Mass-like lesion in the bone marrow of a dog

Marion Cambrelin[1], Kate Pennick[2], Brittany Lucchetti[3], Emily Rout[1], R. Adam Harris[1]A Russell Moore[1]

[1]Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA
[2]IDEXX Laboratories Inc, Westbrook, Maine, USA
[3]CharlestonVeterinary Referral Center, Charleston, South Carolina, USA

Marion Cambrelin, DVM: Marion.cambrelin@colostate.edu

Specimen

EDTA whole blood, peripheral blood film, bone marrow aspirate smear, bone marrow core

Signalment

Dog, Goldendoodle, 11-year-old, neutered male

History

The dog had a 4-month history of presumptive immune-mediated thrombocytopenia and was treated with prednisone (1.6 mg/kg/day), which was tapered to 0.27 mg/kg/day over this period by the referring veterinarian. The platelet count, however, never exceeded 91 K/µL (RI 120-412 K/µL). He was ultimately referred for evaluation of waxing and waning lethargy, severe progressive thrombocytopenia, and a new-onset moderate non-regenerative anemia.

Clinical Findings

On presentation to the referral hospital, the dog was mildly hyperthermic (39.4°C) with quiet mentation, mild firm swelling of the left tarsus, ventral abdominal ecchymoses, and dorsal erythema and bruising. Complete blood count (Sysmex XN900V-1-L) revealed a moderate non-regenerative anemia (RBC 3.5 M/µL; RI 5.8-8.9 M/µL; HCT 25.8%; RI 41.0-60.0%; hemoglobin 8.5 g/dL; RI 14.6-21.7 g/dL, reticulocytes 18 K/µL; RI 21-140 K/µL), neutropenia (0.2 K/µL; RI 3.0-9.7 K/µL), lymphocytosis (6.1 K/µL; RI 1.0-4.2 K/µL), monocytosis (1.0

K/µL; RI 0.1-0.7 K/µL), thrombocytopenia (22 K/µL; RI 120412 K/µL), and circulating unclassified cells (0.8 K/µL).

Blood film evaluation by a clinical pathologist identified approximately 60% unclassified cells (4,860 cells/µL). These cells were about the size of a medium to large lymphocyte (12-18 micrometers diameter) with round to occasionally indented or lobulated nuclei, finely granular chromatin, variably distinct nucleoli, and scant to mildly increased amounts of light blue cytoplasm (Figure 1). Some cells contained coarse, magenta to purple cytoplasmic granules. These findings raised concern for hematopoietic neoplasia, and bone marrow evaluation was pursued. SNAP™ 4Dx™ Plus Test results (IDEXX Laboratories, Westbrook, Maine, USA) were negative for *Anaplasma spp.*, *Ehrlichia spp.*, *Borrelia burgdorferi*, and *Dirofilaria immitis*. Comprehensive tick-borne PCR testing was also performed and was negative for *Anaplasma spp.*, *Ehrlichia spp.*, *Babesia spp.*, *Bartonella spp.*, *Mycoplasma spp.*, *Rickettsia spp.*, *Borrelia burgdorferi*, and *Dirofilaria immitis*.

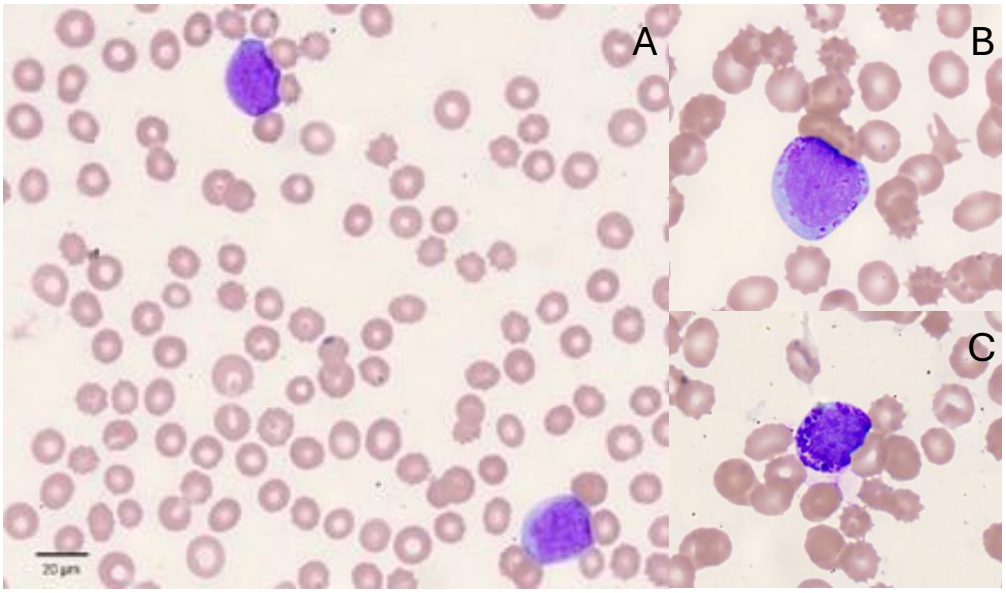


Figure 1. A-C, Blood film demonstrating unclassified cell population. Some of these cells contained coarse magenta to purple cytoplasmic granules (B-C). Wright Giemsa, 100x objective.

Bone marrow aspirate smears (Figure 2) were moderately to highly cellular with numerous particles. Megakaryocytes were mildly decreased (approximately 1–2 per particle) but exhibited appropriate maturation. Hematopoietic elements were nearly effaced by a dominant atypical

round cell population consisting primarily of cells that were about the size of a small to intermediate lymphocyte (8-12 micrometers diameter). These cells had a high nuclear to cytoplasmic (N:C) ratio, a scant rim of pale basophilic cytoplasm, coarsely clumped chromatin, and variably prominent nucleoli. An additional smaller subset of atypical cells was present, characterized by a larger size (approximately 12-18 micrometers diameter), a round to ovoid nucleus, coarsely clumped chromatin, and more expanded cytoplasm. Many cells within both populations contained abundant coarse magenta to purple intracytoplasmic granules, and similar granules were also present free in the background. Low numbers of immature erythroid precursors were identified. The background contained moderate blood contribution, platelet clumps, and lysed cellular debris.

Alkaline phosphatase (ALP) reactivity was observed in the majority of the small to intermediate cells (approximately 70-80% showing positive cytoplasmic reactivity) and in approximately 40-50% of the larger cells (Figure 3).

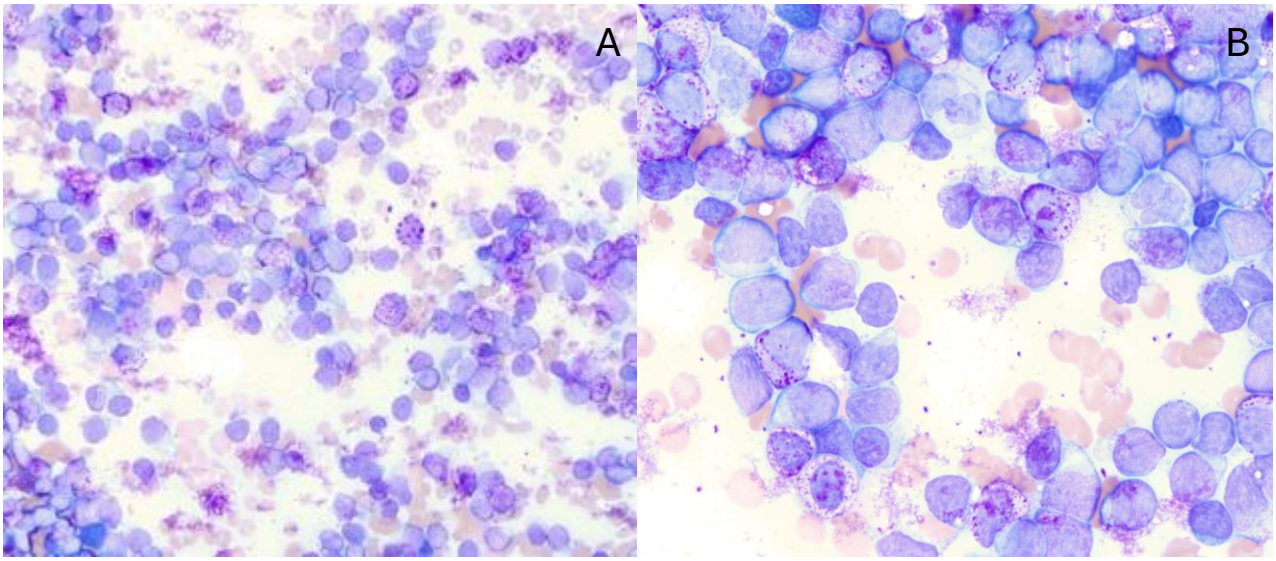


Figure 2. A-B, Bone marrow aspirate smears demonstrating cells with coarse magenta to purple intracytoplasmic granules. Wright-Giemsa stain, 50x objective (A) and 100x (B).

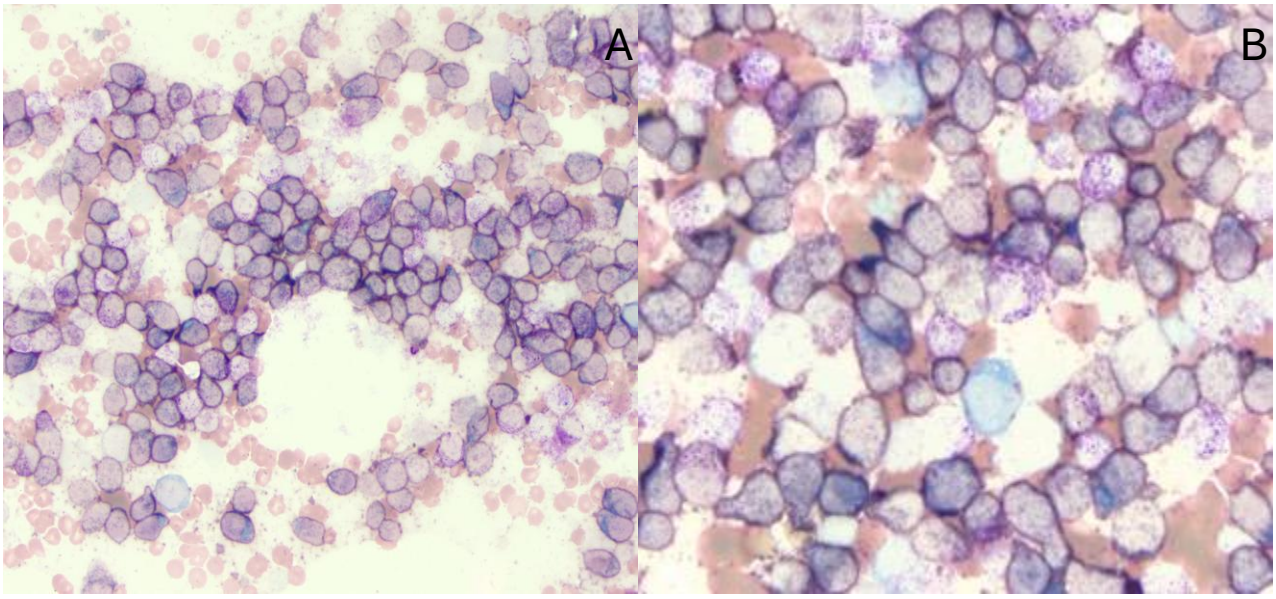


Figure 3. Bone marrow aspirate smear with ALP reactivity. Wright-Giemsa stain, 50x objective (A) and 100x objective (B). Both small to intermediate and the larger cells exhibit ALP reactivity.

The bone marrow core biopsy was markedly hypercellular, with approximately 10-20% adipose tissue within the intertrabecular regions. Consistent with the aspirate findings, normal hematopoietic tissue was effaced by a dominant round cell population exhibiting two morphologies. The subpopulation corresponding to the small to intermediate round cells in the aspirate predominated and was characterized by a high N:C, coarsely clumped chromatin, and 1-3 distinct nucleoli. Interestingly, the subpopulation corresponding to the larger cells in the aspirate formed morphologically distinct aggregates throughout the marrow, which was most apparent at a lower magnification. These cells had more abundant cytoplasm, central to eccentric nuclei, and clumped nuclear chromatin. In contrast to the aspirate, cytoplasmic granules were not evident in the core biopsy sections (Figure 4). The nuclear features of the two populations were still considered to be similar, which suggested a relationship between them and raised the possibility of maturation within a single neoplastic lineage.

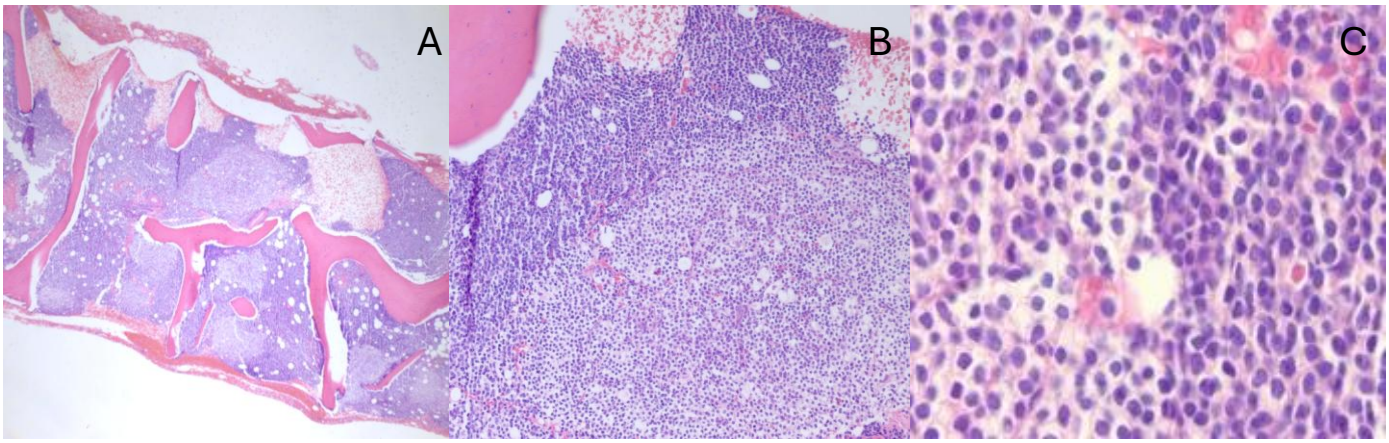
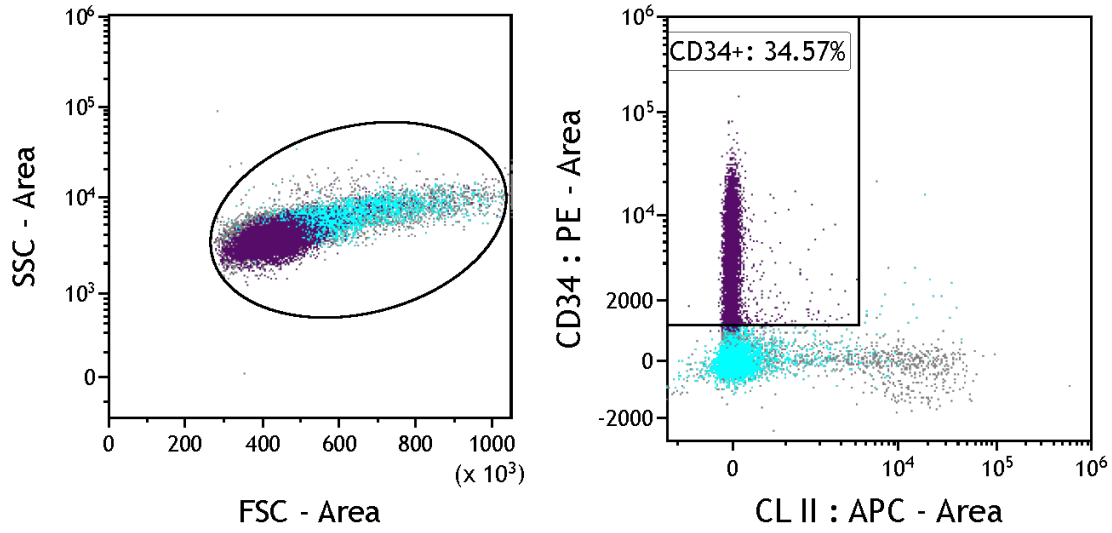


Figure 4. A-C. Bone marrow core, H&E 4x (A), 20x (B), 100x (C).

Routine immunophenotyping by flow cytometry was performed on the bone marrow aspirate (Figure 5). 34.6% of the cells (purple) expressed CD34 and had low forward scatter. A subset of the CD34+ cells co-expressed CD18. A separate population of cells (24.3%; teal) with higher forward scatter expressed CD14, CD18, and low levels of class II.



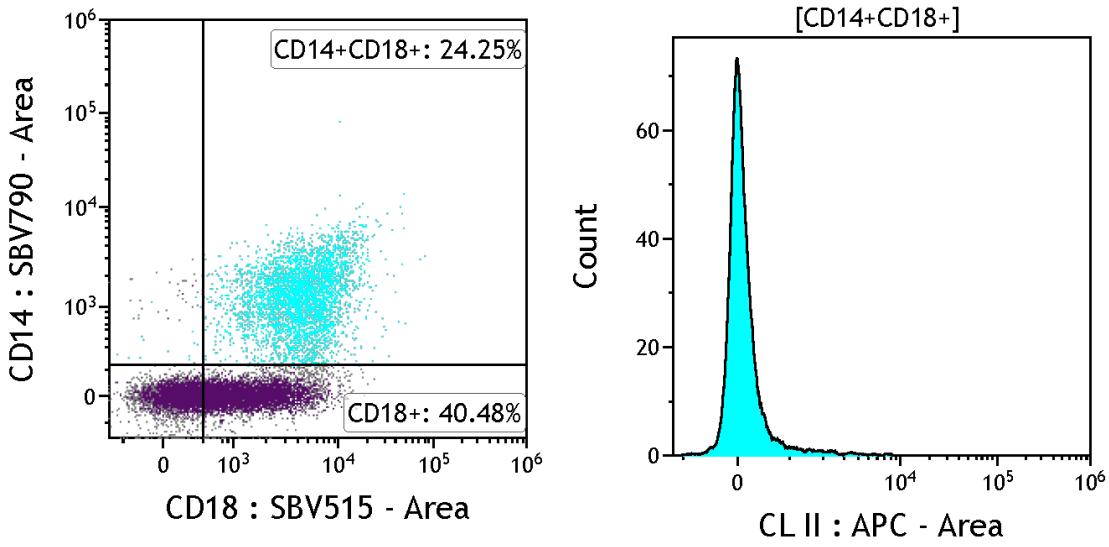


Figure 5. Flow cytometry plots of the bone marrow aspirate. CL II, class II major histocompatibility complex. FSC, forward scatter. SSC, side scatter.

Questions:

1. What are reasonable diagnoses for this neoplasm based on aspirate, core, and flow cytometry data?

2. What additional immunohistochemical or special staining would be most helpful to better characterize the two cell populations described?

3. How would you best explain the discrepancy in granulation between the aspirate and core samples?