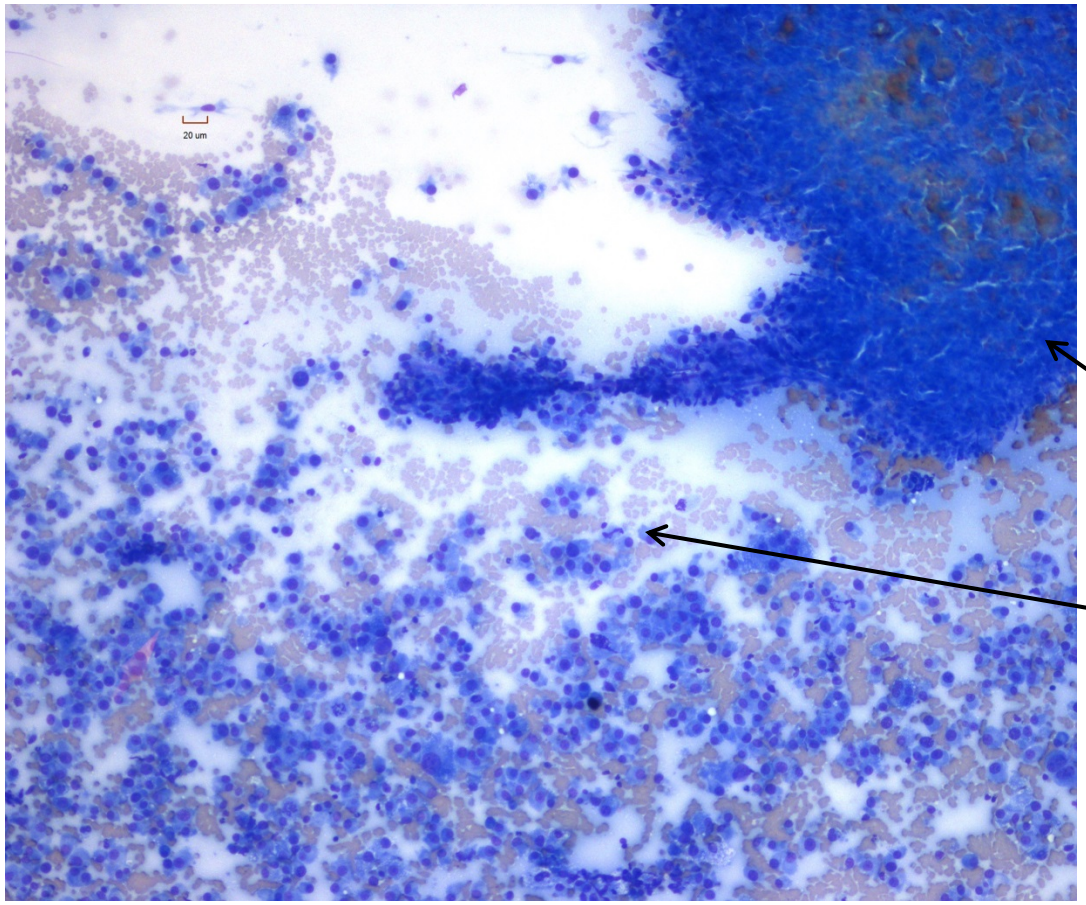
8 year old male neutered Golden Retriever

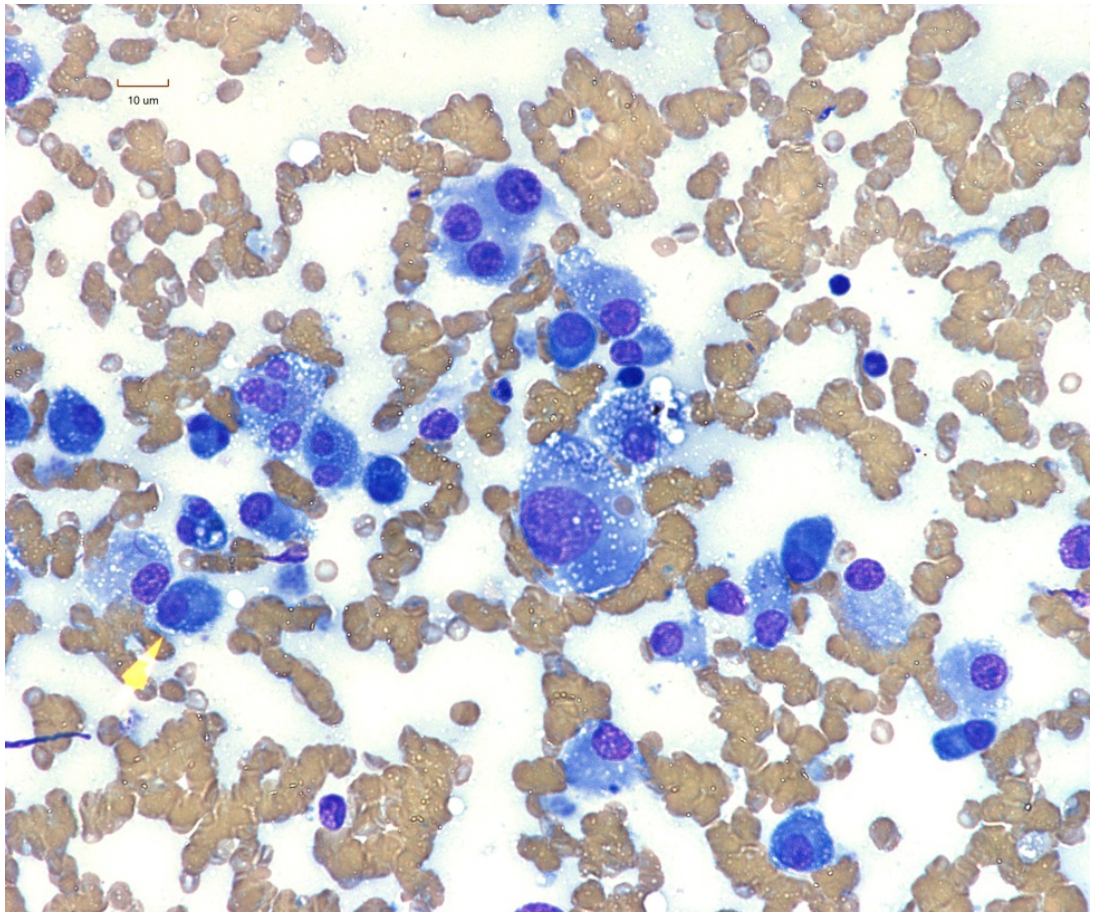
Swelling near proximal humerus, lytic lesion seen on radiographs

Aspirate of lytic bone lesion 10x view



- Sample is highly cellular
- Cells are grouped in large aggregates and scattered individually

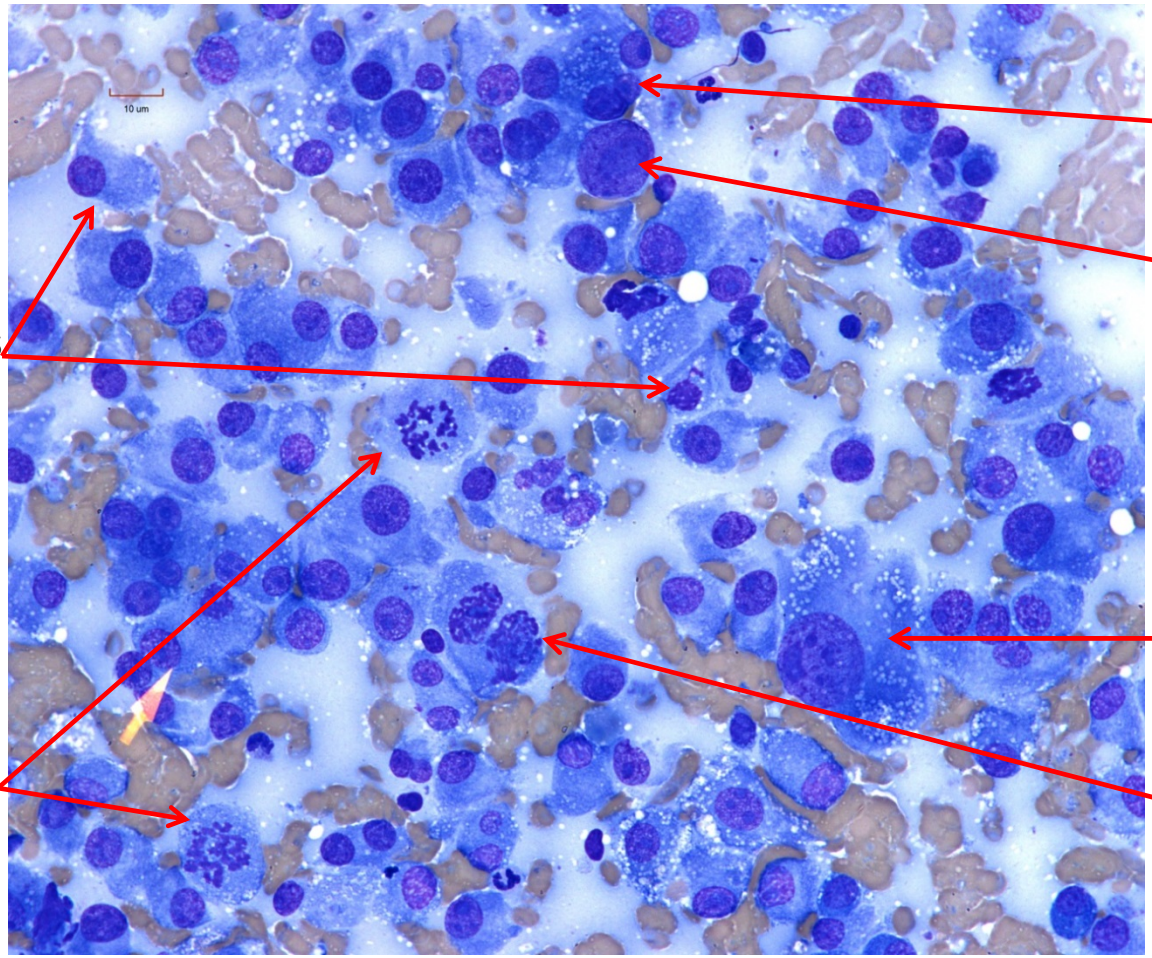
Aspirate of lytic bone lesion 40x view



From a slightly closer view the discrete nature of the cells can be appreciated

Aspirate of lytic bone lesion
40x view

Can you find the features of atypia in these cells??



Multinucleation

Abnormal nucleoli

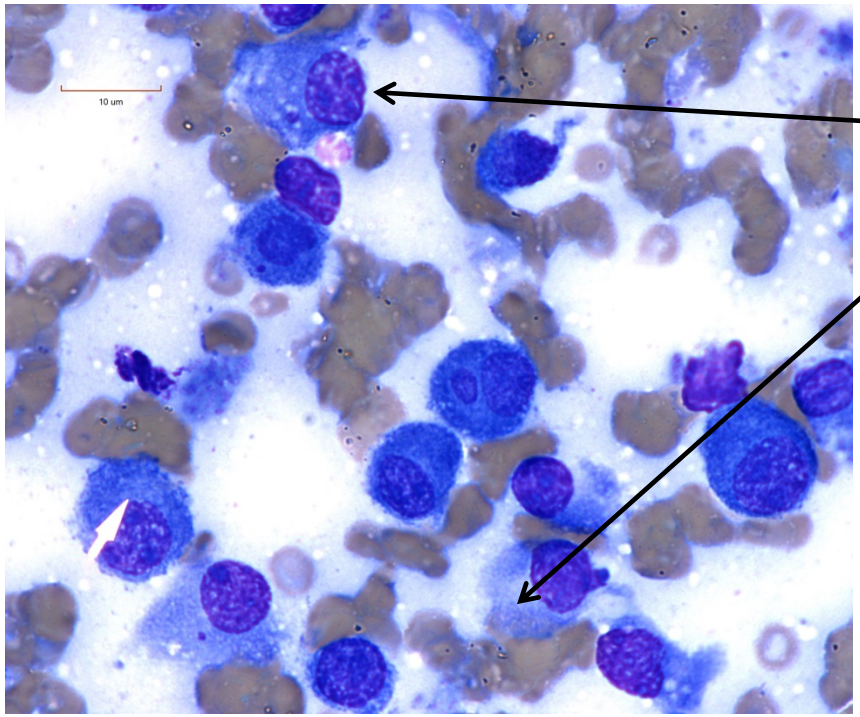
Variable N:C ratios

Karyomegaly
(marked anisocytosis and anisokaryosis)

Abnormal mitosis

Coarse chromatin patterns

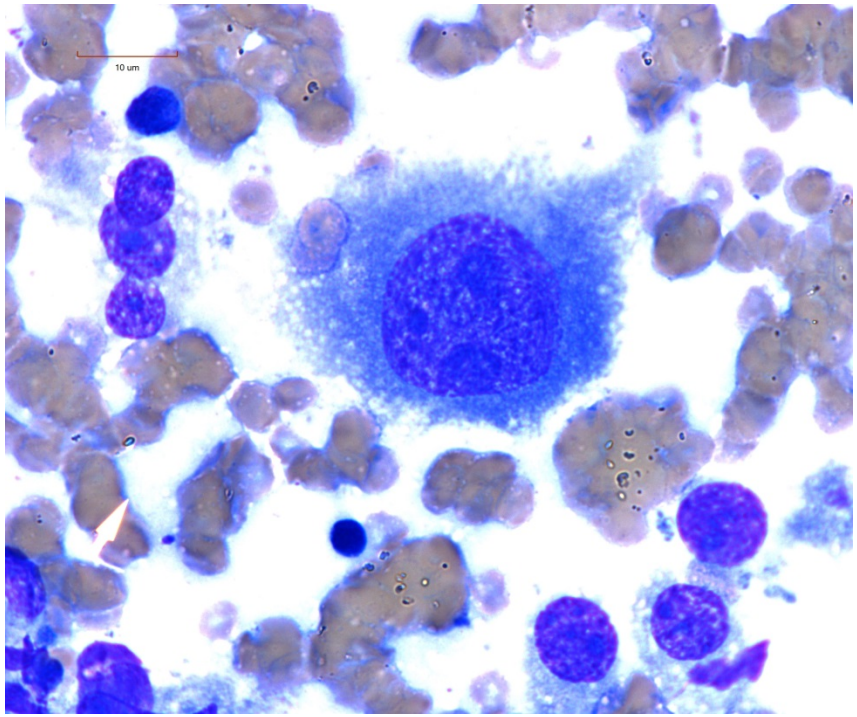
Aspirate of lytic bone lesion 100x View



- Other morphologic features include:
  - Deeply basophilic cytoplasm
  - Eccentric nuclei
  - Prominent golgi zones

- These features are most suggestive of osteoblastic origin (osteosarcoma). Other differentials included histiocytic sarcoma, hemangiosarcoma, synovial cell sarcoma and plasma cell tumor

Aspirate of lytic bone lesion 100x



- **What stain can be used on cytologic samples to confirm osteoblastic origin**?

What stain can we use to rule in or out an osteosarcoma?

- "*Alkaline phosphatase* stain": actually the application of 5-bromo, 4-chloro, 3-indolyl phosphate/nitroblue tetrazolium **(NBT/BCIP)** substrate which reacts with ALP
- Bone is the only *connective tissue* shown to produce ALP in dogs[1]. This makes ALP expression a useful marker to differentiate **osteosarcomas** from other **mesenchymal** neoplasms
  - **when a diagnosis of neoplasia is certain, as both normal and reactive osteoblasts will express ALP!!

- When used appropriately, this stain has been shown to have:

	Sensitivity	Specificity
Previously stained slides[1]	100%	89%
Unstained slides[2]	88%	94%

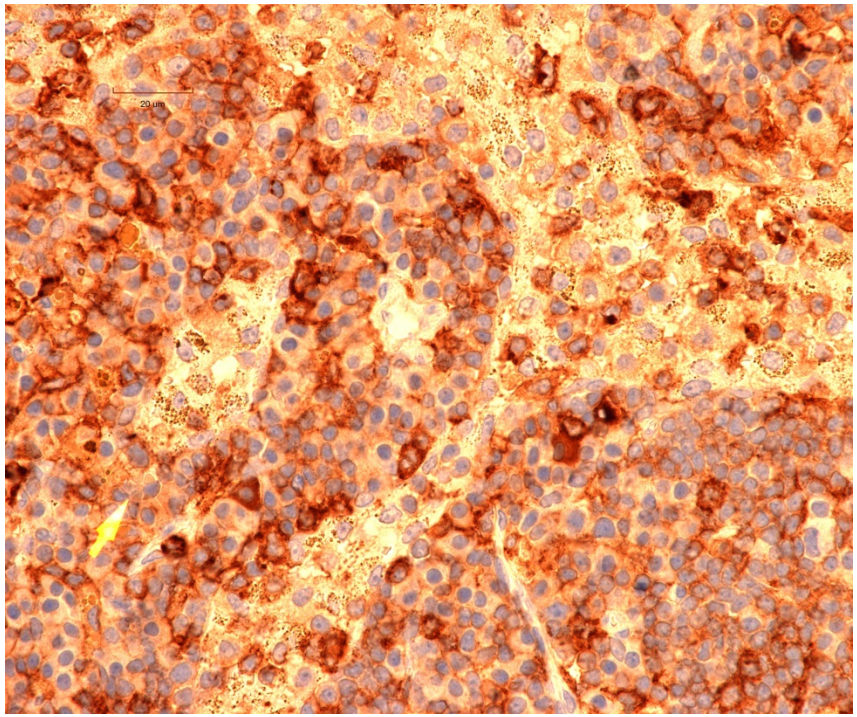
- On this case, an ALP was **negative** on unstained slides
- While this result did not completely rule out an osteosarcoma, it makes this differential less likely
- The affected forelimb of the patient was amputated and submitted for histopathologic examination

- On histopathologic examination, sheets of pleomorphic cells with discrete borders were seen (similar to cytologic sample).
- Though a cytologic origin could not be definitively ascertained, **histiocytic sarcoma** was suspected.
- What immunohistochemical marker can be used to identify cells of histiocytic origin?

CD18

- CD18 is a panleukocyte marker[3]
- Often CD18 is used to confirm histiocytic origin, but since all leukocytes will stain positive, lymphoid origin must be considered (can rule out by staining for CD3 and CD79a, etc). This was deemed unnecessary in this case because morphologically the cells did not demonstrate lymphoid characteristics (i.e. most had relatively low N:C ratios, eccentric nuclei etc).

CD18 staining was markedly positive on histologic section from proximal humerus



- Note the prominent cytoplasmic staining with CD18
- This confirmed the the neoplasm was of histiocytic origin

Histiocytic sarcoma

- When using IHC/ICC markers (CD18) or special stains (ALK), it is important to remember that oftentimes, these stains only confirm ***lineage*** of a particular cell population, and **not neoplasia itself.**

- Determination of neoplasia must be based on the complete pathologic and/or clinical picture

References

1. Barger A, Graca R, Bailey K, et al. Use of alkaline phosphatase staining to differentiate canine osteosarcoma from other vimentin-positive tumors. Vet Pathol, March 2005 42: 161, p.42-2-161.
2. Ryseff, J. K. and Bohn, A. A. Detection of alkaline phosphatase in canine cells previously stained with Wright–Giemsa and its utility in differentiating osteosarcoma from other mesenchymal tumors. Vet Clin Pathol, September 2012; 41: 391–395.
3. Fernandes, N. J. et al. Immunohistochemical and Histochemical Stains for Differentiating Canine Cutaneous Round Cell Tumors. Vet Pathol July 2005; 42:437–445 (2005).