	x103/µl
Band	(9%) 7.7	(0%) 0	n/a	x103/µl
Lymph	(3%) 2.6	(32%) 0.6	4 +/- 2.2 (0.9-13.1)	x103/µl
Mono	(0%) 0	(6%) 0.1	0.3 +/- 0.3 (0-1.6)	x103/µl
Eos	(9%) 7.7	(17%) 0.3	1.2 +/- 0.9 (0-5.1)	x103/µl
Baso	(3%) 2.562	(2%) 0.04	0.4 +/- 0.3 (0-1.5)	x103/µl
Others	(46%) 39.284	(32%) 0.6	0	x103/µl
nRBC	2	3	n/a	/100 WBC

* Reference intervals are from “Exotic animal formulary”, by Carpenter J.W., Elsevier 4th ed. 2012

CASE OUTCOME:

The clinical status of the animal continued to deteriorate with worsening of depression, anorexia and lethargy. The hedgehog died the day after the second CBC was performed and necropsy was performed. Histologic sections of bone marrow collected post mortem.

QUESTIONS:

What are the possible differentials and what features would you use to reach a definitive diagnosis?

What stains would you use to confirm the cell(s) of origin?

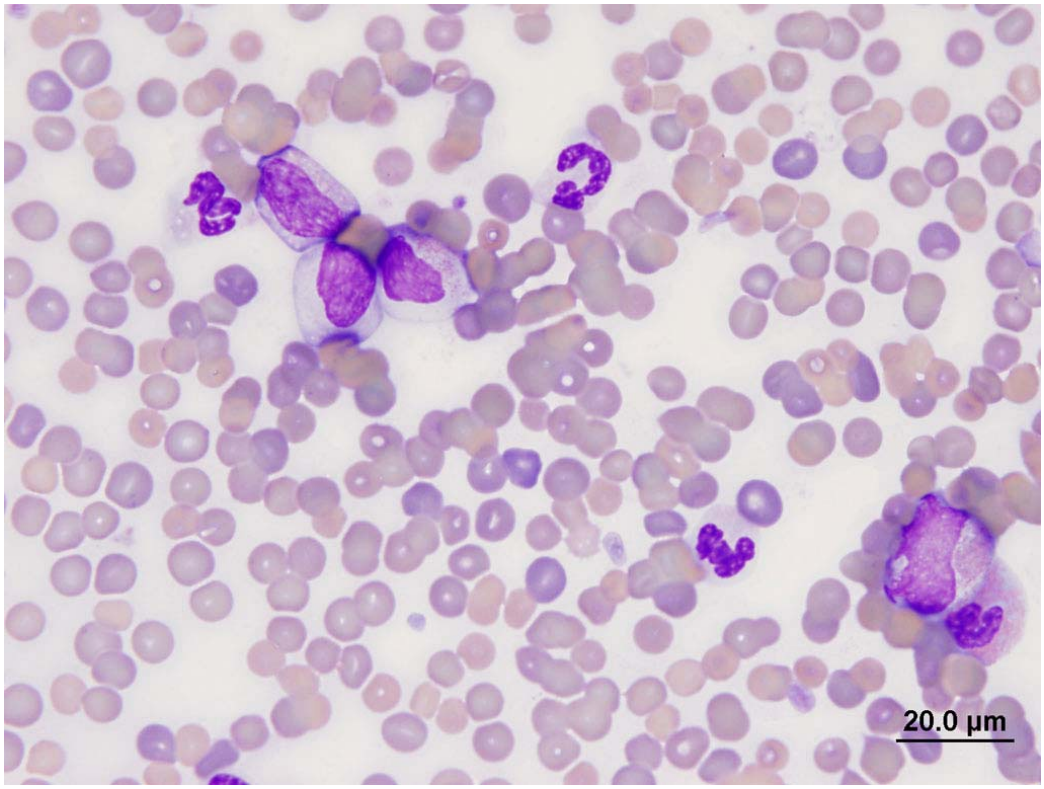

Figure 1
Peripheral blood from a hedgehog. Modified Romanowsky stain, Original magnification X1000

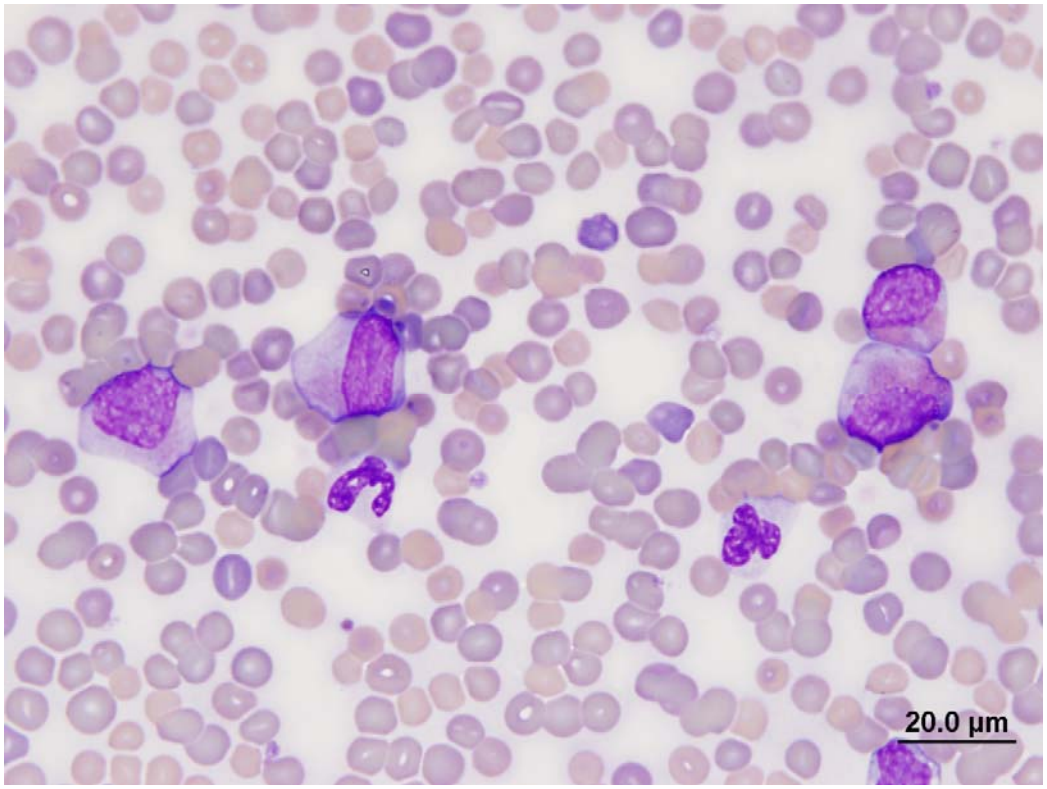

Figure 2
Peripheral blood from a hedgehog. Modified Romanowsky stain, Original magnification X1000

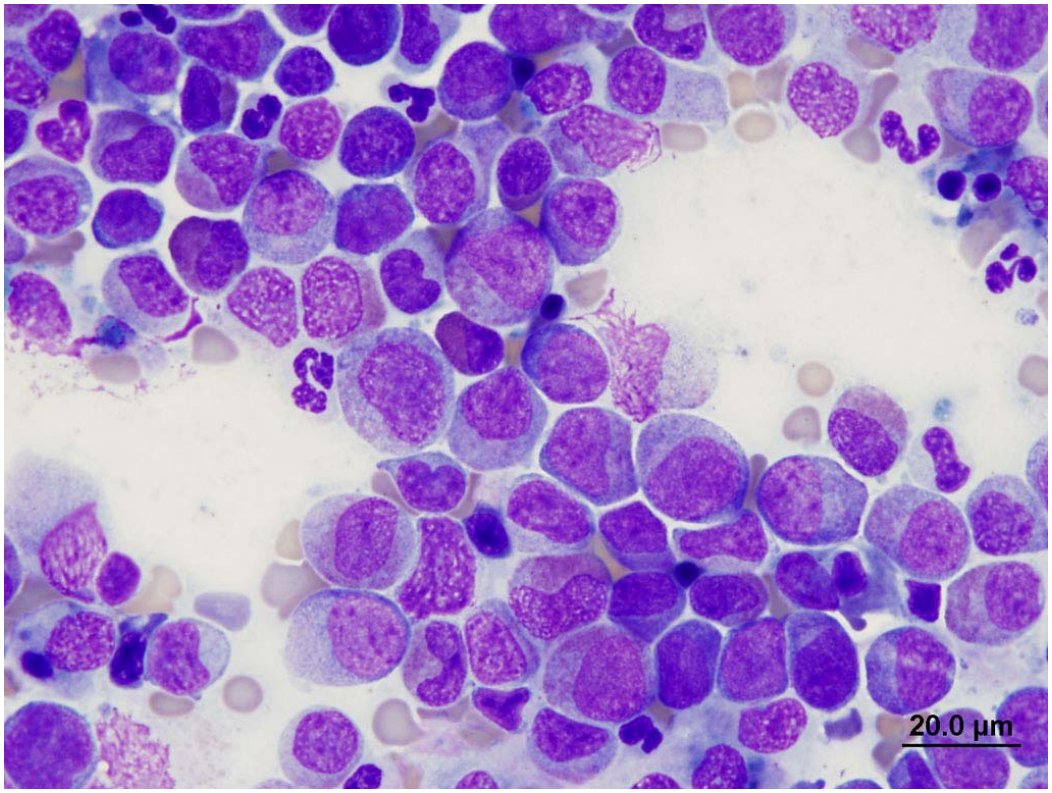

Figure 3
Bone marrow fine needle aspirate from a hedgehog. Modified Romanowsky stain, Original magnification X1000

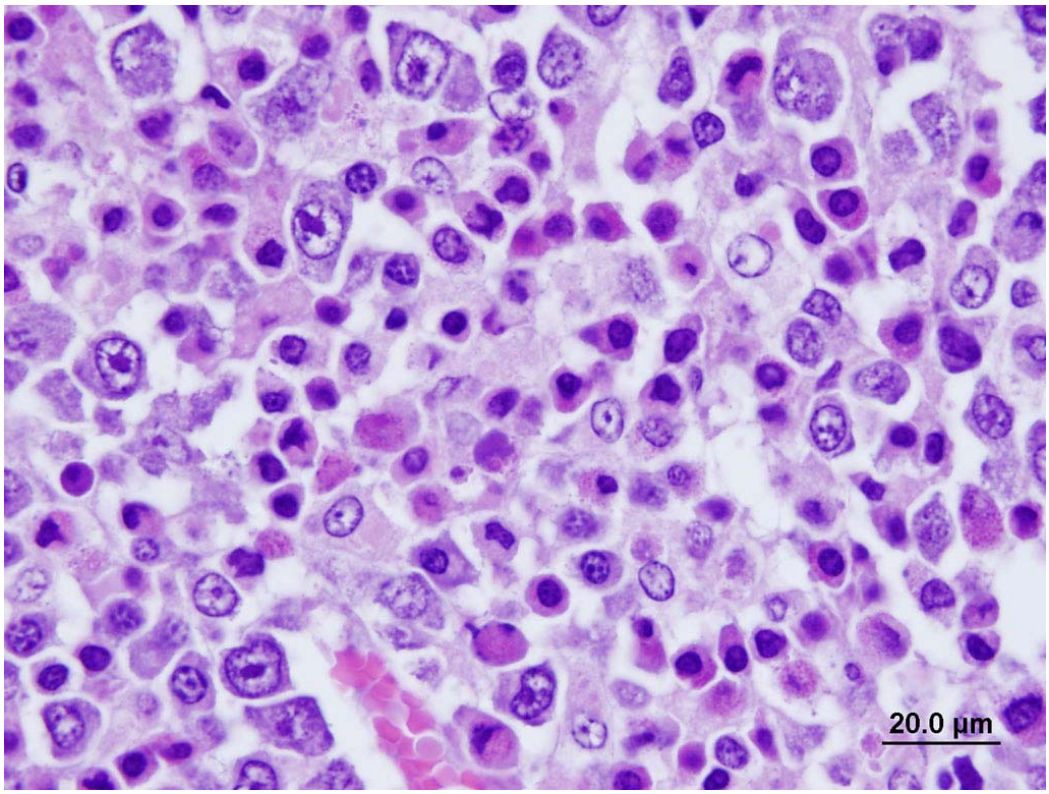

Figure 4
Histologic section of bone marrow from a hedgehog. Hematoxylin and eosin, Original magnification X1000

Further investigations and discussion:

BLOOD SMEAR EXAMINATION:
The cells called "others" are large (15 to 30 microns in diameter), round to oval cells with high N:C ratio. They have a moderate, lightly to moderately basophilic cytoplasm often filled with moderate to high numbers of small, eosinophilic granules. The nucleus is round to oval, and has an open, ropy, chromatin pattern. The cells classified as eosinophils comprise mature eosinophils, banded eosinophils and eosinophilic metamyelocytes.

BONE MARROW EXAMINATION:
The bone marrow is hyperplastic with approximately 95-100% cells and 0-5% adipose tissue. Iron deposits are not readily visible. Megakaryocytes are adequate to mildly decreased, are normally distributed and have a regular maturation. The M:E ratio is approximately 40 to 50: 1 with a large expansion in the myeloid line and a relative erythroid hypoplasia. The vast majority of the cells are immature myeloid cells ranging from promyelocytes to myelocytes, many of which contain variable numbers of round eosinophilic granules. Low numbers of cells have nuclear to cytoplasmic maturation asynchrony and mild cellular dysplasia (large cells with banded nucleus). Myeloblasts comprise 10-15% of the nucleated cells while banded and segmented granulocyteas are present in low numbers. Erythroid cells are scattered and comprised approximately 5% of the nucleated cells. Occasional small lymphocytes and rare well-differentiated plasma cells are also seen.

INTERPRETATION/DIAGNOSIS:
Eosinophilic leukemia

ADDITIONAL FINDINGS:
The circulating cells classified as “others” on the CBC resembled the granulated cells observed in the bone marrow. Fine needle aspirates of the cervical mass were consistent with lymph node aspiration with high numbers of the eosinophilic precursors similar to those seen in circulation. The lack of an orderly maturation or well-organized left shift in the granular cells suggested these were neoplastic eosinophils, and not part of an inflammatory response. Chemotherapy and corticosteroids were administered after the CBC on day 1, and treatment greatly reduced the number of circulating leukocytes (Table 1). All organs examined at necropsy were diffusely infiltrated by cells similar to those seen in the marrow and peripheral blood. Luna stain was applied to a section of paraffin embedded bone marrow and showed moderate staining of cytoplasmic granules. Alkaline phosphatase stain was applied to an unstained bone marrow cytology slide and while more immature neoplastic cells were negative, low numbers of more mature cells demonstrated cytoplasmic positive staining. The neoplastic cells were positive for Luxol fast blue (LFB) stain and the granules were solely occasional positive to Periodic acid-Schiff (PAS). Cells present in unstained cytology slides of bone marrow were negative for Sudan IV. Stain results are summarized and compared to eosinophils staining patterns of dogs and cats[1] in table 2.

Table 2

Stain	Hedgehog (our case)	Cats[1]	Dogs[1]
Luna	+	+	+
Alk Phos stain	+ (mature cells)	+	-/+
Luxol Fast Blue	+	+	+
PAS	-/+	-/+	-/+
Sudan (IV or Black)	- (IV)	- (Black)	+ (Black)

DISCUSSION:

Eosinophilic leukemia is a rare neoplasm of the myelopoietic line that has been described in a variety of animals and in humans[2-5]. Eosinophilic leukemia should be differentiated from hypereosinophilic syndrome. Both conditions are rare but have been most frequently observed in cats[2,3]. In both conditions myeloid hyperplasia with an eosinophilic predominance is seen in the bone marrow and high numbers of eosinophils are present in the peripheral blood[1]. The predominant features to differentiate these two conditions, extrapolated from reference 2, are summarized in Table 3.

Table 3

	Hypereosinophilic syndrome	Eosinophilic leukemia
Cellular morphology in bone marrow and blood	No morphologic abnormalities	Atypical cells can be present
Cellular maturity in blood	Rare immature cells	Increased immature cells
Total leukocyte count	50,000–75,000 cells/µL	75,000-150,000 cells/µL
Bone marrow M:E ratio	< 10	> 10
Eosinophil maturity in bone marrow	Mature eosinophils predominate	Immature eosinophils can predominate
PCV	Mildly decreased (average of 34%)	Moderately decreased (average 25%)
Eosinophilic infiltration heart and mediastinum	Not usually seen	Possible
Maturity of infiltrating cells	Normally mature	Can be immature

The presence of skin mites in this case could have contributed to a hypereosinophilia as eosinophils are commonly seen in parasitic and allergic reactions[5]. However, the predominance of promyelocyte and myelocyte eosinophils both in the peripheral blood and in the bone marrow, the marked increase in the M:E ratio, the dysplastic changes observed, and the infiltration of immature cells in all the organs were diagnostic for eosinophilic leukemia. Poorly differentiated eosinophils may resemble immature myeloid cells of neutrophil, basophil or monocyte origin. For this reason additional stains may be necessary for a definitive differentiation. Luna stain is generally used to improve visibility of eosinophils in histologic sections[6]. Surprisingly only a moderate percentage of cells in the bone marrow of this hedgehog showed marked positivity. The lack of widespread, marked positivity was attributed to either altered granule content in this neoplastic population or differences in hedgehog eosinophil staining properties. LFB is also considered helpful in the identification of eosinophils[1]. In this case, the cytoplasm of the neoplastic cells was positive for LFB. Solely a low percentage of cytoplasmic granules were PAS positive. This stain has demonstrated inconsistency also in dogs and cats[1].

Eosinophils in this hedgehog were negative for Sudan stain, showing similarity with cats eosinophils[1].Leukocyte alkaline phosphatase activity has been demonstrated in the eosinophils of both cats and dogs[3,7]. Alkaline phosphatase activity was seen also in the majority of the more banded and segmented cells in the bone marrow of this hedgehog. A specific eosinophil peroxidase monoclonal antibody has been evaluated in dogs[5], however, to the authors' knowledge, the validity of this antibody has not been verified in other domestic animals. The presence of immature circulating cells has been demonstrated in 2 cases of feline eosinophilic leukemia by positivity to CD45[3] and CD34[8] antibodies in circulating eosinophils. Electron microscopy of the neoplastic cells is pending and results will be presented during the podium presentation.

REFERENCES:

1. Raskin R. Cytochemical Staining, in Schalm's Veterinary Hematology, 6th ed, 2010 Blackwell Pubblishing: Ames, Iowa. p. 1141-1156
2. Valli V.E. Chronic myeloproliferative diseases, in Veterinary Comparative Hematopathology, 2007 Blackwell Pubblishing: Ames, Iowa. p. 436-440
3. Gelain M.E., Antoniazzi E., Bertazzolo W., Zaccolo M., Comazzi S. *Chronic eosinophilic leukemia in a cat: cytochemical and immunophenotypical features*. Vet Clin Pathol, 2006;35:454–459
4. Port D.C. and Richter W.R. *Eosinophilic leukemia in a Syrian Hamster*. Vet Pathol.1977;14:283-286
5. Morris D.D., Bloom J.C., Roby K.A.W. et al, *Eosinophilic myeloproliferative disorder in a horse*. J Am Vet Med Assoc 1984;185(9):993-6
6. Gomes P., Torres S.M.F., Plager D.A., Jesses C.R. and Lee J.J. *Comparison of three staining methods to identify eosinophils in formaline-fixed canine skin*. Vet Dermatol, 2013; 24: 323–372
7. Iazbik M.C., Couto C.G. *Morphologic characterization of specific granules in Greyhound eosinophils*. Vet Clin Pathol, 2005;34:140–143
8. Sharif H., Nassiri S.M., Esmaelli H. and Khoshnegah J. *Eosinophilic leukaemia in a cat*. J Feline Med Surg, 2007;9:514-517