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
• Biphasic 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)

*Modified from: Textbook of Veterinary Anatomy by Dyce, Sack & Wensing, 3rd edition (2002), Saunders*
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
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
Permeative lysis – indicates an aggressive lesion
Osteosarcoma - dog

Permeative lysis

Pallisading to spiculated periosteal reaction – likely reactive bone
“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

Reactive osteoblasts – ALP stain
Osteosarcoma – ALP stain
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
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”
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
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
Reactive bone in a repairing fracture
Reactive or neoplastic?

Early fracture callus in a cat
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

Evaluation of radiographs → Fine-needle aspiration cytology

If suspicious of 1° bone sarcoma - amputate limb

Submit limb and draining lymph node to pathology laboratory → Pathologist or lab technician selects multiple sites for histopathology (+/- decalcification)

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
FNA from skull mass

Reactive bone or multilobular tumour of bone?
FNA from skull mass

Variability suggests malignancy
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
Diagnosis: Multiple myeloma

FNA collected during post mortem examination
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