

Skeletal tumours in domestic animals - *a challenge for the diagnostic pathologist*

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Presentation outline

- What are the challenges?
- Overview of osteosarcoma
- Diagnosis of osteosarcoma
- Differentiation from other skeletal lesions
- Conclusions



Why focus on osteosarcoma?

- Common tumour, especially in dogs
- Highly malignant
- Early & accurate diagnosis crucial
- Differentiation of osteosarcoma from other neoplastic and non-neoplastic skeletal lesions can be difficult

Few tumours put as much pressure on the pathologist

Possible consequences of misdiagnosis

False positive dx → inappropriate amputation, chemotherapy or euthanasia

False negative dx → metastatic spread to lungs, pathological fracture

In either situation, the pathologist and/or surgeon are at risk of litigation

Challenges for the pathologist

- Inadequate clinical history
- Sample size – core biopsies for histology often small and may not be representative
- Sample handling – e.g. delay in fixation, decalcification may alter cell morphology
- Cytological preparations may be poorly cellular, haemodilute or non-diagnostic

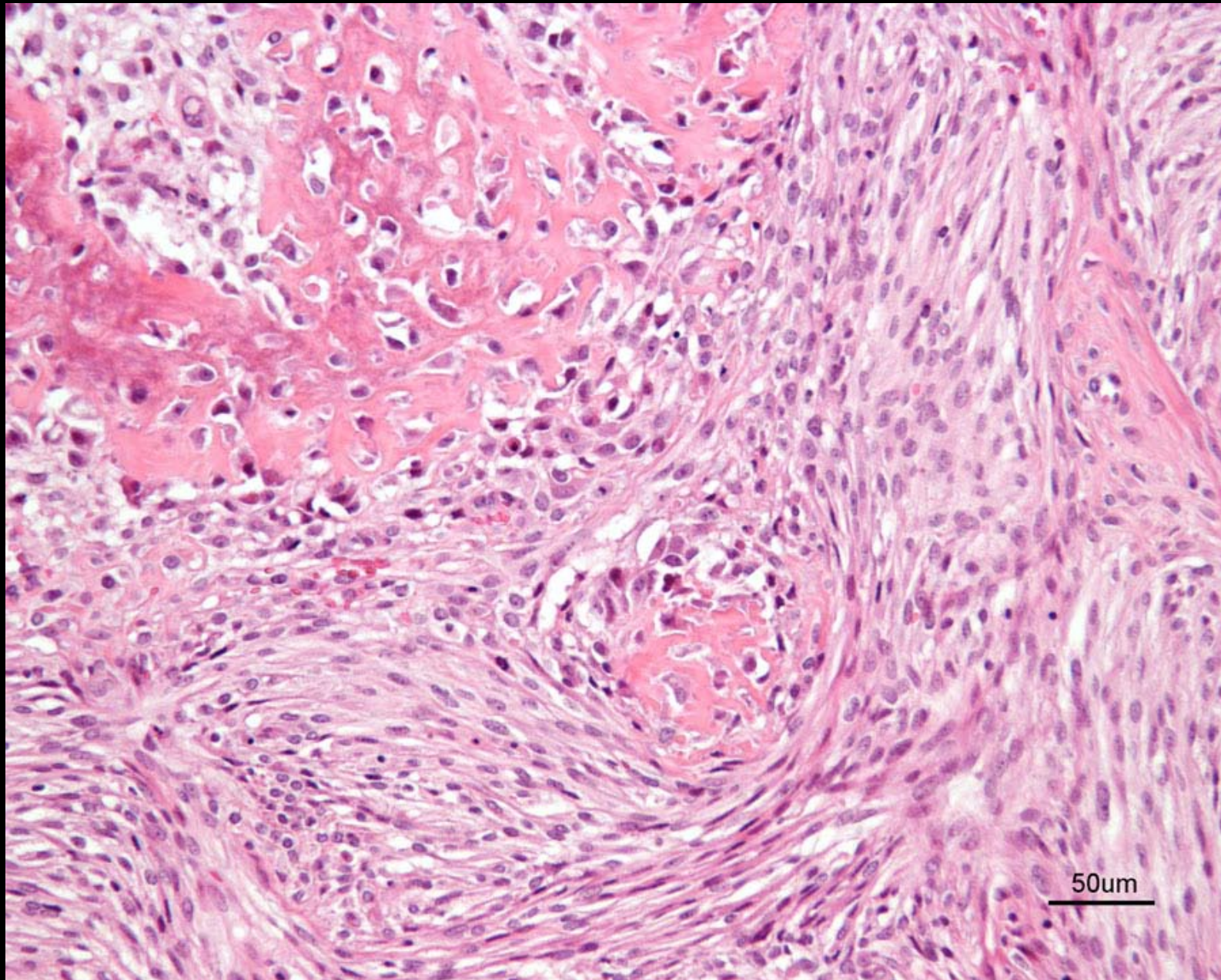
Complicating factors

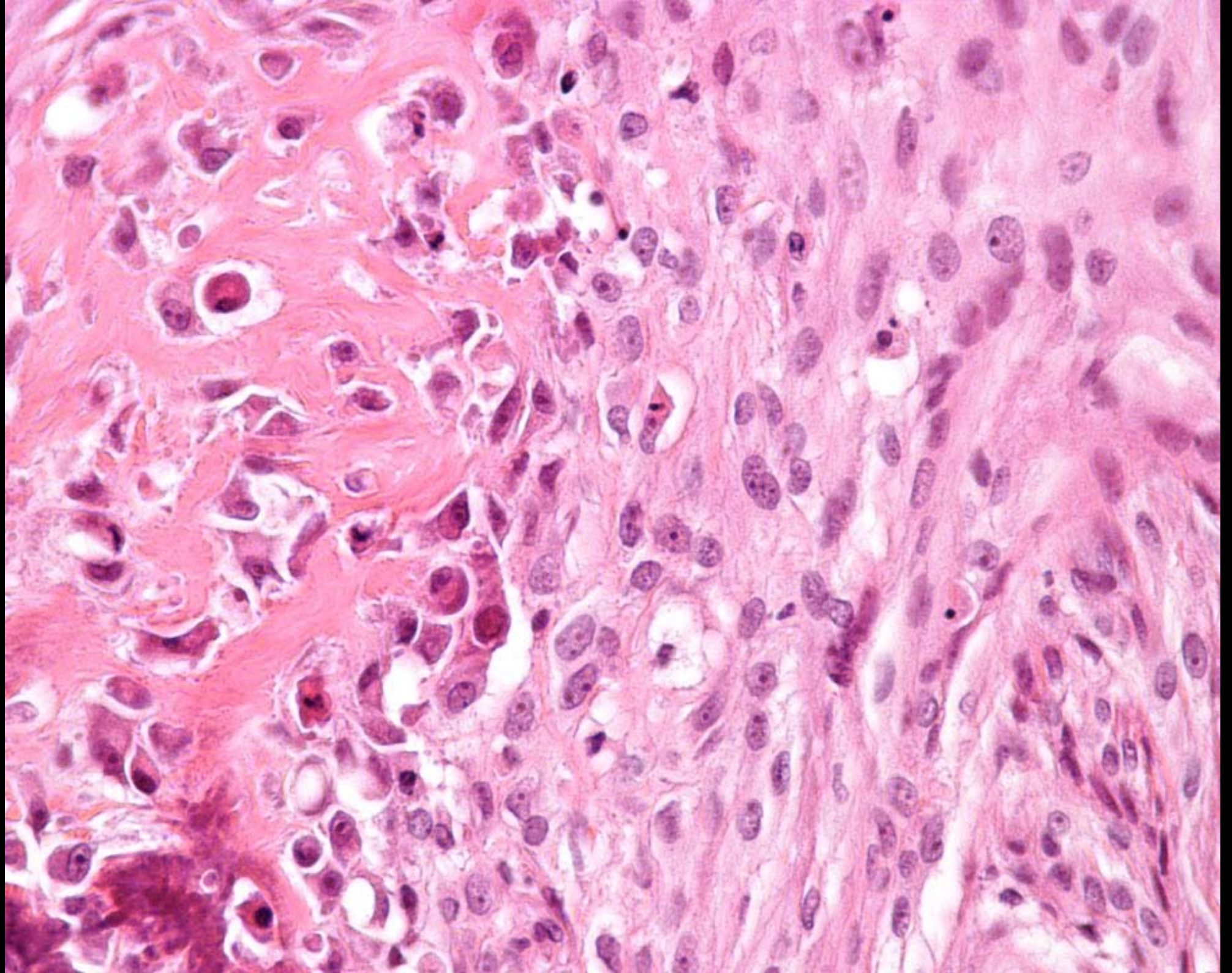
- Proliferation of new, woven bone, is a normal response to skeletal injury
- Cytologically, hyperplastic (reactive) osteoblasts closely resemble osteoblasts from well differentiated osteosarcomas
- Repairing fractures may contain a mix of highly reactive mesenchymal cells
- Aggressive tumours contain both neoplastic and reactive osteoblasts

Further complications

- Pathological fractures often occur at sites of benign or malignant skeletal tumours
- Several non-skeletal tumours can induce metaplastic bone formation (e.g. mammary, thyroid and salivary carcinomas, melanoma, malignant PNSTs)
- Bone in these tumours can undergo malignant transformation

Osseous metaplasia in a malignant peripheral nerve sheath tumour





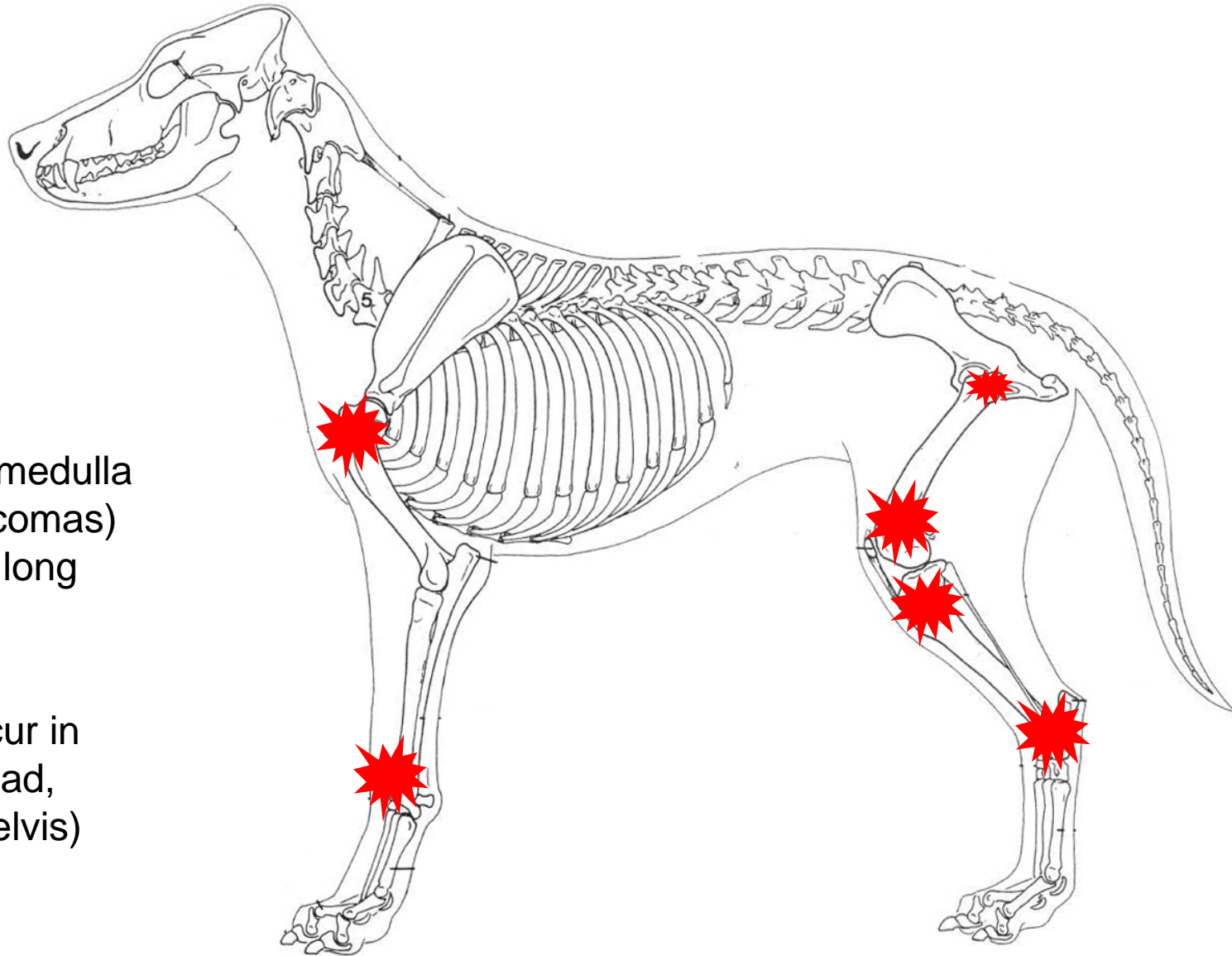
Overview of osteosarcoma

- Common in dogs & cats
- Biphaseic age distribution in dogs
- Medium-sized and large breeds predisposed
- Highly malignant – but behaviour varies between types and locations
- Metastasise haematogenously to lungs – less often to regional lymph nodes

Predilection sites for canine osteosarcoma

Most originate in medulla
(central osteosarcomas)
in metaphyses of long
bones

BUT 20-25% occur in
axial skeleton (head,
ribs, vertebrae, pelvis)



Overview of osteosarcoma

- Predilection sites less well defined in cats but appendicular skeleton favoured
- In dogs, osteosarcomas may be induced by bone infarcts, metal implants or chronic inflammation
- Rarely diagnosed in other domestic species – some published reports questionable

Gross features

- May be lytic, productive, or mixed
- Rapid destruction of metaphyseal bone and cortex, especially in lytic forms
- Telangiectatic forms resemble haemangiosarcoma
- Periosteal reaction consistent but variable
- Seldom extend into joints
- Pathological fracture may be present

Lytic



Productive



Telangiectatic



Periosteal reaction



Radiography of skeletal lesions can assist pathologists, as well as clinicians and surgeons

Valuable alternative to gross assessment – good indication of bone destruction and presence of fractures

Radiographic features

- Osteosarcomas are aggressive tumours – permeate and destroy adjacent bone
- Lytic or productive (or mixed)
- Thinning of cortical shadow → complete cortical destruction
- Poorly defined margins

Radiographic features

- Periosteal reaction – not necessarily proportional to cortical destruction
- Codman's triangle – reactive bone at angle between cortex and elevated periosteum (*not specific for osteosarcoma*)
- Rapid progression – can be of diagnostic value

Osteosarcoma - dog

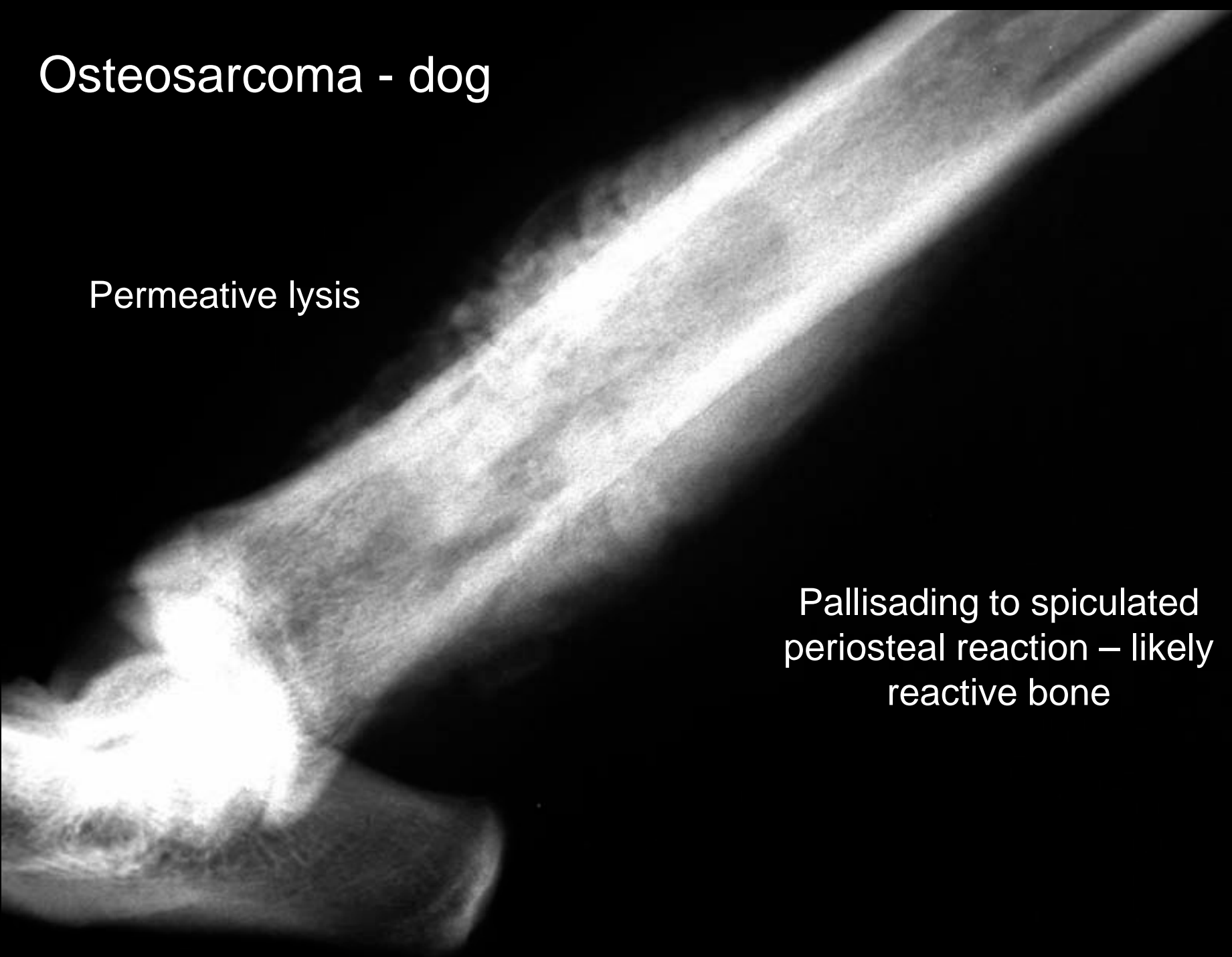
Permeative lysis – indicates
an aggressive lesion



Osteosarcoma - dog

Permeative lysis

Pallisading to spiculated
periosteal reaction – likely
reactive bone



Osteosarcoma



Aneurysmal bone cyst



“Moth-eaten” lysis (left) and
“geographic” lysis (right) - suggestive
of less aggressive lesions

Adult dog with lytic lesion and
periosteal reaction in mid-shaft
radius – wrong location for
osteosarcoma

This was a metastatic
biliary carcinoma



Diagnosis of osteosarcoma

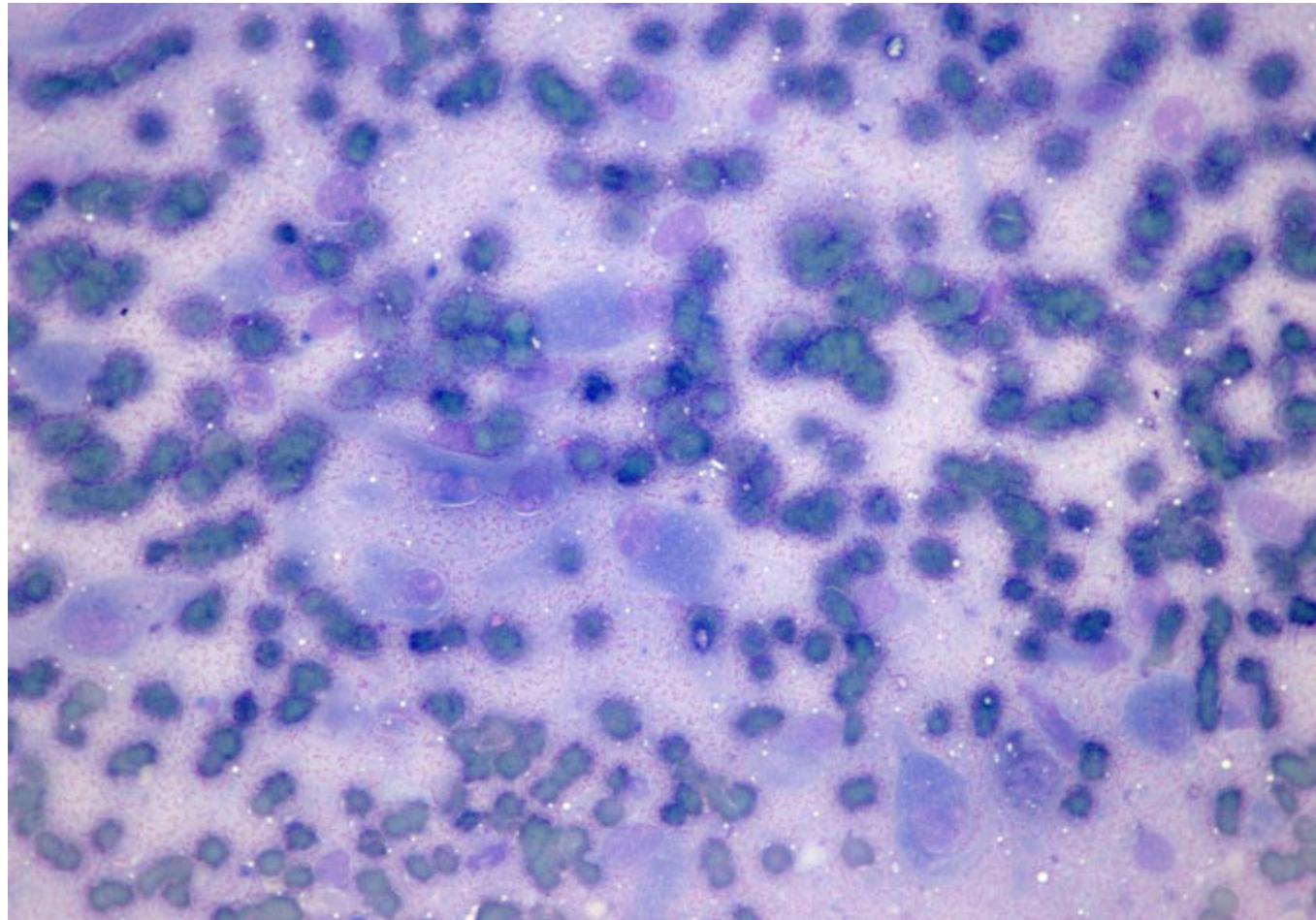
- Confirmation requires either **cytology** or **histology**
- Cell detail best in cytological preparations
- Architecture best in histological sections – but can be unreliable in small biopsies

Diagnoses can be made using either alone, but the two in combination are much more powerful

Cytology of osteosarcoma

Smears should be prepared routinely at time of biopsy
(but keep them away from formalin fumes)

FNA from suspect
osteosarcoma - exposed to
formalin fumes before
staining



Cytology of osteosarcoma

- Good preparations can be obtained by ultrasound-guided needle aspiration or rolling biopsies on slides before fixation
- Osteosarcomas generally yield cells more readily than other sarcomas
- Preparations from telangiectatic forms are usually haemodilute

Cytology of osteosarcoma

- May enable definitive diagnosis of malignancy without need for histology
- FNAs yielded diagnostic cytological preparations in 32 of 36 cases in one study (Britt *et al*, 2007)
- Differentials include chondrosarcoma, fibrosarcoma, synovial sarcoma, haemangiosarcoma, plasma cell tumour

Alkaline phosphatase stain

- Valuable advance in cytological diagnosis of osteosarcoma
- Requires unfixed, unstained smears
- Reliably identifies cells as osteoblasts – BUT, stains both reactive and neoplastic cells

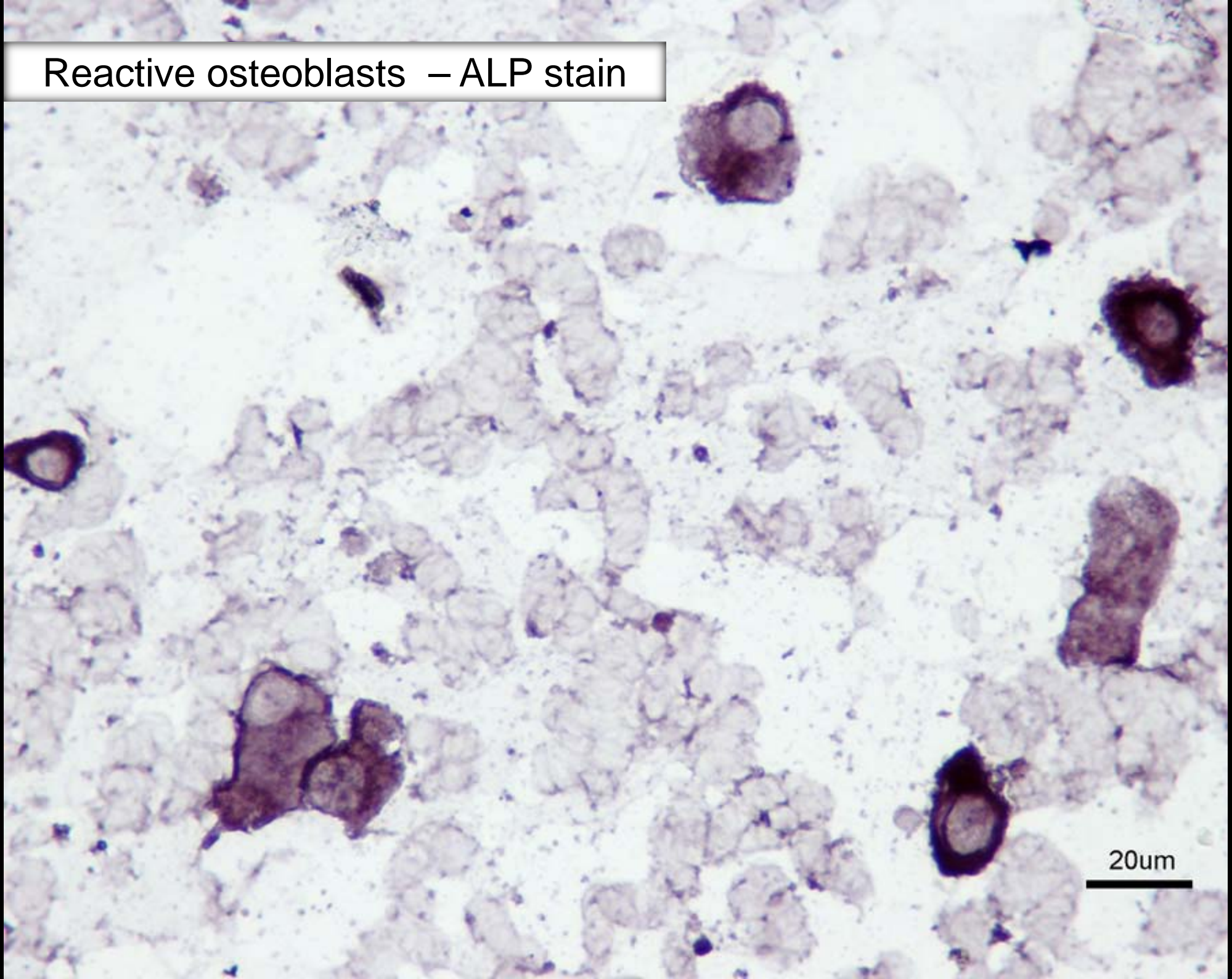
Alkaline phosphatase stain

- Osteoblast cell membrane stains black/brown
- Sensitivity 100%
- Specificity 89%
- Can exclude dx of OSA if malignant cells are negative

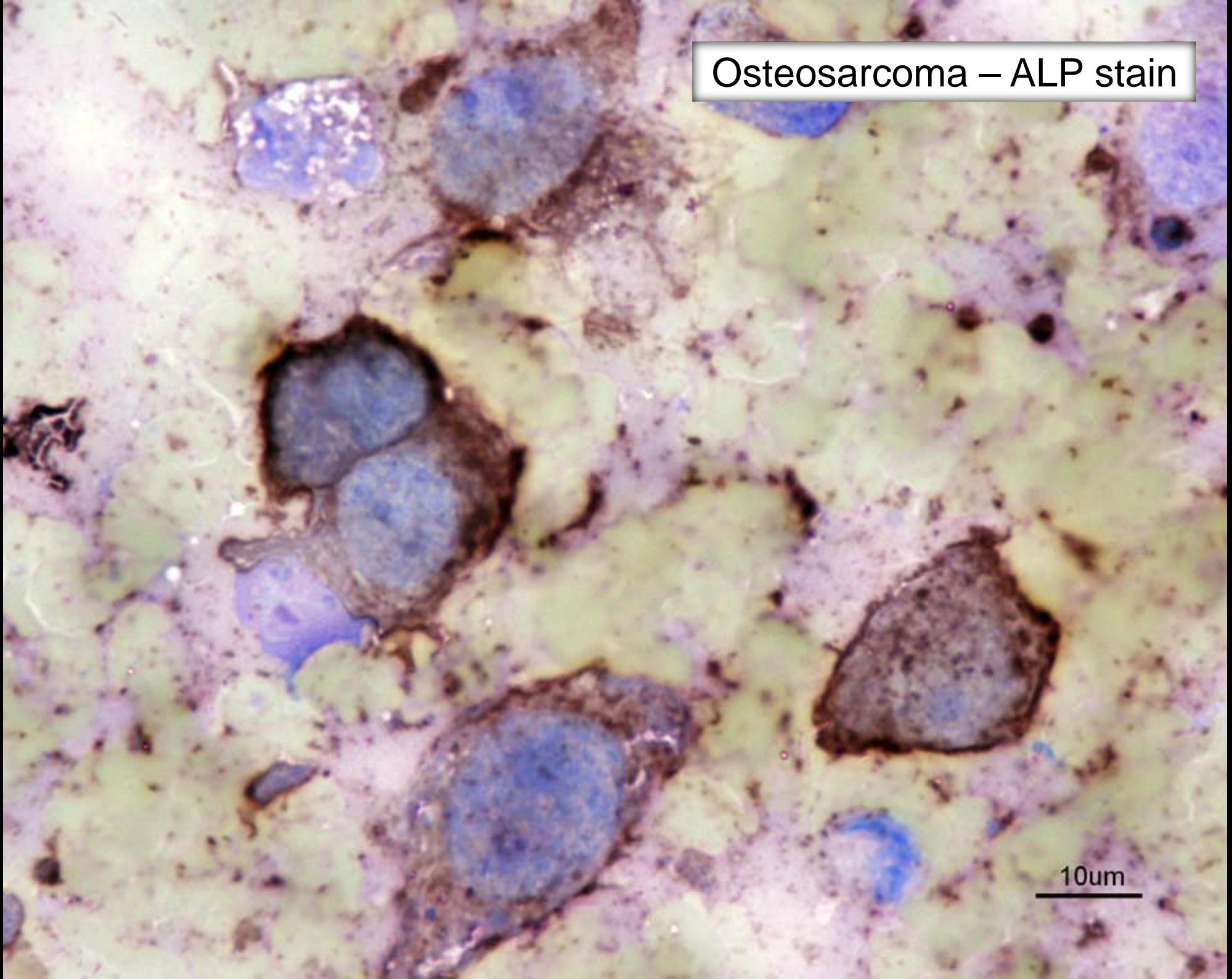
A. Barger *et al*, Vet Pathol 42:161–165 (2005)



Reactive osteoblasts – ALP stain



Osteosarcoma – ALP stain

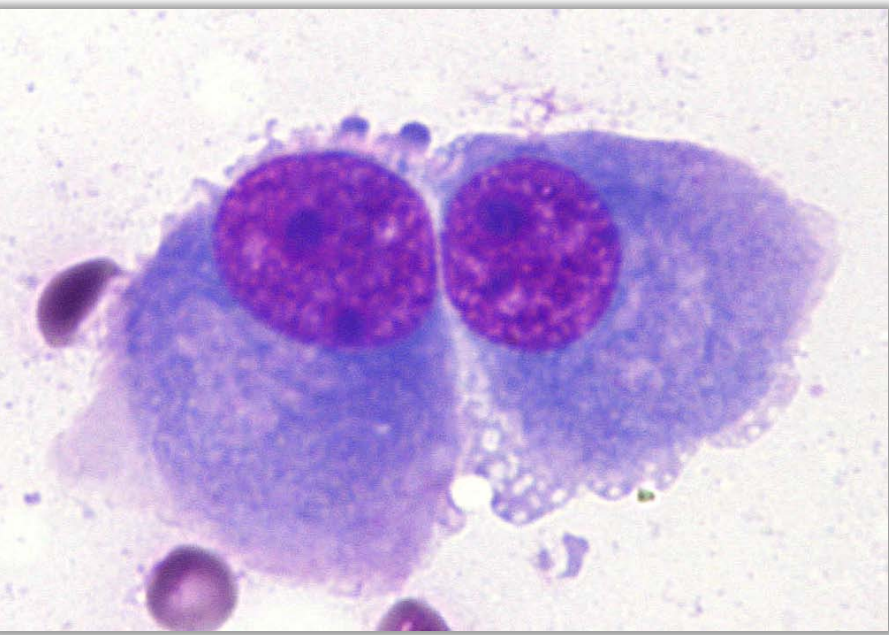


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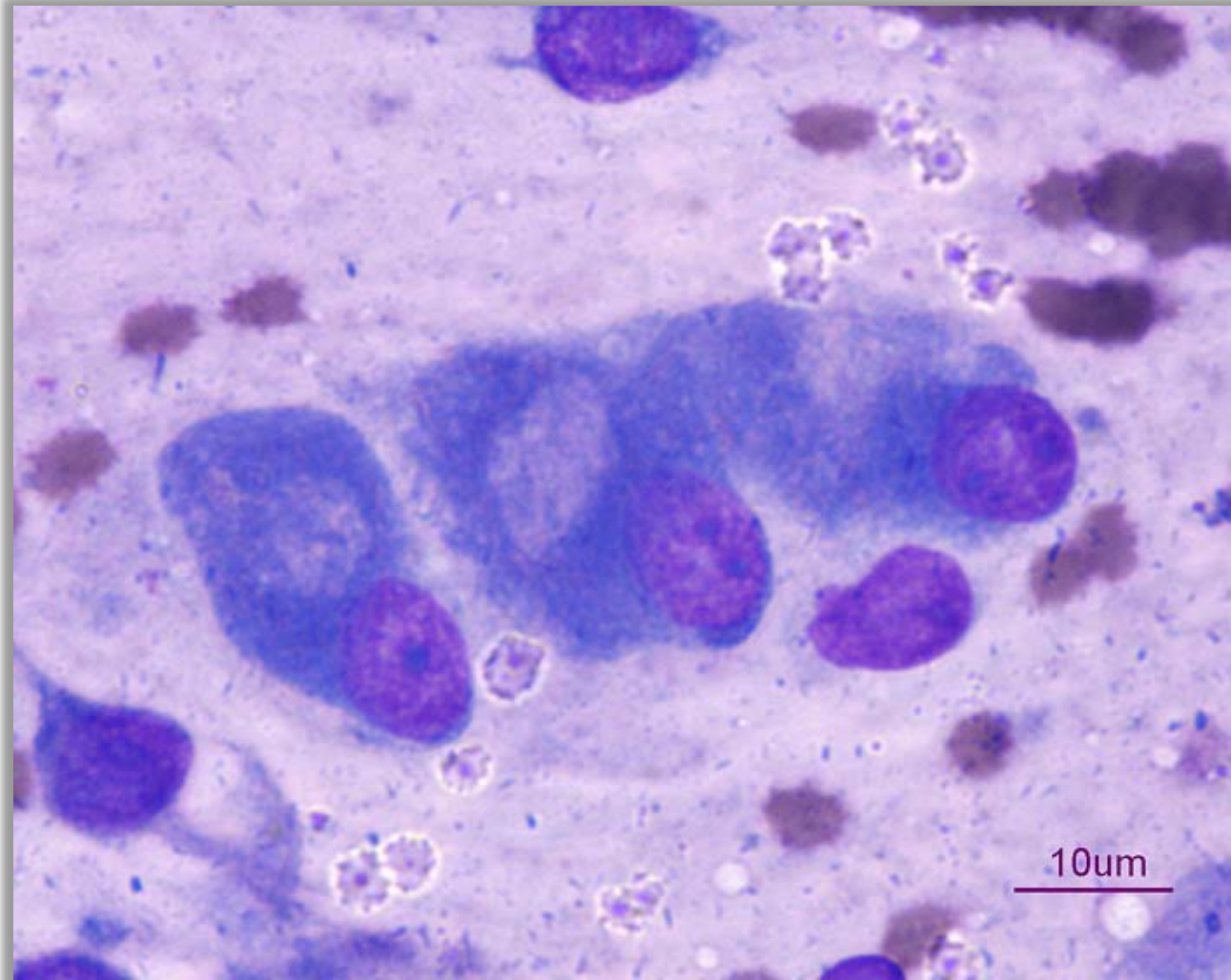
Cytology of reactive bone

- Preparations usually have low cellularity
- Osteoblasts relatively uniform but some anisocytosis and anisokaryosis acceptable
- Mitotic figures and osteoclasts may be present

Reactive osteoblasts

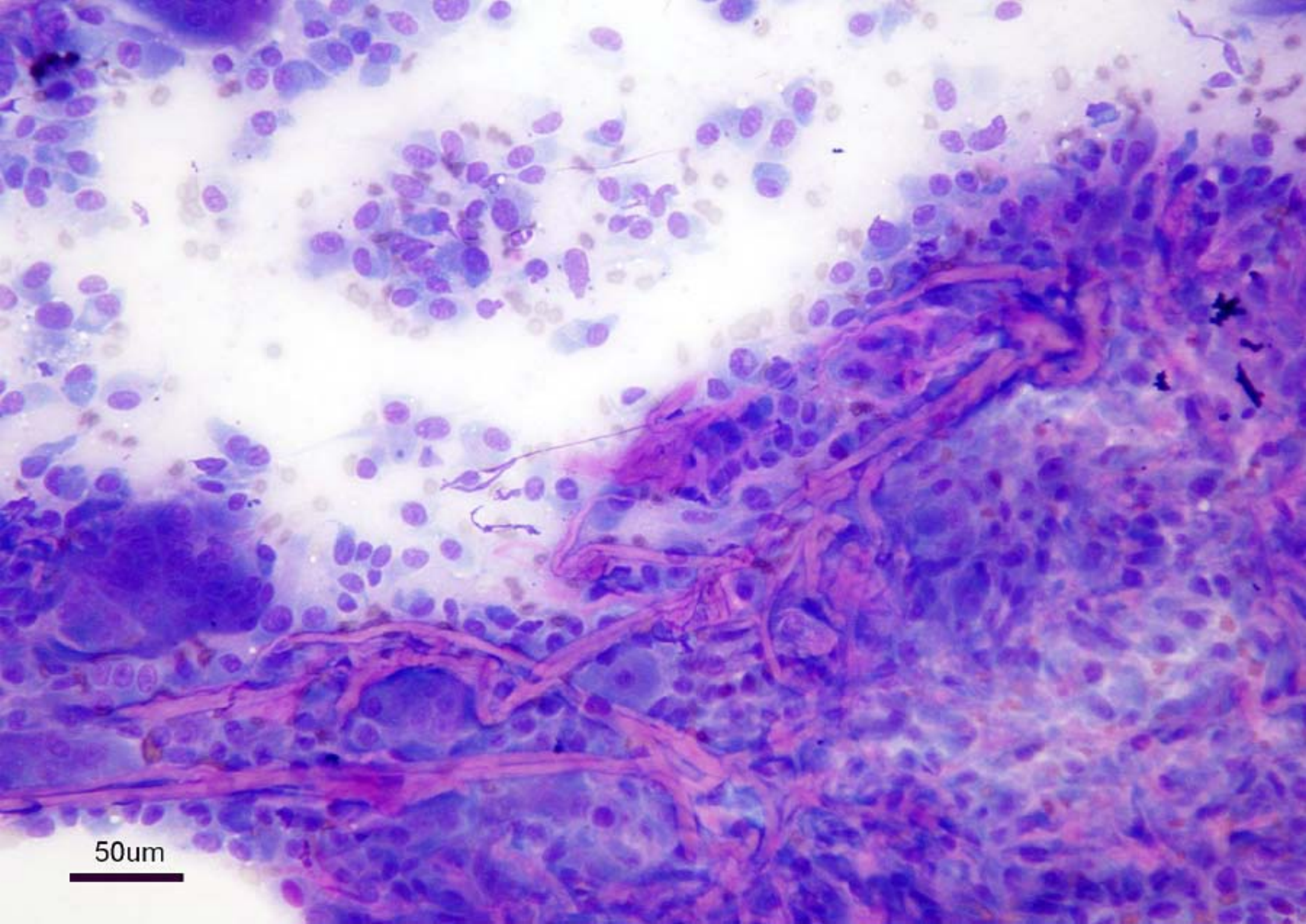


- Eccentric nuclei
- Basophilic cytoplasm
- Golgi zone
- Prominent, but small nucleoli

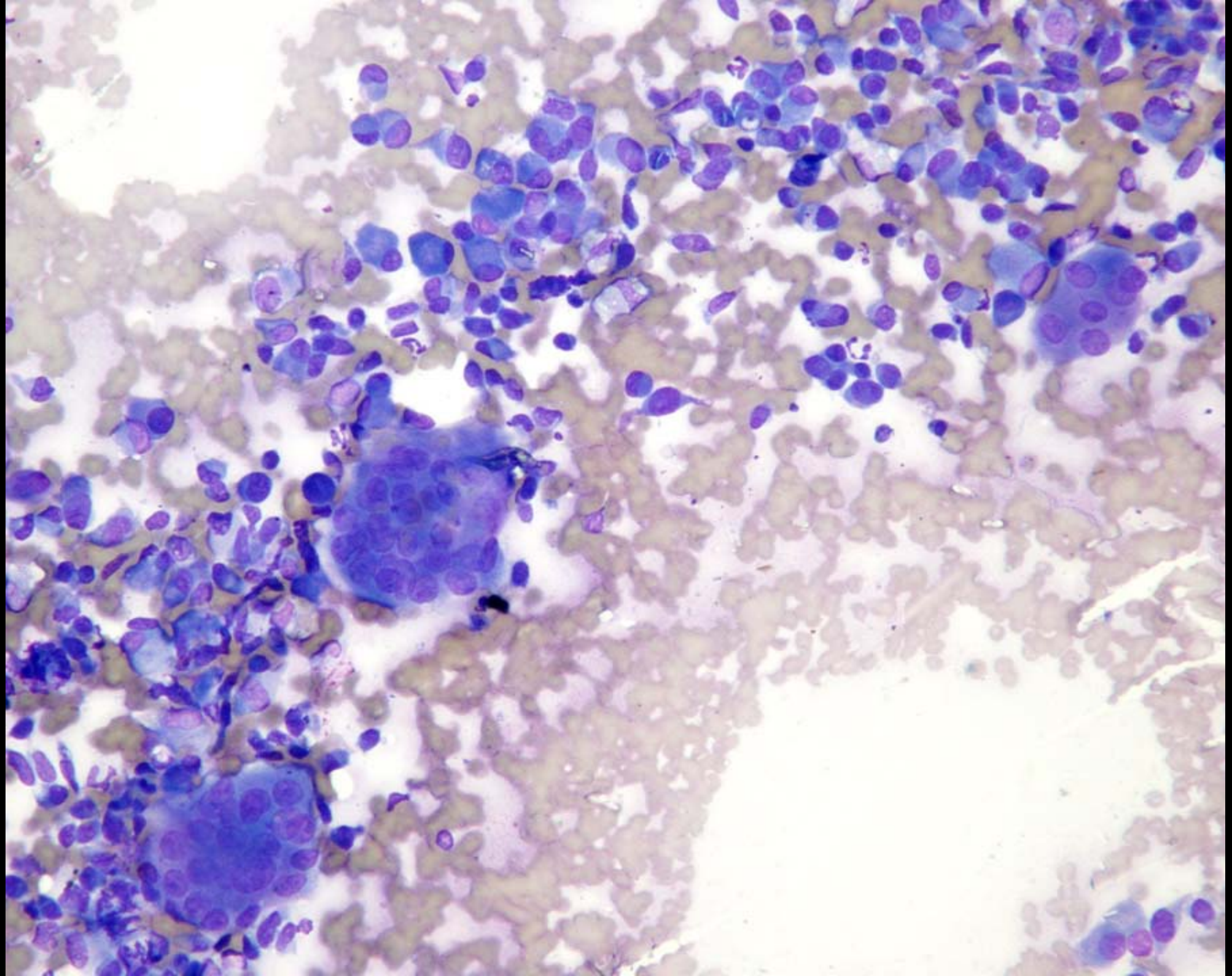


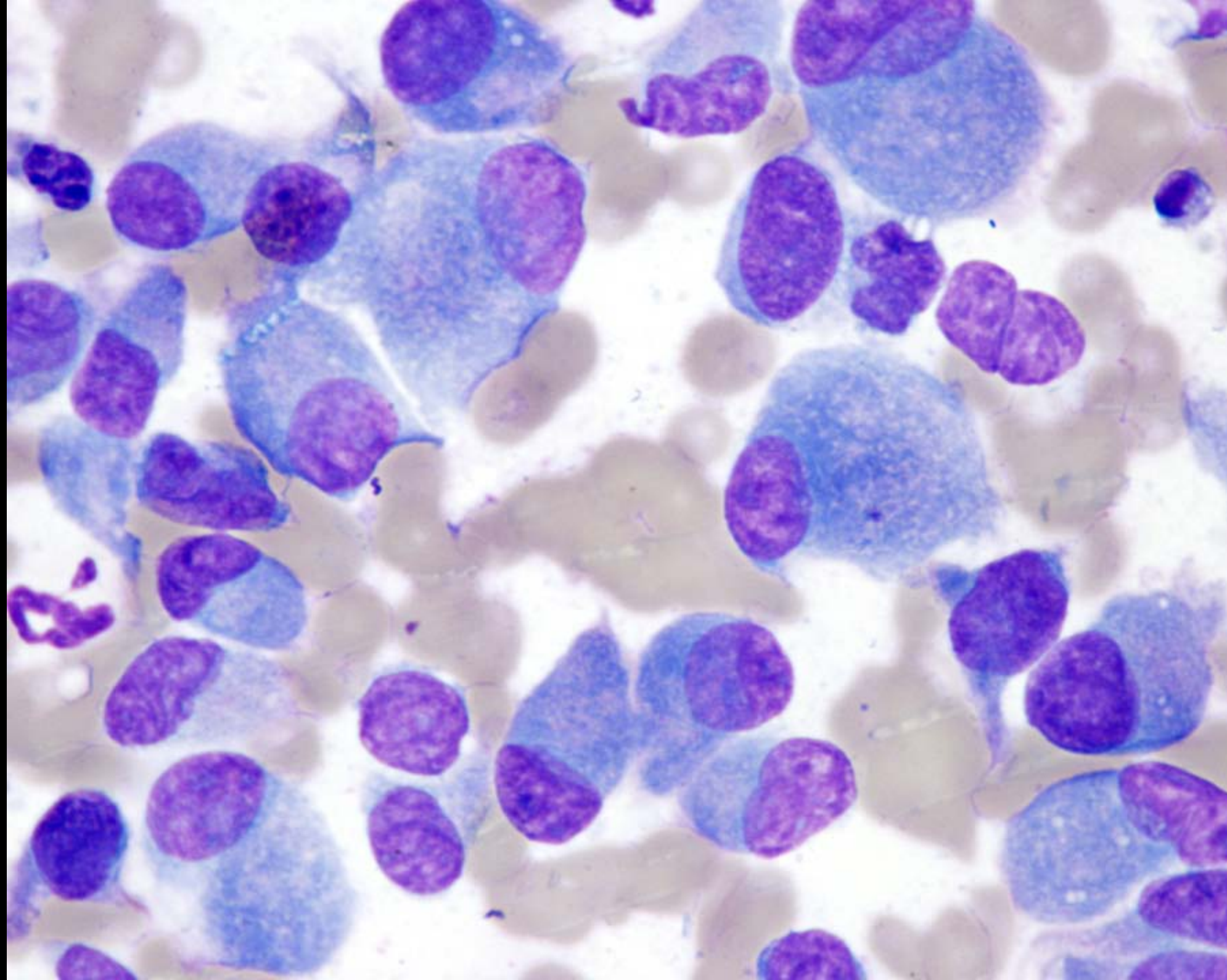
Cytology of osteosarcoma

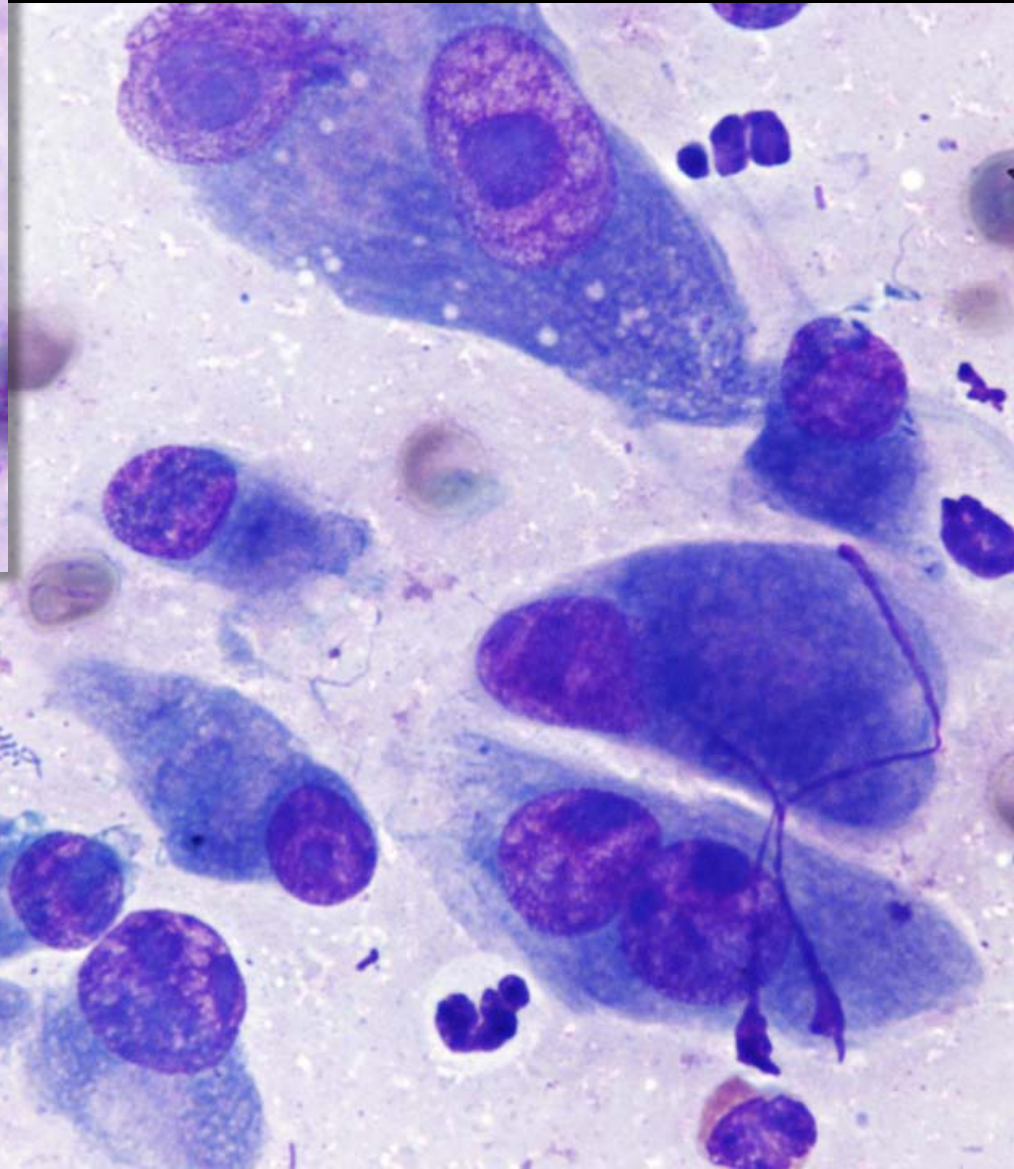
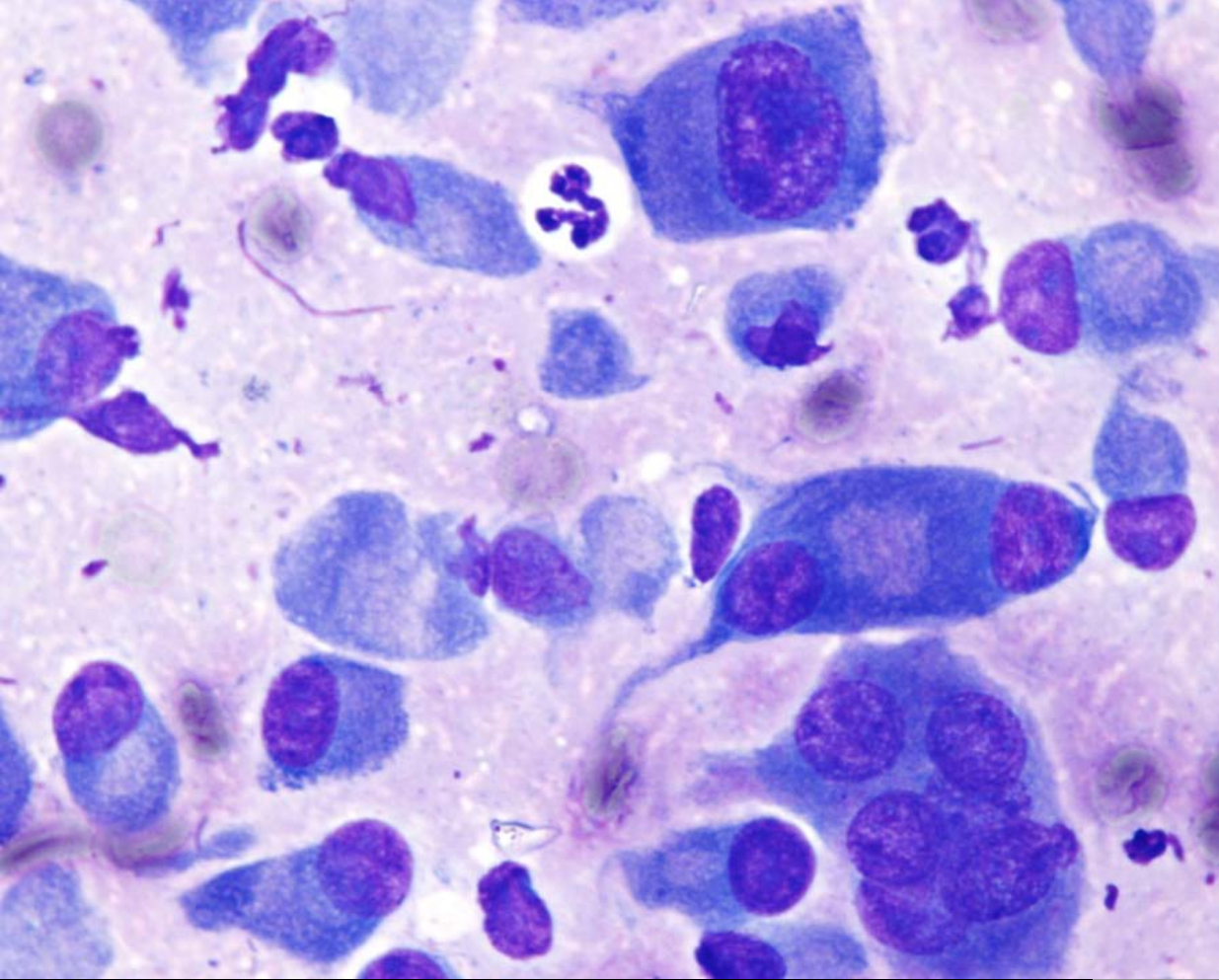
- High cellularity
- Variability in osteoblastic cell population
- Frequent mitoses, often abnormal
- Coarse chromatin pattern, nuclear molding
- Multinucleate forms (excluding osteoclasts), often with variable nuclear size

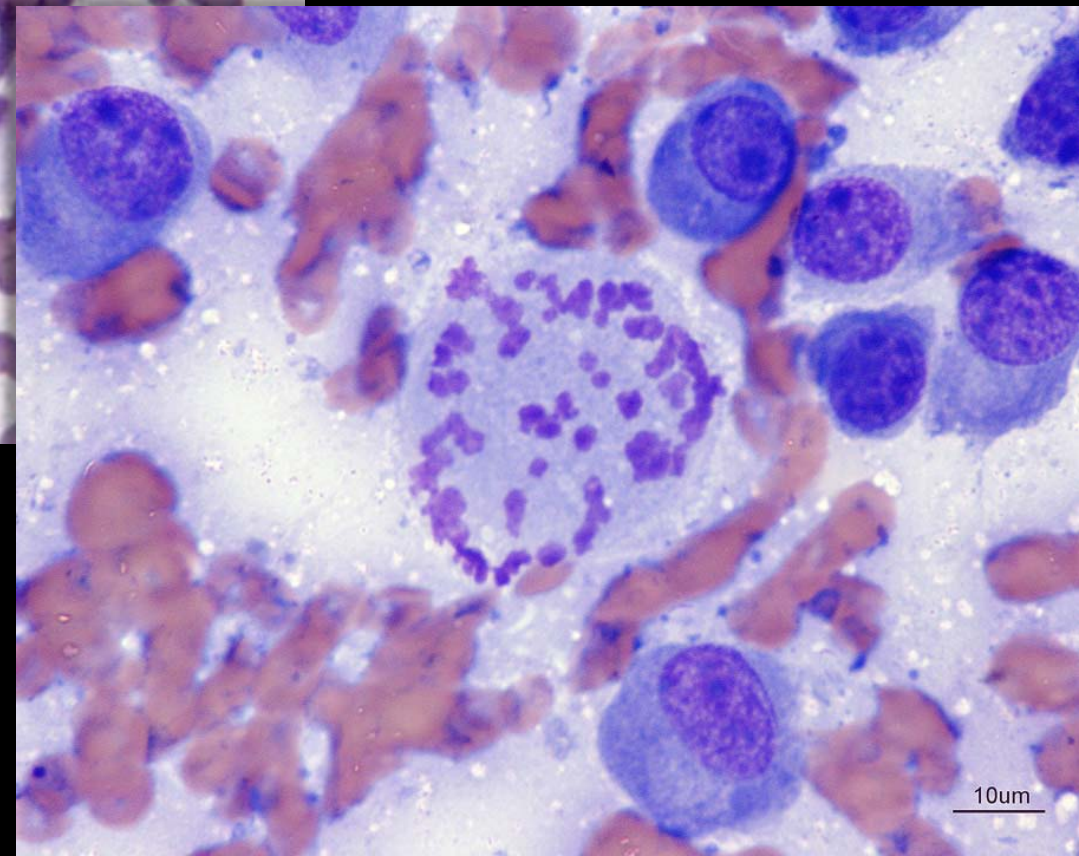
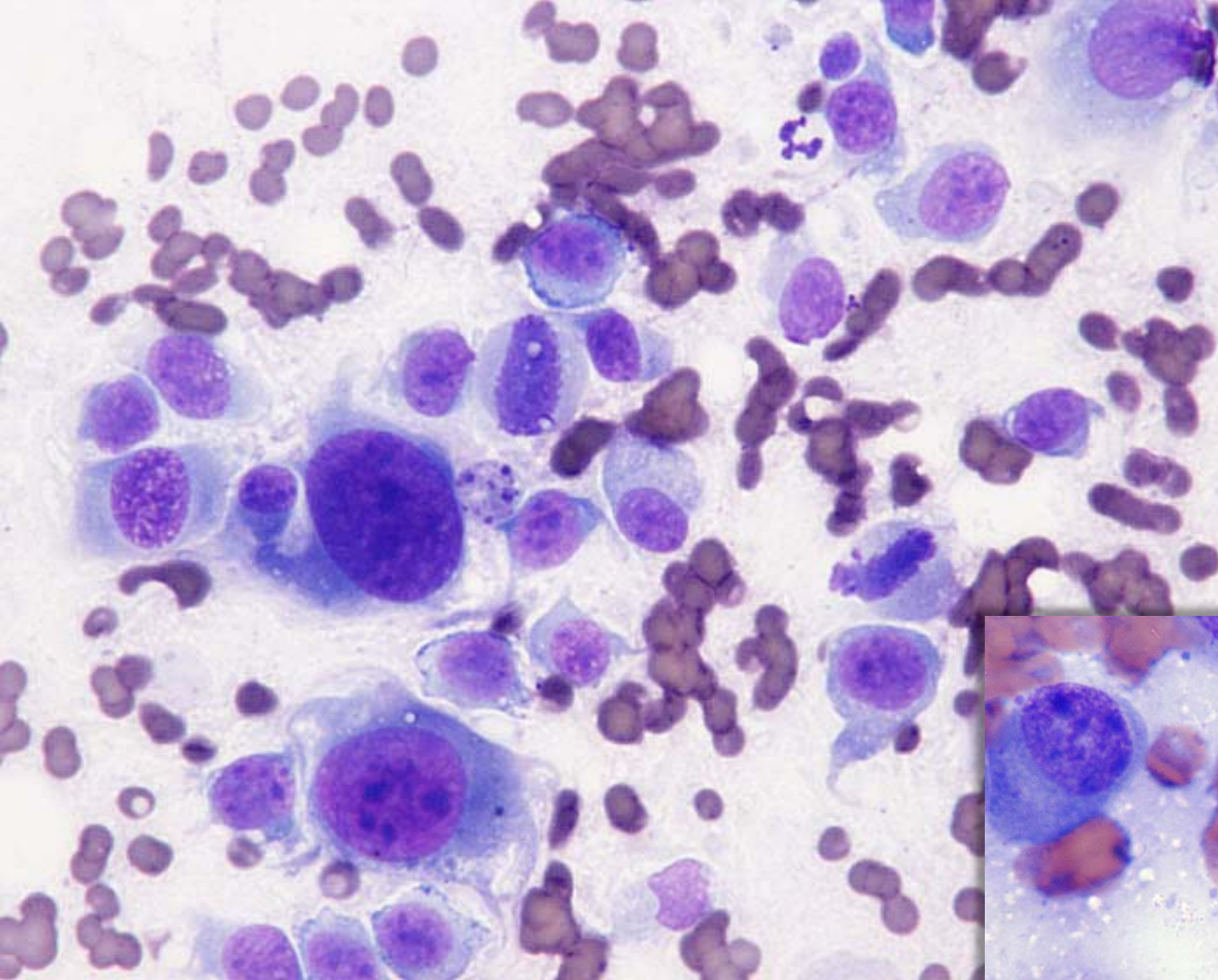


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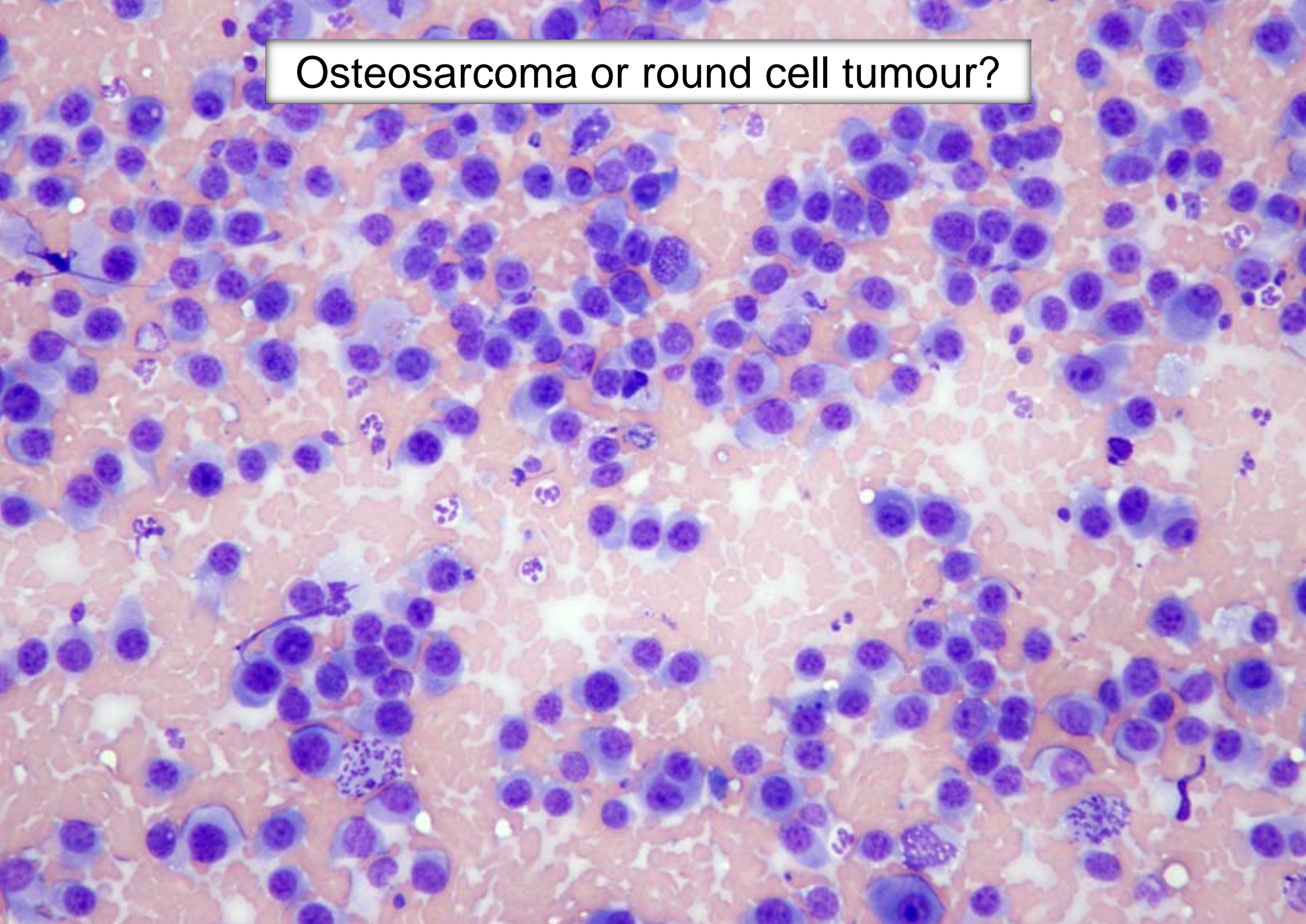


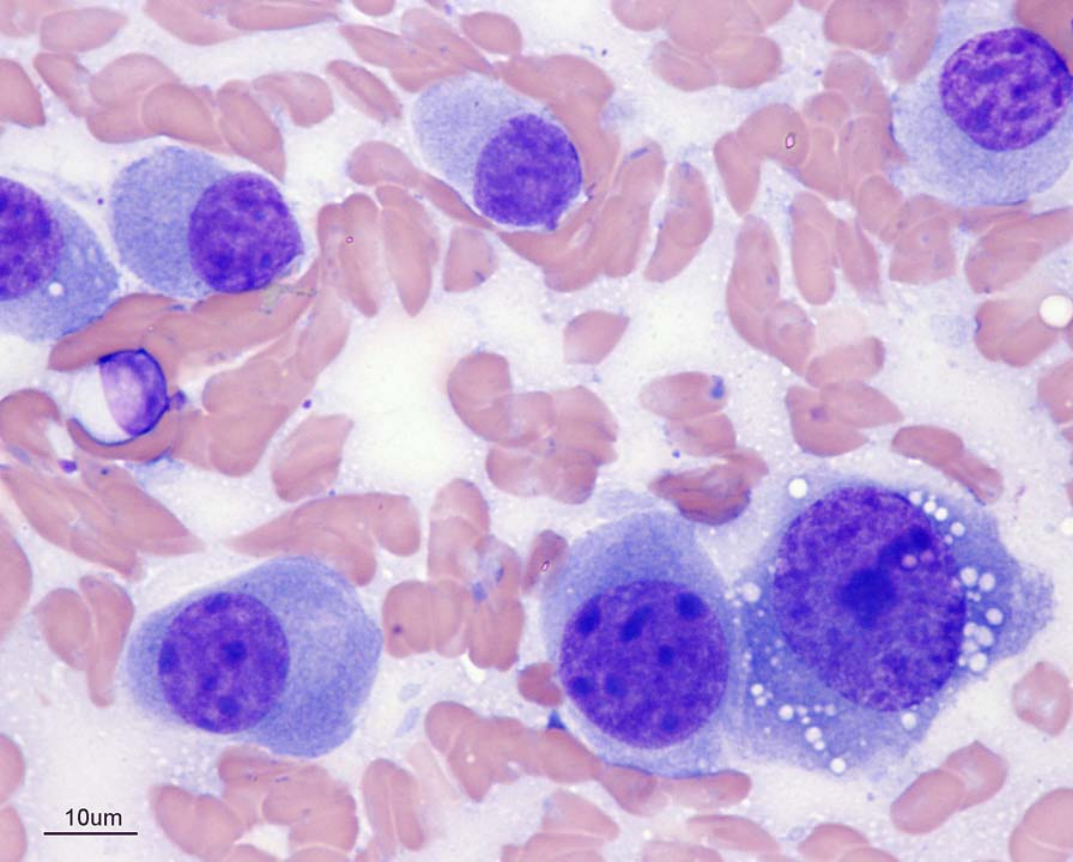


Words of warning!!

- Cytological diagnosis of osteosarcoma requires a good clinical history
- If you are not sure about the origin of the cells don't commit yourself
- Malignant osteoblasts can closely resemble other tumour cells (e.g. other sarcomas, melanoma, plasma cell tumour)

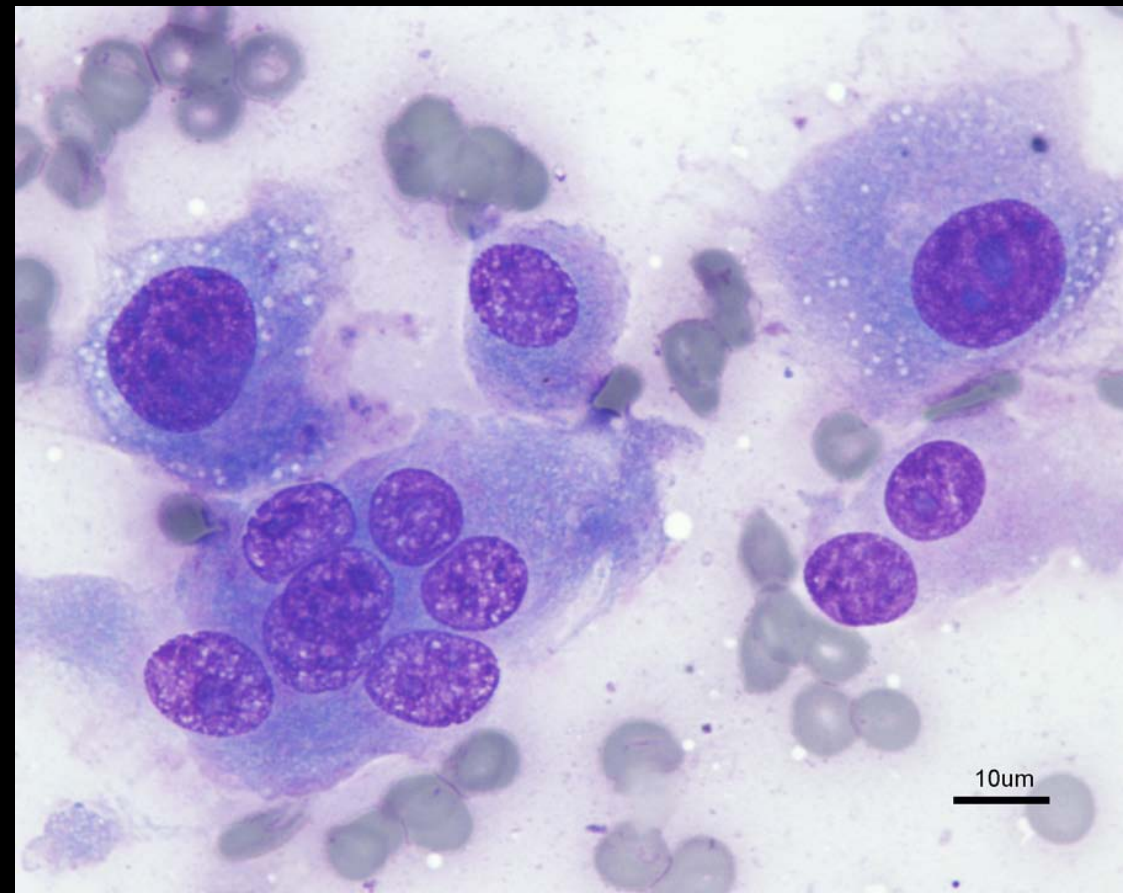
Osteosarcoma or round cell tumour?



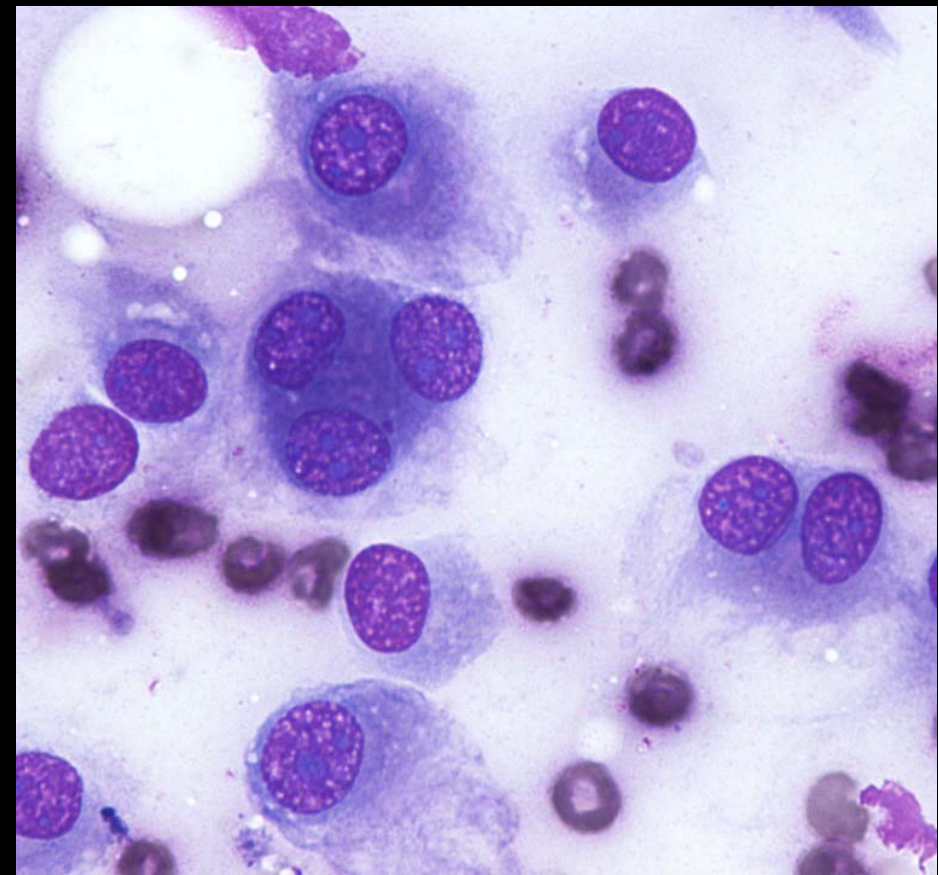
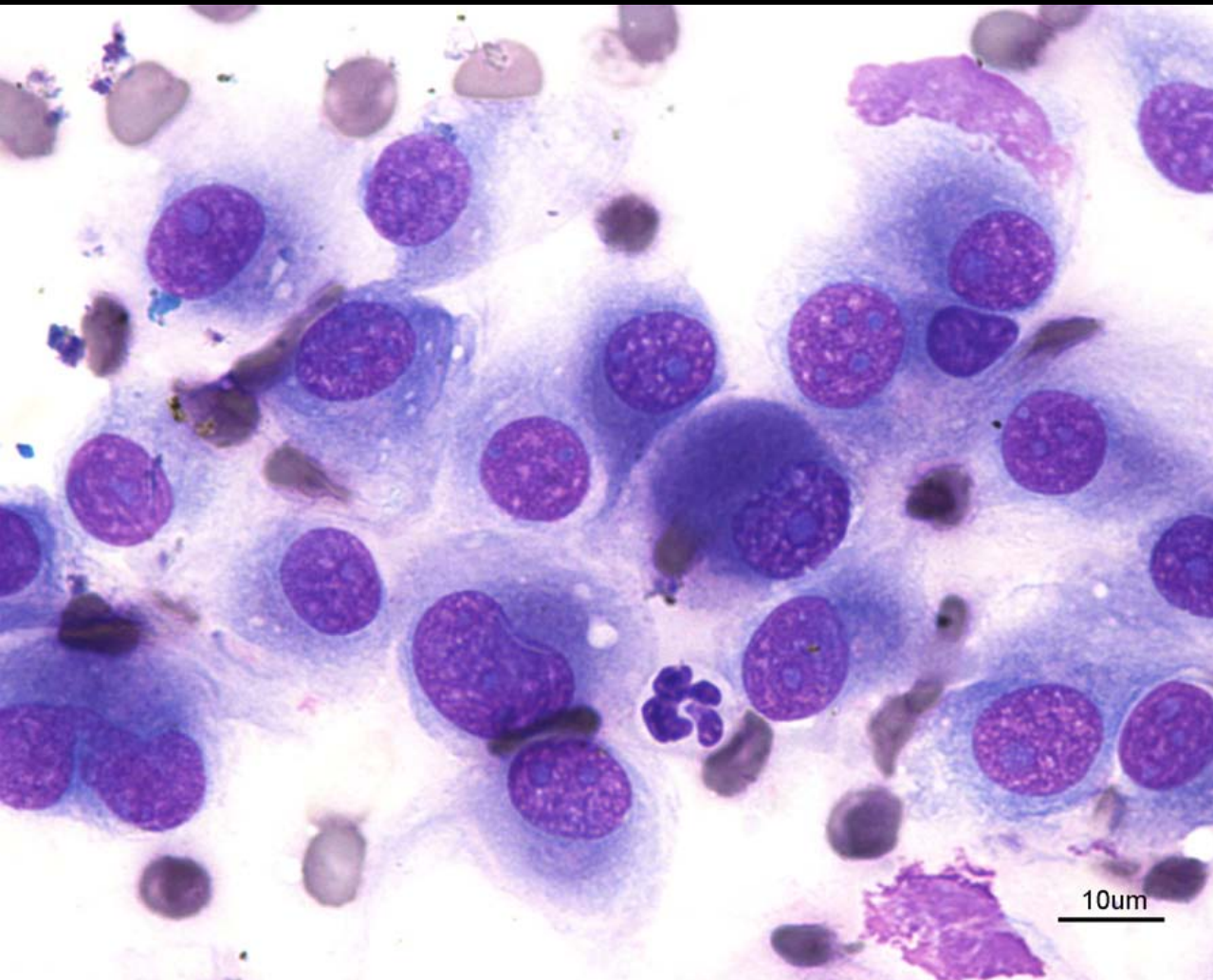


Osteosarcoma - distal radius of a
5yr Pyrenees Mountain dog
(same case as previous slide)

Amelanotic melanoma –
naso-oral mass in a
Miniature Schnauzer



FNA from a 12 yr old Boxer with a history of a
“mass on its antebrachium”



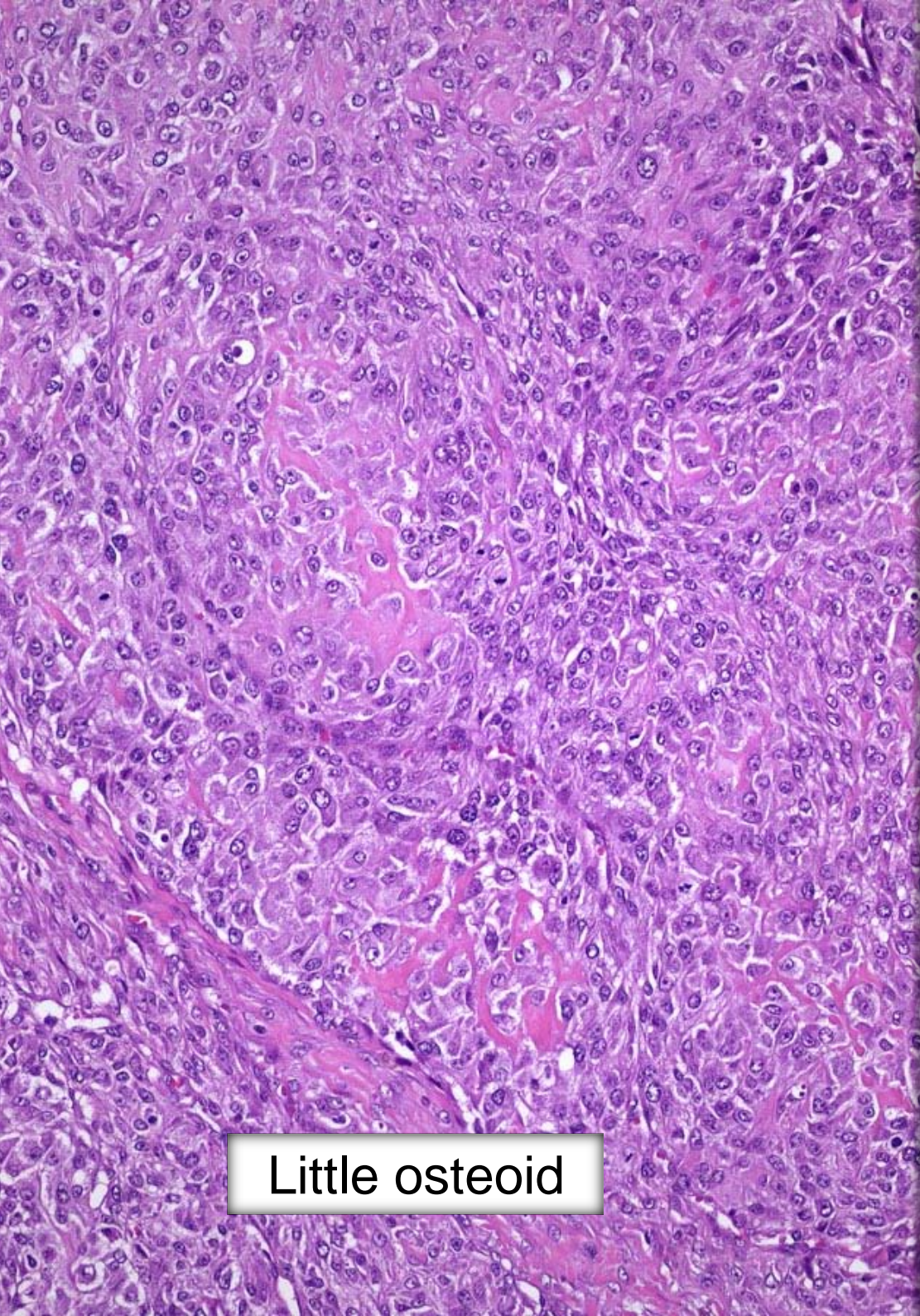
A histological slide showing a dense population of spindle-shaped cells with elongated, hyperchromatic nuclei. The cells are arranged in a disorganized, fascicular pattern, characteristic of a soft tissue sarcoma. The background is a pinkish-purple stain, likely hematoxylin and eosin (H&E).

Diagnosis: Soft tissue sarcoma

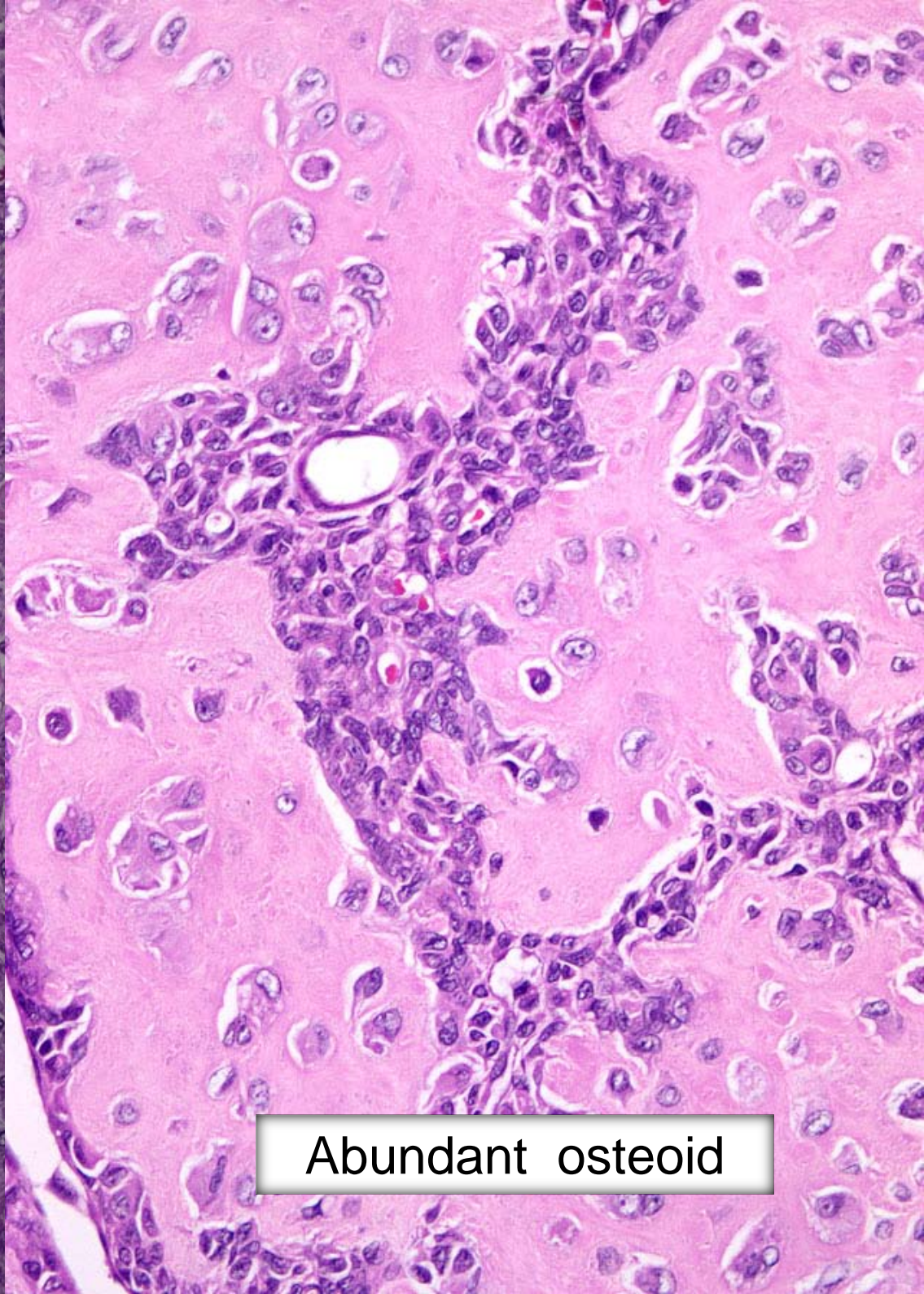
Lack of adequate history could have led to
an incorrect diagnosis of OSA

Histology of osteosarcoma

- Widely considered “gold standard” for osteosarcoma diagnosis – questionable if based on biopsies
- Morphology of malignant osteoblasts varies markedly
- Osteoclasts often present
- Hallmark is production of osteoid by malignant mesenchymal cells – marked variation in amount and pattern of osteoid



Little osteoid



Abundant osteoid

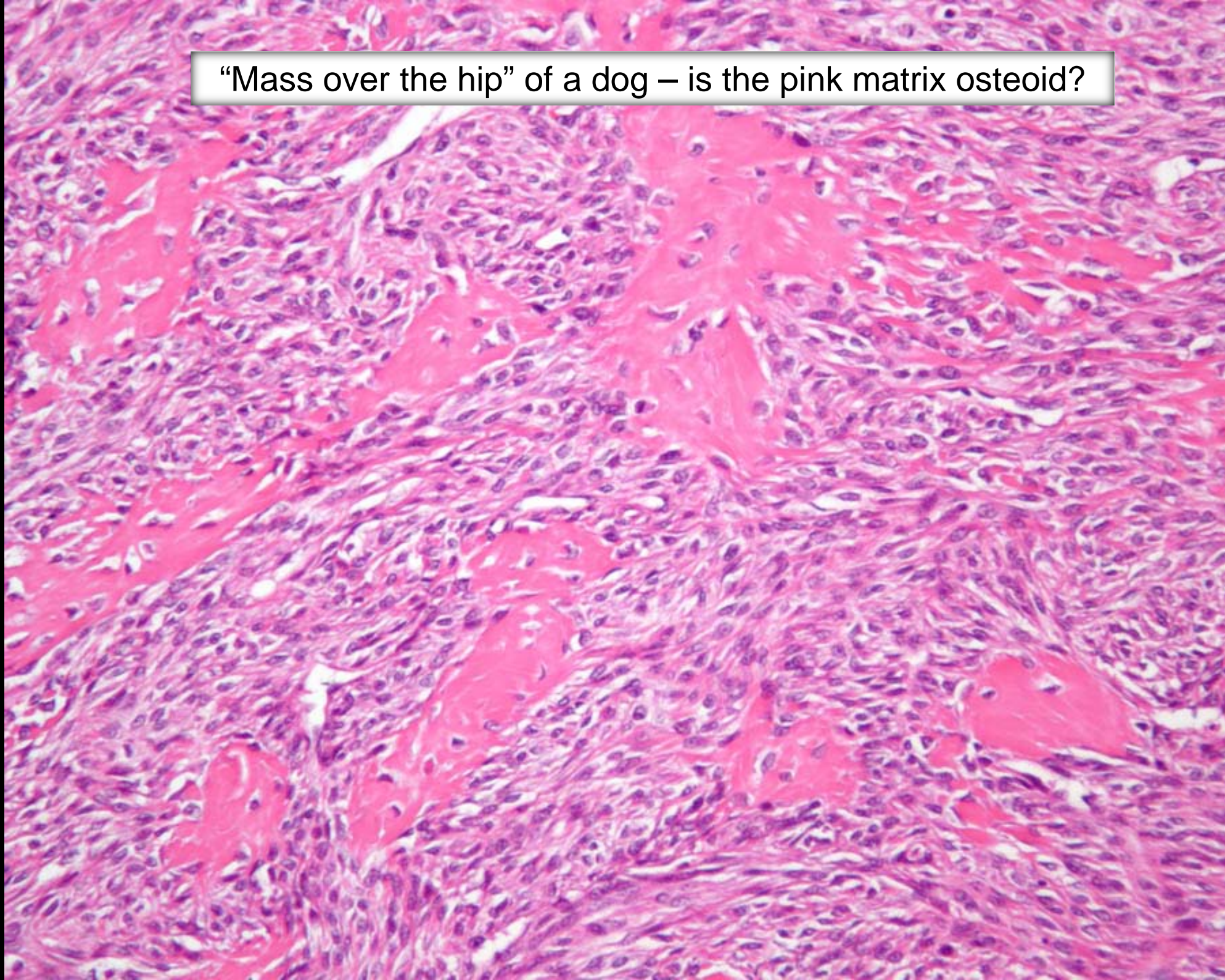
Histology of osteosarcoma

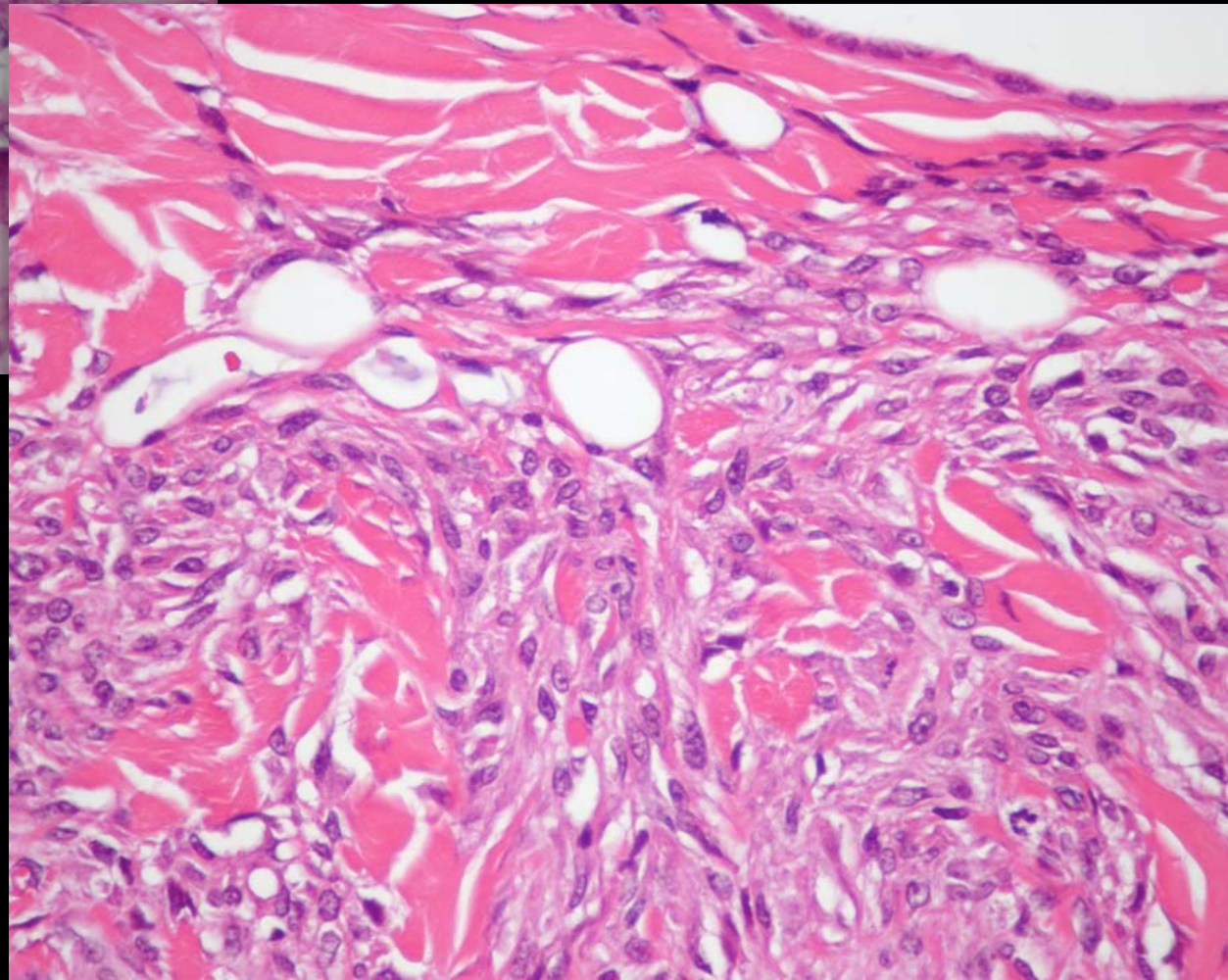
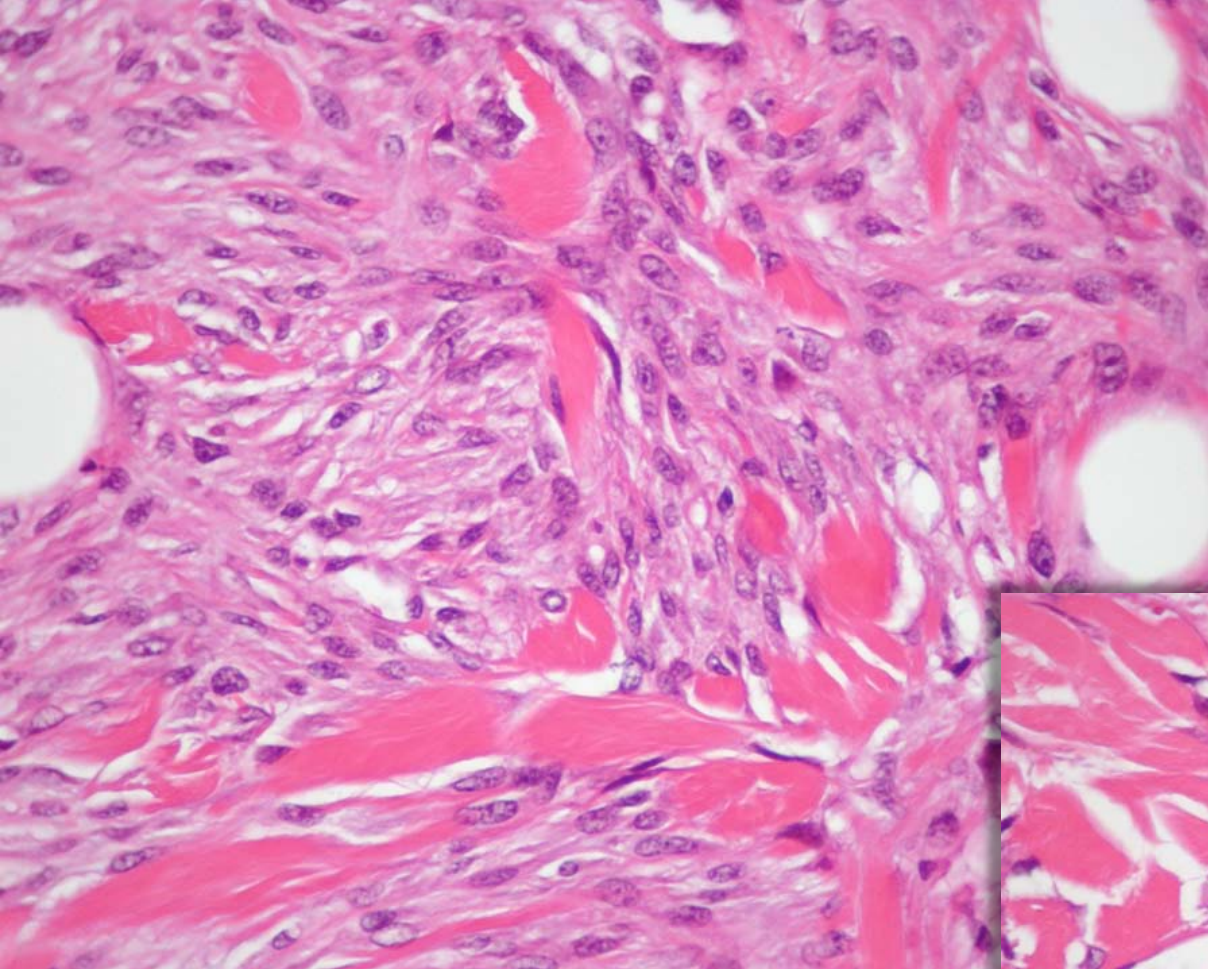
Two important questions:

1. Is the pink material osteoid?
 - less fibrillar than collagen
 - if it is mineralised it is probably osteoid
 - special stains are of little value
2. Are the cells producing it malignant?

If the answer is yes to both questions then the diagnosis is most likely osteosarcoma

“Mass over the hip” of a dog – is the pink matrix osteoid?

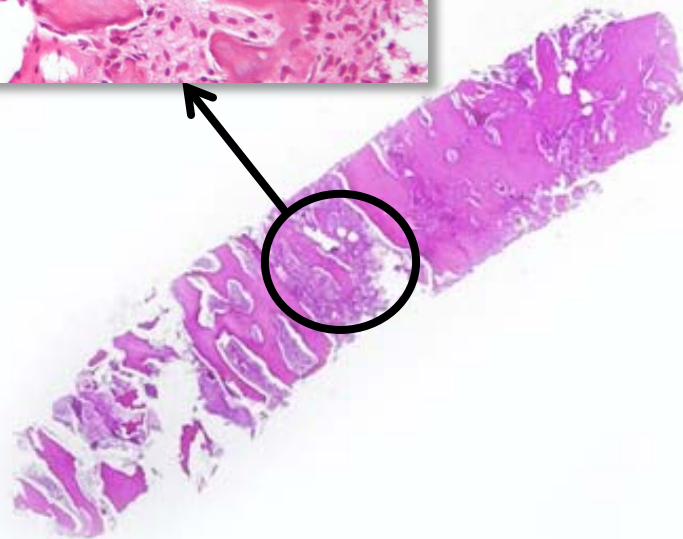
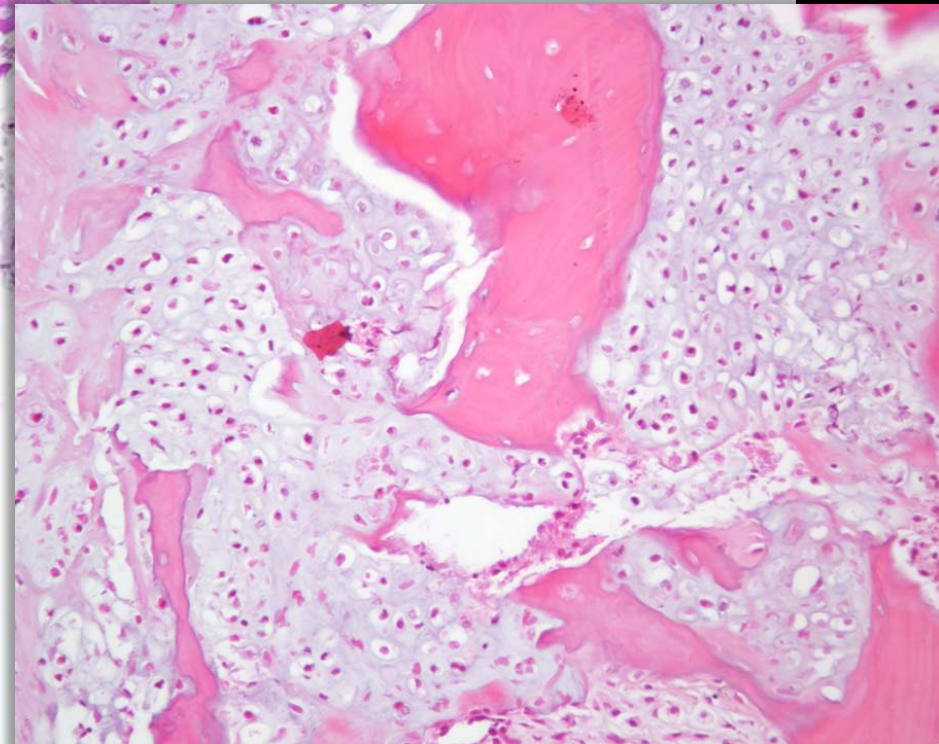
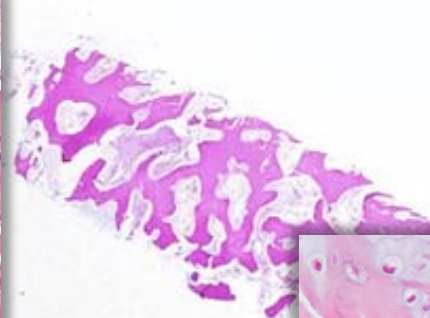
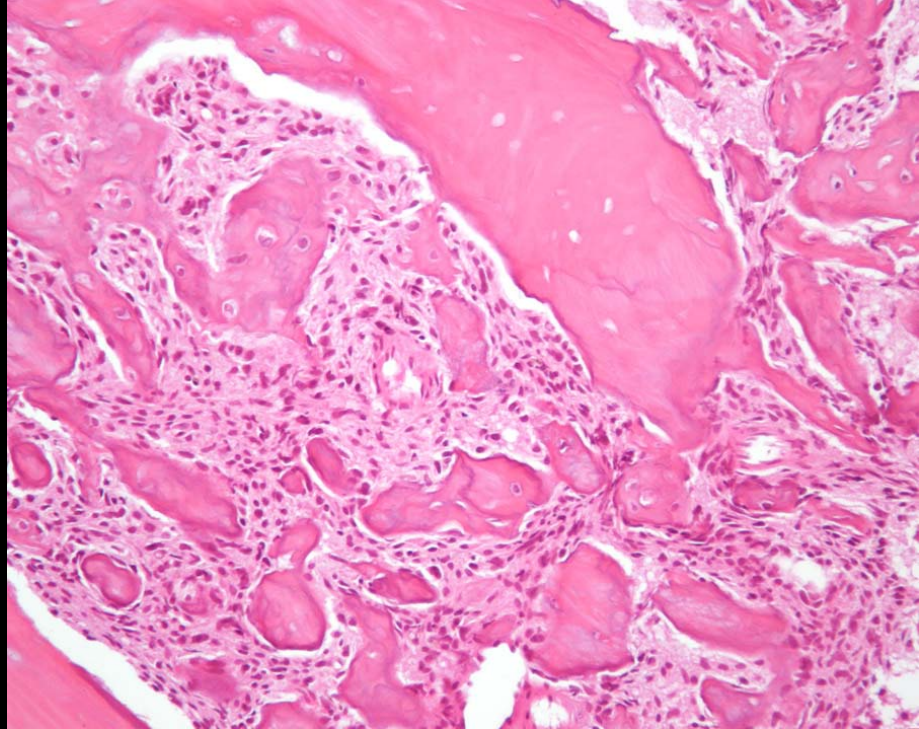




Diagnosis: Soft tissue
sarcoma infiltrating dermal
collagen

Histology of osteosarcoma

- Several sub-types recognised
 - Poorly differentiated
 - Chondroblastic
 - Fibroblastic
 - Telangiectatic
 - Giant cell
 - Classification based on cell types is seldom possible when examining small biopsies
- May be a combination of these

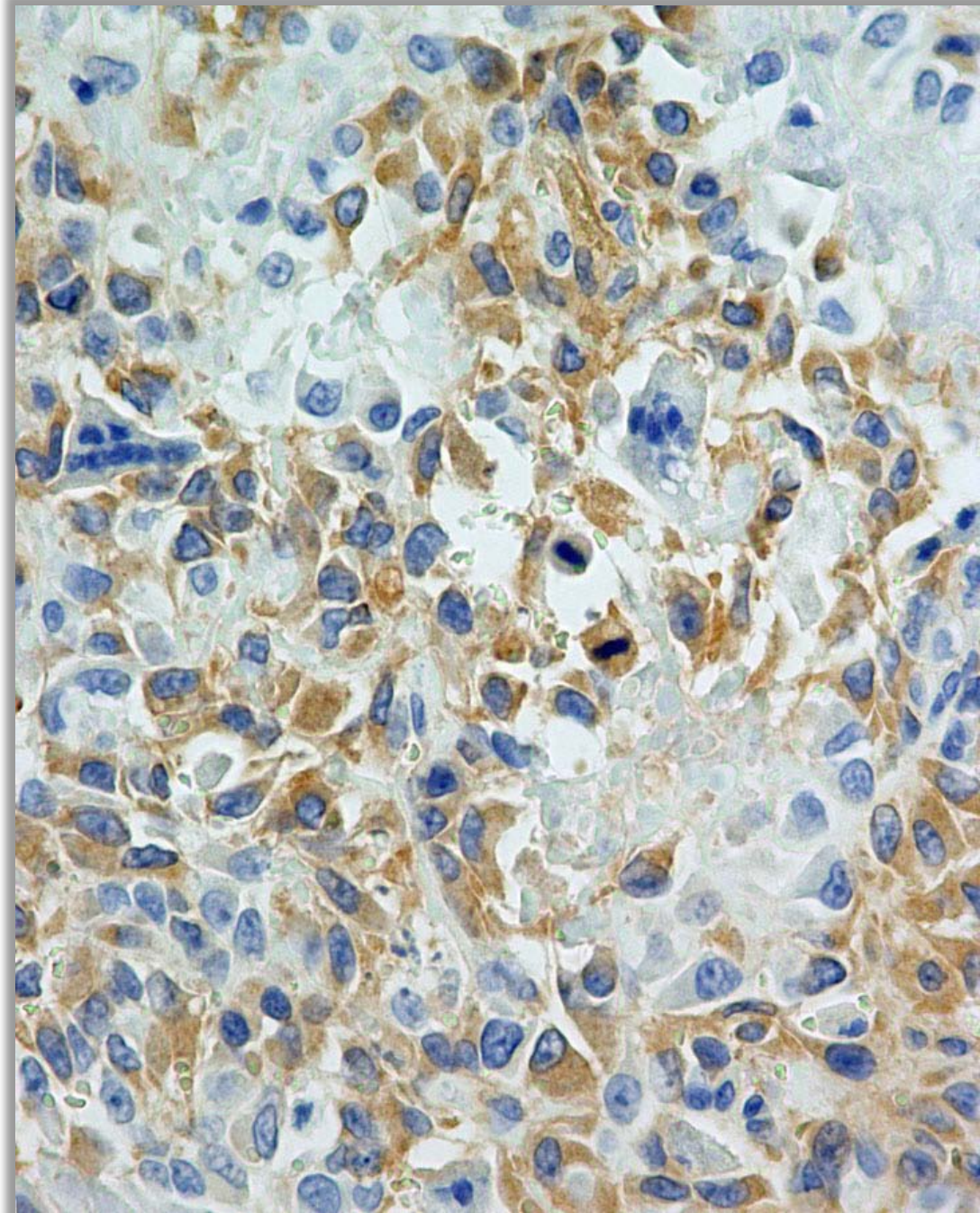
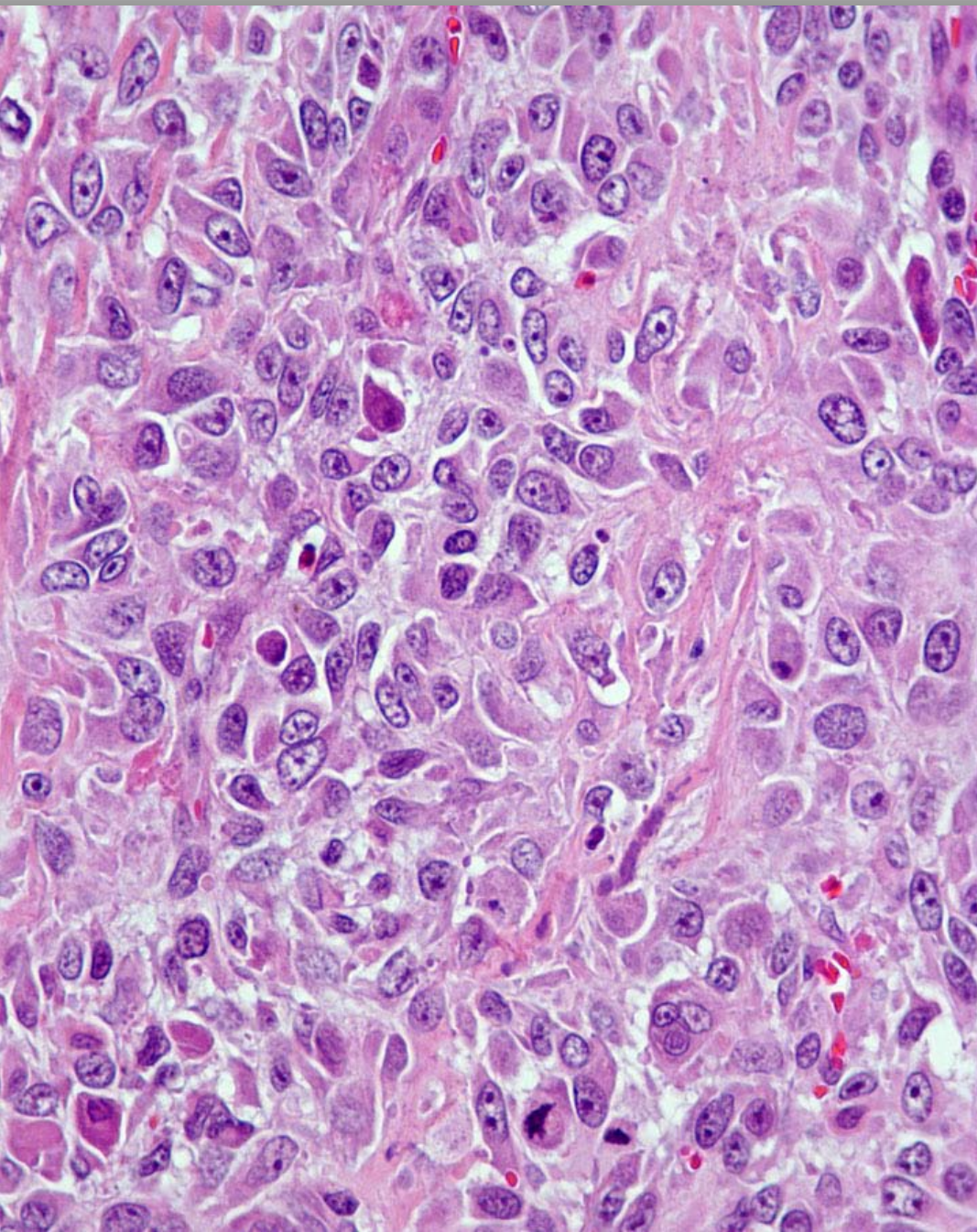


The primary aim when examining bone biopsies should be to decide if the lesion really is an osteosarcoma – not which sub-type it might be

Immunohistochemistry – is it useful?

- Bone specific proteins (e.g. osteocalcin & osteonectin)
- Osteocalcin 70-80% sensitive & 100% specific
- Osteonectin highly sensitive but low specificity
- Can not differentiate between reactive and neoplastic osteoblasts
- Can not reliably differentiate between fibroblastic osteosarcomas and fibrosarcomas in human patients

Immunohistochemistry - osteocalcin



Histology of reactive & neoplastic bone

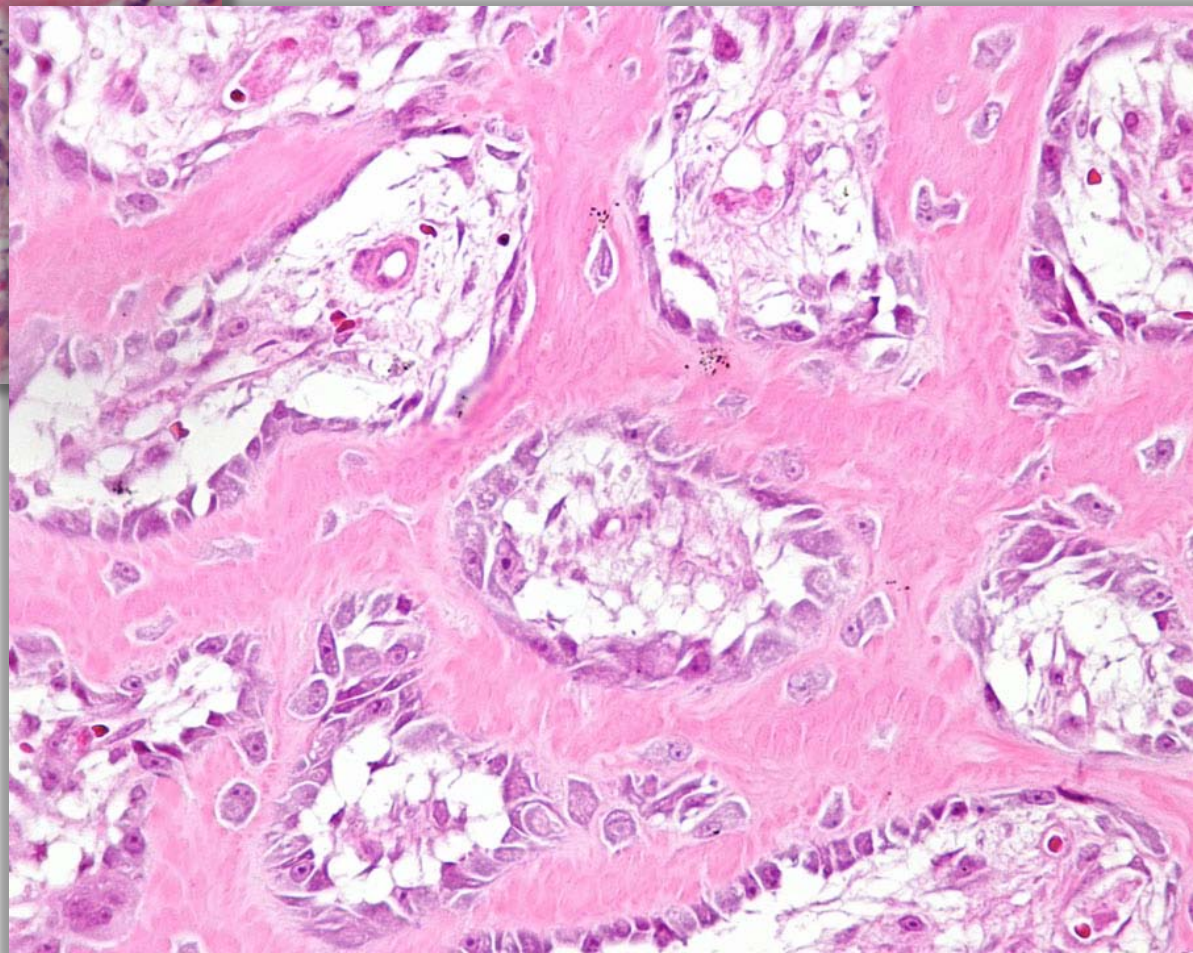
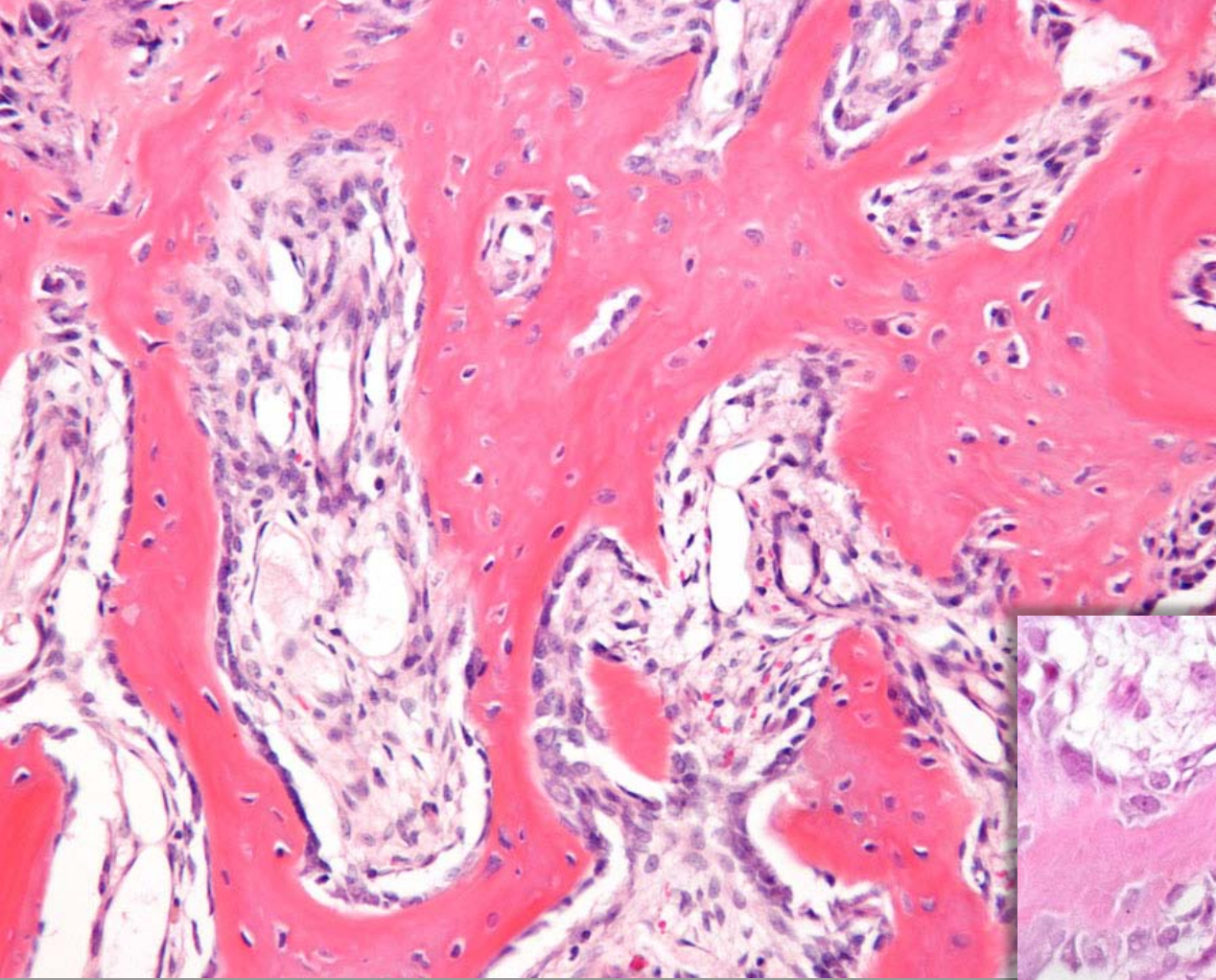
Reactive

- Woven bone
- Trabeculae usually interconnected
- Typically anchored to adjacent lamellar bone
- Osteoblasts relatively uniform
- Trabeculae lined by single layer of osteoblasts

Neoplastic

- Woven bone
- “Trabeculae” usually not interconnected
- Often not anchored to adjacent lamellar bone
- Osteoblasts often highly variable
- Tumour cells typically fill inter-trabecular spaces

Reactive bone



A histological section of bone tissue stained with hematoxylin and eosin (H&E). The image shows a dense network of bone trabeculae, which are the structural units of bone. These trabeculae are composed of osteons, the basic functional units of bone. The osteons are separated by narrow spaces called osteoid spaces. The bone tissue is characterized by a high density of osteocytes, which are the mature bone cells. The overall appearance is one of a highly organized and mineralized tissue structure. The text "Reactive bone in a repairing fracture" is overlaid on the bottom left of the image.

Reactive bone in a
repairing fracture



Reactive or
neoplastic?

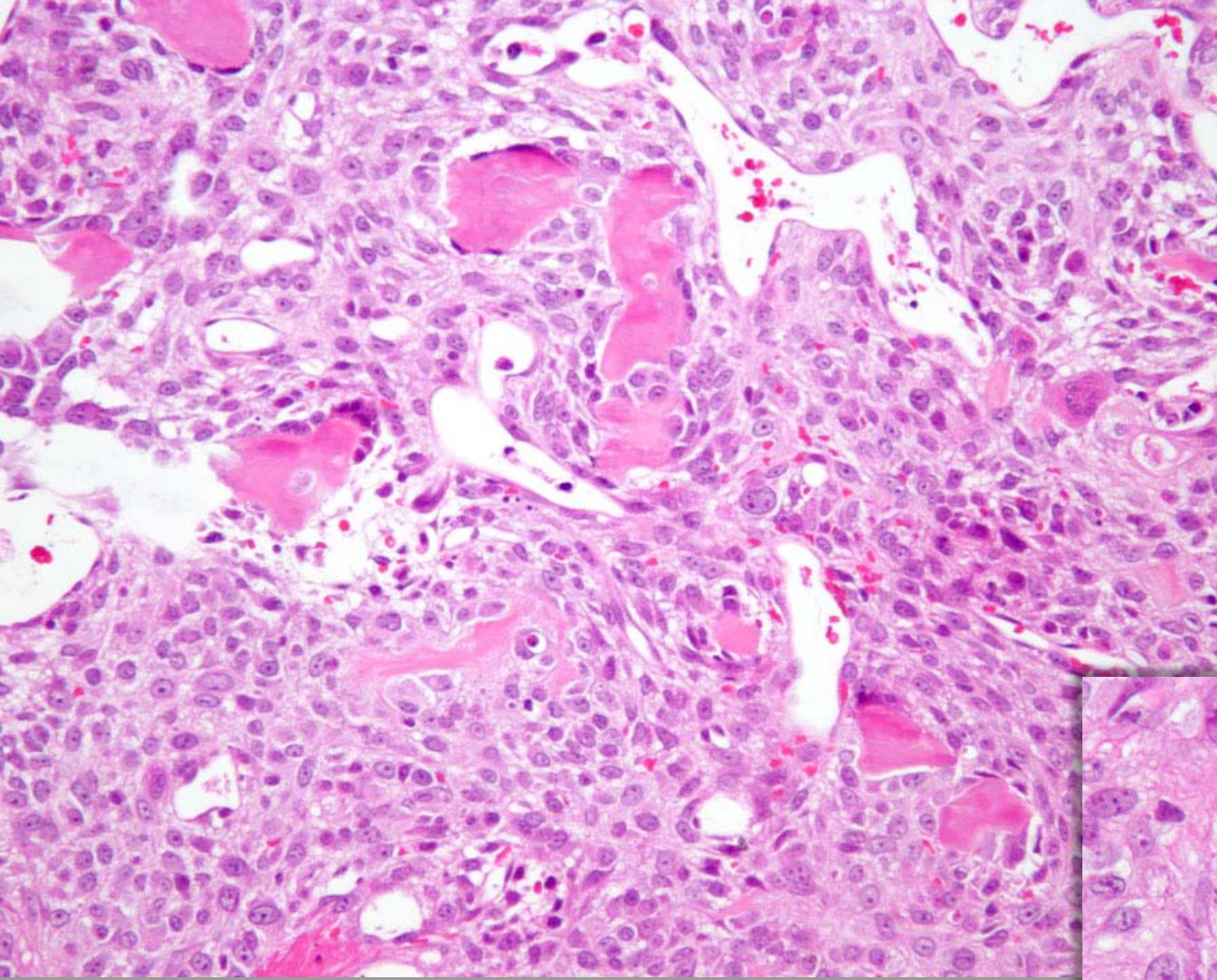
This histological image shows a low-power view of a tissue section stained with hematoxylin and eosin (H&E). The tissue is characterized by a dense population of cells with purple nuclei and pink cytoplasm/extracellular matrix. There are several elongated, somewhat irregular structures that appear to be developing or organizing, possibly representing early stages of bone formation or cellular differentiation within the fracture callus.

Early fracture callus
in a cat

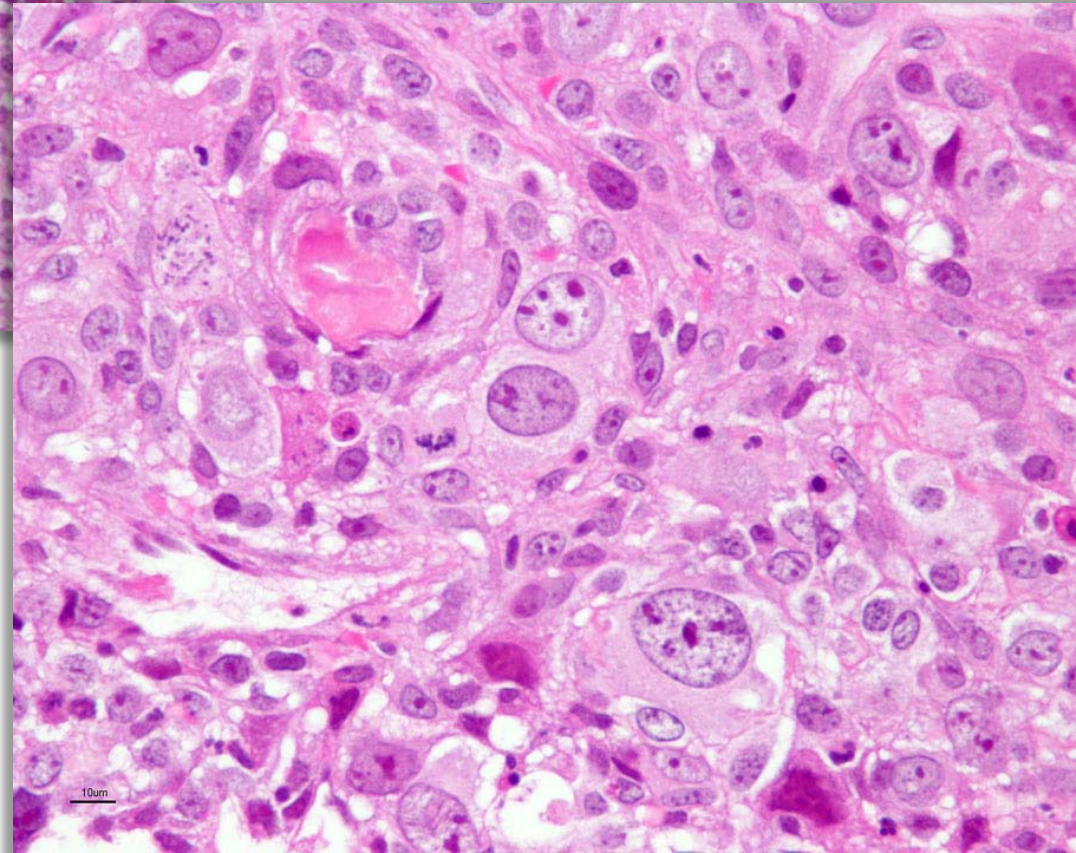


This high-magnification histological image shows a detailed view of the tissue from the fracture callus. The cells are densely packed, with many having large, dark purple nuclei. The pink-stained extracellular matrix is prominent, showing a complex, fibrous structure. The overall appearance is one of active cellular processes and tissue remodeling, consistent with the early stages of bone healing.

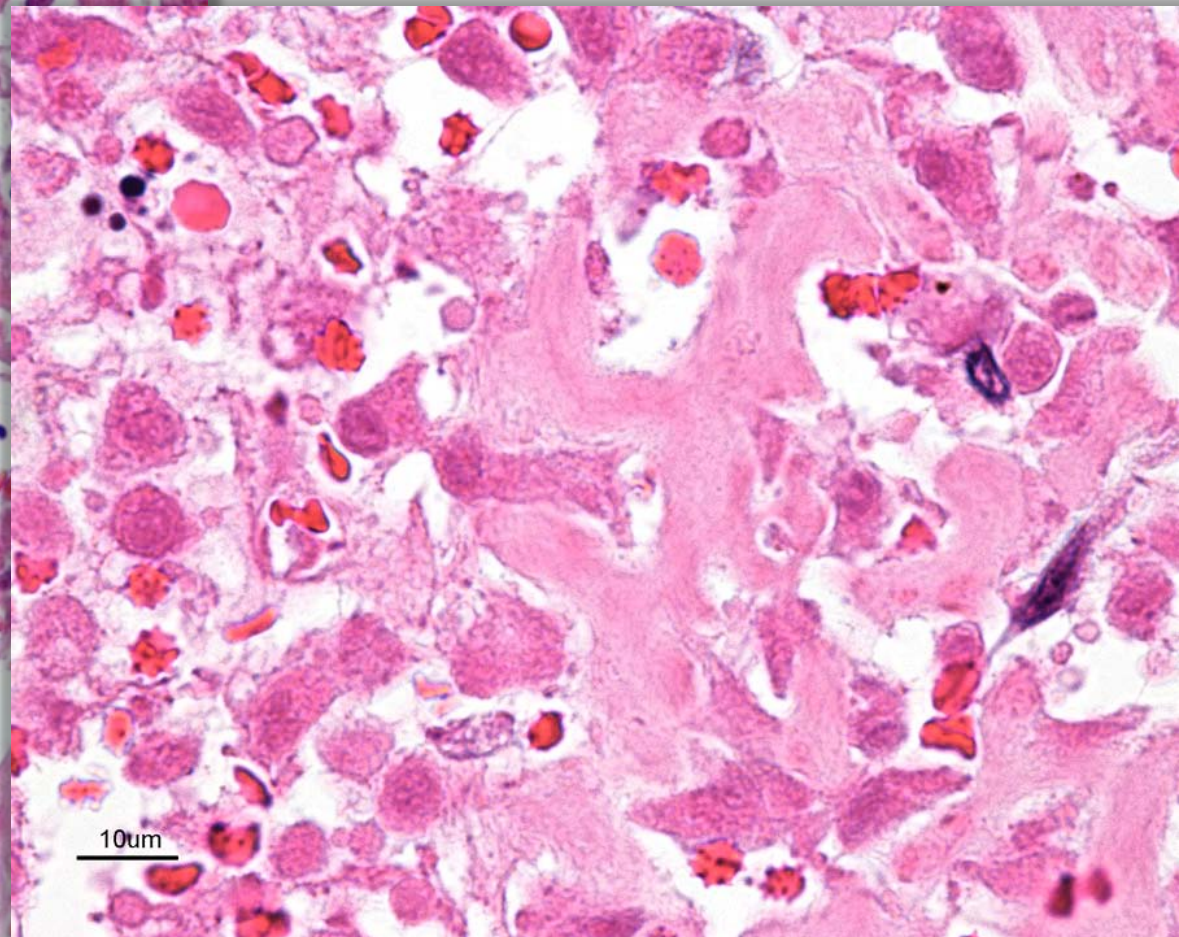
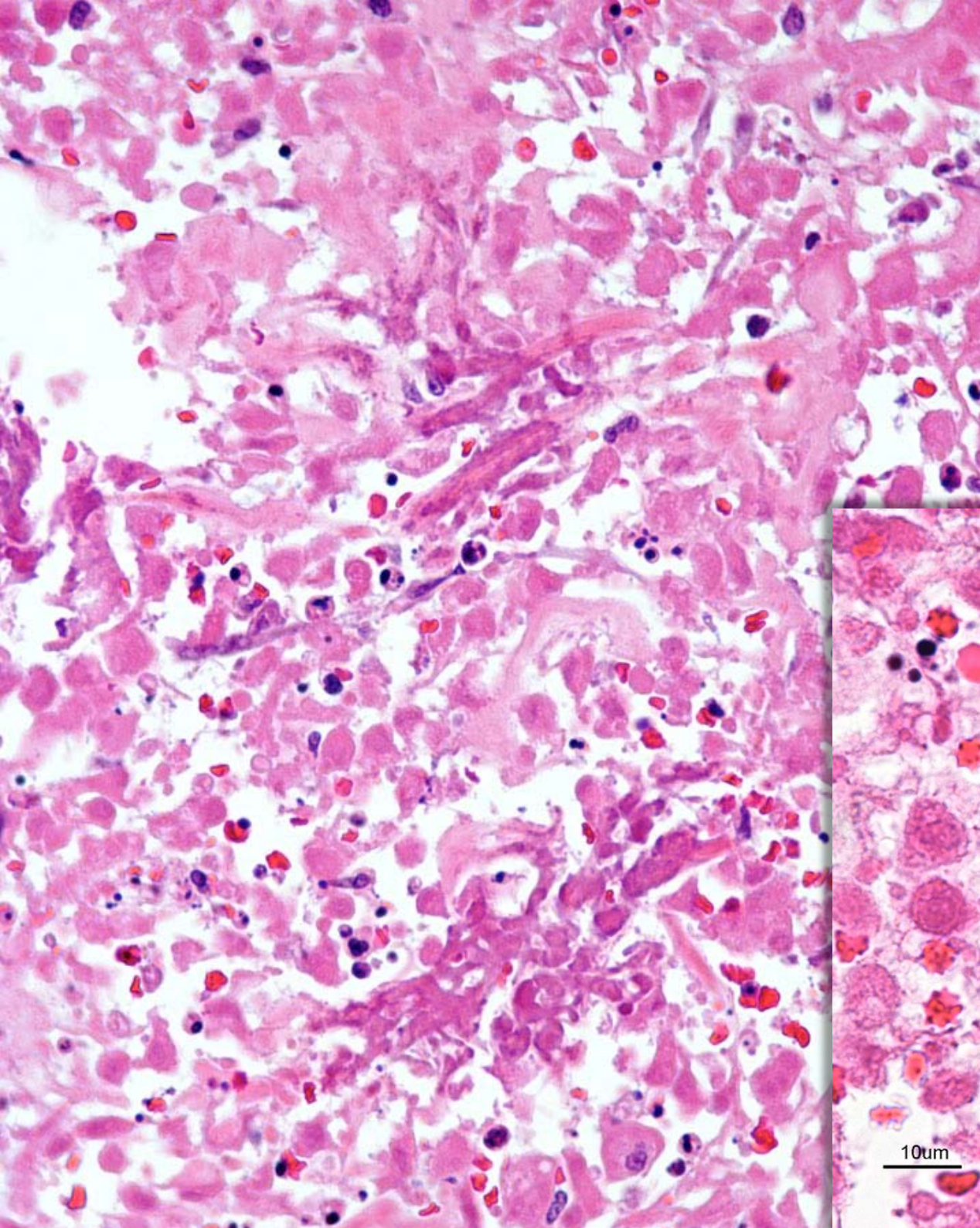
Osteosarcoma



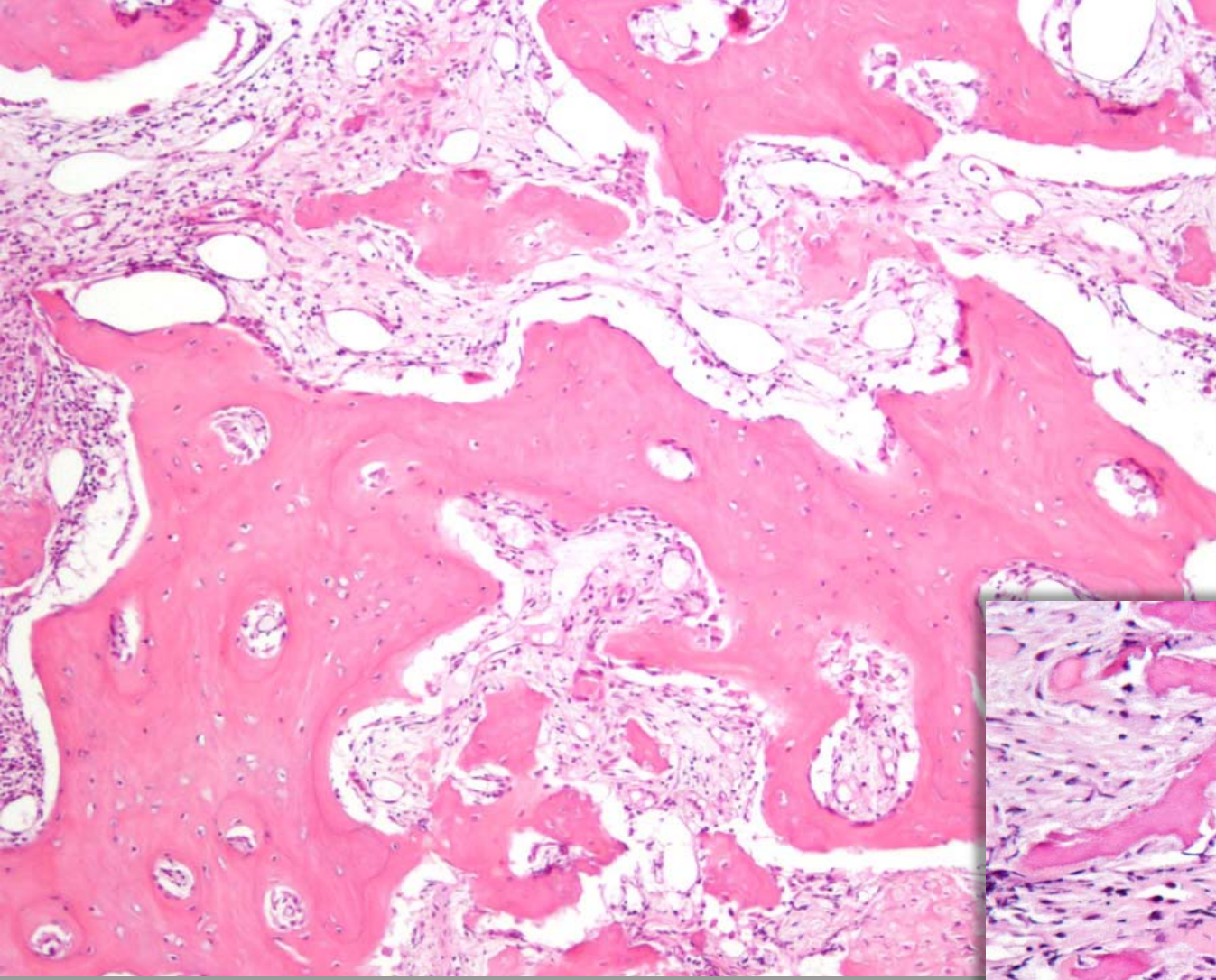
Highly variable mesenchymal
cells producing osteoid



Necrotic biopsy tissue from osteosarcoma

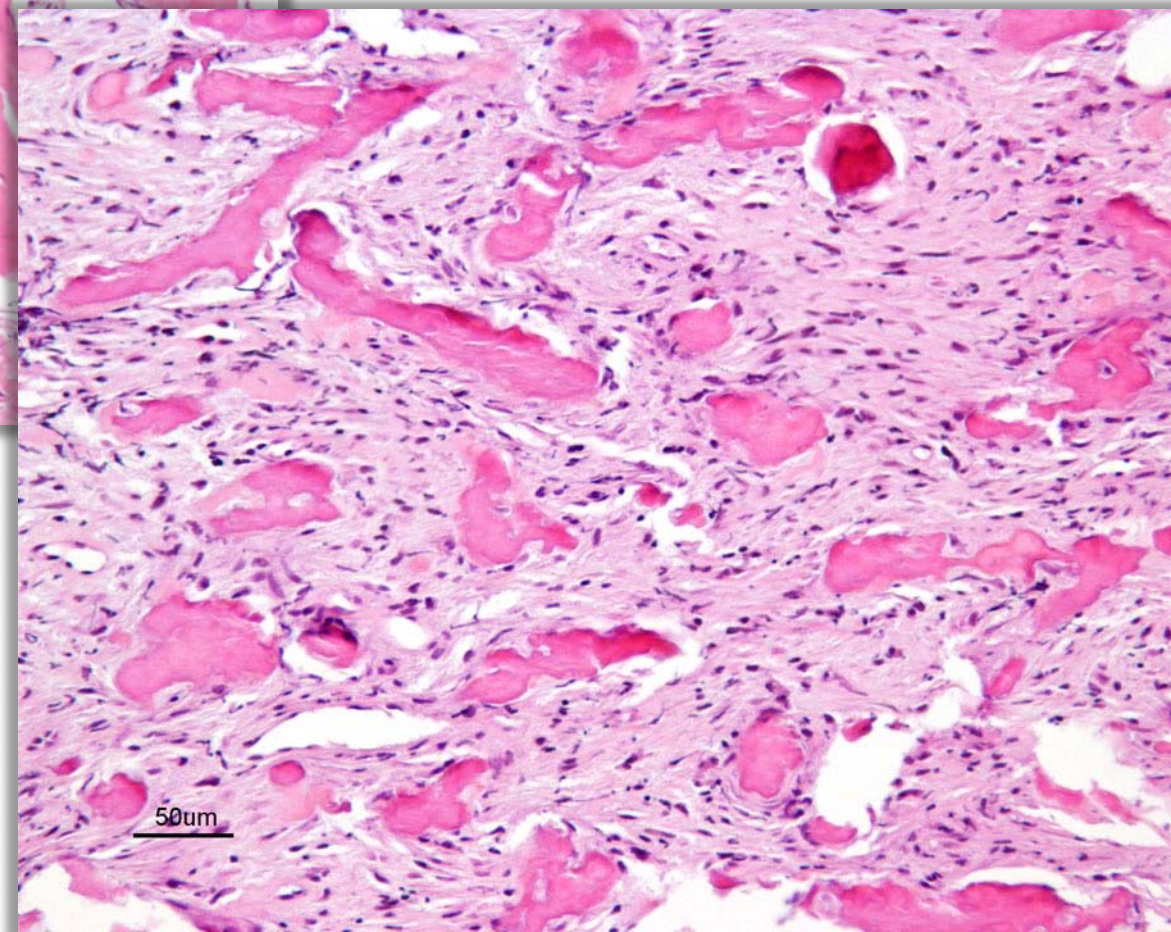


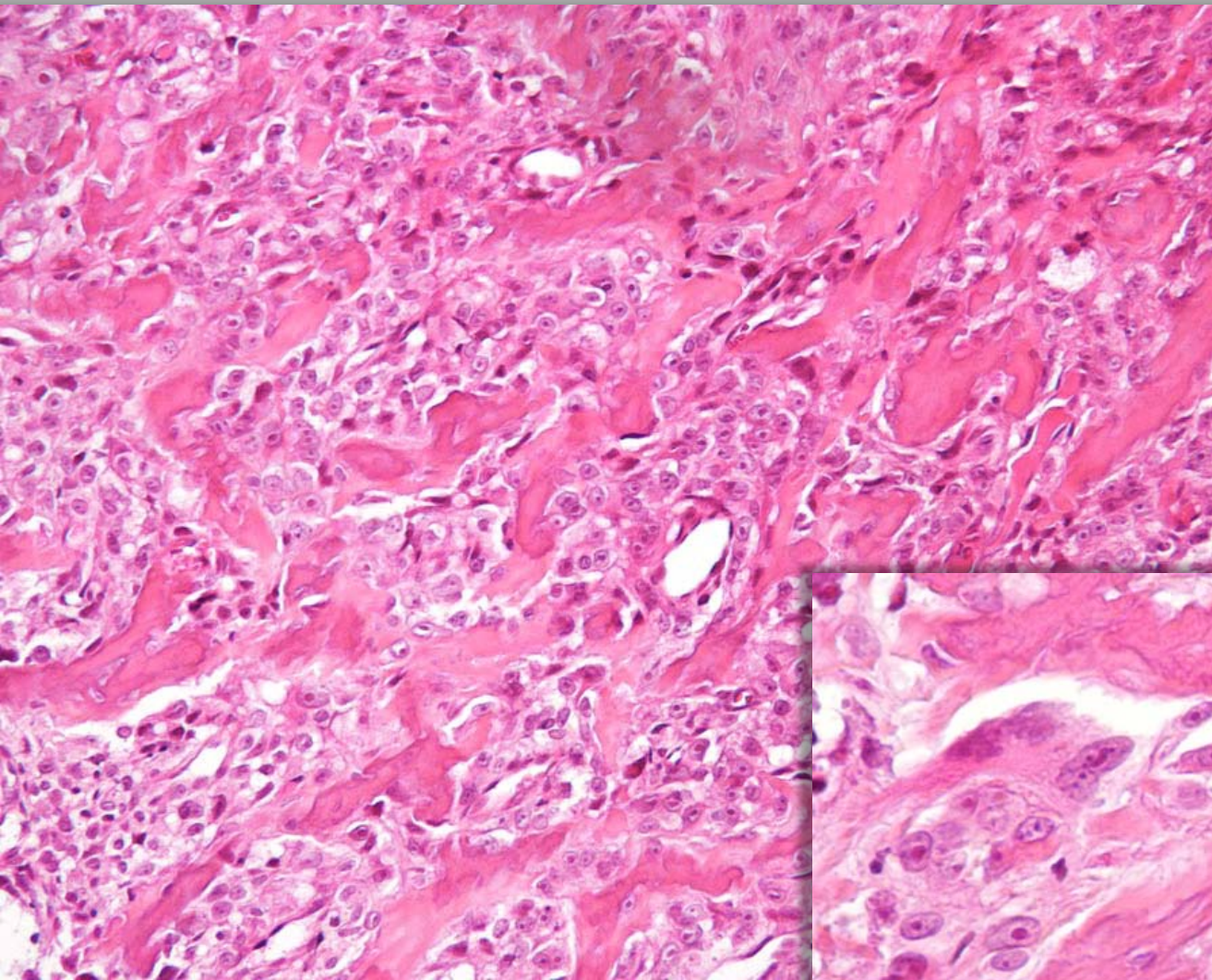
Biopsy specimens from
proximal humerus of a
7-year-old Labrador



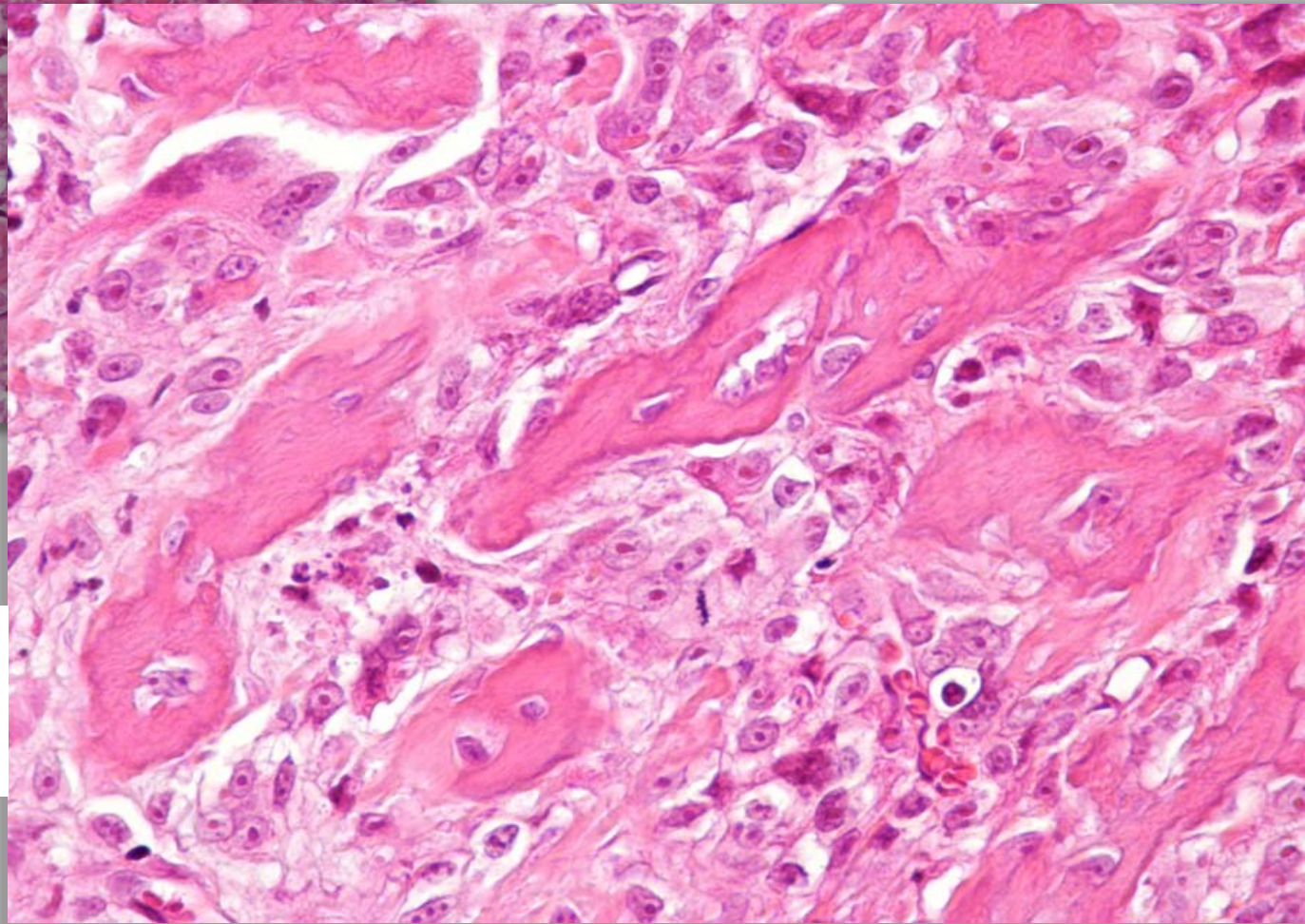
Reactive bone

Tumour?



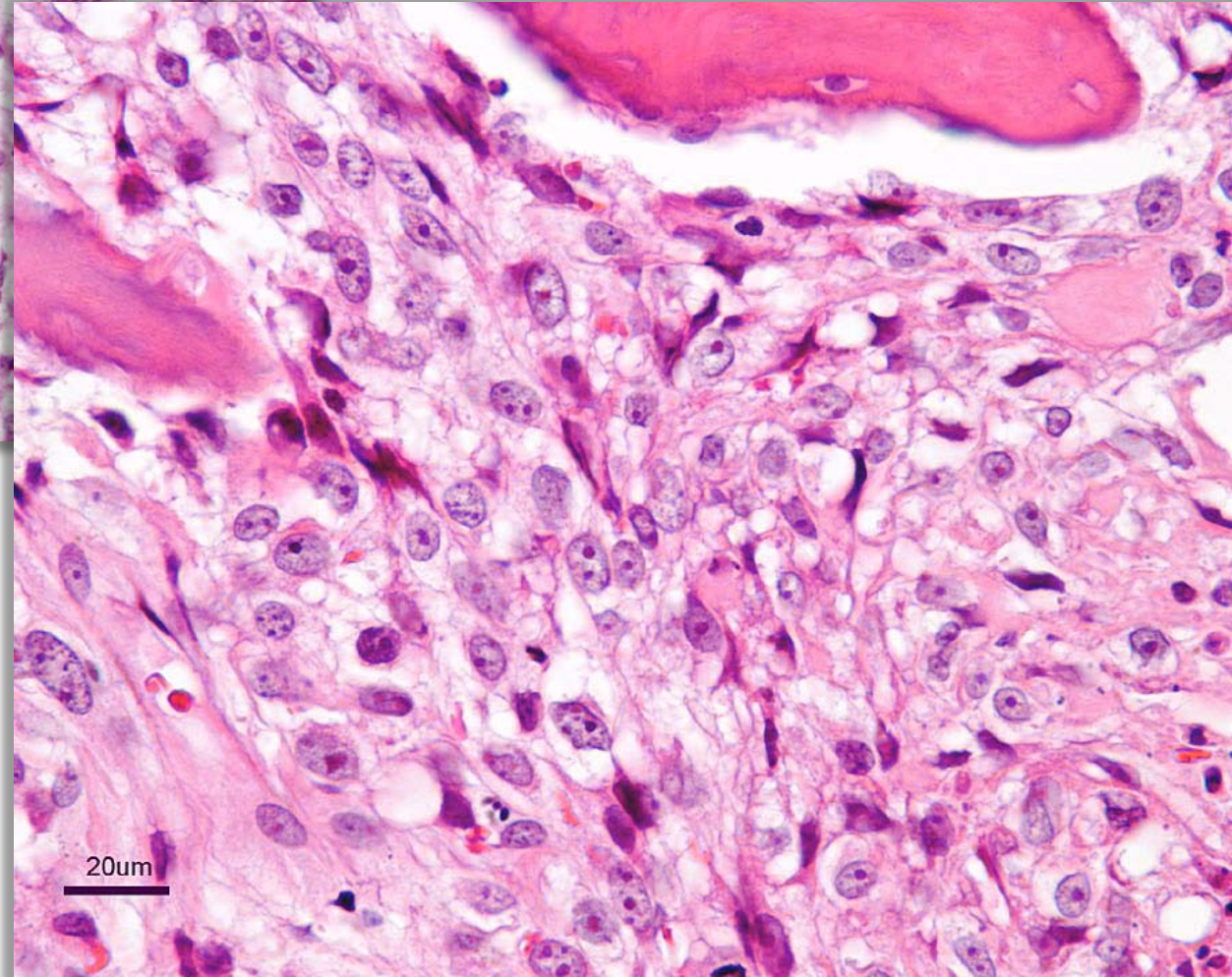
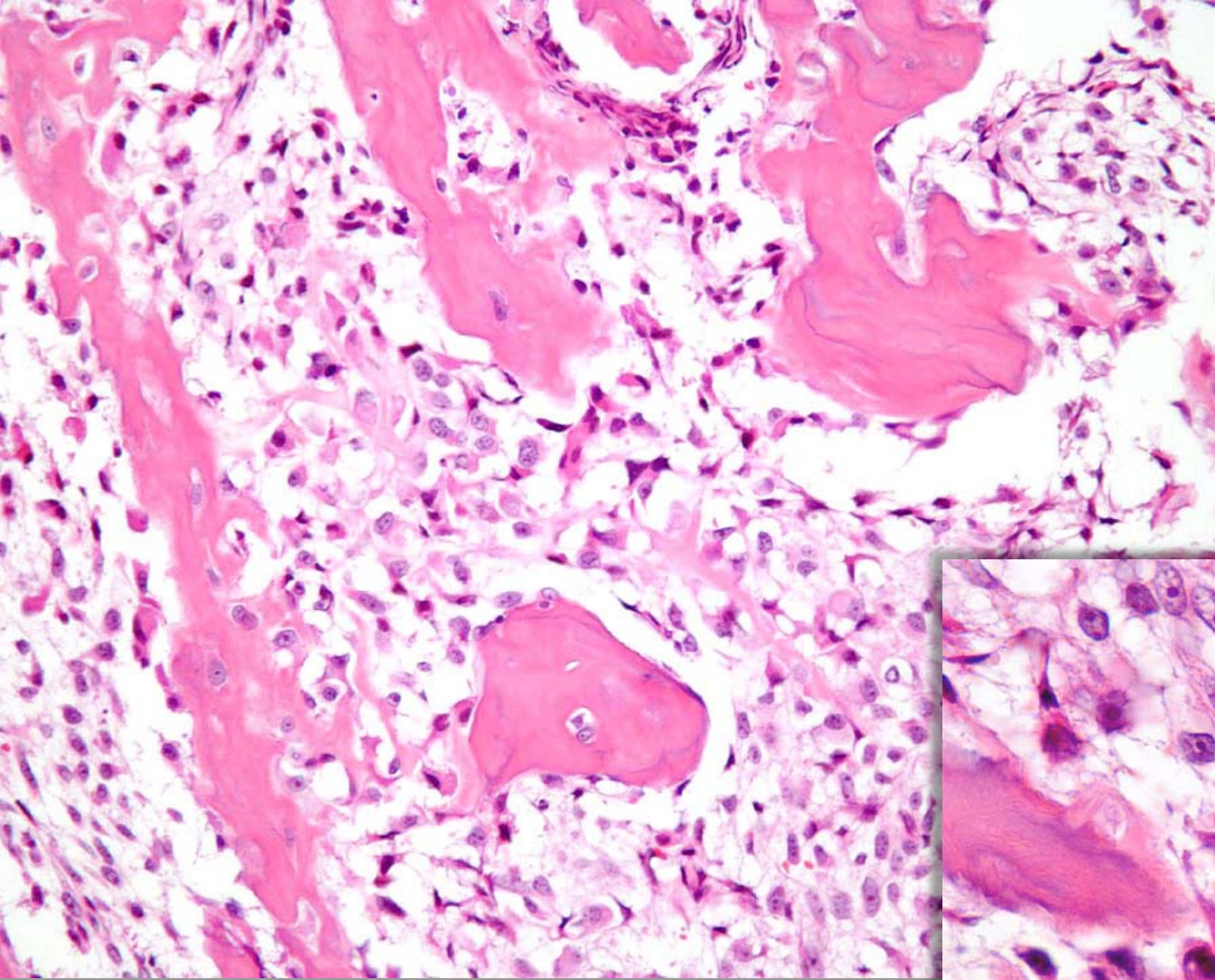


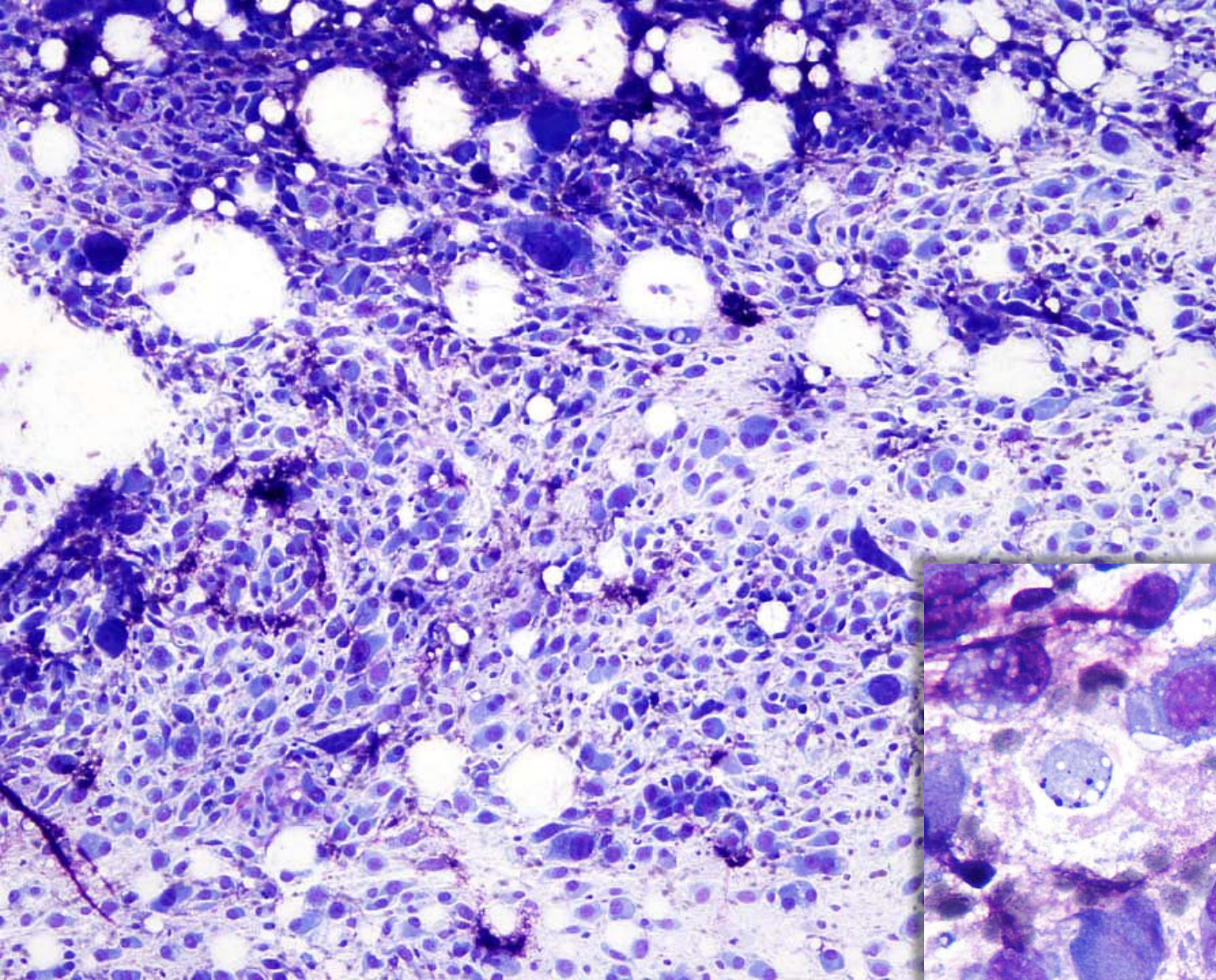
Further specimens from
the same lesion



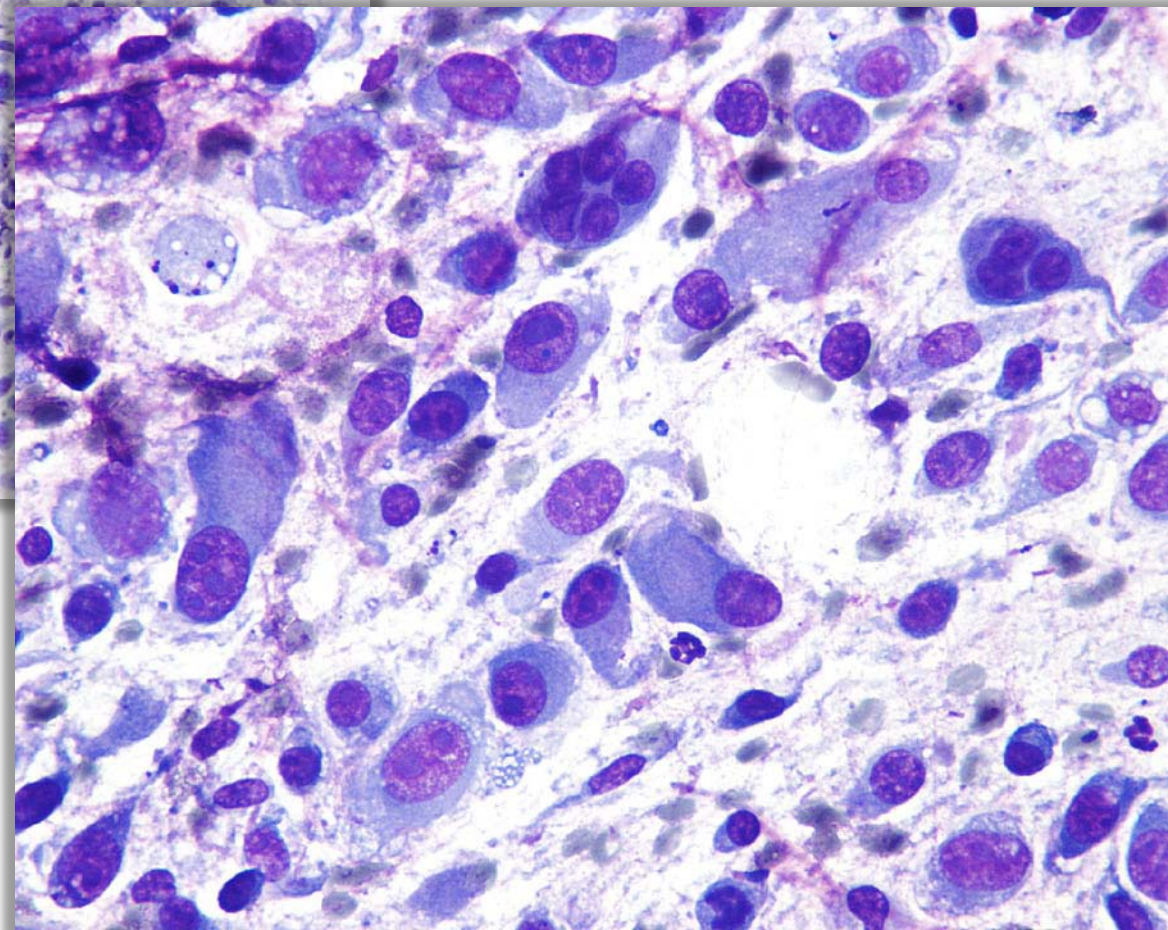
Diagnosis:
Osteosarcoma

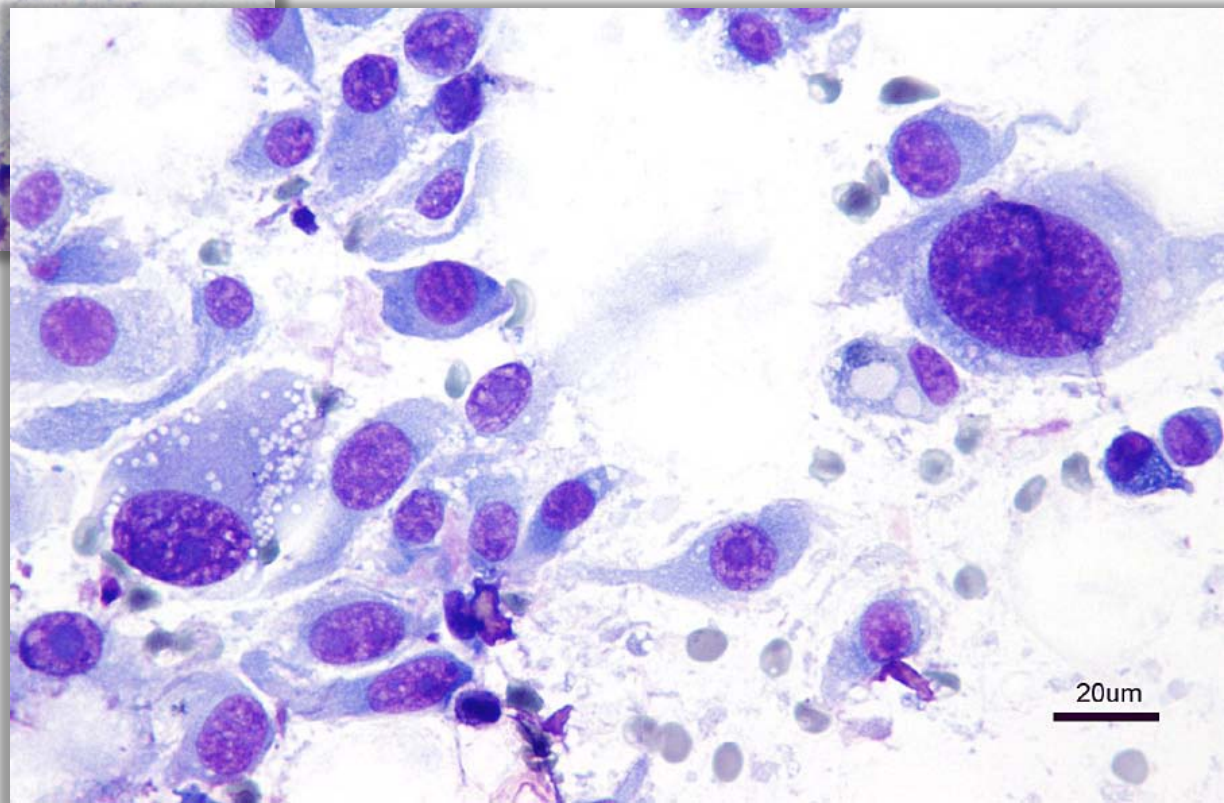
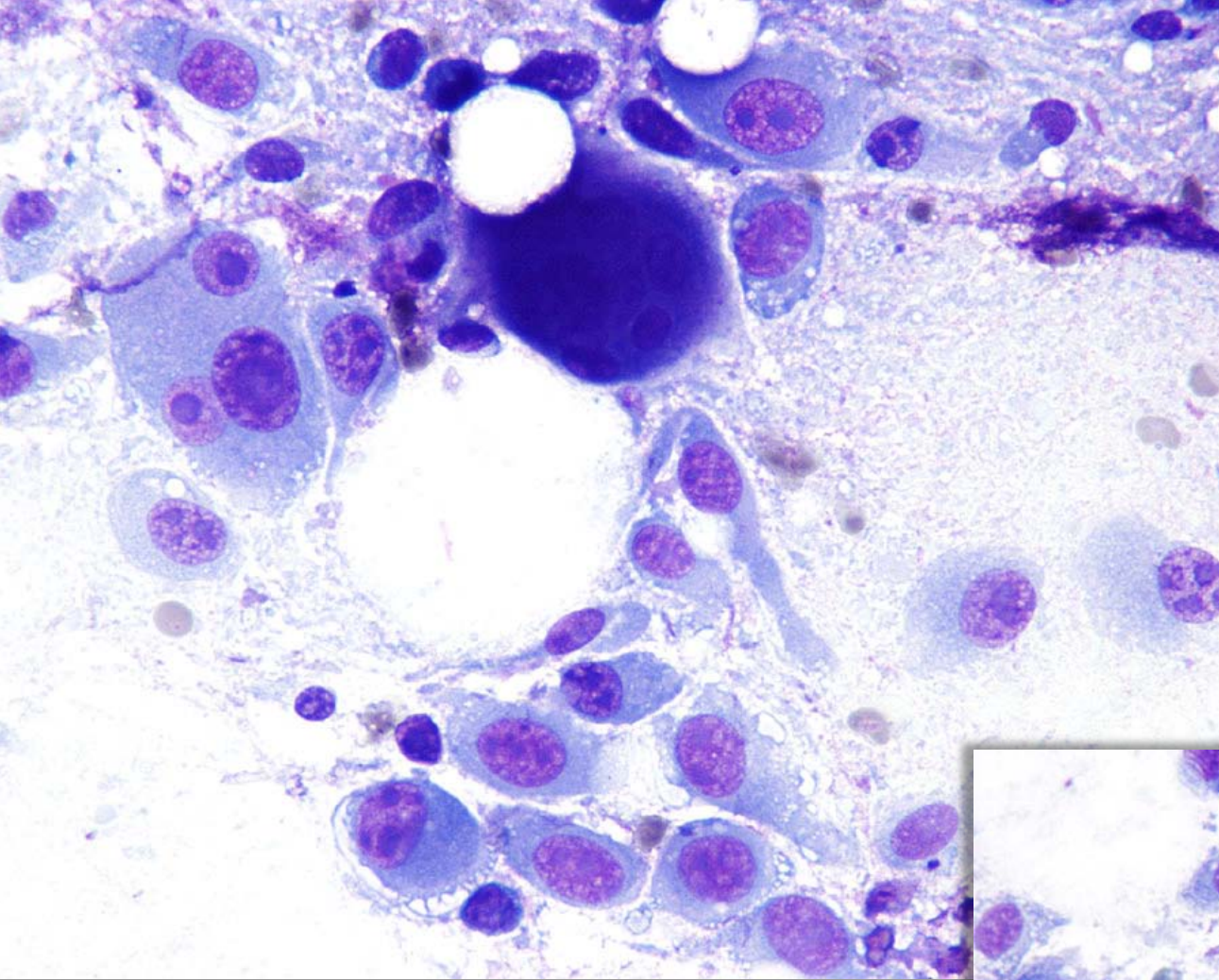
Biopsy from mass on hip of an 11- year-old Labrador





Cytology from
the same case





Diagnosis: Osteosarcoma

Cytology will often provide extra assurance that the cells producing the matrix are malignant



BUT, even if there is no evidence of
osteosarcoma cytologically or
histologically, do not exclude the
diagnosis - especially if the history
and radiology are suggestive

Grading of osteosarcomas – is it useful?

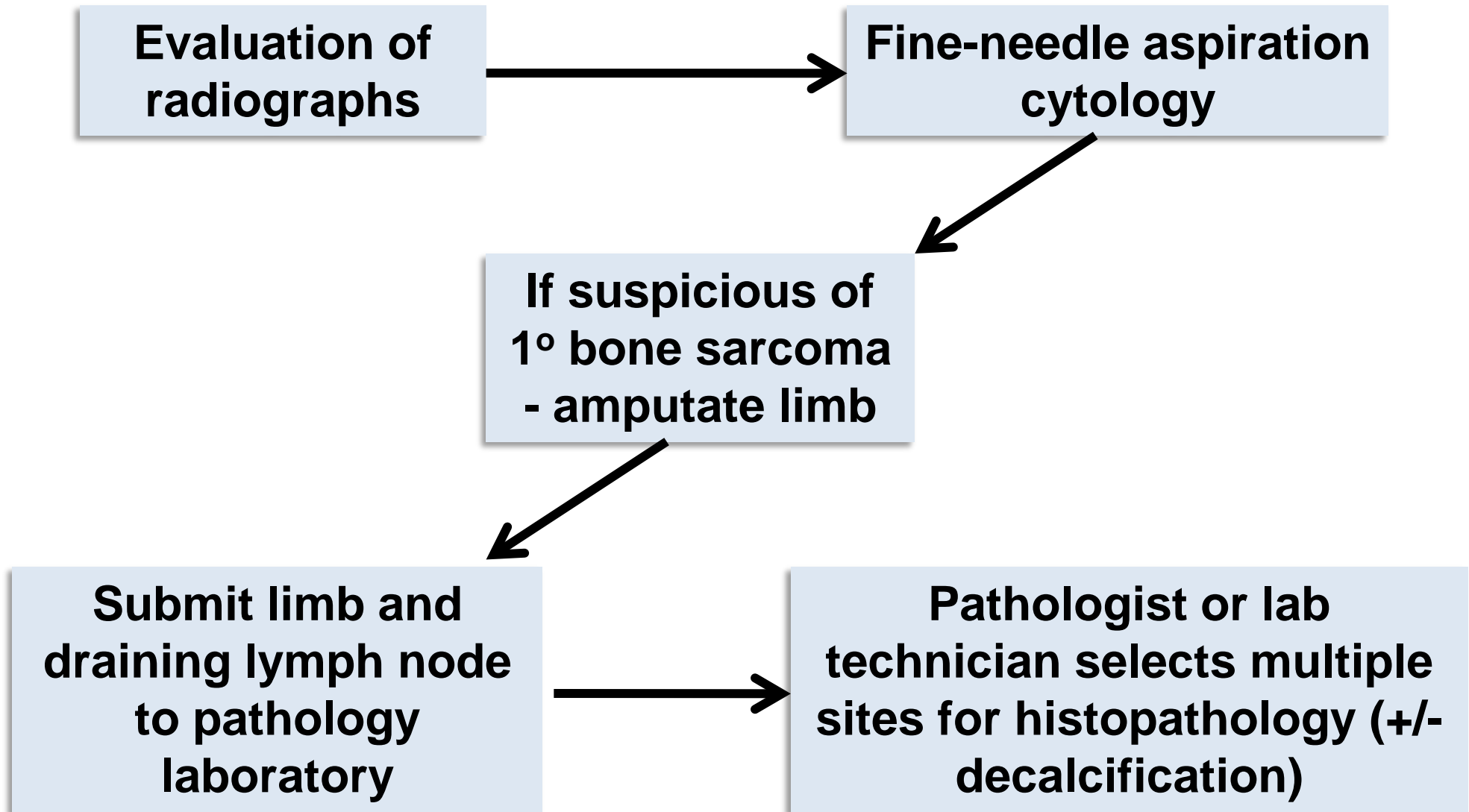
- Grading system (I-III) for dogs proposed by Kirpensteijn *et al* (*Vet Pathol*, 39: 240-246, 2002)
- Based on cellular pleomorphism, **mitotic rate**, tumour matrix, cellular density, tumour necrosis, # of multinucleated giant cells, **vascular invasion**
- Reduced survival time associated with Grade III osteosarcoma

Grading of osteosarcoma

BUT

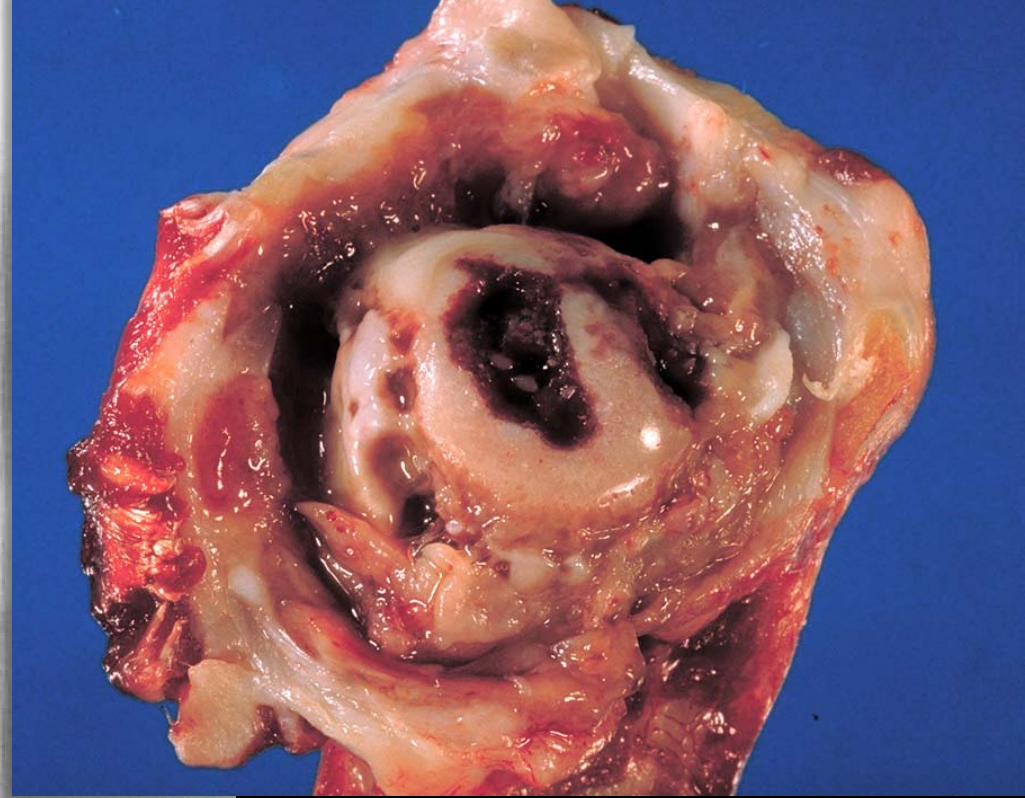
- Questionable value in biopsies due to small sample size & marked variability
- 75% of osteosarcomas in the study were Grade III anyway
- Grading only likely to be useful if sample size allows reliable assessment (e.g. amputations)

New paradigm for suspected bone sarcomas

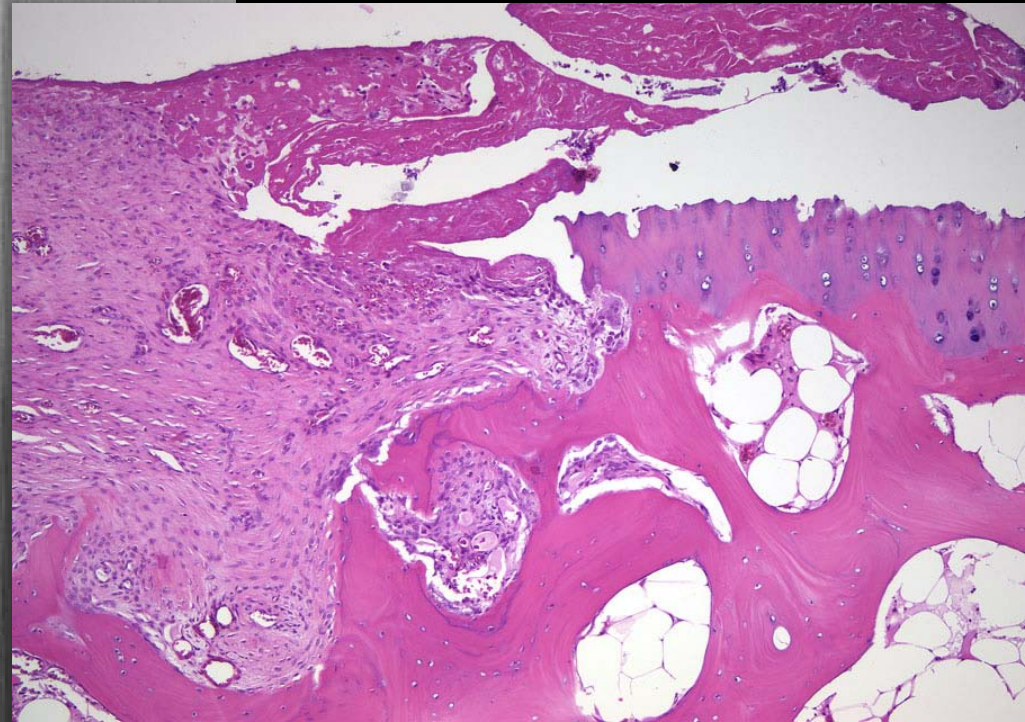


Details courtesy of Dr Paul Stromberg, The Ohio State University

Lytic lesions in the
proximal humerus of an 8-
year-old Akita



Erosive rheumatoid-like arthritis

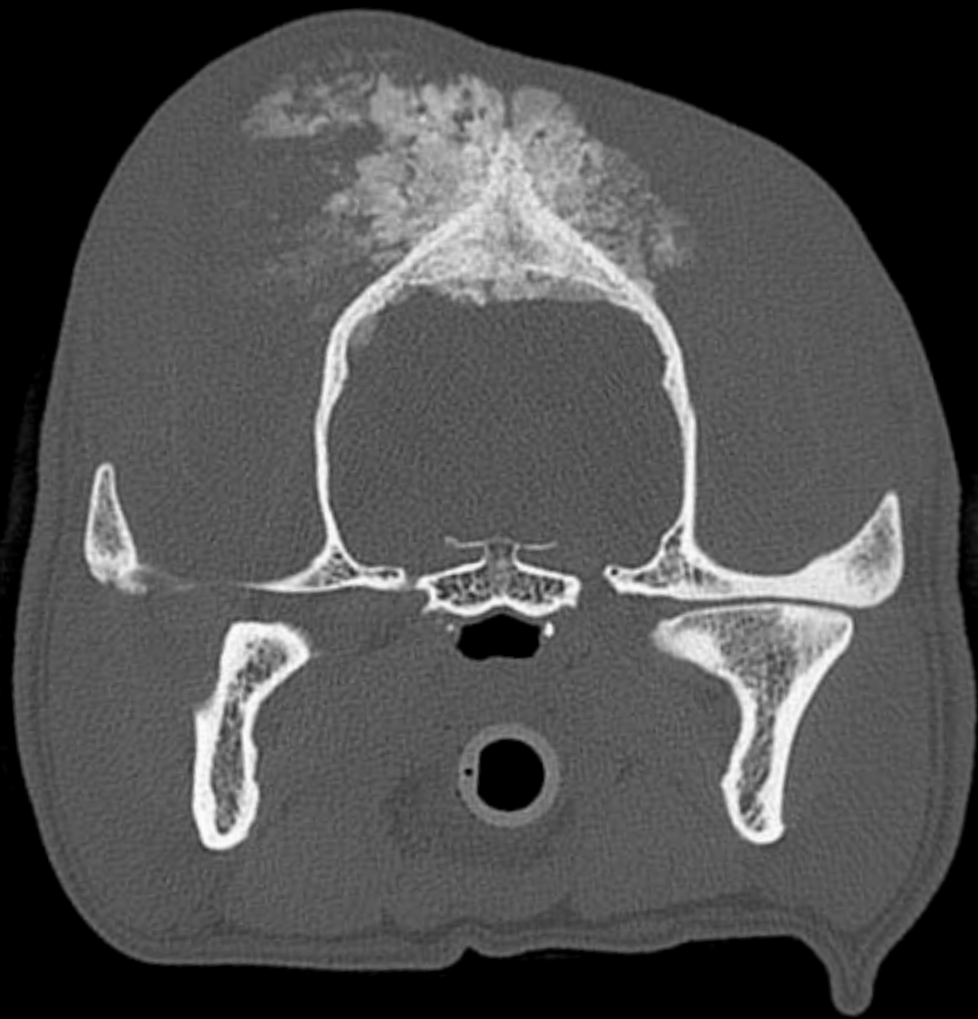


Other differentials



5-year-old Boxer – firm mass on skull





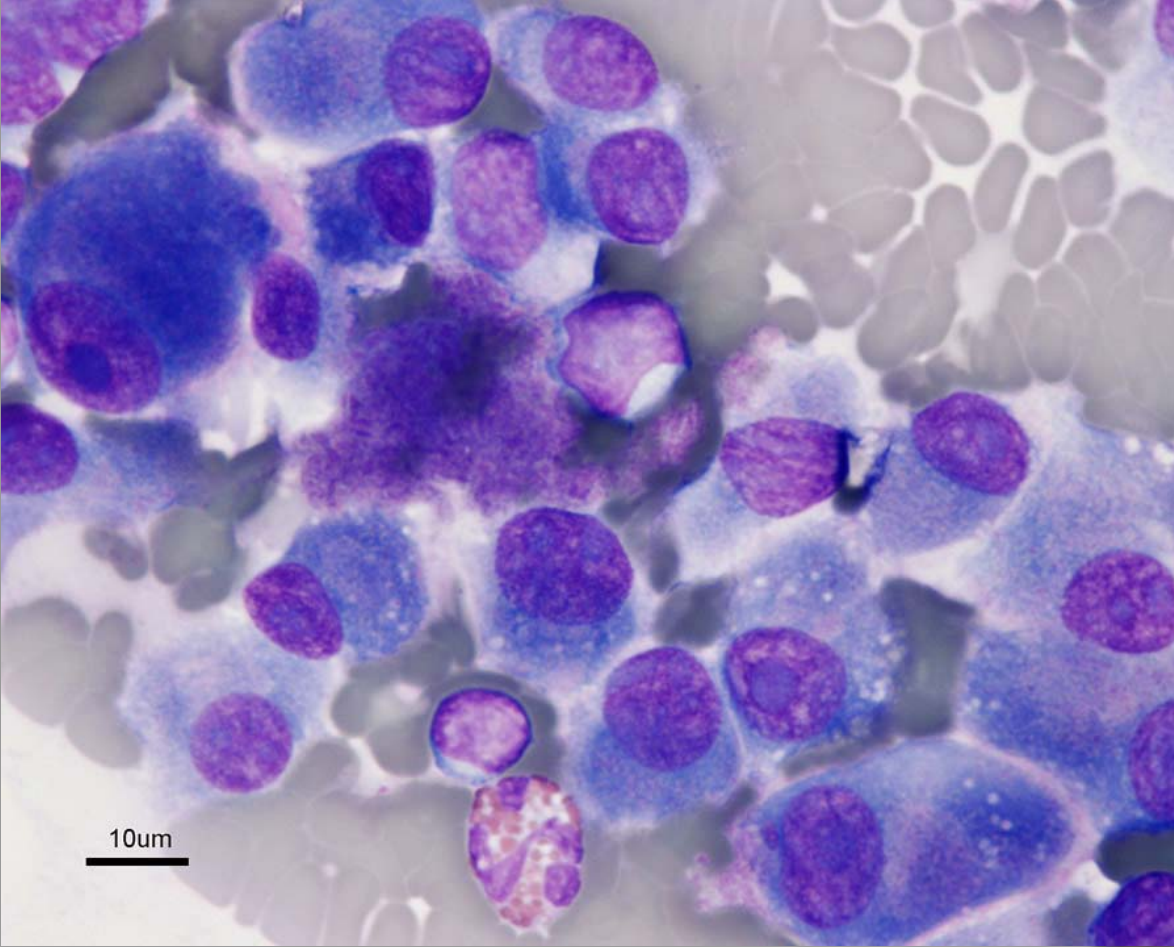
*Images courtesy of Dr
Devon Thompson*



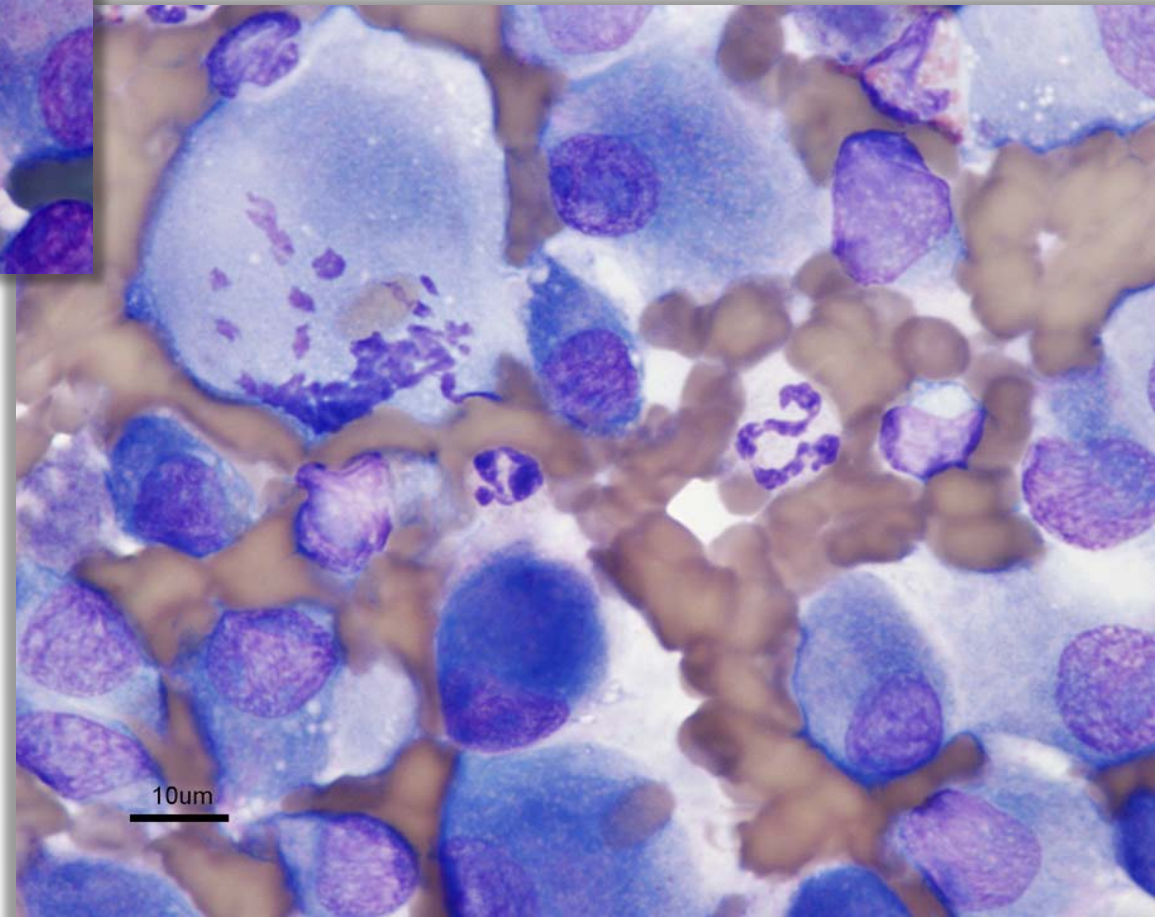
FNA from skull mass

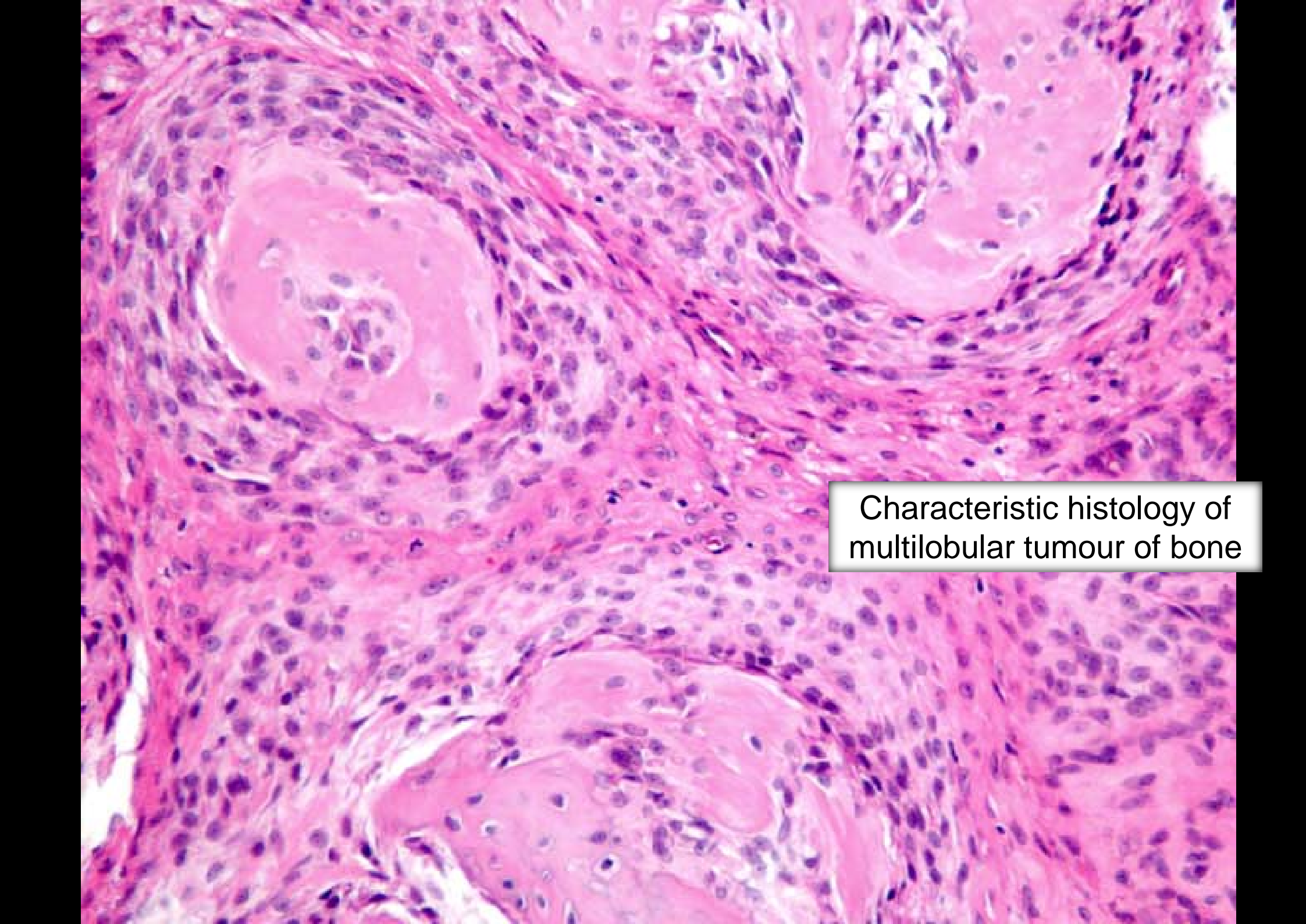
Reactive bone or multilobular
tumour of bone?

FNA from skull mass

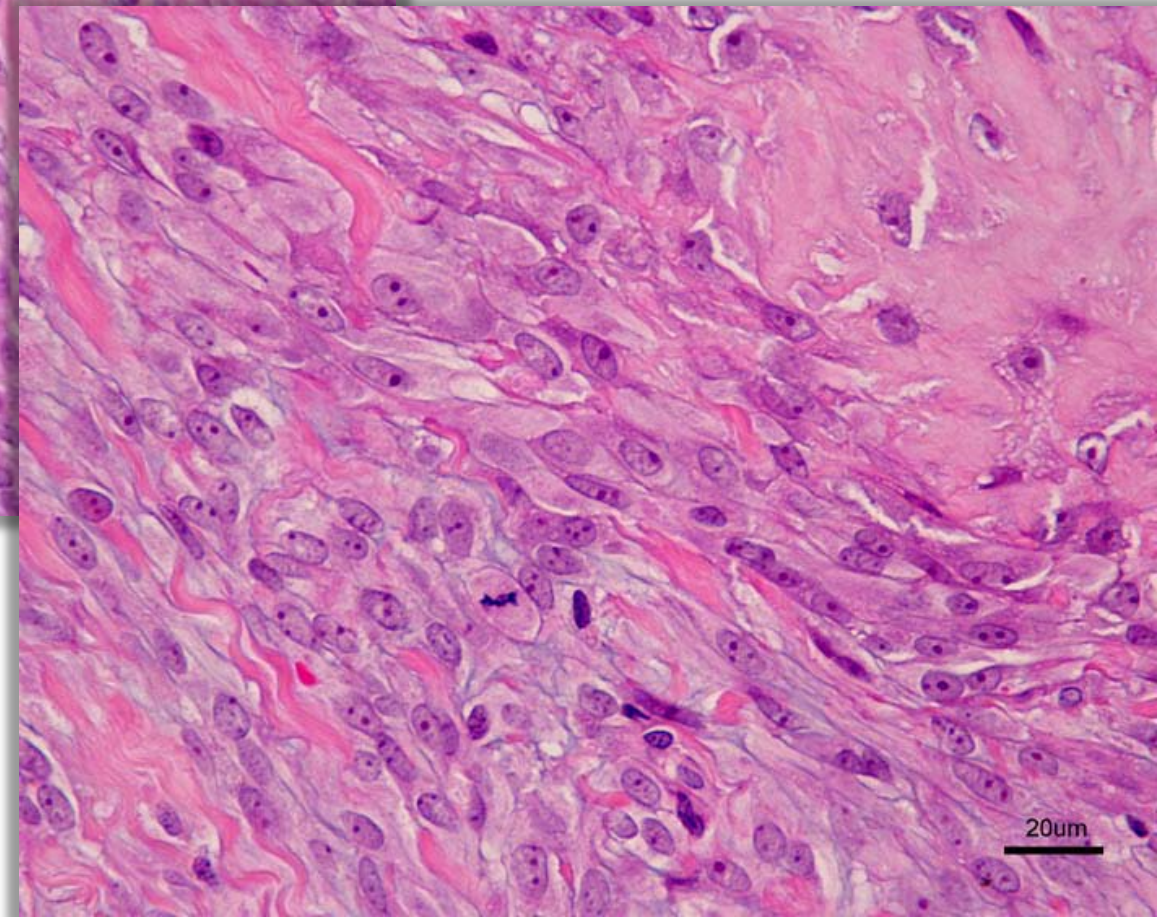
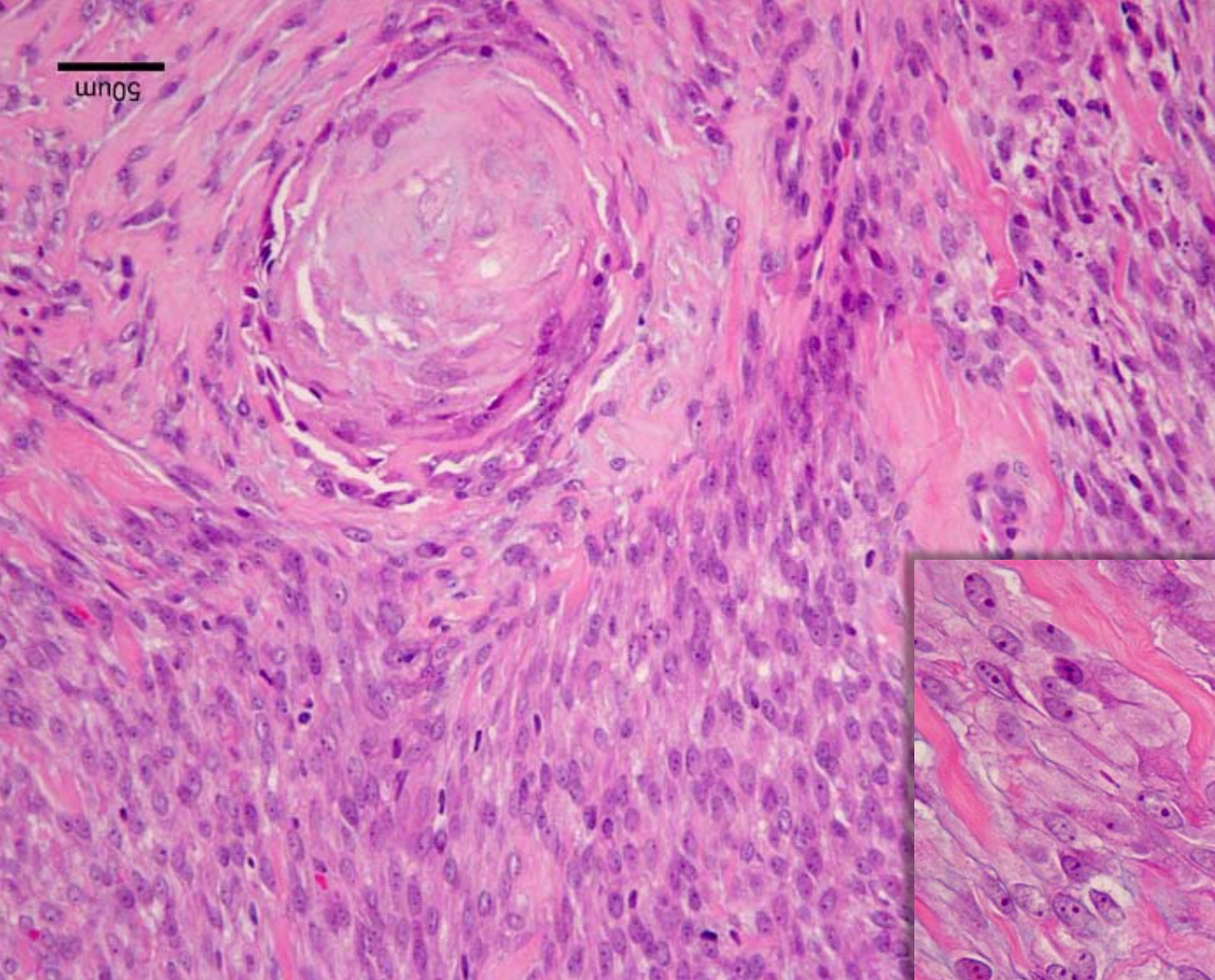


Variability suggests
malignancy



A histological section of a multilobular bone tumor, stained with hematoxylin and eosin (H&E). The image shows several large, pale, eosinophilic lobules of tumor tissue, which are separated by thin layers of cellular stroma. The lobules have a characteristic "chicken wire" appearance, with a network of fine, dark-staining lines representing the tumor's internal architecture. The surrounding stroma is densely cellular, with many small, dark-staining nuclei. The overall pattern is characteristic of a multilobular tumor of bone, such as a chondrosarcoma or a giant cell tumor of bone.


Characteristic histology of
multilobular tumour of bone



Malignant transformation
of multilobular tumour

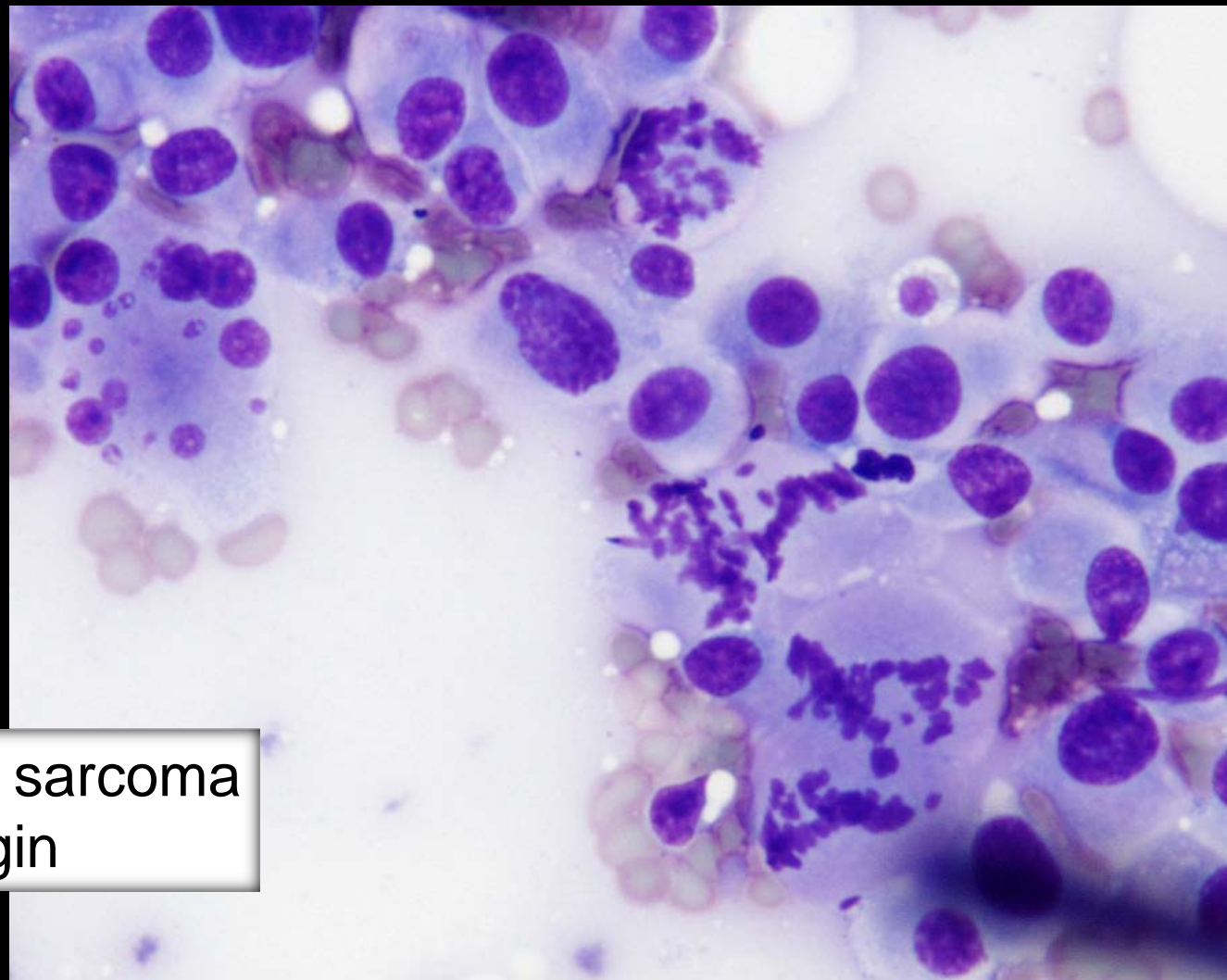
Multilobular tumour of bone
with chondroid matrix



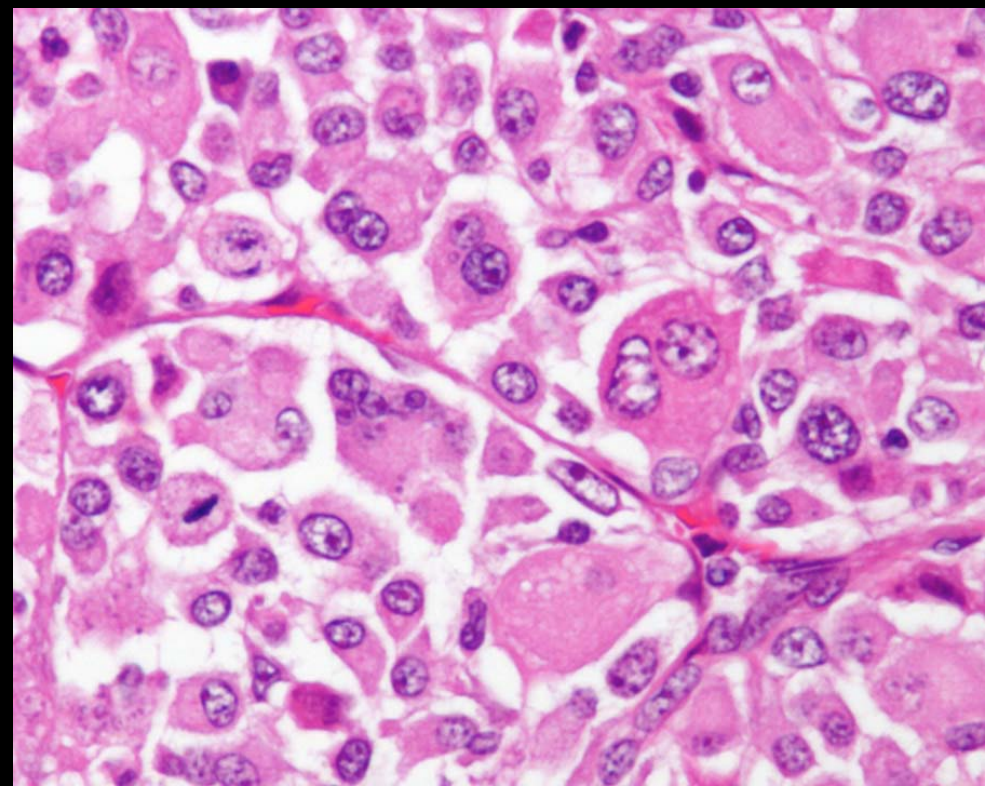
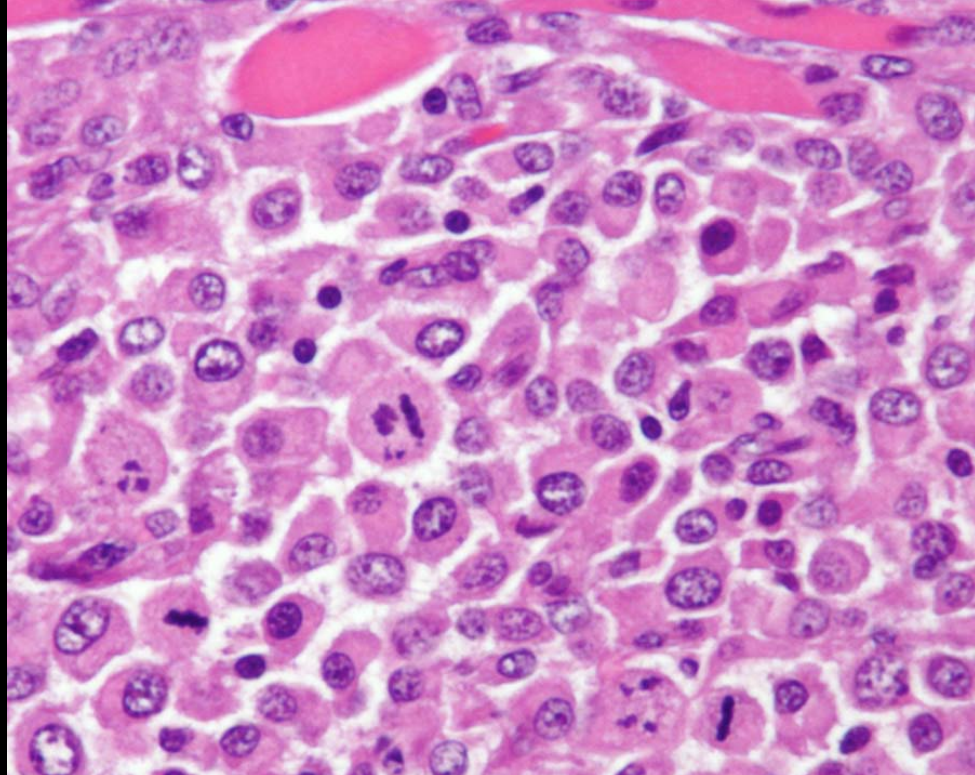
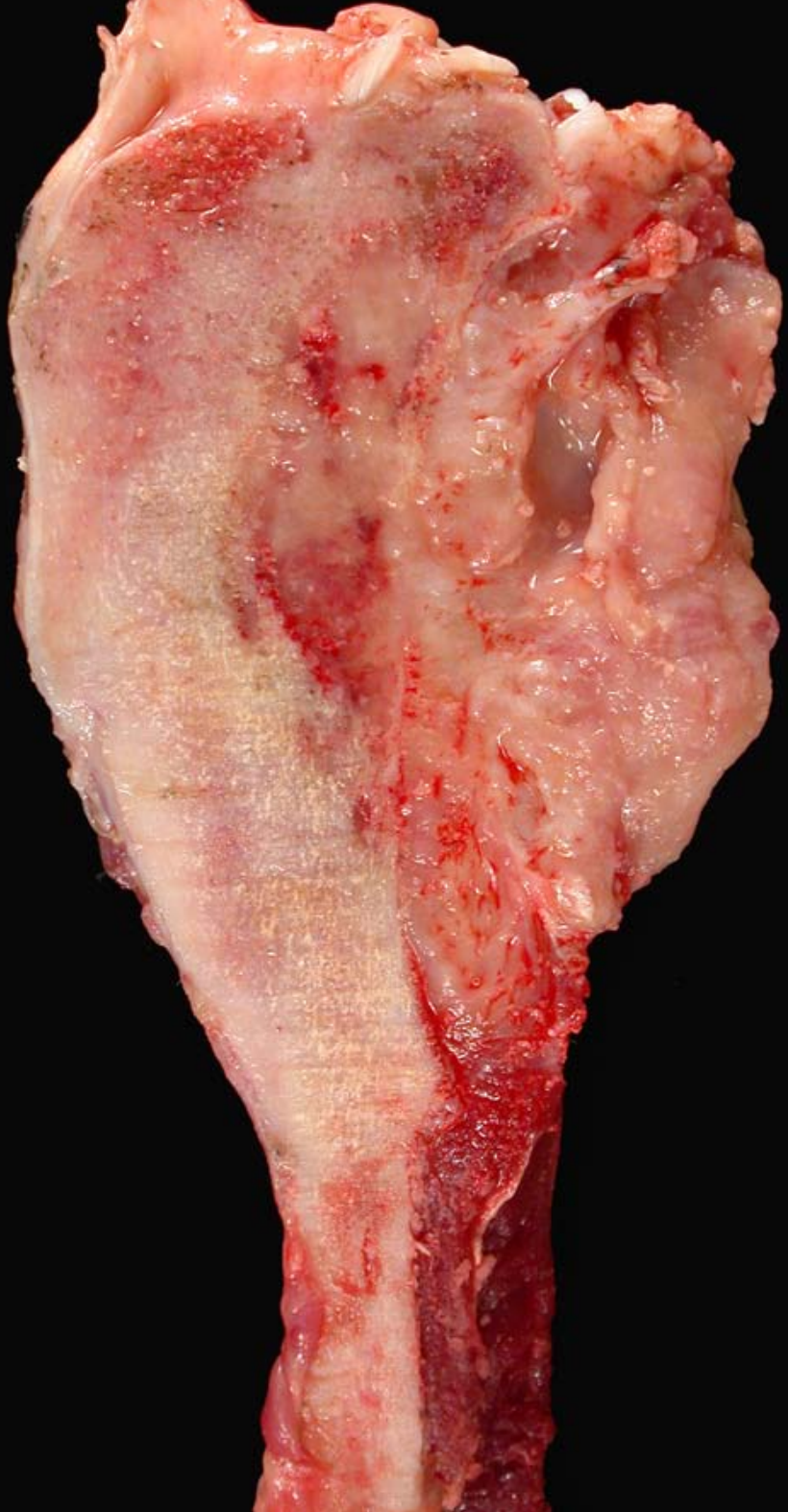


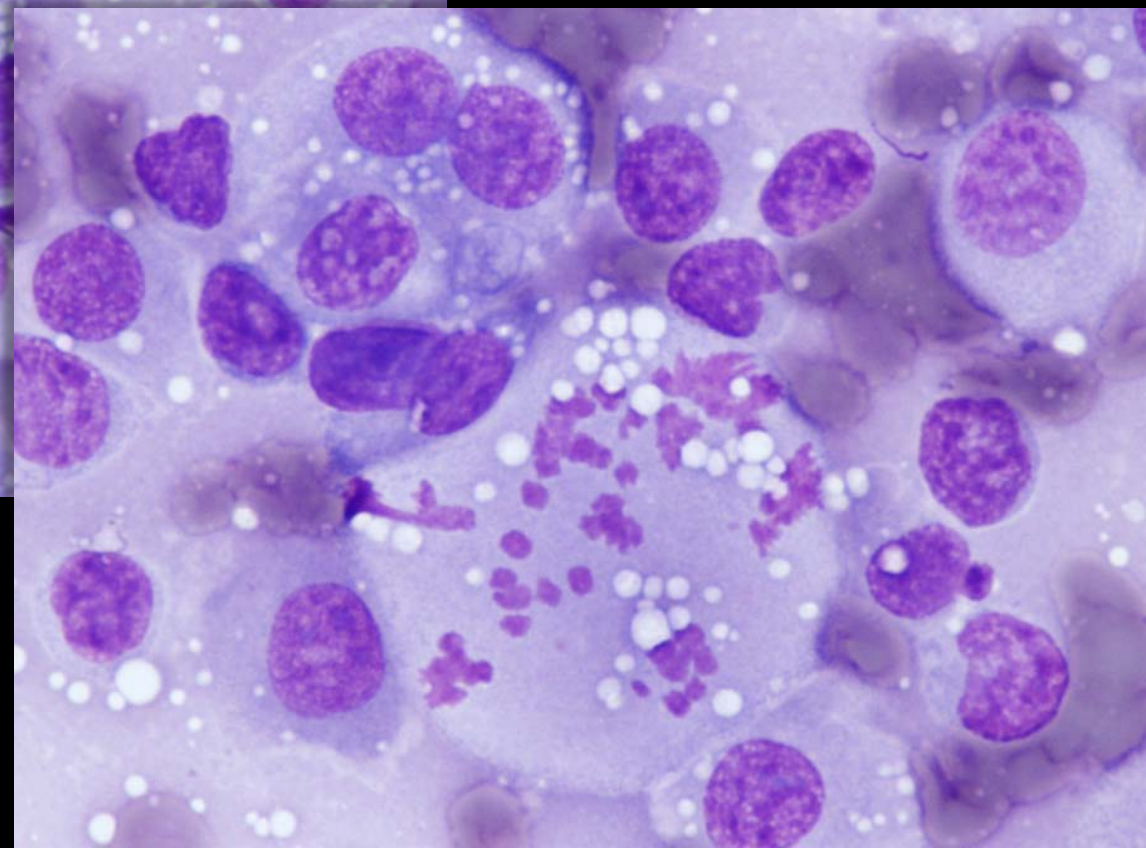
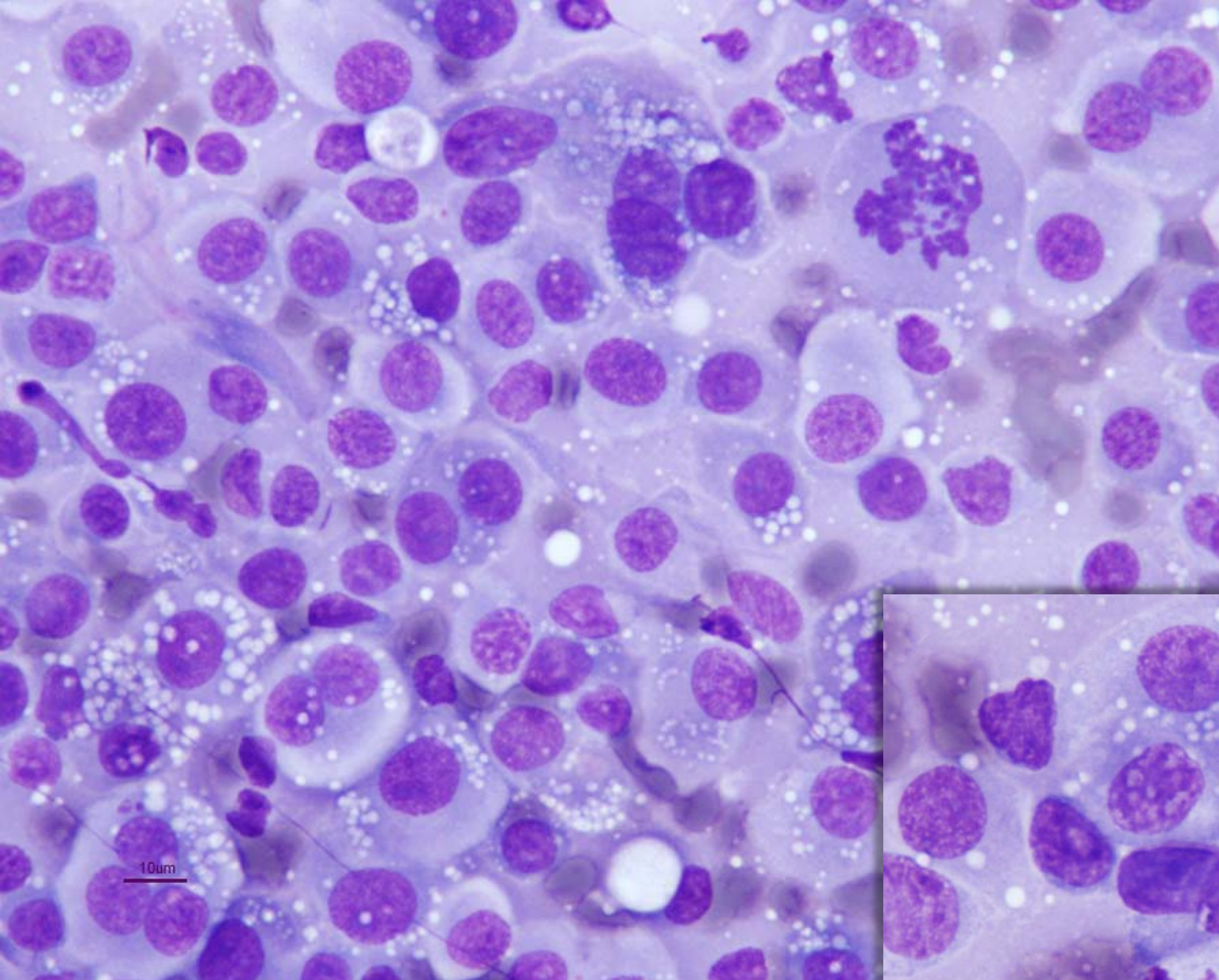
Adult flat-coated retriever - lytic lesion
involving proximal tibia and adjacent
muscles

The popliteal lymph node was also enlarged



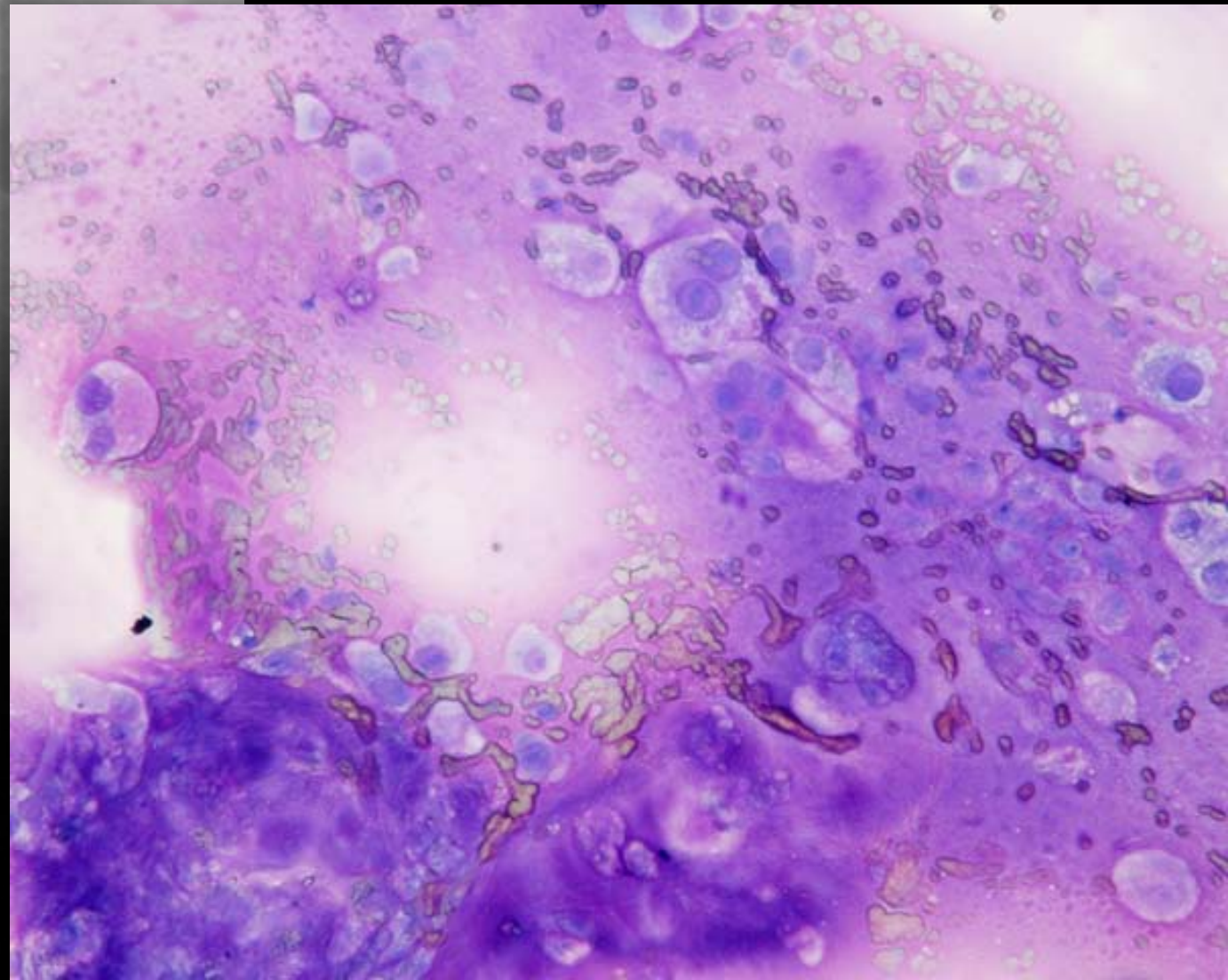
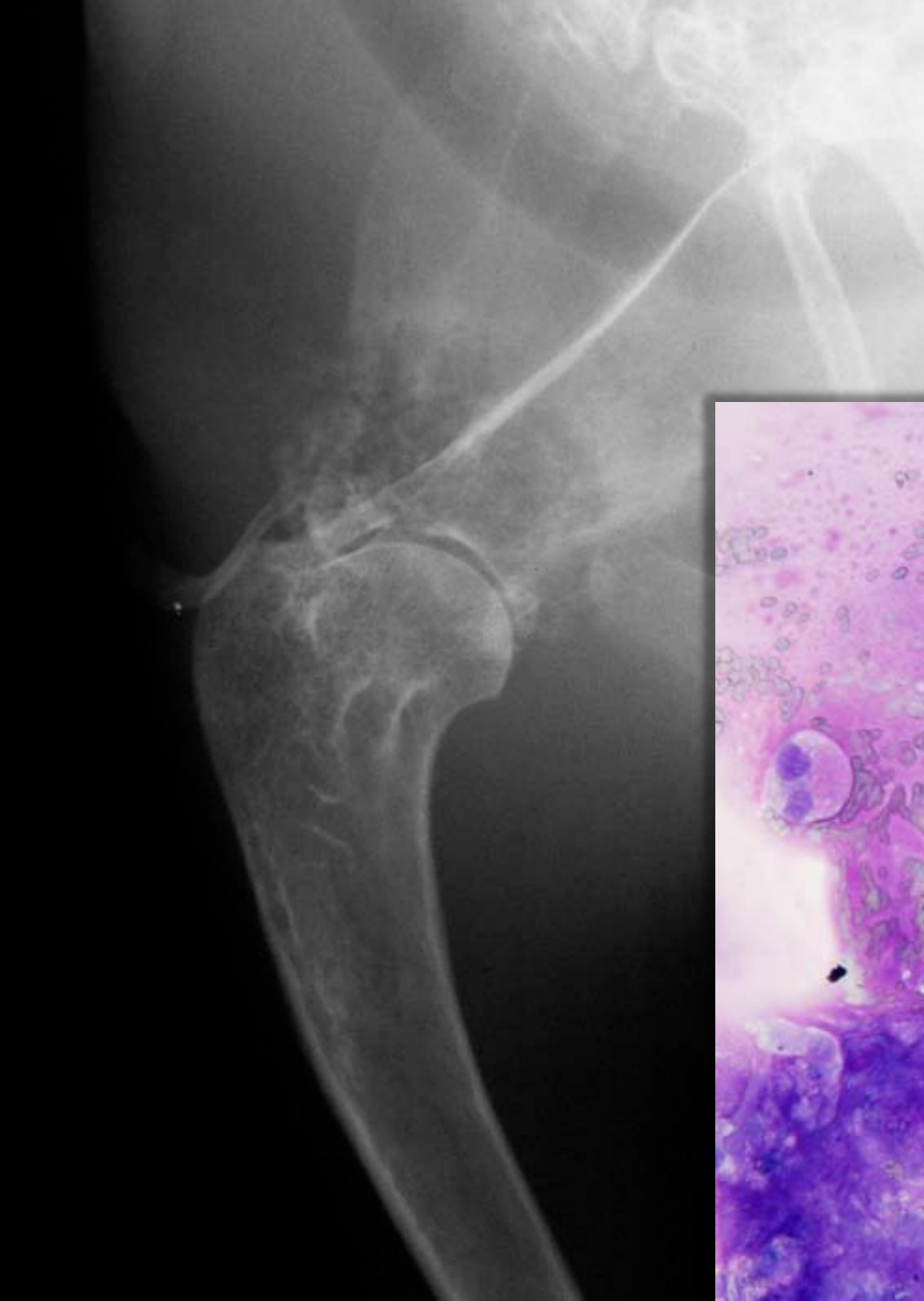
Diagnosis: Histiocytic sarcoma
of synovial origin

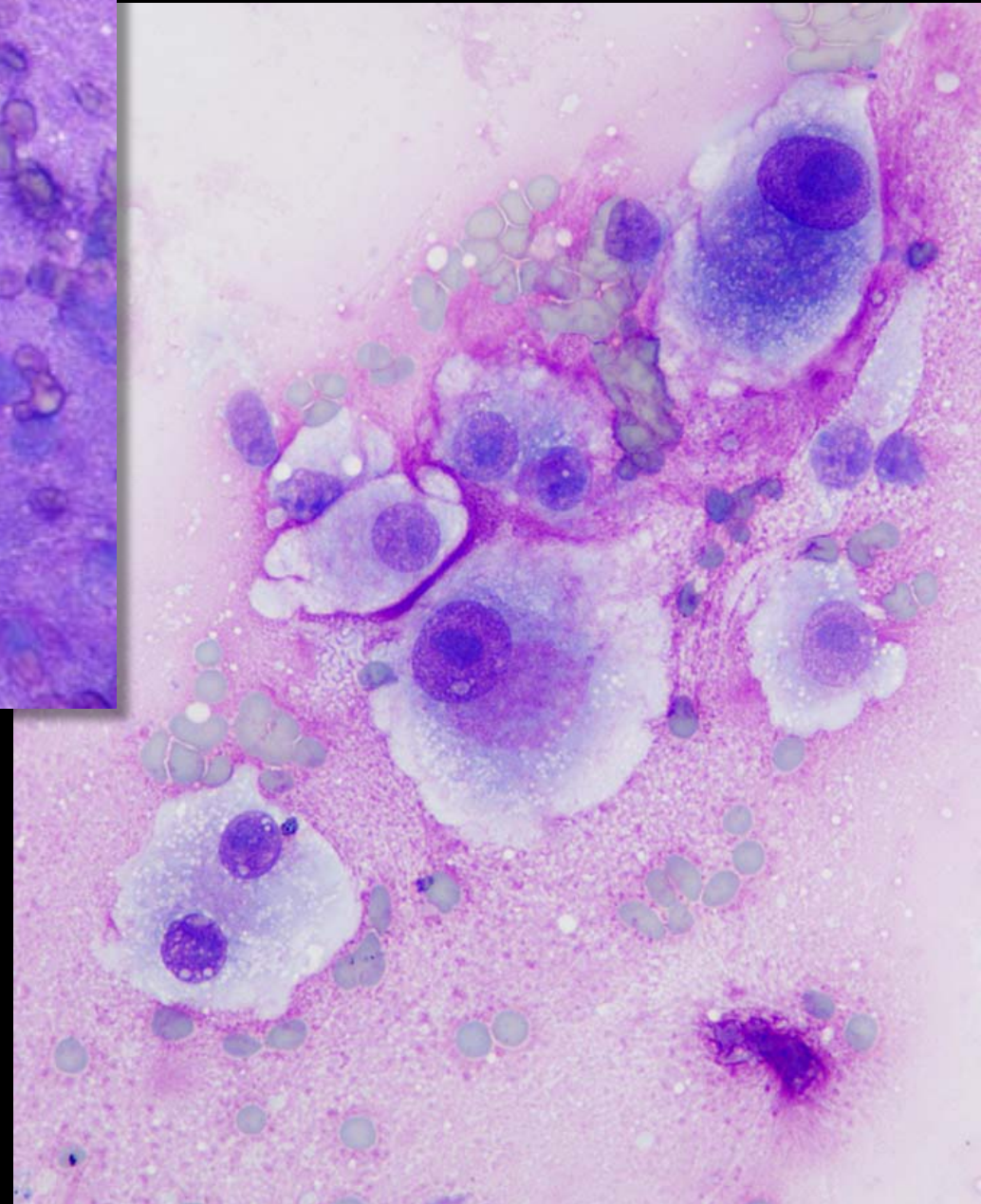
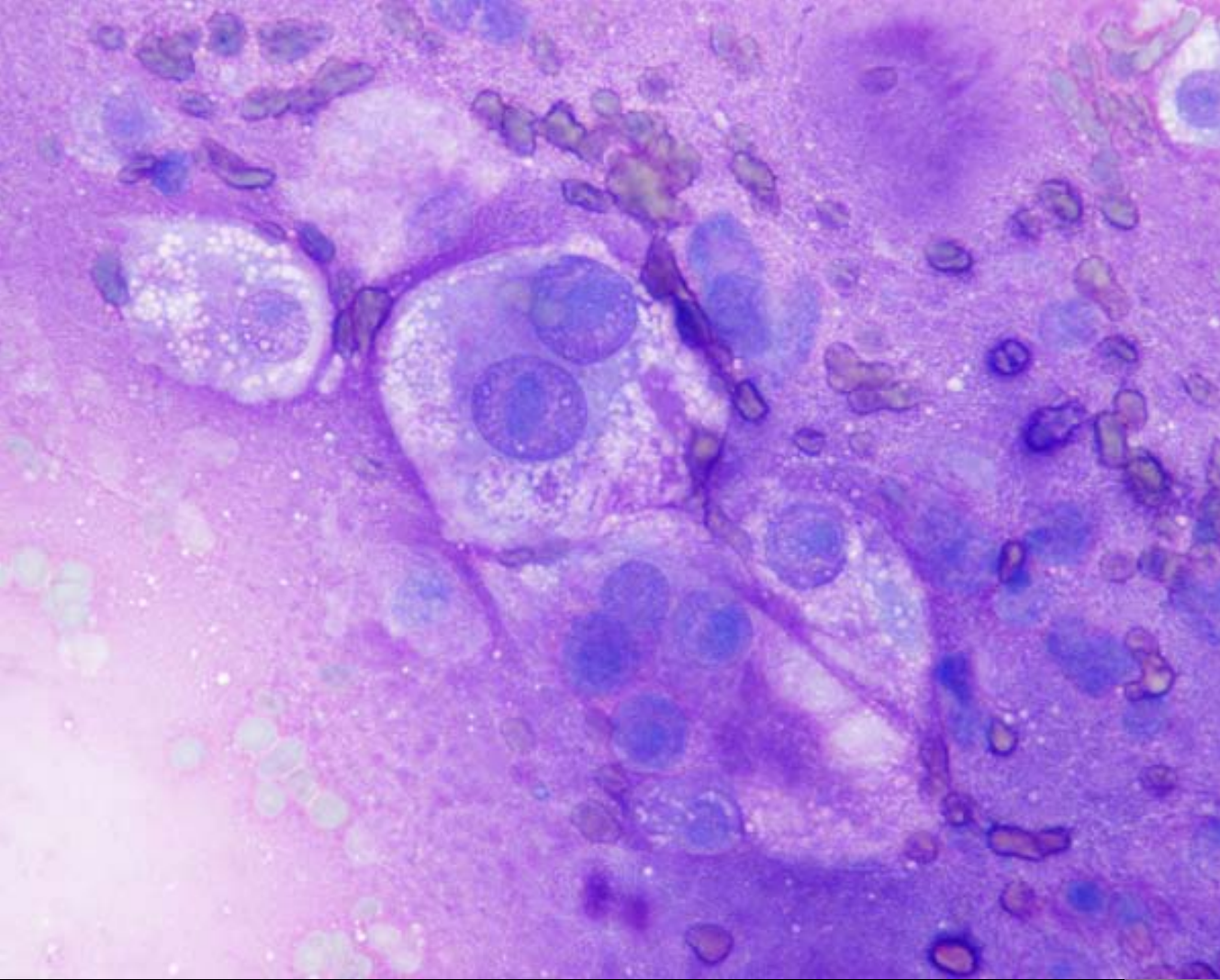




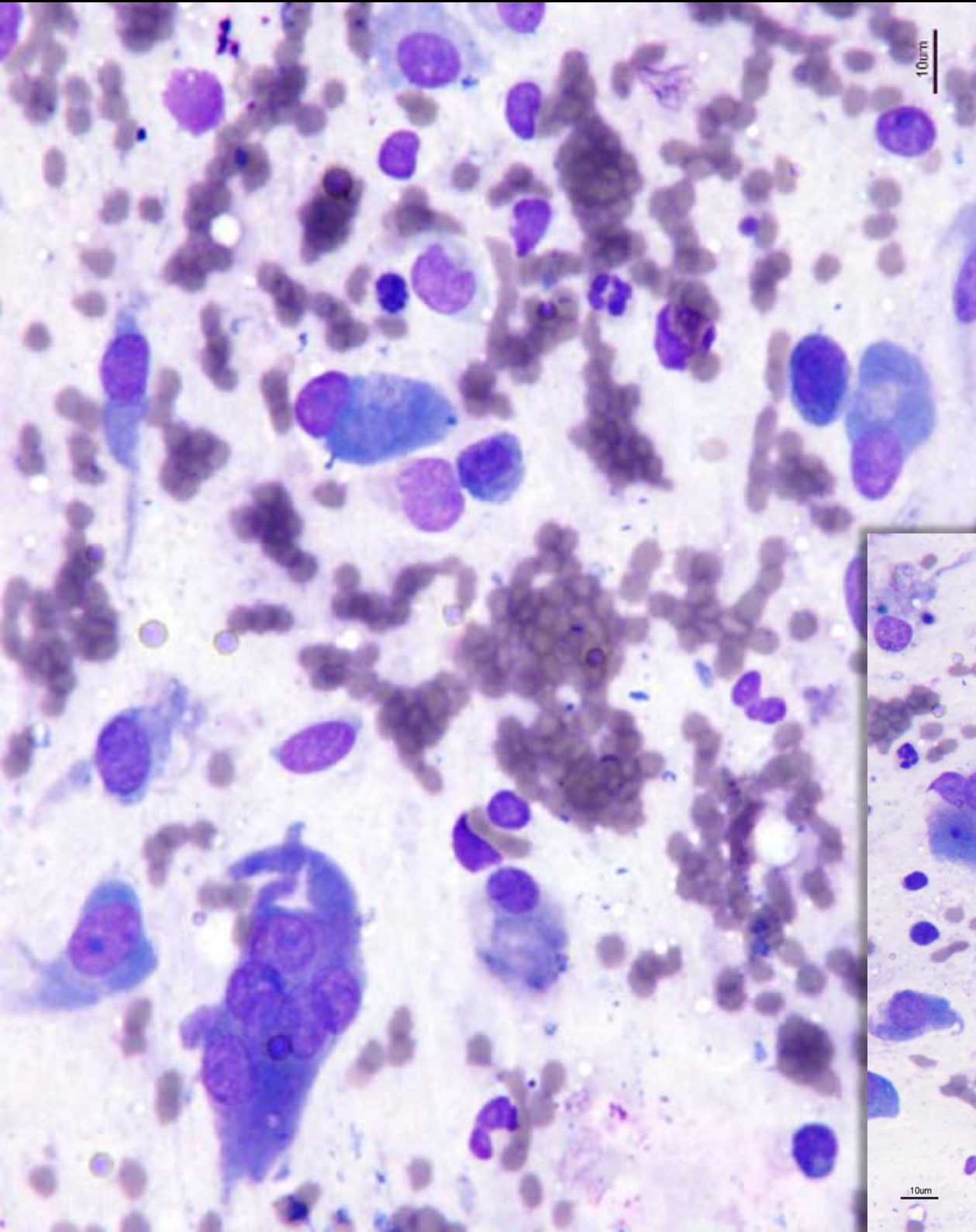
Synovial origin histiocytic
sarcoma

Lytic lesion in the
scapula of a cat

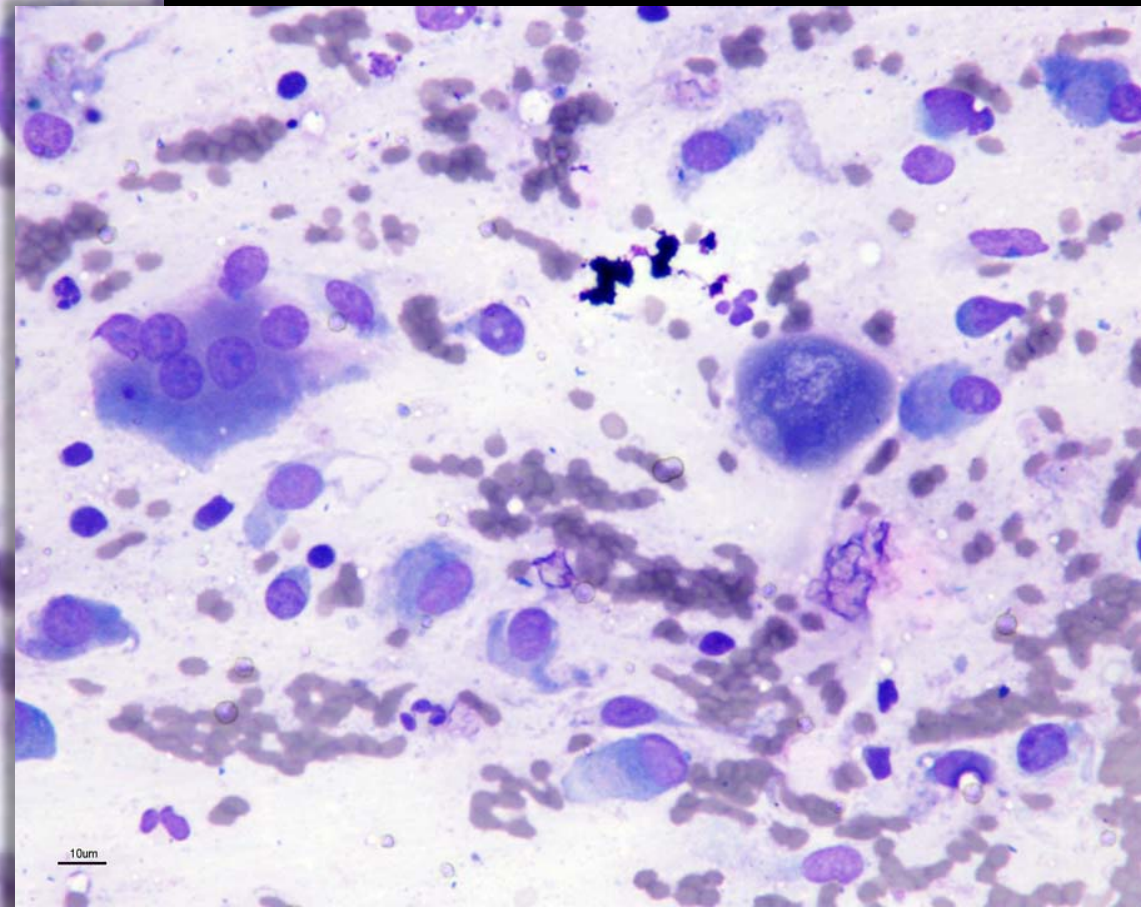


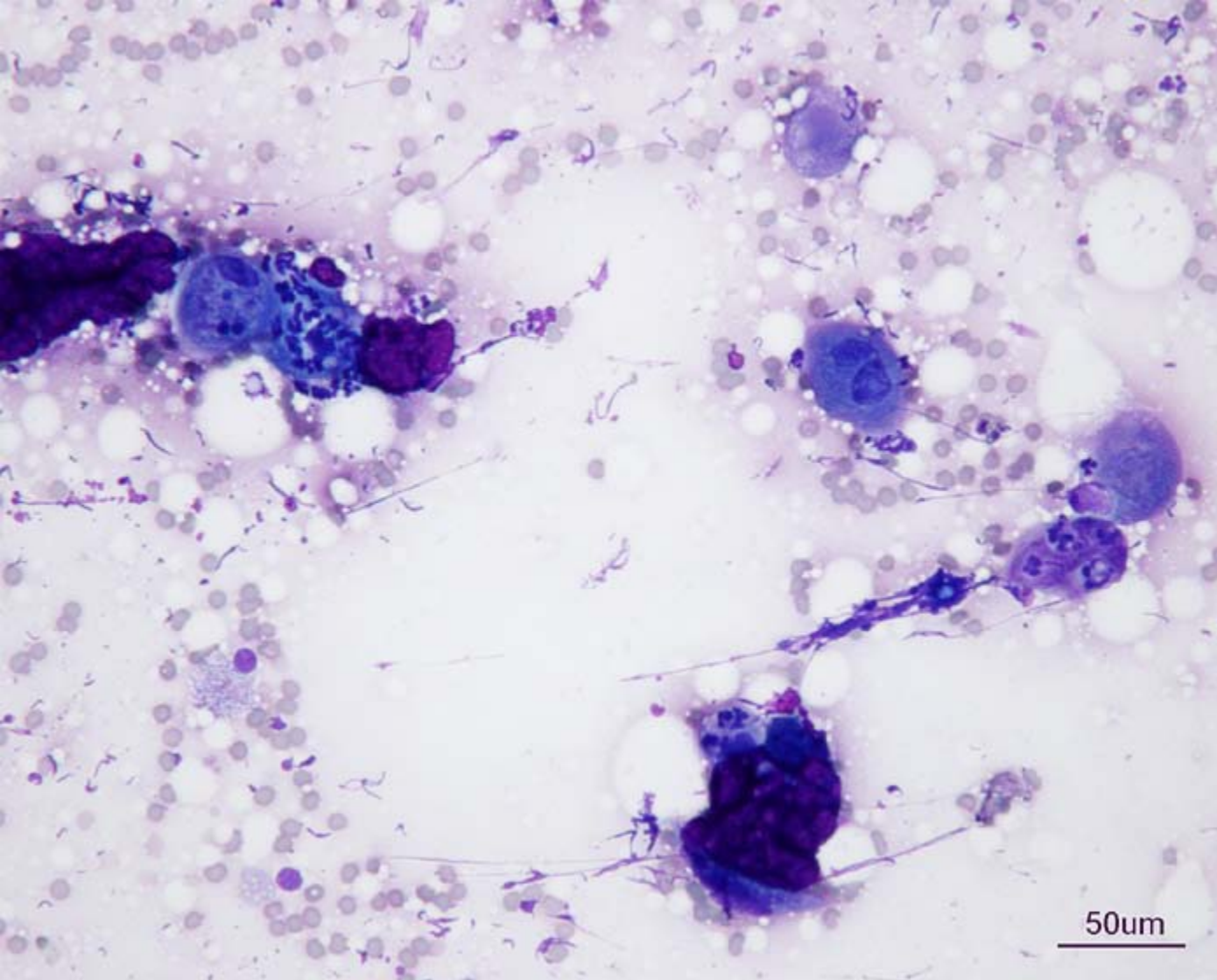


Diagnosis: Chondrosarcoma

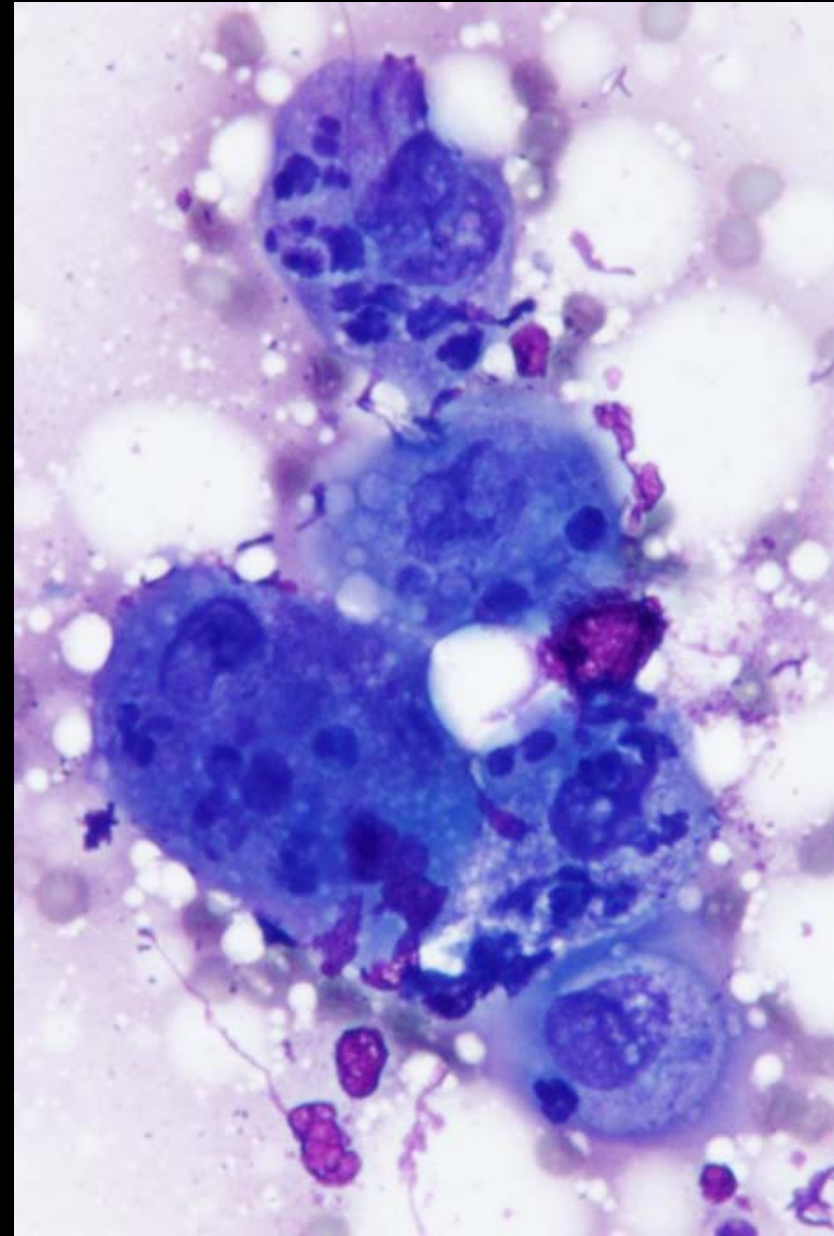


FNA from a mass in the
mandible of an adult cat





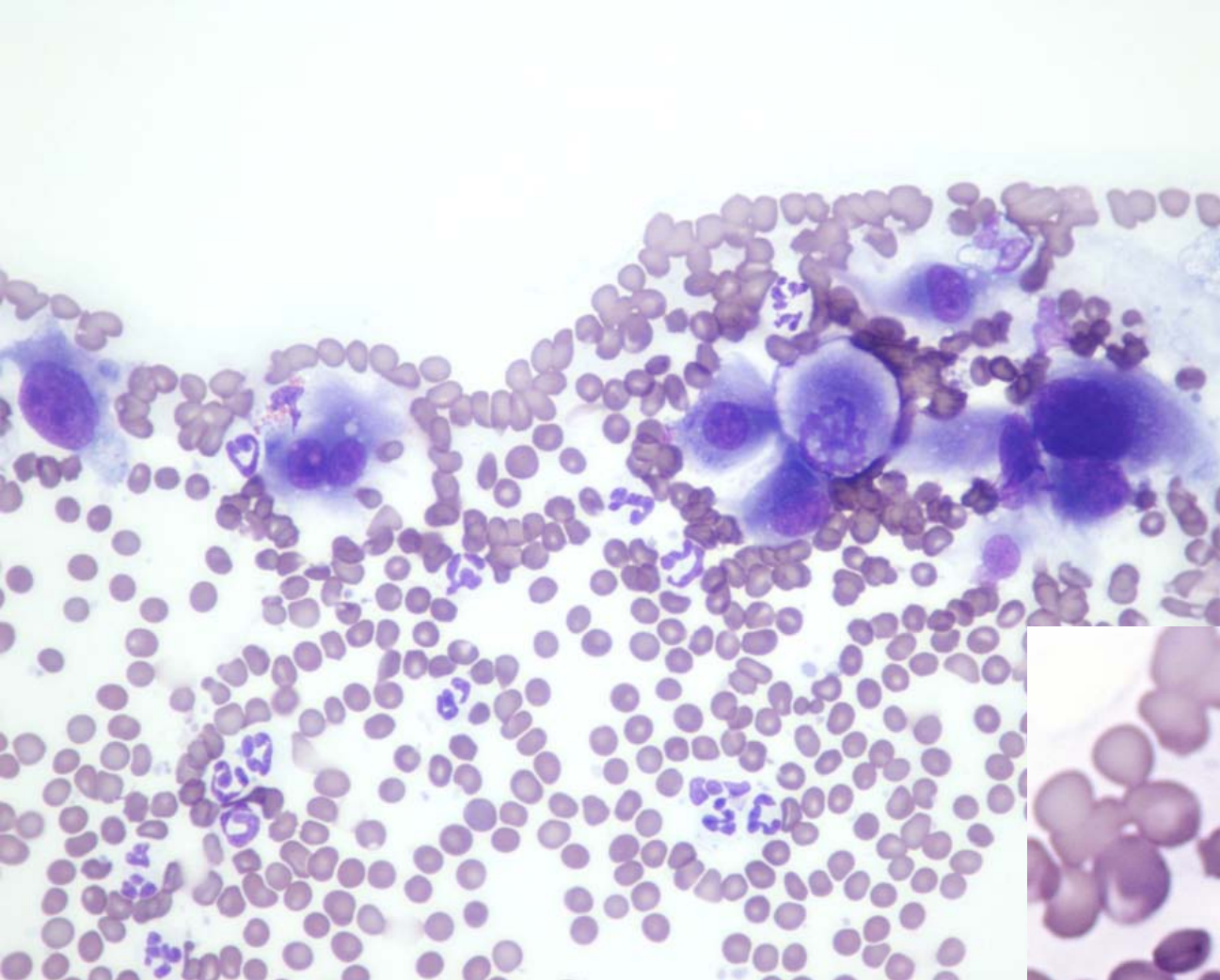
Diagnosis: Squamous cell carcinoma
invading mandibular bone



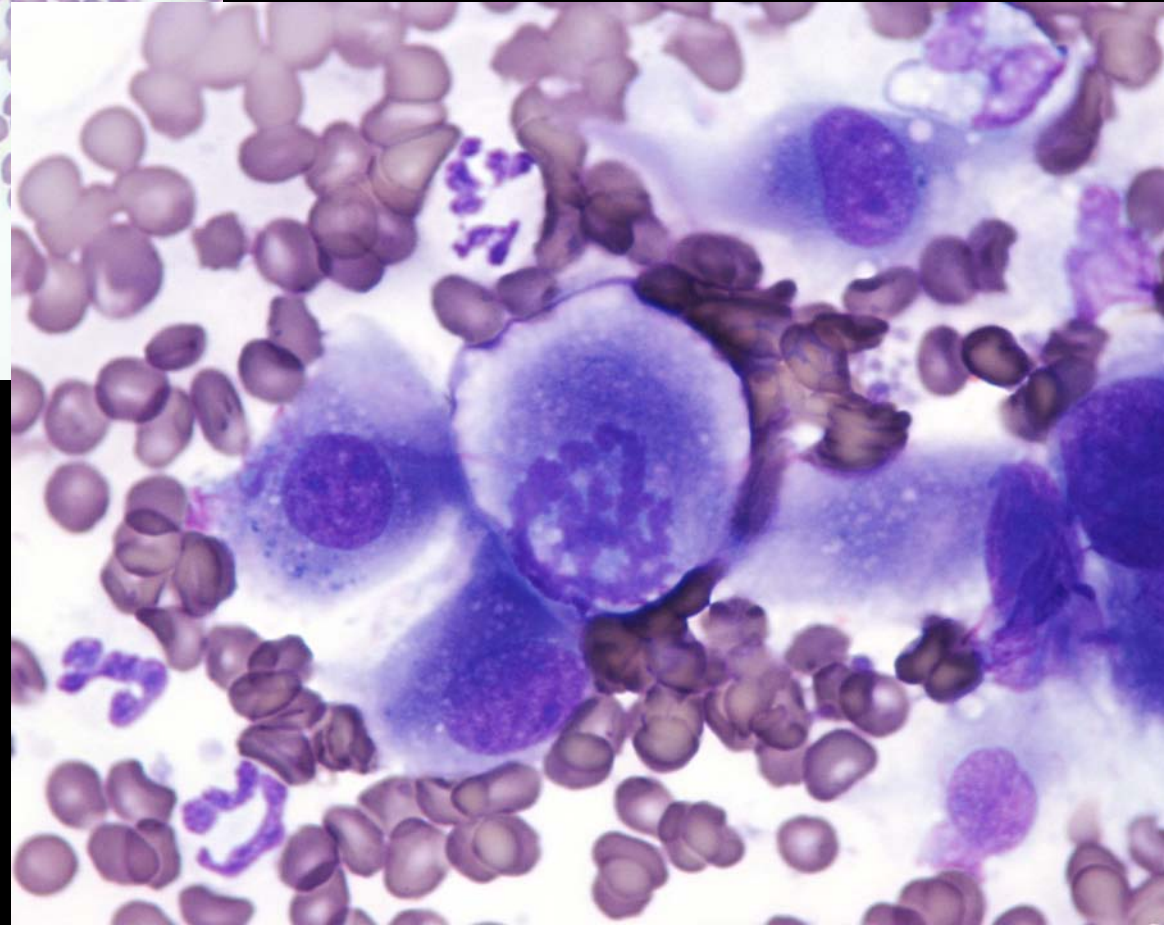
Lytic lesions in proximal tibia of a 12-year-old German Shepherd

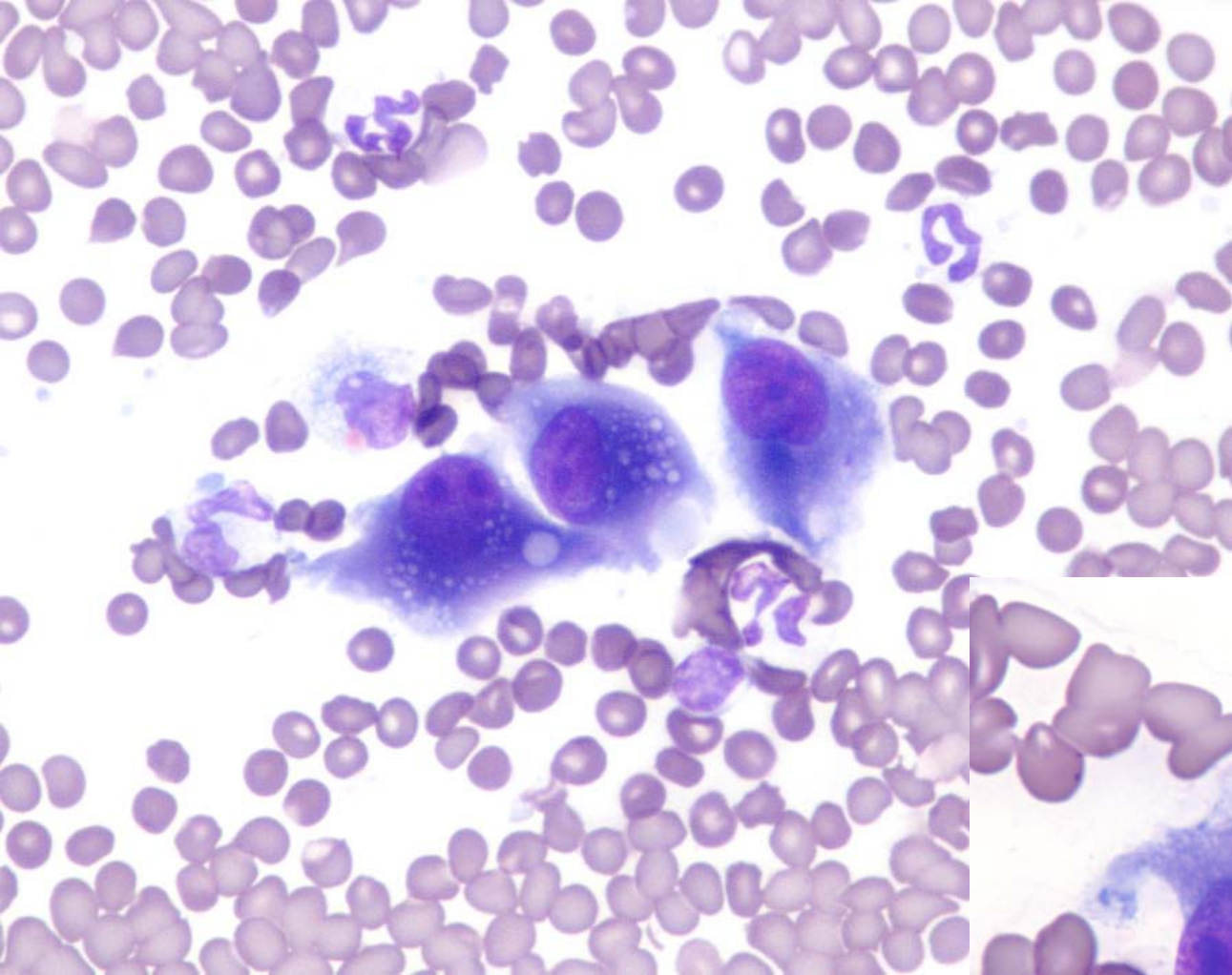


FNA cytology



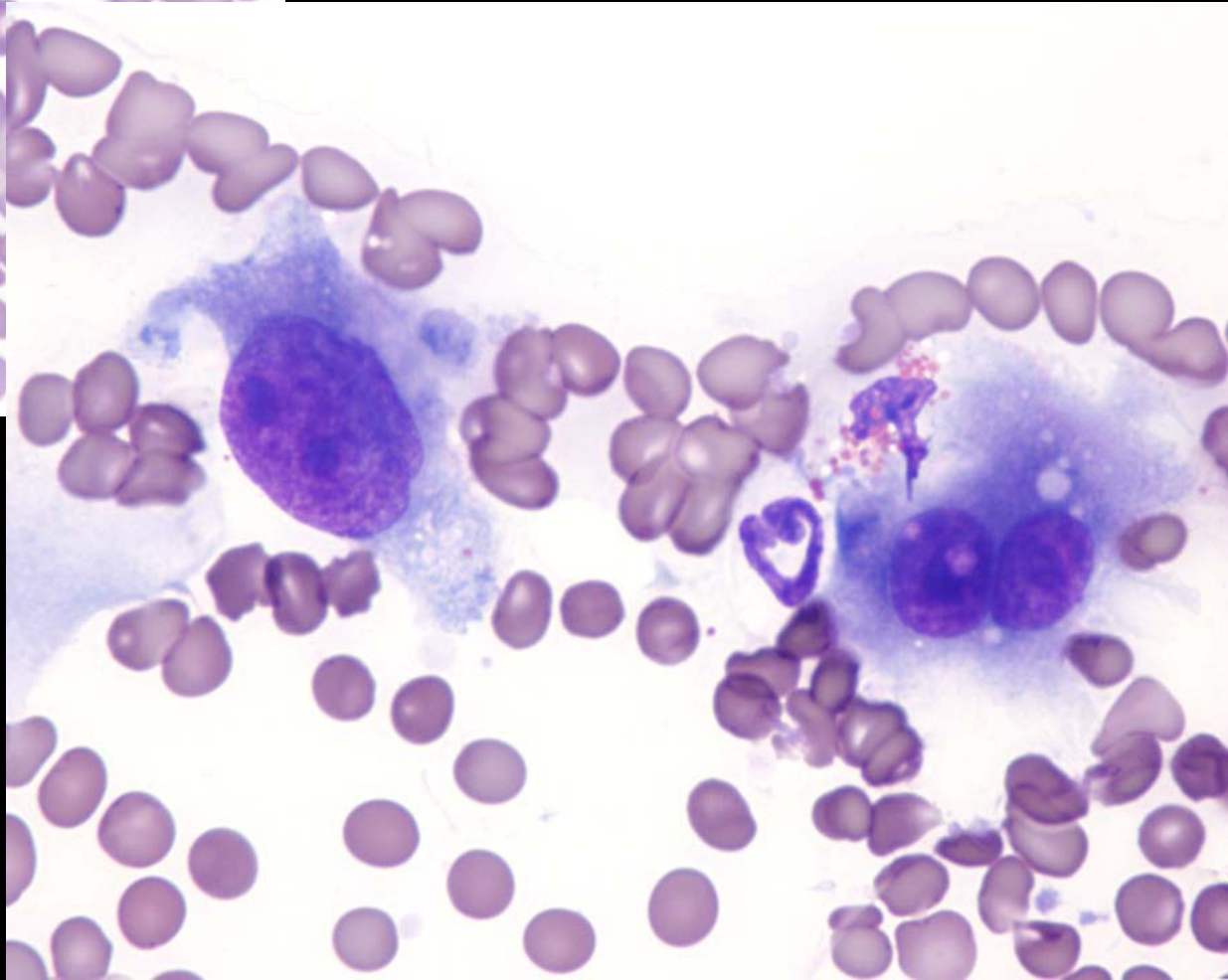
Indicates malignancy –
but is it osteosarcoma?

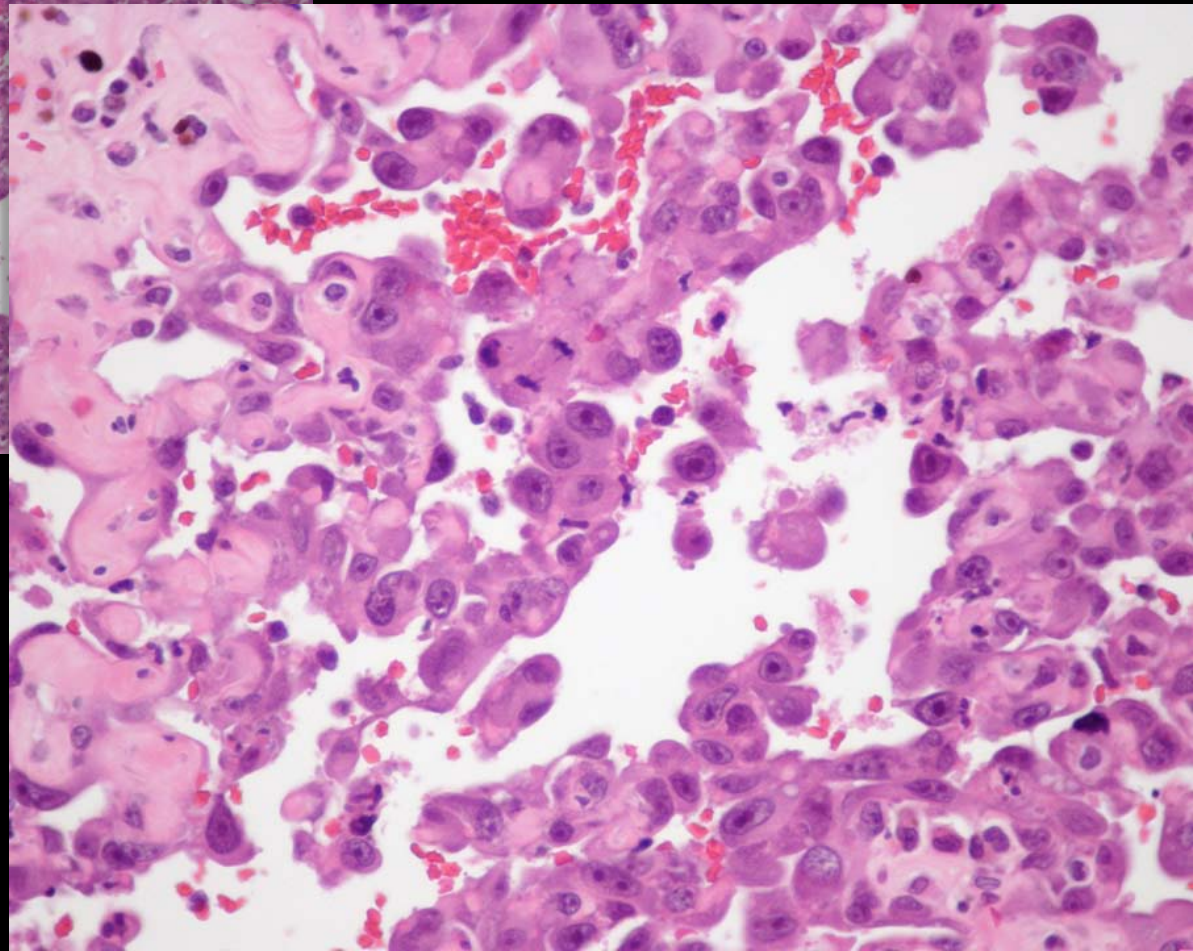
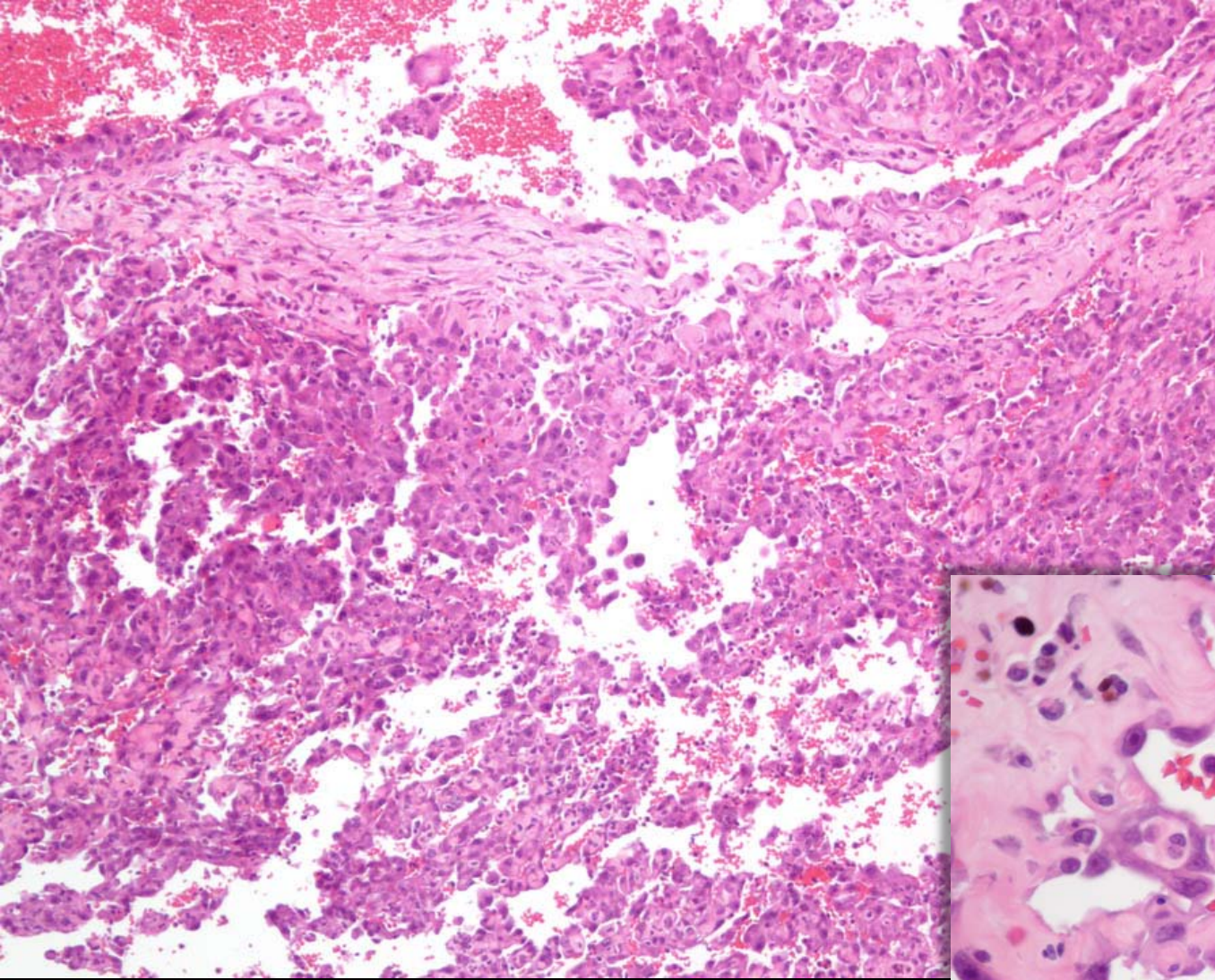




Primary differentials at this stage were telangiectatic osteosarcoma and haemangiosarcoma

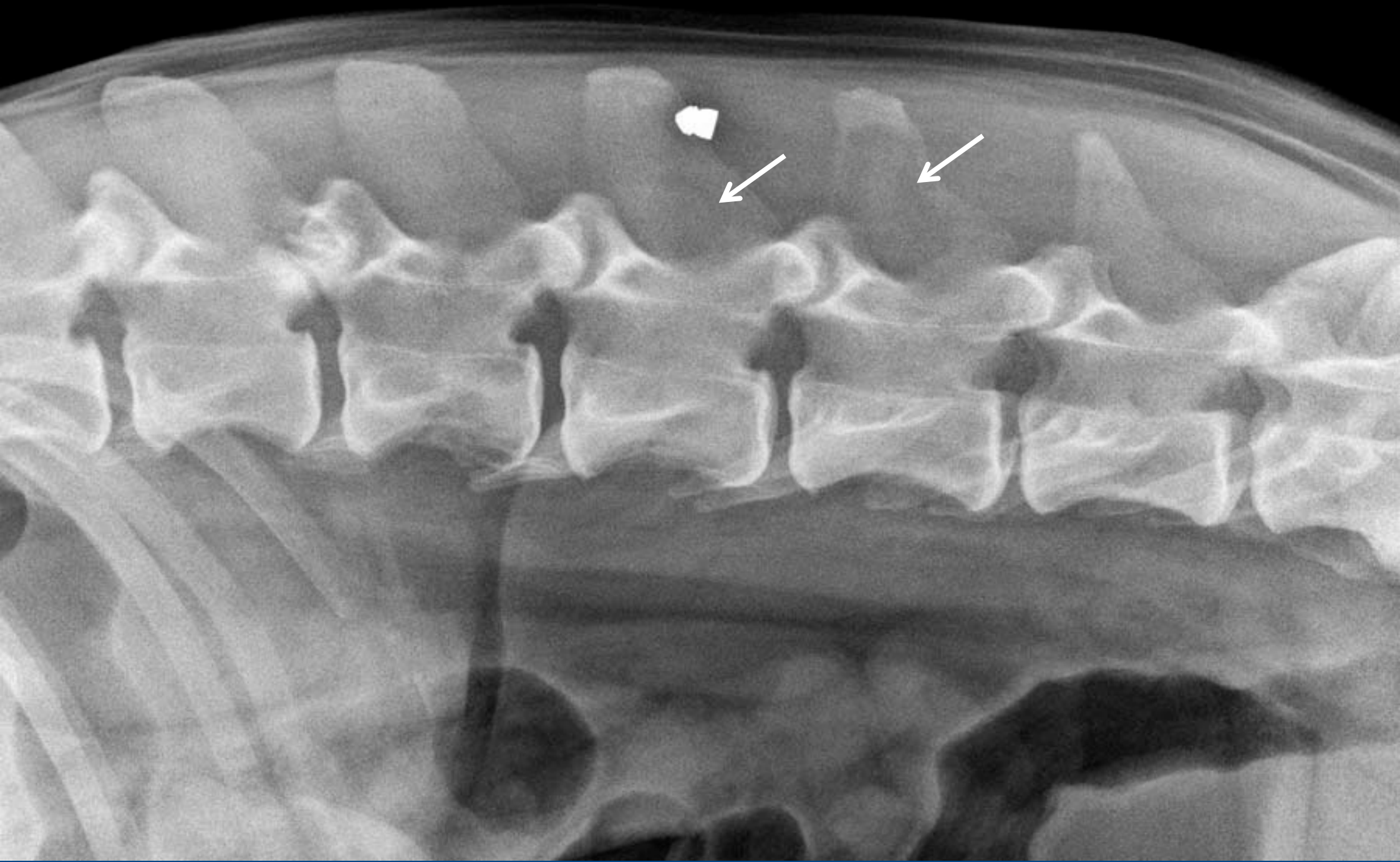
ALP staining would have allowed differentiation





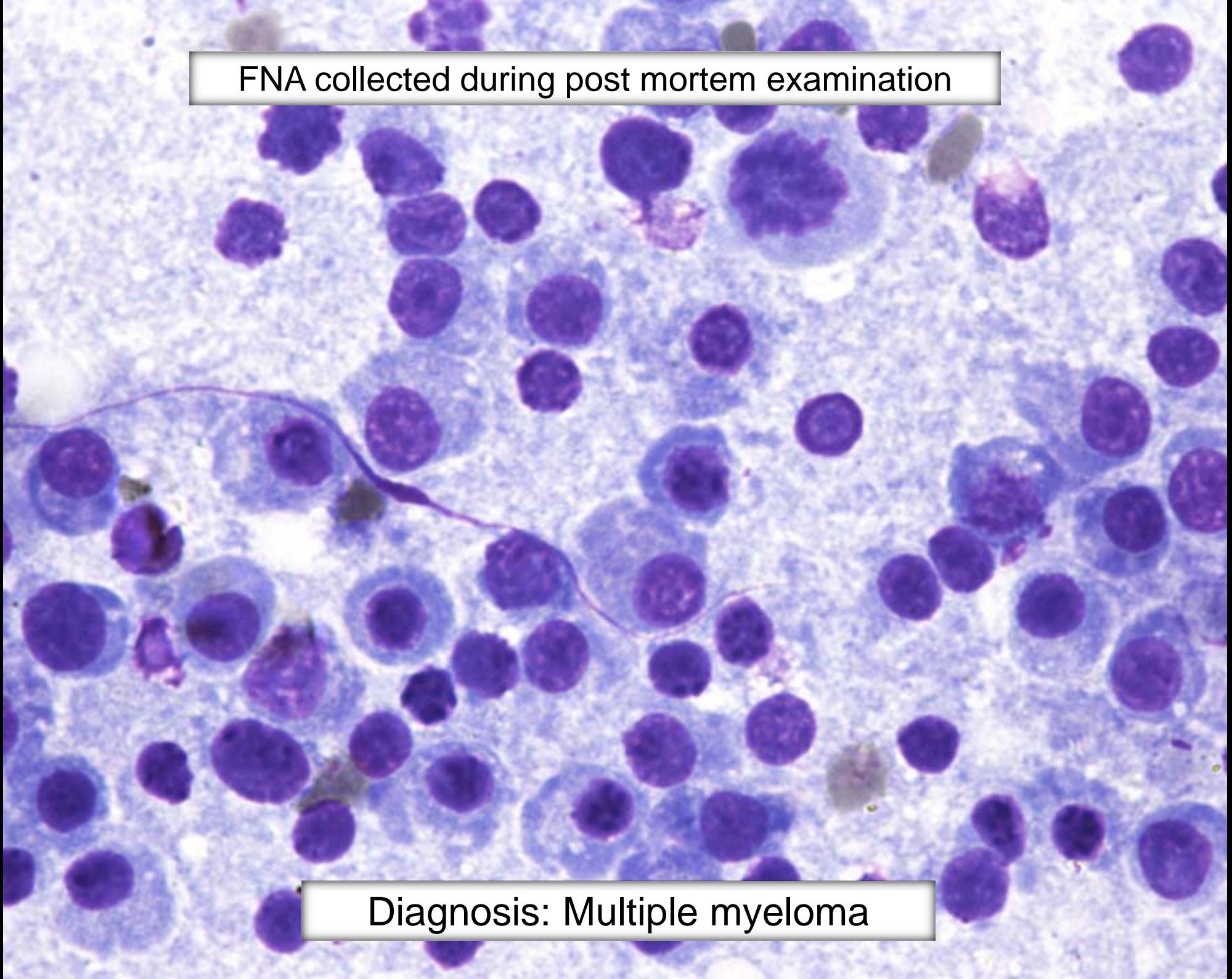
Diagnosis:
Haemangiosarcoma

Lytic lesions in vertebrae of an 8-year-old dog

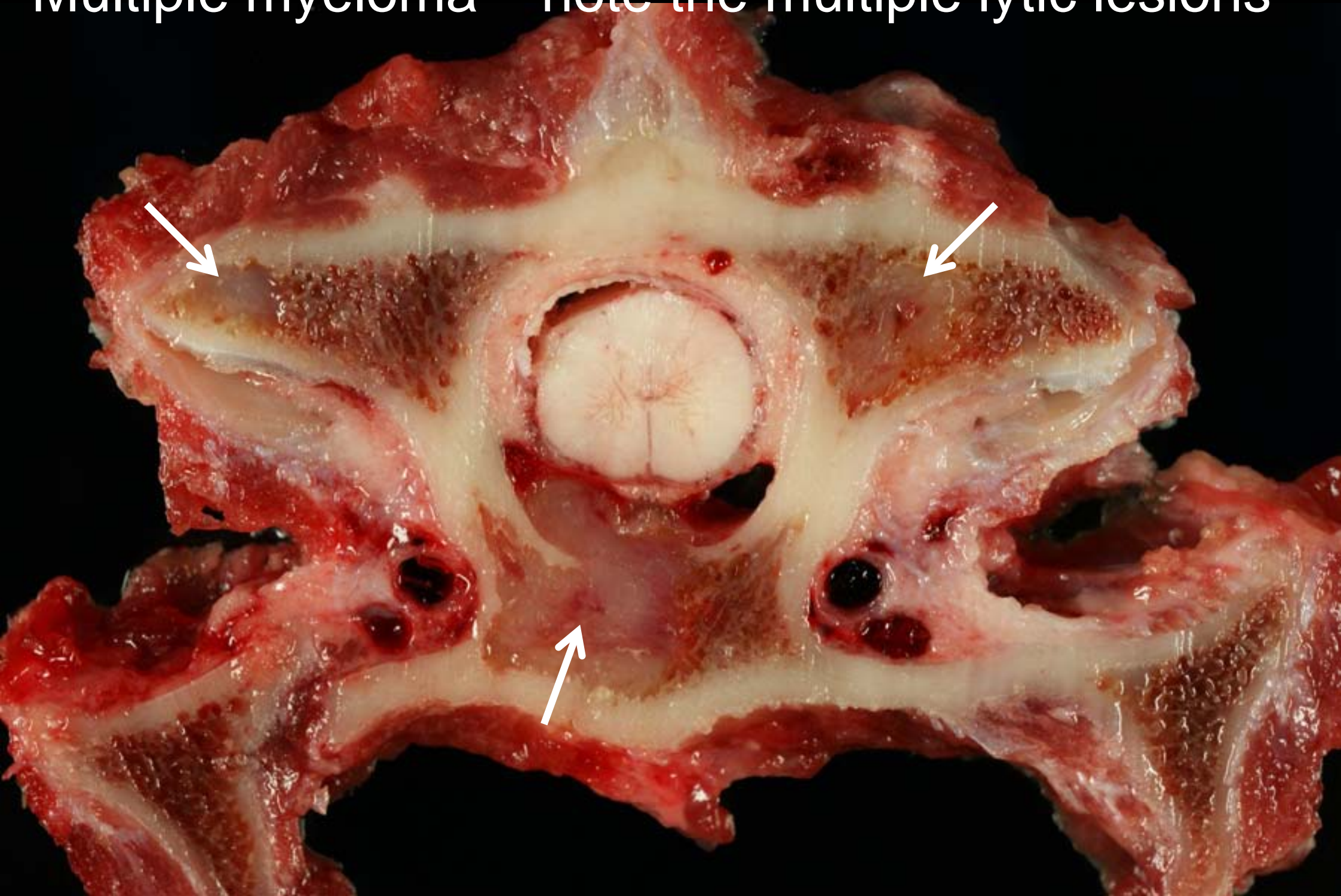


FNA collected during post mortem examination

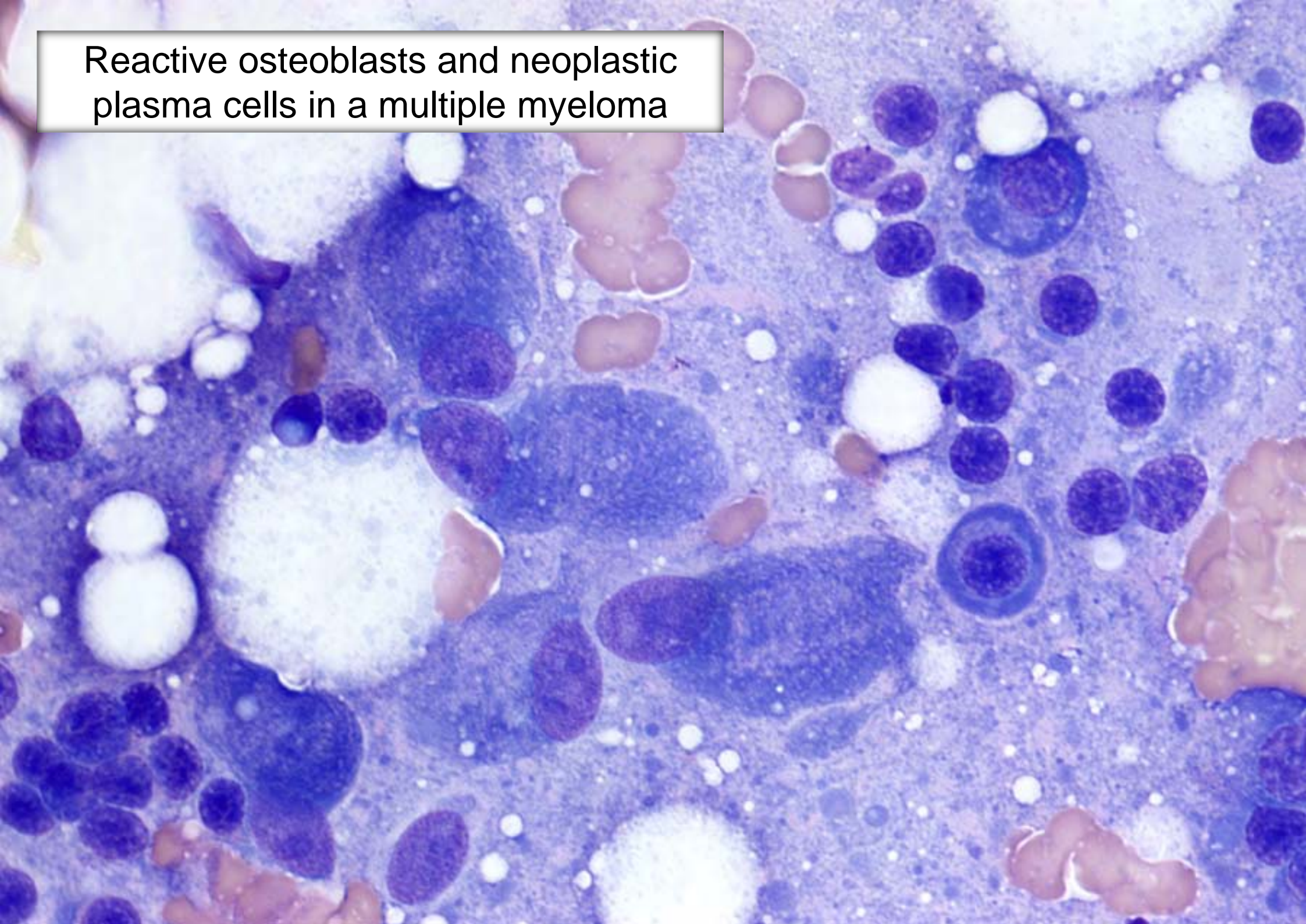
Diagnosis: Multiple myeloma



Multiple myeloma – note the multiple lytic lesions



Reactive osteoblasts and neoplastic plasma cells in a multiple myeloma



Conclusions

- In spite of new technology and diagnostic methods, definitive diagnosis of osteosarcoma using core biopsies remains a challenge
- Radiology can help greatly to raise or lower the level of suspicion
- Cytology (especially with ALP staining) may be as reliable as histology in the diagnosis of osteosarcoma from biopsy samples



Recommendations

- Make sure the diagnosis is consistent with the clinical history and radiographic changes
- Be cautious about committing yourself to a diagnosis if the history or sample is inadequate

**Only make a definitive diagnosis if you are
able to defend it**