A bad case of anemia

Dorothee Bienzle
Department of Pathobiology
University of Guelph, Guelph ON CANADA

Signalment
Mixed breed dog, 9.6 kg, male, neutered, 6.9 years

History
- Poor appetite for ~1 month, recent weight loss
- Referring veterinarian identified anemia and neutropenia on blood work
- Prescribed 10 mg of prednisone (~1 mg/kg) twice a day plus sucralfate
- Serologic testing for *Ehrlichia*, heartworm, *Borrelia* and *Anaplasma* was negative
- Recheck at referring veterinarian after one week: Dog had lost 1 pound, was lethargic and drinking frequently
- Referred to the Ontario Veterinary College at the University of Guelph

Physical Examination
- Weight 8.6 kg, body condition score 3/9
- Very quiet, mucus membranes very pale, grade I/II cardiac murmur
- Otherwise unremarkable

Laboratory Results – Day 1

Test	Result		Reference Interval
WBC	14.1	x 10 ^9/L	4.9-15.4
RBC	0.8 L	x 10 ^12/L	5.8-8.5
Hb	22 L	g/L	133-197
HCT	0.08 L	L/L	0.39-0.56
MCV	96 H	fL	66-75
MCH	28 H	pg	21-25
MCHC	290 L	g/L	321-360
RDW	26.6 H	%	11-14
Platelets	101 L	x 10 ^9/L	117-418
MPV	22.2 H	fL	7-14
Plateletcrit	0.23	%	0.14-0.47
T.S. Protein	81 H	g/L	55-75
Seg Neutrophil Count	7.19	x 10 ^9/L	2.9-10.6
Band Neutrophil Count	1.97 H	x 10 ^9/L	0.0-0.3
Lymphocyte Count	2.96	x 10 ^9/L	0.8-5.1
Monocyte Count	1.13 H	x 10 ^9/L	0.0-1.1
Rubricyte Count	0.85 H	x 10 ^9/L	0.0-0.0
Polychromasia	10-15		

Reticulocytes 270.7 x10^9/L
Reticulocytes 34.7%
Direct DAT 1:32

Numerous spherocytes
Platelet clumping

Test	Result		Reference Interval
Hemolysis	Negative		
Lipemia	Negative		
Calcium	2.50	mmol/L	2.50-3.00
Phosphorus	1.19	mmol/L	0.90-1.85
Magnesium	1.0	mmol/L	0.7-1.0
Sodium	151	mmol/L	140-154
Potassium	4.5	mmol/L	3.8-5.4
Chloride	111	mmol/L	104-119
Carbon Dioxide	16	mmol/L	15-25
Anion Gap	**28 H**	mmol/L	13-24
Na:K Ratio	34		29-37
Total Protein	**77 H**	g/L	55-74
Albumin	31	g/L	29-43
Globulin	**46 H**	g/L	21-42
A:G Ratio	**0.67 L**		0.7-1.8
Urea	**15.7 H**	mmol/L	3.5-9.0
Creatinine	70	umol/L	20-150
Glucose	6.8	mmol/L	3.3-7.3
Cholesterol	**2.54 L**	mmol/L	3.60-10.20
Total Bilirubin	**8 H**	umol/L	0-4
Conjugated Bilirubin	**4 H**	umol/L	0-1
Free Bilirubin	**4 H**	umol/L	0-3
Alkaline Phosphatase	98	U/L	22-143
Steroid-induced ALP	31	U/L	0-84
GGT	0	U/L	0-7
ALT	25	U/L	19-107
CK	148	U/L	40-255
Amylase	542	U/L	299-947
Lipase	120	U/L	25-353
Calculated Osmo	312	mmol/L	

Specimen type	Test	Result	
Urine	Colour	Yellow	
	Clarity	Slightly cloudy	
	Volume Submitted	40.0	mL
	Specific Gravity	1.013	
	pH	7.5	
	Multistix Protein	3+	
	Glucose	Negative	
	Ketones	Negative	
	Bilirubin	1+	
	Blood	Trace	
	Urobilinogen	3.2	umol/L
	Leukocytes	None Seen	/400 x
	RBC	Occasional	/400 x
	Epithelial Transitional	Occasional	/400 x
	Fat	1+	
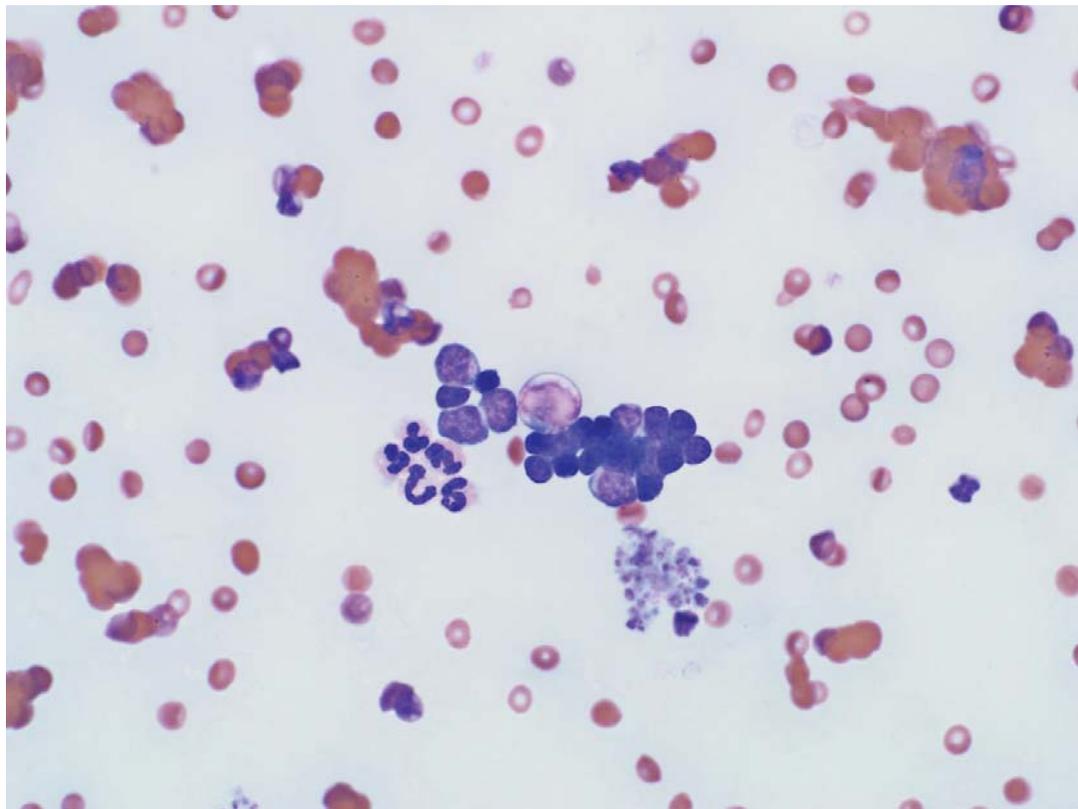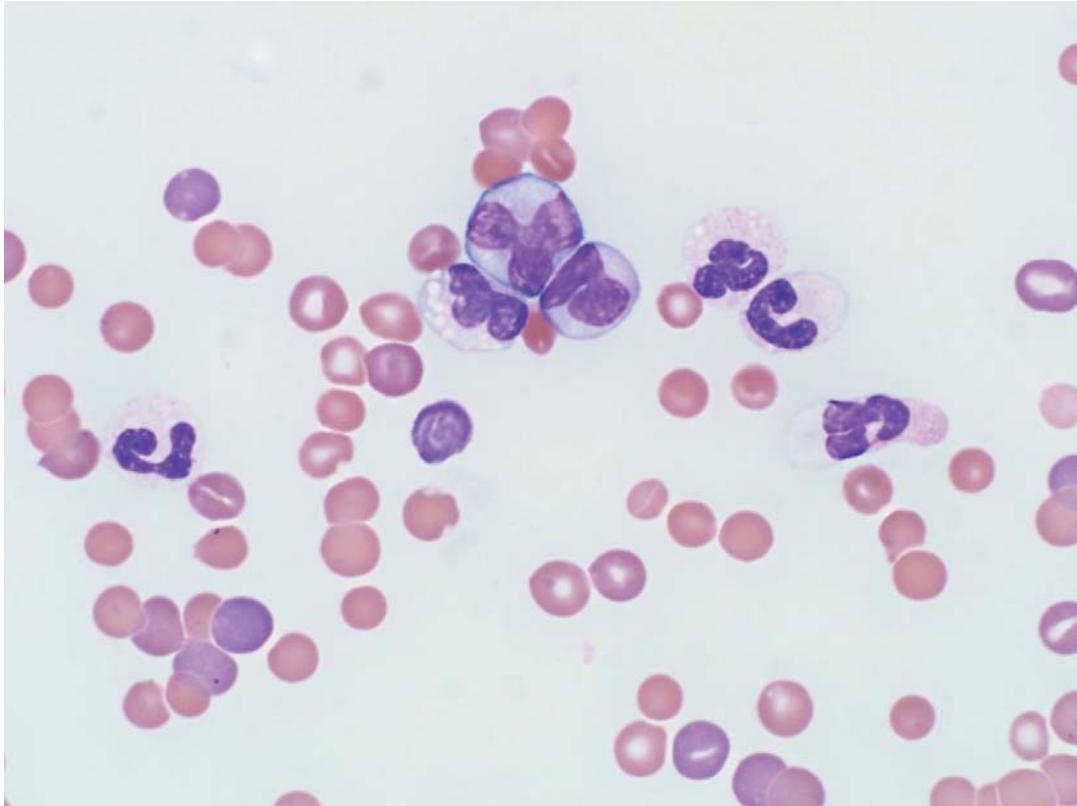
Initial Treatment Plan
Blood transfusion (packed RBC) since increased respiratory rate
Cerenia 1 mg/kg IV
Famotidine 0.5 mg/kg IV
Dexamethasone 0.25 mg/kg IV
Clopidogrel 1/4 tab (75 mg tab) PO
Trazodone 50 mg PRN
Sucralfate 500 mg PO
Cyclosporine 6 mg/kg

Other Diagnostic Test Results
Abdominal ultrasound:
1. Diffuse hyperechoic hepatic parenchyma; likely non-specific, unlikely neoplasia
2. Multiple hypoechoic nodules throughout the spleen, up to 8mm in size; likely lymphoid hyperplasia or extramedullary hematopoiesis

Thoracic radiographs: no abnormalities.

Laboratory Results – Day 4

Test	Result		Reference Interval
WBC	8.4	x 10 ^9/L	4.9-15.4
RBC	**1.5 L**	x 10 ^12/l	5.8-8.5
Hb	**34 L**	g/L	133-197
HCT	**0.10 L**	L/L	0.39-0.56
MCV	69	fL	66-75
MCH	23	pg	21-25
MCHC	338	g/L	321-360
RDW	**24.2 H**	%	11-14
Platelets	**62 L**	x 10 ^9/L	117-418
MPV	**18.8 H**	fL	7-14
Plateletcrit	**0.12 L**	%	0.14-0.47
T.S. Protein	63	g/L	55-75
Seg Neutrophil Count	4.87	x 10 ^9/L	2.9-10.6
Band Neutrophil Count	**0.59 H**	x 10 ^9/L	0.0-0.3
Lymphocyte Count	2.27	x 10 ^9/L	0.8-5.1
Monocyte Count	0.17	x 10 ^9/L	0.0-1.1
Rubricyte Count	**0.50 H**	x 10 ^9/L	0.0-0.0
Polychromasia	5-10		
Reticulocytes	151	$x10^9/L$	5-80
Reticulocytes	11.5%		

Occasional spherocytes
Mild RBC agglutination

Test	Result		Reference Interval
Hemolysis	Negative		
Lipemia	Negative		
Calcium	**2.22 L**	mmol/L	2.50-3.00
Phosphorus	**0.79 L**	mmol/L	0.90-1.85
Magnesium	0.7	mmol/L	0.7-1.0
Sodium	**139 L**	mmol/L	140-154
Potassium	5.0	mmol/L	3.8-5.4
Chloride	107	mmol/L	104-119
Carbon Dioxide	16	mmol/L	15-25
Anion Gap	21	mmol/L	13-24
Na:K Ratio	**28 L**		29-37
Total Protein	62	g/L	55-74
Albumin	**26 L**	g/L	29-43
Globulin	36	g/L	21-42
A:G Ratio	0.72		0.7-1.8
Urea	4.9	mmol/L	3.5-9.0
Creatinine	52	umol/L	20-150
Glucose	6.2	mmol/L	3.3-7.3
Cholesterol	**1.84 L**	mmol/L	3.60-10.20
Total Bilirubin	**7 H**	umol/L	0-4
Conjugated Bilirubin	**3 H**	umol/L	0-1
Free Bilirubin	**4 H**	umol/L	0-3
Alkaline Phosphatase	98	U/L	22-143
Steroid-induced ALP	43	U/L	0-84
GGT	1	U/L	0-7
ALT	31	U/L	19-107
CK	113	U/L	40-255
Amylase	377	U/L	299-947
Lipase	117	U/L	25-353
Calculated Osmo	279	mmol/L	

"Micah" was discharged on day 5 with a diagnosis of primary immune-medicated hemolytic anemia (IMHA) and the following medications:

Prednisone 20 mg (~2 mg/kg) once per day
Clopidogrel 20 mg (~2 mg/kg) once per day
Cerenia 16 mg – give as needed to control nausea or inappetence
Sucralfate 2 mL of 200 mg/mL oral suspension every 8 hrs

Follow-up
- CBC performed by referring veterinarian on day 8 was essentially unchanged from discharge
- On **day 22** Micah started to become lethargic, drinking more, panting frequently and consistently sleeping
- She was taken to her family veterinarian who noted weight gain and discharged Micah with RC recovery food to continue her weight gain
- On **day 23** Micah was unable to stand and taken the local emergency clinic
- In-clinic CBC: hematocrit 3.5 L/L, platelets 65 x10^9/L and reticulocytes 182 x10^9/L
- Referred back to OVC

Re-assessment – Day 23
- Mucus membranes where white/jaundiced, HR 200, RR 36
- Auscultation and palpation: no abnormal findings
  Weight 6.9 kg, body condition score 2/9, lactate 18 mmol/L
- Treated with 0.25 mL/kg dose of iv dextrose and packed RBC transfusion

Laboratory Results – Day 26
- Leptospira microscopic agglutination test: negative for *L. icterohemorrhagica*, *L. hardjo*, *L. grippotyphosa*, *L. pomona*, *L. canicola;* low titer for *L. autumnalis*
- Liver aspirate: Mild hepatocellular vacuolar change and trilineage hematopoiesis

Test	Result		Reference Interval
WBC	5.9	x 10 ^9/L	4.9-15.4
RBC	**1.2 L**	x 10 ^12/l	5.8-8.5
Hb	**27 L**	g/L	133-197
HCT	**0.08 L**	L/L	0.39-0.56
MCV	73	fL	66-75
MCH	24	pg	21-25
MCHC	321	g/L	321-360
RDW	**21.9 H**	%	11-14
Platelets	**58 L**	x 10 ^9/L	117-418
MPV	**21.8 H**	fL	7-14
Plateletcrit	**0.13 L**	%	0.14-0.47
T.S. Protein	66	g/L	55-75
Seg Neutrophil Count	4.54	x 10 ^9/L	2.9-10.6
Band Neutrophil Count	**0.41 H**	x 10 ^9/L	0.0-0.3
Lymphocyte Count	**0.41 L**	x 10 ^9/L	0.8-5.1
Monocyte Count	0.24	x 10 ^9/L	0.0-1.1
Immature Count	**0.06 H**	x 10 ^9/L	0.0-0.0
Rubricyte Count	**0.24 H**	x 10 ^9/L	0.0-0.0
Polychromasia	2-5		
Anisocytosis	2+		
Reticulocytes	106	x10^9/L	5-80
Reticulocytes	9.2%		

Rare spherocytes
Mild RBC agglutination
Occasional metamyelocytes

Hemolysis	Mild		
Lipemia	Negative		
Total Protein	62	g/L	55-74
Albumin	**25 L**	g/L	29-43
Globulin	37	g/L	21-42
A:G Ratio	**0.68 L**		0.7-1.8
Urea	8.6	mmol/L	3.5-9.0
Glucose	6.0	mmol/L	3.3-7.3
Cholesterol	**3.43 L**	mmol/L	3.60-10.20
Total Bilirubin	**8 H**	umol/L	0-4
Alkaline Phosphatase	107	U/L	22-143
Steroid-induced ALP	43	U/L	0-84
GGT	0	U/L	0-7
ALT	**1484 H**	U/L	19-107
Bile Acid	**21 H**	umol/L	0-6
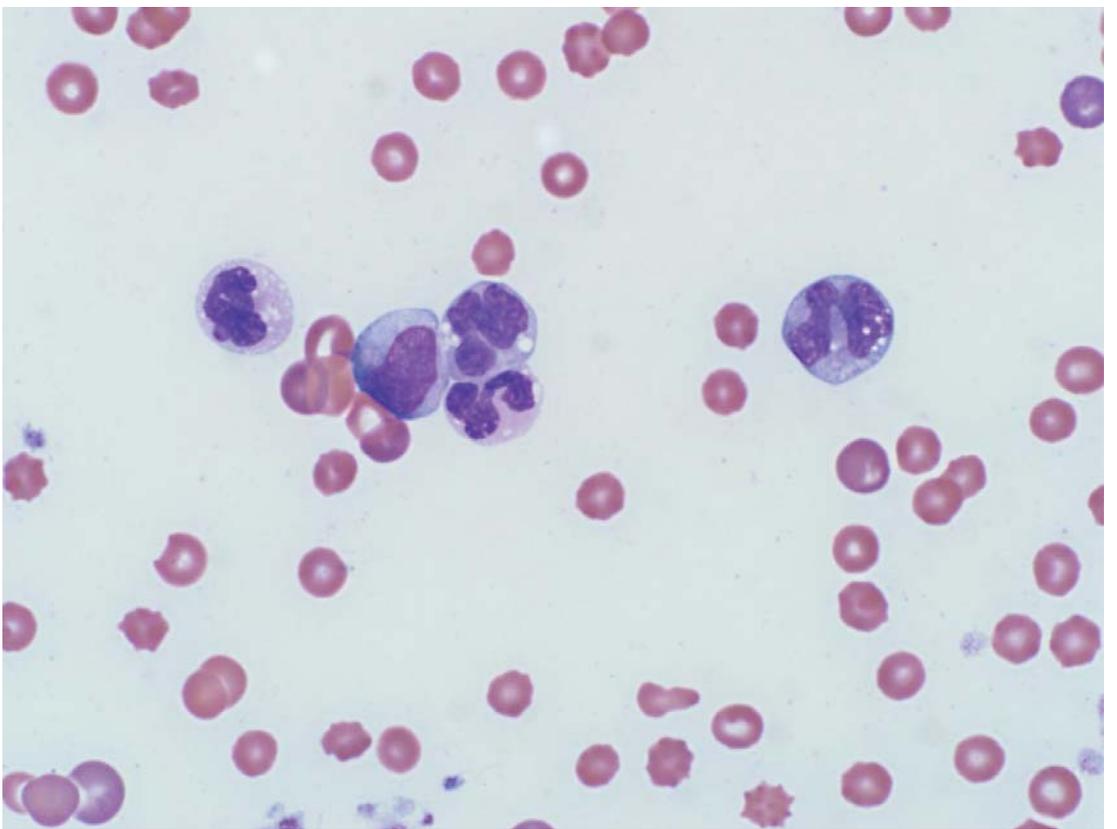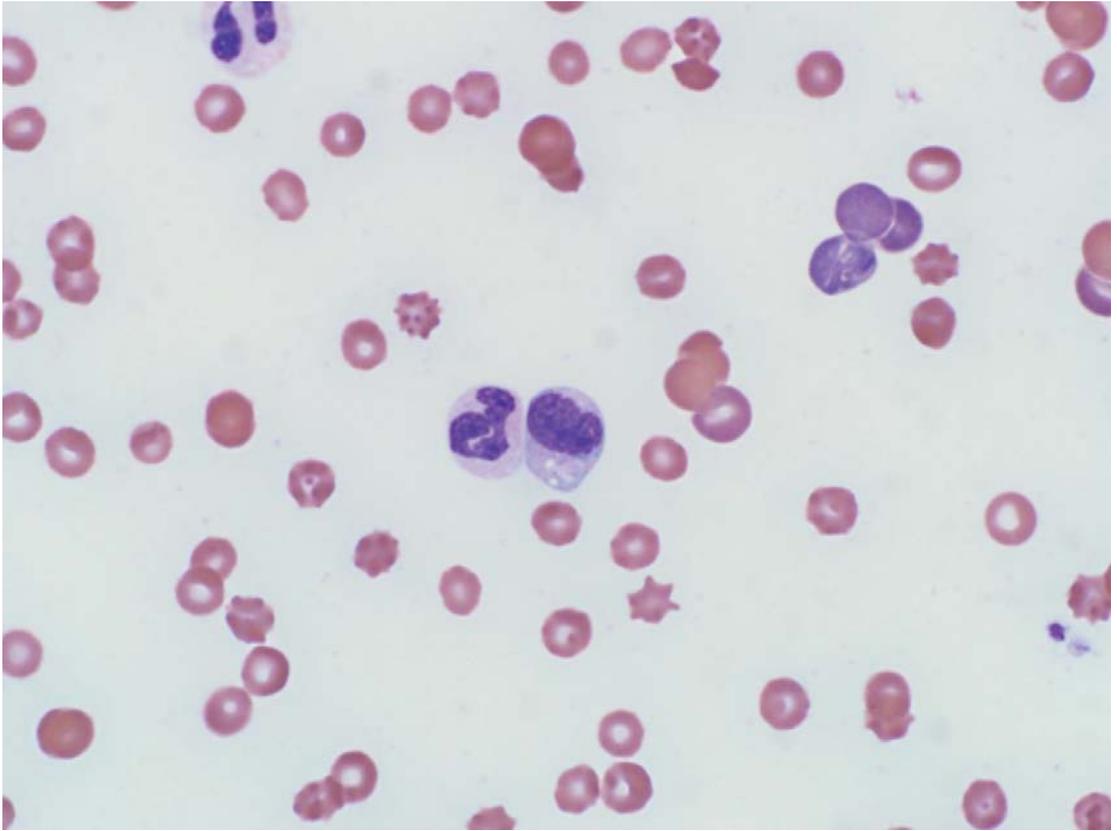
Other Tests – Day 27

Sternal bone marrow aspirate (no sedation):
"The sample is hemodiluted and poorly cellular with no particles. Individual rubricytes, granulocytic precursors and megakaryocytes were observed but neither cell maturation nor distribution can be assessed. The sample may not be representative of the hematopoietic tissue."

Questions:

1. What are the differential diagnoses in this dog?
2. Have the differential diagnoses changed since the first episode of anemia?
3. What additional diagnostic steps should be performed?
4. What is the prognosis for this dog at this point?

Outcome:
Micah received another packed RBC transfusion and was anesthetized for a humeral bone marrow core biopsy and aspirate.

## Humeral bone marrow aspirate
"Hemodilute sample without bone marrow particles and with only rare individual hematopoietic cells."

Bone marrow core

MPO

Iba-1

MHC II
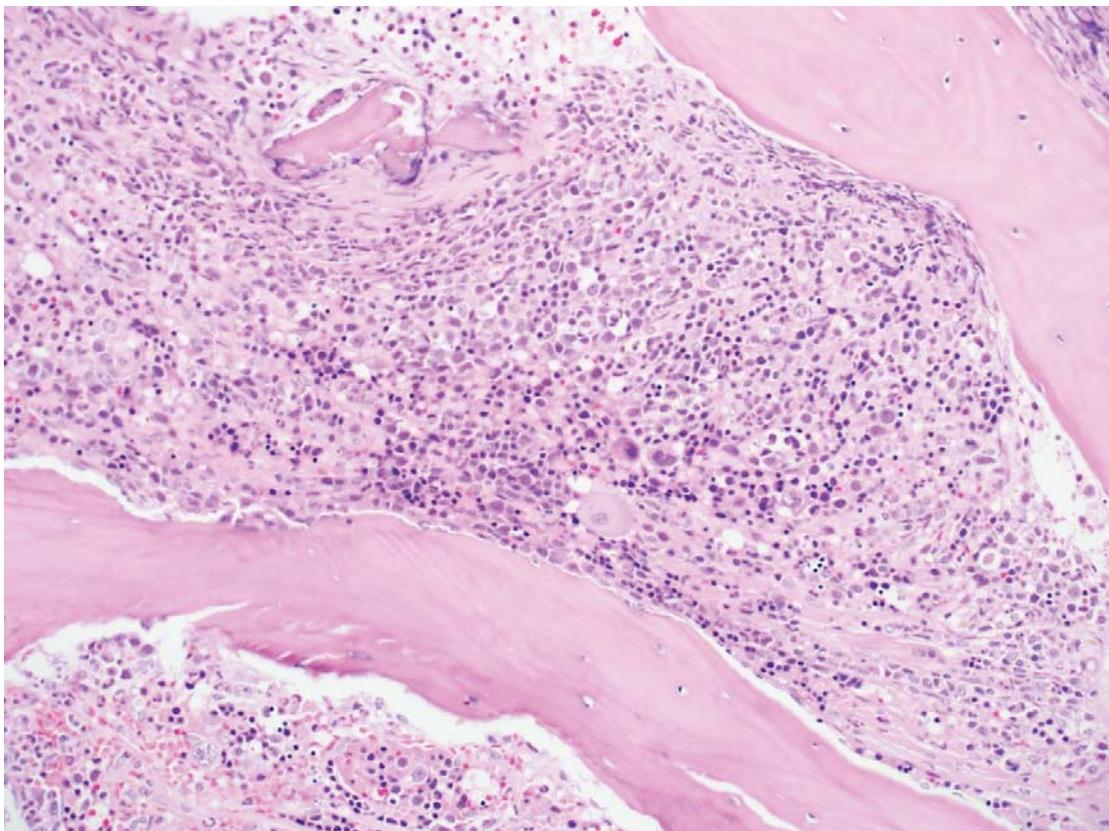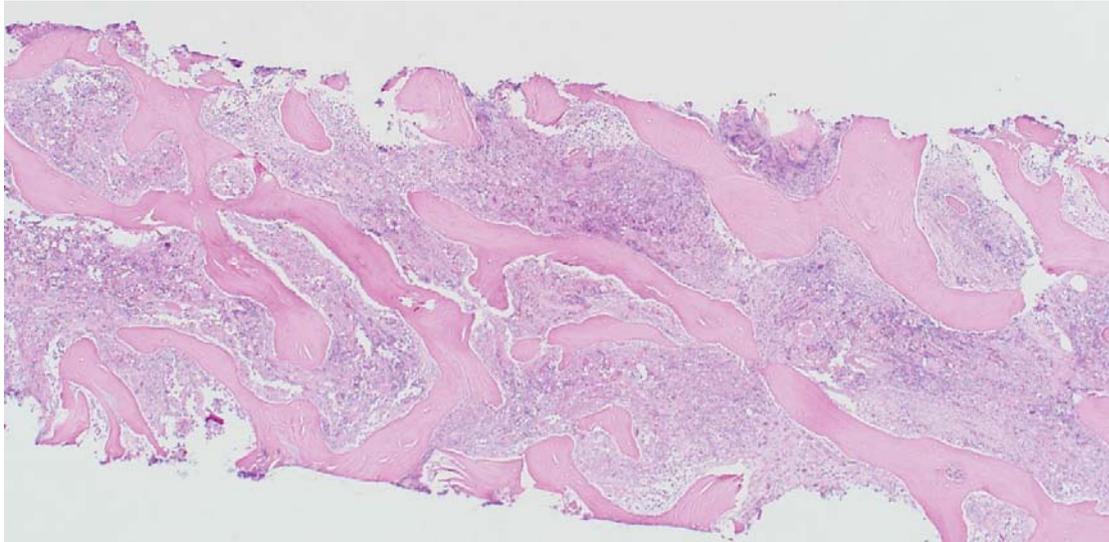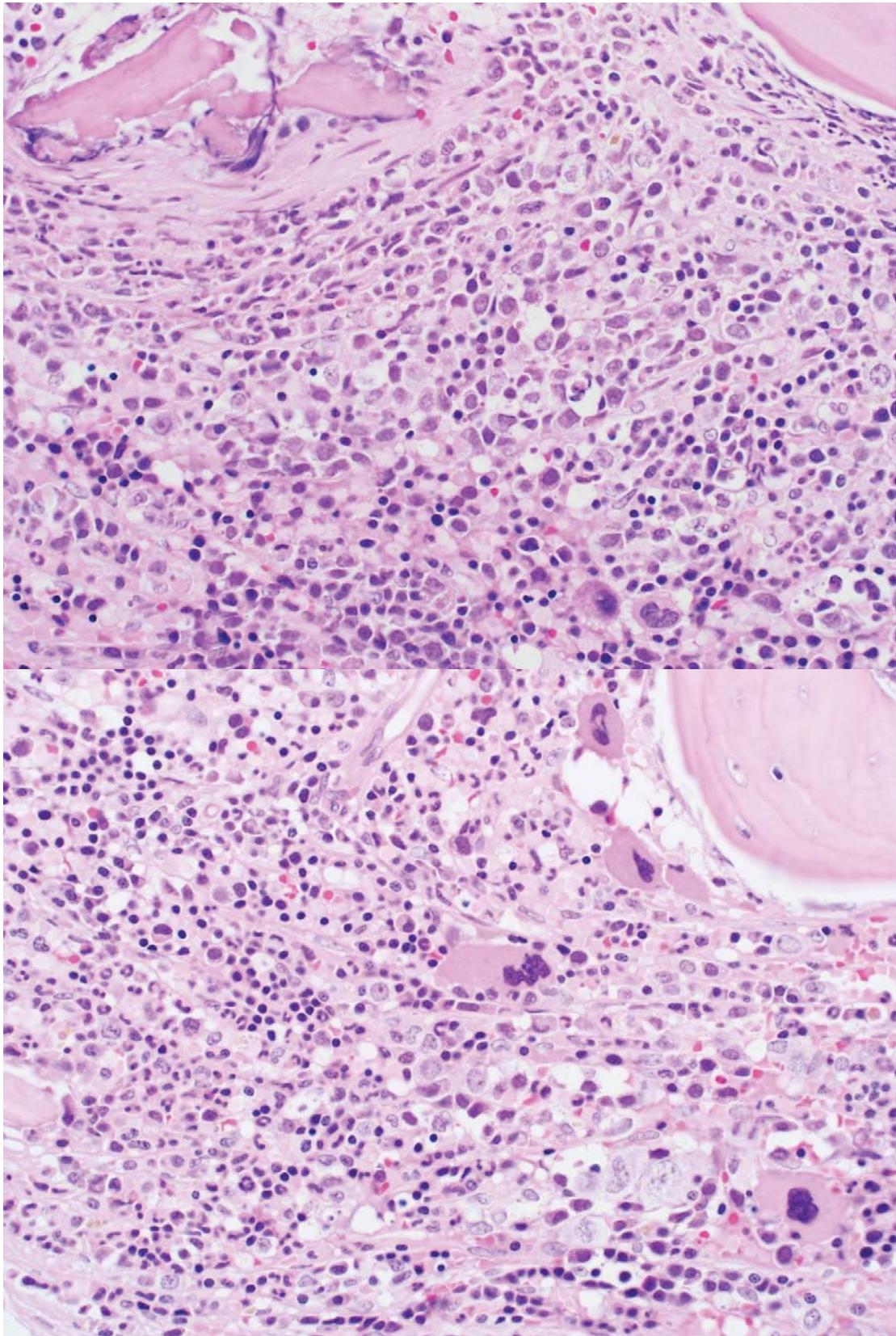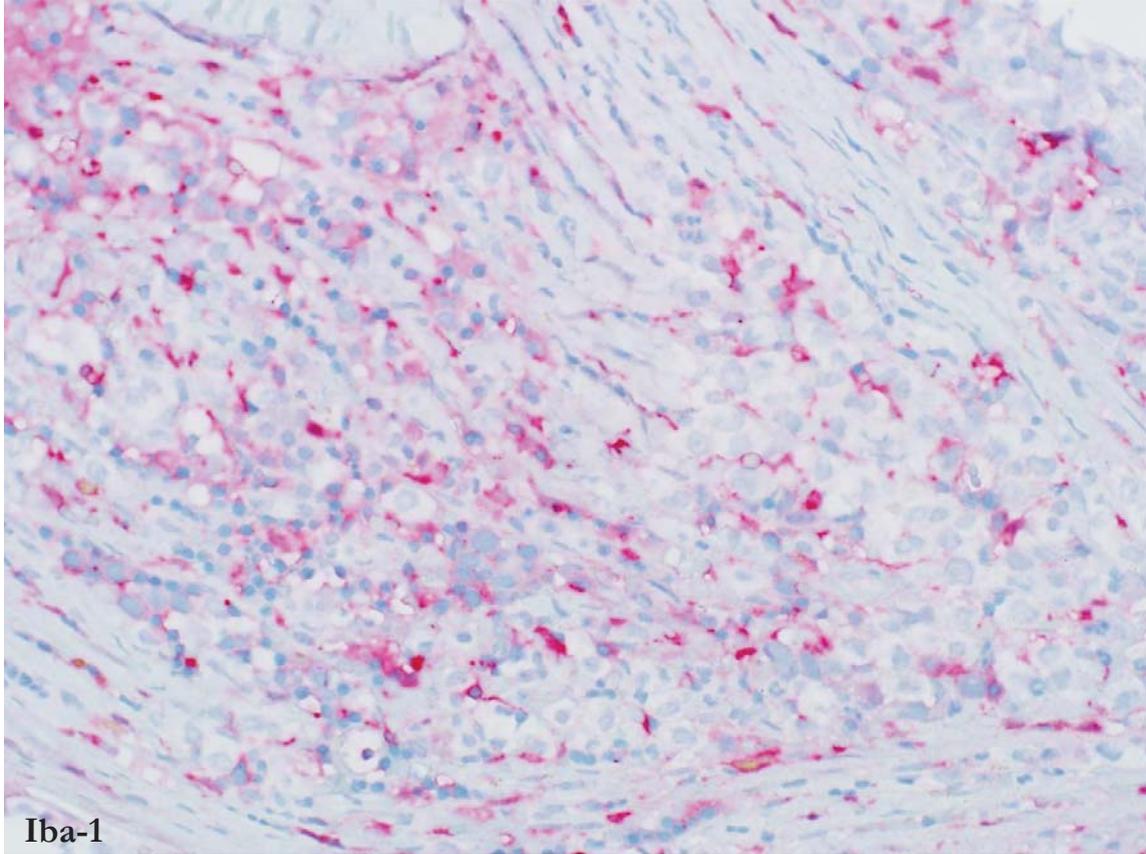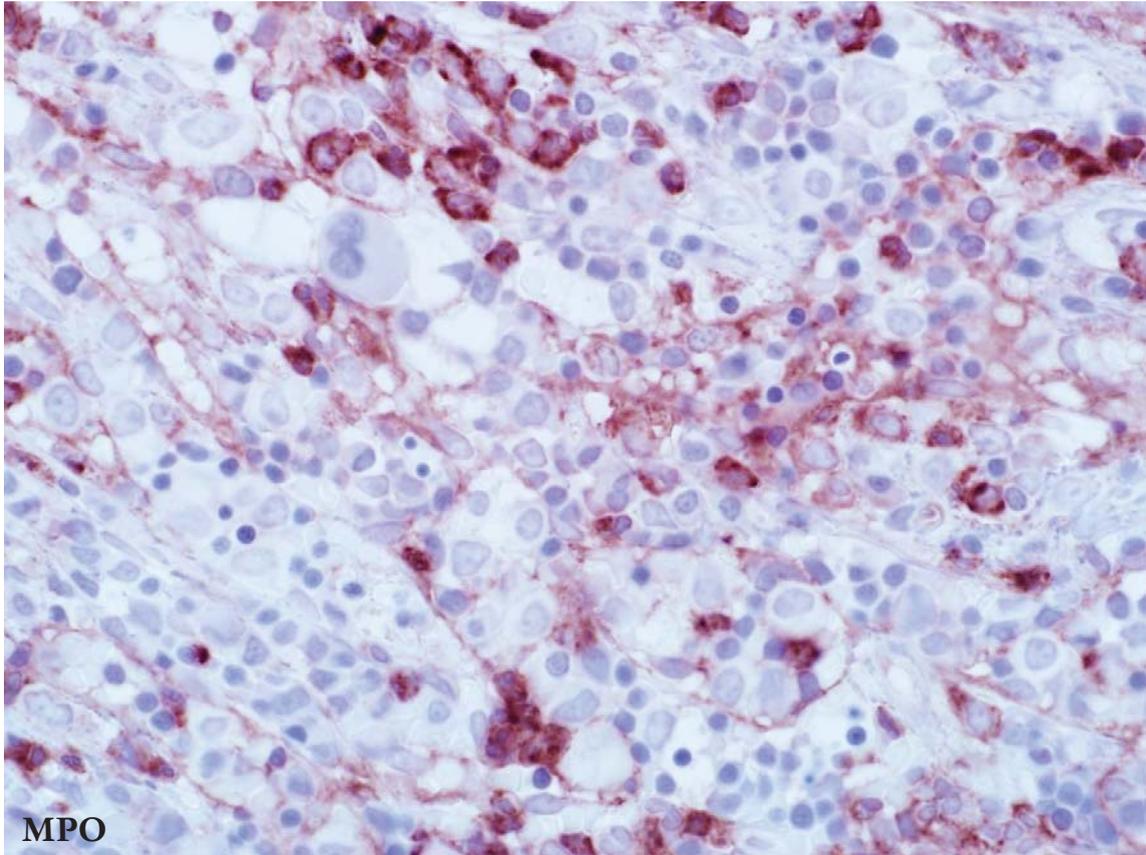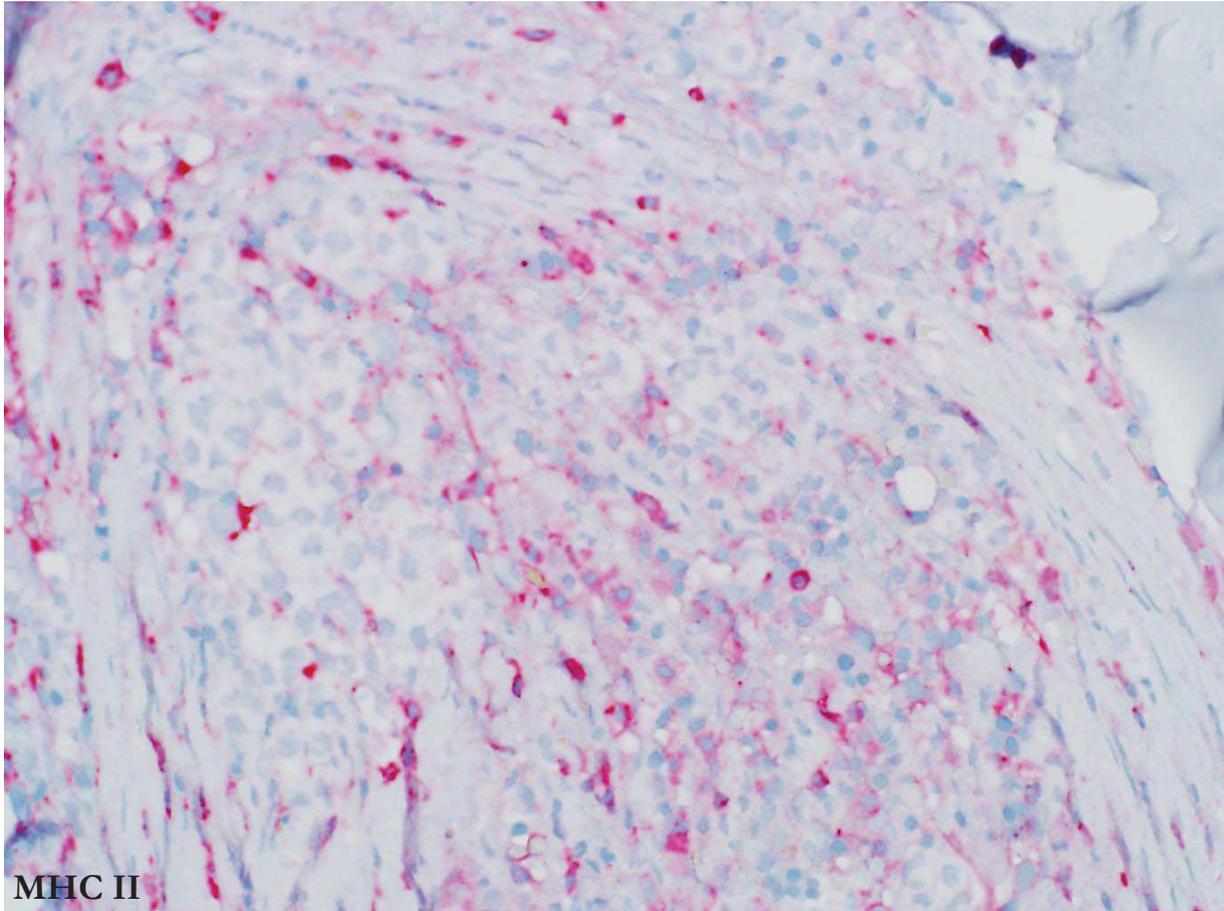
The bone marrow core consisted of 9 intertrabecular spaces free of artifact. It is 70% cellular and with increased cellular density. The remaining inter-trabecular space is occupied by fibrous tissue and few adipocytes. Blast cells of indeterminate lineage, with large round/oval/indented vesicular nuclei and a thin rim to moderate amount of clear to lightly eosinophilic cytoplasm comprise ~25% of marrow cells. The granulocytic/erythrocytic ratio is approximately 3:1, with scattered clusters of hyperchromatic erythrocytic precursors, band cells, and fewer segmented neutrophils. Megakaryocytes were clustered dysplastic with micromegakaryocytes and absent nuclear lobation. Coarse iron stores were minimal.

<u>Interpretation: Acute myeloid leukemia with myelofibrosis and megakaryocytic dysplasia</u>

"Micah" received 2 injections of rabbit anti-dog thymocyte serum, and was discharged on day 30 with the following medications:

Prednisone, cyclosporin, clopidrogel, Cerenia, doxycycline

His prognosis was considered guarded before the bone marrow biopsy due to relapse of anemia, and guarded in light of the bone marrow biopsy findings. He was euthanized on Day 58 due to anorexia, emaciation and inability to get up.

Discussion

This dog was noted to be "ill" for one month prior to first identification of severe anemia. Some of the initial laboratory findings were consistent with immune-mediated anemia (spherocytosis, agglutination, marked regeneration) but he did not have leukocytosis or more than mild hyperbilirubinemia. The patient had leukocyte agglutination and also platelet microaggregates throughout the blood smear, which is unusual in dogs with primary immune-mediated hemolytic anemia (IMHA). Most dogs with IMHA have marked leukocytosis and marked hyperbilirubinemia.[1] Treatment with immunosuppressive dosages of glucocorticoids for 6 weeks did not improve the anemia in this dog. There was intermittent thrombocytopenia but it was never very severe. Review of sequential blood smears showed increasingly abnormal appearing leukocytes, which triggered the Pathologist to consider that this may not be an "ordinary" IMHA but rather that there may be underlying primary bone marrow disease.

A diagnostic sample of bone marrow could not be aspirated from either the sternum or humerus by an experienced operator; therefore, myelofibrosis was considered likely. Dysplastic megakaryocytes and rubriblasts were among the few hematopoietic cells that were retrieved. An excellent core biopsy was obtained, which confirmed fibrosis which ranged from fine fibers to areas with mature collagen fibers. Blast cells were unevenly distributed and comprised between 20 and 90% of cells in different regions of the section. There were maturing rubricytes and granulocytes in some areas, but failure of erythropoiesis was the likely cause of death. The nature of the blasts was not identifiable with myeloperoxidase (MPO), ionized calcium binding adaptor molecule 1 (Iba1) or major histocompatibility class (MHC) class II immunohistochemistry, which was not unexpected since these proteins are expressed on relatively differentiated neutrophils, macrophages and antigen-presenting cells, respectively.

Persistent and treatment-refractory severe anemia in conjunction with dysplastic leukocytes in blood, dysplastic megakaryocytes in bone marrow, and excess bone marrow blasts, formed the basis for diagnosis of acute myeloid leukemia (AML) with dysplasia and fibrosis in this case. Approximately 25% of humans diagnosed with AML have had a prior hematological disorder such as immune hemolytic anemia or thrombocytopenia, and all horses with acute leukemia (either myeloid or lymphoid) in two case series have prior agglutinating anemia.[2,3,4] Data regarding the frequency of IMHA or immune-mediated thrombocytopenia (ITP) preceding a diagnosis of AML in dogs are lacking.

Clonal hematopoiesis is a common finding in older humans without hematopoietic disease, but poses an increased risk for development of hematopoietic neoplasms such as myelodysplastic syndrome (MDS) and AML.[5] Persistent cytopenia or dysplasia are also recognized risk factors for development of MDS and AML.[5] The features of the leukemia in this dog were most similar to what is termed acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) in humans.[6] Neoplasms of this type have a high frequency of certain cytogenetic and genetic abnormalities.[6] The genetics of AML in dogs are undetermined, and therefore it is unclear at this point whether such knowledge would help in diagnosis or prognosis.

In summary, we should carefully evaluate cytopenic animals regarding theduration of cytopenia, possible infectious or toxic causes, response to therapy and presence of dysplasia, and we should consider etiologies other than autoimmunity as potential causes of persistent agglutinating anemia.
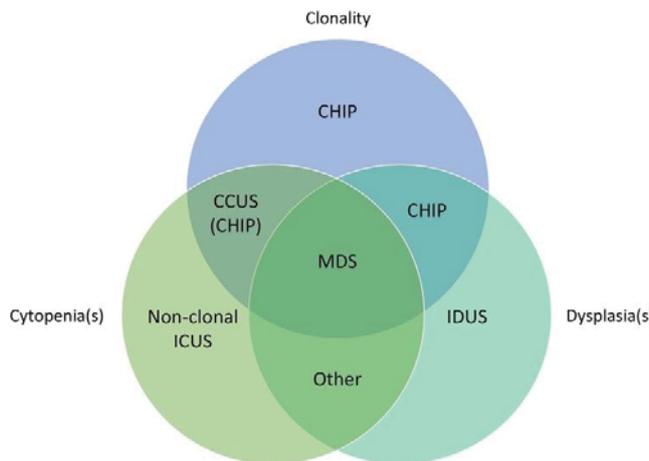
Relationship between clonal hematopoiesis and related entities with respect to morphological dysplasia, cytopenia(s) and clonality (not to scale). CHIP = clonal hematopoiesis of indeterminate potential; CCUS = clonal cytopenia of undetermined significance; MDS = myelodysplastic syndrome; ICUS = idiopathic cytopenia of undetermined significance; IDUS, idiopathic dysplasia of undetermined significance

Adapted from: Capo-Chichi J-M, et al. Emerging patterns in clonal haematopoiesis. J Clin Pathol 2019;0:1–7

1. Garden OA, et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. J Vet Intern Med. 2019 Mar;33(2):313-334
2. Becker H, et al. Tracing the development of acute myeloid leukemia in CBL syndrome. Blood. 2014 Mar 20;123(12):1883-6.
3. Cooper CJ, et al. Acute Leukemia in Horses. Vet Pathol. 2018;55(1):159-172
4. Barrell EA, et al. Acute leukemia in six horses (1990-2012). J Vet Diagn Invest. 2017;29(4):529-535
5. Capo-Chichi J-M, et al. Emerging patterns in clonal haematopoiesis. J Clin Pathol 2019;0:1–7
6. Vardiman J, Reichard K. Acute Myeloid Leukemia With Myelodysplasia-Related Changes. Am J Clin Pathol 2015;144:29-43