Canine Appendicular Osteosarcoma Guideline
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Introduction: This Guideline focuses on central appendicular osteosarcoma (OSA), the most common and microscopically variable primary bone sarcoma of dogs; it is not intended for feline OSA or canine OSA arising on the bone surface (periosteal or parosteal), in extraskeletal tissue, or within the axial skeleton. This Guideline provides additional diagnostic information not found in the QRG for canine OSA. The only piece of data needed by an oncologist from the pathologist to aid with clinical management of canine appendicular OSA is a definitive diagnosis, despite the description of various histologic subtypes of OSA in the literature. It is imperative to differentiate OSA from other neoplastic and non-neoplastic bone lesions due to the consequences of misdiagnosis (inappropriate surgical/chemotherapeutic intervention or euthanasia). When an adequate sample is provided and the key histologic features are observed, diagnosing OSA can be straightforward; however, when a small or poor-quality sample is received or if there is inadequate information to determine the precise sample location, diagnostic challenges arise. In an ideal setting, prior to characterizing the microscopic features of a bone tumor, knowledge of the signalment and a review of the clinical history is required. Presence or absence of pulmonary metastases should be established before sampling and results reported to the pathologist. Access to medical imaging or the radiology report for the bone lesion as well as a detailed description of the tumor location and extent can add to the weight of evidence toward or away from a diagnosis of OSA.[1] Therefore, this Guideline focuses on making an **accurate diagnosis** which involves integrating tumor characteristics beyond the microscopic features. Diagnostic confidence increases with increasing sample size, number, and quality. It is paramount for the diagnostic pathologist to recognize that the biopsy may not be representative of the entire bone tumor/lesion; if they believe this is the case, the clinician must be informed so they can develop a plan to resample.

A **differential diagnosis table** is included at the end of this Guideline. First, patient and lesion details that can provide evidence to support/refute a diagnosis of OSA are listed. Bullet points mostly include details about OSA which are not captured within the differential diagnosis table; the text and table should be used together. Additional details and references will be provided in the full Protocol for canine OSA, which is currently under development.

Patient details for collection: signalment (age, breed, weight, geographic location), travel history, lifestyle, pertinent medical history (previous fracture or metallic implant or osteomyelitis or radiation therapy site, progression of lameness, presence of other orthopedic conditions, prior/concurrent neoplasia)

- OSA is common in middle-aged to older dogs, like other primary malignant bone neoplasms; therefore, age is more useful for differentiating OSA from non-neoplastic conditions, but OSA also has a smaller peak in incidence at 18-24 months.
- OSA is most common in the metaphysis; it occasionally originates at sites of previous trauma, chronic inflammation, infarction, metallic implant, and prior radiation therapy.
- OSA has a predilection for large-breed (heavy) dogs.

Consideration of recent diagnostic results: previous incisional biopsy or cytology [alkaline phosphatase (ALP) staining; *details below*], medical imaging of lesion, chest radiographs

- OSA produces an aggressive bone lesion that is commonly both osteolytic and osteoproductive and induces periosteal reaction.
- Diagnostic imaging demonstrates extent of periosteal reaction and bone lysis, as well as lesion location/extent, and aids with rate of progression when serial images are available; if taken post-sampling, the quality of biopsy site can be assessed.
- Codman's triangle radiographically, is also observed with trauma and osteomyelitis.
- Periosteal reaction is inevitable with any lesion that induces cortical lysis.
- Necrotic tissue can be mineralized (mineralization ≠ bone formation).

Lesion distribution/location/extent details for collection and consideration: bone(s) affected, bone region(s) affected (epiphysis, metaphysis, diaphysis; cortex, trabecular bone, surface), involvement of other structures [soft tissue, lymph node (LN), joint (via invasion of subchondral bone then articular cartilage vs. cortical surface then under joint capsule insertion vs. osseous insertion site of intracapsular-extrasynovial cruciate ligaments)], presence of a fracture

- Top 5 predilection sites represent > 90% of OSA = distal radius > proximal humerus > distal femur > proximal and distal tibia. Carpus/tarsus and more distal sites represent ≤ 10% of OSA. OSA accounts for ≤ 7% of malignant digital neoplasms.
- OSA most commonly arises centrally (intramedullary); surface versions also occur in OSA (parosteal: long bones; periosteal: long bones & axial bones), chondrosarcoma (periosteal: typically flat bones, reported in long bones), and fibrosarcoma (periosteal: most common in bones of the head).
- OSA does not typically invade through the subchondral bone and articular cartilage but can occasionally invade the joint via the other routes listed above.

Consideration of sample provided: core/needle biopsy/aspirate (± pre/post sampling imaging), curettage, partial amputation, full amputation, unfixed or fixed, inclusion of LN

- There is risk of misdiagnosis with tangential/oblique/fragmented samples through lesions in the differential diagnosis table, as well as others: granulation tissue, osteophyte/exostosis, subperiosteal hematoma, previous biopsy tract, wall of bone abscess or hematoma (fracture), active stage of canine panosteitis, metaphyseal osteopathy, hypertrophic osteopathy, and osteochondroma.

- Cytologic preparations must be from intramedullary lytic areas.[2] Radiographically lytic areas tend to be cell-rich and osteoid-poor; therefore, these are the best areas to sample, which can be aided via ultrasound guidance.[3]
- Biopsies from central lytic areas plus the zone of transition/edge of lesion have the best likelihood of obtaining a diagnosis.
- The regional LN is affected in < 5% of OSA cases and must be searched for and reported on in all samples where it has not been identified pre-submission by the surgeon. One paper suggests that LN metastases are associated with decreased survival time[4] but another found no correlation with outcome.[5] Additional data and validation are required to determine whether OSA in a LN correlates with outcomes.
- If an unfixed sample is received, take impression films for ALP (NBT/BCIP) staining (*details below*).

Microscopy: evaluate/consider sample quality (over/under demineralization, necrosis, cellularity/tumor area, fragmentation, orientation), report standard microscopic features for neoplasms (mitotic count, lymphovascular invasion (soft and strict criteria), surgical margins).[6,7]

- Identifying osteoid production by malignant mesenchymal cells is the defining diagnostic feature for OSA histologically. OSA can vary widely in histologic appearance, also containing regions with cartilaginous differentiation, fibrous differentiation, blood-filled cavities, or various combinations of these phenotypes, as well as other phenotypes which are rare; however, these histological subtypes do not affect clinical decisions. For features that aid with differentiation from other bone lesions, *see the differential diagnosis table*.
- Cytologic diagnosis correlates better with the histologic diagnosis when the diagnosis is neoplasia (90% agreement); diagnostic agreement for non-neoplastic diagnoses is low (~27%).[8] Cellularity of the sample significantly affects the likelihood of agreement with histology (low cellularity = poorer correlation).
- Cytology can differentiate neoplastic from non-neoplastic lesions with high accuracy. When matrix is abundant, aspirates will be less cellular. Necrotic cells and debris can be common in OSA.
- Combining cytological findings with signalment, tumor location, and medical imaging should be sufficient to report a definitive diagnosis or alternatively, to recommend resampling or histology.
- *Differentiating reactive bone from bone produced by neoplastic cells*:
    - *Reactive bone* – maturation is orderly; trabeculae of bone become thicker and interconnect, trabeculae may be connected to pre-existing bone, thicker trabeculae are lined by a single layer of osteoblasts, osteocytes encased at regular intervals, early in the process spaces between trabeculae filled with loose assembly of undifferentiated mesenchymal cells, no atypical mitotic figures. Note, pre-existing trabeculae of bone can become entrapped within the advancing neoplastic cells, this bone is usually lamellar, rather than woven (as is newly formed reactive bone and the bone produced by neoplastic mesenchymal cells).

- *Bone produced by neoplastic cells* – no maturation of bone; trabeculae do not usually interconnect in a well-structured manner, bone is usually not connected to pre-existing lamellar bone, spaces between trabeculae filled with pleomorphic mesenchymal cells, irregularly spaced embedded neoplastic cells (if present), cellular pleomorphism with frequent or atypical mitotic figures, osteoid can be various colors: pale eosinophilic and glassy through to basophilic which may be globular to diffuse.

Surgical margin evaluation:
For limb-sparing – minimum of four samples that include soft tissue and bone from each end of the bone are evaluated.
Full limb amputation – histological assessment at the excision margin is not necessary if there is at least one joint present between the tumor and the surgical margin. For OSA located in proximal femur and proximal humerus, soft tissue margins should be assessed due to possible local infiltration; recommend at least four sites. It would be helpful if the surgeon indicated the closest margin they observed or any specific points along the excision margin they would like evaluated histologically.

Diagnostic aids:
- Positive ALP (NBT/BCIP) staining, cytologic preparation: sensitivity 88-100% and diagnostic specificity 67-94%[3,9–11]; unstained films are best (can use previously stained films). Osteoblasts stain positive; the stain cannot differentiate benign vs. malignant lesions. For OSA, it is essential to identify the cells as mesenchymal[12] with features of malignancy (i.e. sarcoma diagnosis). Positive ALP staining is reported in other canine neoplasms: melanoma, pulmonary epithelial, multilobular tumor of bone, anaplastic sarcoma, and gastrointestinal stromal tumor (*also see table*).[3,9–11]
- Immunohistochemistry (IHC): Limited utility beyond a few specific diagnoses (*see table*); no IHC marker should be used in isolation. Excessive demineralization can interfere with tissue/cellular detail; use of acid solutions can alter immunoreactivity, which must be considered when performing IHC. ALP IHC is positive in OSA but is also variably positive in chondrosarcoma, fibrosarcoma, and histiocytic sarcoma.[13]

Outcome prediction informed by histology or clinical pathology: published prognostic indicators include histologic grade[14,15], histologic subtype, serum ALP activity, and the presence of local LN metastasis. Unfortunately, none have been appropriately validated nor do they perform consistently. The only reliable negative prognostic indicator is the presence of distant metastases at diagnosis (~ 15% of cases) or development of distant metastases (> 85% of cases), most commonly identified in lung. Microscopic features are not predictive of outcome. However, just the microscopic diagnosis of OSA is sufficient for the clinician to integrate this information with all the other known data for the patient and discuss prognosis and treatment with the owners.

Future investigations should evaluate a wide array of clinical, pathological, and molecular parameters correlated with accurate clinical outcome data to determine what, if any, further testing beyond a microscopic diagnosis will help predict outcome and or guide clinicians.

VETERINARY CANCER GUIDELINES & PROTOCOLS

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	Breed				Distribution/Location/Extent							Sample R/O DDx		Bone Imaging								Histology									Histology/Cytology					Cytology				IHC			
	small	medium	large	giant	monostotic	polyostotic	epiphyseal	metaphyseal	diaphyseal	cortical destruction	plus involvement of ST	joint invasion through subchondral bone	small samples inadequate	minimum microscopy required	xray inadequate to differentiate from OSA	aggressive lesion	osteolytic	cortical destruction	beyond thinning	osteoproductive	periosteal reaction	rapid rate change	pathologic fracture	malignant mesenchymal cells	permeates medullary spaces	osteoid produced by neoplastic cells	cartilagenous matrix	myxoid matrix	fibrous matrix	blood-filled spaces lined by malignant cells	single layer cells lining osteoid	multinucleated cells	more than rare mitoses	atypical mitoses	anisokaryosis	atypia mononuclear	high cellularity	perinuclear clearing	neutrophils	ALP staining	vimentin	osteocalcin	MUM1	CD31/factor VIII
Reactive bone: response to adjacent lesion -> trauma, neoplasia, inflammation etc.; endosteal or periosteal; osteoclasts small uniform nuclei (vs MN tumor cells)					✓	(✓)						shallow/ tangential capture reactive bone only miss deep lesion		many types of lesions will have adjacent reactive bone or overlying periosteal new bone formation, which will eventually mineralize									✖	✓	✓	(✓)	✖	✖	✖	✓	✓	(✓)	✖	✓	✓	✖	(+)				
					polyostotic when 1° lesion is and with hypertrophic or metaphyseal osteopathy																		spicules osteoid surrounded undifferentiated mesenchymal cells -> thicker woven bone lined single layer osteoblasts -> osteocytes regular spacing in slit-like lacunae; interconnected trabeculae attached pre-existent lamellar bone																					
Osteosarcoma (OSA): 80-90% of 1° bone neoplasms; bone enlargement and swelling in adjacent soft tissue; osteoid can be elusive and can require a hunt; can have MN tumor cells and/or osteoclasts	(✖)	(✓)	✓	✓	✓	(✖)	(✓)	✓	(✓)	(✓)	✖					✓	✓	✓	✓	✓	✓	(✓)		✓	(✓)	✓	(✓)	(✓)	(✓)	(✓)	✖	✓	✓	✓	✓	✓	✓	(✓)	✖	(+)	(+)	(+)	(-)	(-)
					~60:40 = forelimb:hindlimb; non-metaphyseal consider DDx or previous injury/implant/RT site											variable mineralization, not all bone matrix produced by the neoplasm will mineralize									bone lacks maturation, trabeculae (±) interconnected; b/w trabeculae = filled malignant mesenc.; matrices variable								typically marked atypia; >1 large irregular nucleoli; ↑ matrix = ↓ cells; MN cells sparse to abundant											
OSA Differential Diagnoses (DDx)																																											
Chondrosarcoma (CSA): ~10% of 1° bone neoplasms; often slower progression than OSA	(✓)	(✓)	✓	(✖)	✓	✖	(✓)	(✓)	(✓)	(✓)	(✓)	✓	histo		✓	✓	✓	✓	(✓)	✓	(✓)	(✓)	✓	✓	(✓)	✖	✓	(✓)	(✓)	✖	✖	✓	(✓)	(✓)	(✓)	(✓)	✓	✖	✖	(±)	(+)	(+)	(-)	(-)
					most common in flat bones										can have bone or mineralization									if bone = via endochondral ossification								typically less atypia/mitoses vs OSA											
Fibrosarcoma (FSA): ~5-7% of 1° bone neoplasms; adult male dogs; can develop after RT (less commonly than OSA)		✓	✓		✓	✖		✓		✓	(✓)	✓	histo		✓	✓	✓	✓	✖	(✓)	(✓)	✓		✓	✓	✖	✖	(✓)	✓	✖	✖	(✓)	(✓)	(✓)	✓	(✓)	✖	✖	✖	(-)	(+)	(-)	(-)	(-)
					prox femur/tibia/ulna, dist radius										mainly lytic; minimal periosteal rxn									matrix can resemble osteoid								cytology limited value for diagnosis											
Giant cell tumor of bone: rare; cells can have >50 nuclei; LVI @ periphery common; can have cavernous spaces					✓	✖	✓	(✓)	✖	(✓)	✓	✓	histo		✓	✓	✓	✓	(✓)	(✓)	✓	(✓)		✖	✖	(✓)	✖	✖	✖	✖	✖	✓	(✓)	✖	(✓)	(✖)	✓	✖	✖	(+)				
					periosteum seldom breached										periosteal rxn uncommon									↑ MN cells vs other neoplasms; scant osteoid, if abundant = OSA																				
Multiple myeloma: <4% of bone neoplasms (1° + 2°); hyperglobulinemia with a mono/biclonal peak					(✓)	✓	(✓)	(✓)	(✓)	✖	✖	✖	cyto		✖	✓	✓	✖	✖	(✖)	✖	(✓)		✖	✖	✖	✖	✖	✖	✖	✖	(✓)	(✓)	✓	(✓)	(✓)	✓	✓	✖	(-)	(-)		(+)	(-)
					in axial too; ± >1 site same bone										typically lacking periosteal rxn									plasmacytoid, variable atypia, cells generally smaller/rounder vs OSA																				
ST neoplasia bone met: ~20% of carcinomas have bone met when check; ST mass identified (not always); >age vs 1° bone	✓	✓	(✓)	(✓)	(✓)	(✓)	✓	✓	✓	(±)	(±)	(✖)	cyto		✓	✓	✓	(±)	✖	(✓)	✓	(✓)		(±)	(±)	✖	✖	(±)	(±)	(±)	✖	(±)	(±)	(±)	(±)	(±)	(±)	(±)	(±)	(±)				
	↑ dogs <25 kg				ribs/vertebrae, prox long bones										(±) for primary neoplasm dependent																														
Bone invasion of non-bone primary: regional LNs can be affected; more common oral neoplasms (SCC, melanoma)					(±)	(±)	(±)	(±)	(±)	✓	(±)	(✖)	cyto		✓	✓	✓	✓	✖	✓	(✓)	(✓)		(±)	(±)	✖	✖	(±)	(±)	(±)	✖	(±)	(±)	(±)	(±)	(±)	(±)	(±)	(±)	(±)				
					occurs with digital SCC										(±) for primary neoplasm dependent																														
Fracture callus: woven chondro-osseous bone; remodel necrotic bone; granulation tissue; no malignant mesenc.					✓	(✓)	(✓)	(✓)	(✓)	(✓)	✖	✓	histo		(✓)	(✓)	✖	✖	✓	✓	✓	✖		✖	(✓)	✖	✓	(✓)	✓	✖	✓	✓	✓	✖	✓	✖	(✓)	(✓)	(✓)	(+)				
															minimal osteoid if peracute fx									zonal maturation osteoid; hemorrhage/hematoma; ↑ hematopoietic precursors																				
Osteomyelitis: travel (region increased risk for specific bacterial/fungal infection?); hematogenous vs implanted					(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)	✓	cyto		✓	✓	✓	(✓)	(✓)	✓	✓	(✓)		✖	(✓)	✖	(✓)	(✓)	(✓)	✖	(✓)	✓	(✓)	✖	✓	(✖)	✓	(✓)	✓	(+)				
					meta/epi ↓ age, diaphysis ↑ age																			reactive bone can be present								organisms present, abundant neuts											
Fibrous dysplasia: rare; ↑ juvenile; poorly organized fibro-osseous/woven trabeculae (thin, curved, lack orientation)					✓	(✓)				✖	✖	(✖)	histo		(✖)	✖	✓	✖	(✓)	(✓)	✖	✓		✖	✖	✖	✖	✓	✖	✖	✖	(✖)	✖	✖	✖	✖	✓	✖	✖					
					↑ skull/jaw, reported long bones										thin rim cortex, ± cystic, ± mineral									replace bone; mesenc. b/w trabec.; reactive bone periph.; mitoses rare/absent																				
Telangiectatic OSA DDx																																											
Telangiectatic OSA: easier to miss when pathologic fracture present	(✖)	(✓)	✓	✓	✓	(✖)	(✓)	✓	(✓)	(✓)	✖					✓	✓	✓	✓	✓	✓	✓		✓	(✓)	✓	(✓)	(✓)	(✓)	✓	✖	✓	✓	✓	✓	✓	✖	(✓)	(✖)	(+)	(+)	(+)	(-)	(-)
																																					hemodilute								
Hemangiosarcoma: ~3% of 1° bone neoplasms; more lesions present (spleen/heart), could be 1° or 2°; <ST		✓	✓		✓	(✓)	✓	✓	(✓)	✓	✓	✓	histo		✓	✓	✓	✓	✖	✓	✓	(✓)		✓	✖	✖	✖	✖	(✓)	✓	✖	(✓)	✓	✓	✓	✓	✖	✖	(✖)	(-)	(+)			(+)
					end tibia/radius/femur/humerus																			some make collagen beams														hemodilute								
Aneurysmal bone cyst: cavernous spaces, septa loosely arranged spindle cells + scattered MNGC + siderophages			✓		✓	(✖)	(✓)	✓	✖	✖	✖	✓	histo		✓	✓	✓	(✓)	✖	✓	✖	(✓)		✖	(✓)	✖	✖	✖	(✓)	✖	✖	✓	(✓)	✖	(✓)	✖	✖	✖	(✖)					
					eccentric in metaphysis										cortex = thin to undetectable									blood-filled spaces have no lining cells														hemodilute								

✓=expected, (✓)=can be present, ✖=not expected, (✖)=rare, (±)=can be present or absent, (+)=positive, (-)=negative, [gray fill]=unknown/inadequate data, ALP=alkaline phosphatase, b/w=between, dist=distal, cyto=cytology, fx=fracture, histo=histology, IHC= immunohistochemistry, LN= lymph node, LVI=lymphovascular invasion, mesenc.=mesenchymal cells, met=metastasis, meta=metaphysis, epi=epiphysis, mod=moderate, MN=multinucleated, MNGC= multinucleated giant cell, neuts=neutrophils, periph.=periphery, prox=proximal, R/O=rule out, RT=radiation therapy, rxn=reaction, SCC=squamous cell carcinoma, ST=soft tissue

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