Canine appendicular osteosarcoma

What are the different treatment options veterinary practitioners have in relation to canine appendicular osteosarcoma, writes Beatriz Belda, Canadian Veterinary Hospital, Doha (Qatar), Ana Lara-Garcia and Pilar Lafuente, Queen Mother Hospital for Animals, Royal Veterinary College, London (UK)

The decision-making process for diagnosis and treatment of canine appendicular osteosarcoma can often be a challenge. The aim of this article is to review and provide an update regarding different techniques available to diagnose and treat this condition in consideration with factors such as patient's status, owner's involvement and prognosis.

INTRODUCTION

Osteosarcoma (OSA) is the most common primary bone neoplasia in dogs, accounting for up to 85% of malignancies originating in the canine skeleton.[1,2] Chondrosarcoma, fibrosarcoma, hemangiosarcoma, histiocytic sarcoma and multilobular osteosarcoma are other differentials for primary appendicular bone tumours. Additional malignant bone lesions some times affecting skeleton are metastatic lesions (often from prostatic, urothelial or mammary gland carcinomas) or those secondary systemic neoplasias, like multiple myeloma, lymphoma or disseminated malignant histiocytosis. However the latter usually differ in their bone distribution pattern from primary bone neoplasia [1,2]

Breeds reported to be at increased risk of OSA development include Dobermans, German Shepherds, Golden Retrievers, great Danes, Greyhounds, Irish Setters, Rottweilers and Saint Bernards. There is evidence of breed-associated heritability in Scottish Deerhounds, retired racing Greyhounds,[3] Saint Bernards and Irish Wolfhounds. Limited genetic diversity, due to selective breeding in some breeds, has clearly contributed to OSA heritability. For example, Scottish Deerhounds have an OSA incidence of 15% with 0.69 autosomal dominant heritability; meaning that almost 70% of OSA cases in this breed are due to heritable traits.[4,5] Higher risk incidence has also been reported in intact males and females.[6] One study reported that male and female Rottweilers undergoing gonadectomy before one year of age, had an approximate one-in-four lifetime risk for bone sarcoma development, and were significantly more likely to develop bone sarcoma than sexually intact Rottweilers.[6,7]

Age at presentation for OSA is bimodal with a small peak at 18 to 24 months of age, and a larger one at seven to nine years of age.[1,2,6]

Canine OSA aetiology is unknown.[2] Regarding physical factors, there is a theory based on circumstantial evidence establishing that, since OSA tends to occur in main weight-bearing bones adjacent to late-closing physes and in heavy dogs, OSA could be associated to multiple minor trauma in the physeal region and subsequent chronic cellular damage leading to malignant transformation. However this theory is not proven. OSA has been associated with metallic implants used in fracture repair, with chronic osteomyelitis and with fractures in which no internal repair was used.[2,8-11]

Metastasis by the time of diagnosis is present in approximately 90% of patients with OSA, most of them with microscopic disease and around 15% gross lesions evident with imaging. The most common sites for metastasis are lungs, bone, and soft tissue. Almost 80% of dogs with OSA will die secondarily to metastatic disease.[2]

HISTORY AND CLINICAL SIGNS

History and clinical signs of patients affected by OSA can be variable but frequent owner complaints include localised limb swelling and/or lameness, more commonly chronic, progressive lameness that might have been responsive to pain killers or non-steroidal anti-inflammatories (NSAIDs). Swelling or an obvious mass may be noted in areas of sparse soft tissue coverage, such as the distal portion of radius or tibia. Large- and giant-breed dogs that present with lameness or localised swelling at metaphyseal sites should be evaluated, with suspicion of OSA as a likely diagnosis. In some instances, acute, severe lameness may occur as a result of pathologic fracture of the bone1 although pathologic fractures account for less than 3% of all fractures seen.[2] A recent study showed that 60% of dogs with OSA had lameness preceding the fracture.[12]

Osteosarcoma has a predilection for metaphyseal regions of long bones, but it can occur at any bone site. The two most common sites for osteosarcoma include metaphyseal regions of distal radius and proximal humerus (away from the elbow), followed by a similar prevalence for distal femur, proximal and distal tibia.[1,2,13]

PROGNOSTIC FACTORS

A wide variety of factors are associated with prognosis. Increased tumour size,[14-16] higher tumour grade and mitotic index[17] or anatomical location, such as humeral surface,[18] have been associated with a poor outcome. Age is associated with a higher mortality, but not with increased risk of developing metastasis.[19] Survival of dogs with OSA distal to the antebrachiocarpal or tarsocrural joints was somewhat longer (median: 466 days) than survival of dogs with OSA of more common appendicular sites.[20]

Clinical stage has been associated with worse outcome. A study with 90 dogs with stage III (detectable metastasis at the time of diagnosis), reported median survival times (MST)[21] of 76 days (range: 0-1,586 days). MST was different depending on metastasis location and treatment used. Patients with bone metastases had longer survival times (132 days) than those with lung (59 days) or lung and other soft tissue (19 days) metastases. If metastatic disease was found in the lymph nodes, then those dogs had short survival times, with a median of only 57 to 59 days, compared to

318 days for dogs without nodal metastases.[21,22] Regarding the treatment used, dogs with stage III disease treated palliatively with radiation therapy (RT) and chemotherapy had a longer survival time (130 days) than dogs treated with surgery.[21] Unfortunately, it is not clear from the few retrospective studies available in this setting, what is the impact in outcome of clinical signs associated to metastasis versus euthanasia decision based on metastasis presence or client motivation to treat.

Elevations in alkaline phosphatase (ALP) has been associated with a poor prognosis, according to several studies.[1,23] Perioperatively, an elevation of total serum (>110U/L) or total bone (23 U/L) ALP isoenzyme has been associated with shorter disease free intervals and survivals. Lack of ALP return to normal limits within 40 days following surgical resection of the primary tumour has been associated to development of earlier metastasis.[23]

Genetic and molecular factors associated with OSA have been studied in human and veterinary medicine. Ezrin, Ron, survivin, VEGF and COX-2 are molecular proteins with strong importance in the disease free interval and survival of dogs with osteosarcoma [24-26] Currently, assessment of expression of these proteins is confined to research and not available for routine use.

DIAGNOSTIC TECHNIQUES AND PATIENT STAGING

A presumptive diagnosis of malignant bone neoplasia, highly suspicious of osteosarcoma can be made based on the location and imaging characteristics of the bone lesion, together with patient's risk factors for this disease. A mesenchymal neoplasia can often be diagnosed by fine needle aspiration and cytology, although a histopathologic evaluation is necessary to confirm the diagnosis.[1]

RADIOGRAPHY

The use of radiography in the diagnostic work up of the affected limb involves orthogonal views and three-view thoracic radiographs to evaluate for pulmonary metastatic disease. Typical appendicular osteosarcoma bone findings include: monostotic lesions (one bone affected), localised at the level of the bone metaphysis. Classically, these lesions show aggressive characteristics such as a lytic pattern of destruction, being a more aggressive, moth-eaten pattern; irregular and poorly defined margins, wide transition zone between the lytic lesion and the normal bone; irregular periosteal reaction with anarchic osteoid formation that can extend to the adjacent soft tissues[27] (see Table 1).

Due to the extension of lytic bone lesions, some patients might present with a pathologic fracture (see Figures 1a, 1b, 2a and 2b).[12,27]

	Aggressive	Non aggressive
Bone lysis pattern	Mottled	Geographic
Cortical destruction	Irregular	Continuous, smooth borders, well defined
Periosteal reaction	Solid pattern	Discontinuous pattern
Transition zone	Extensive, ill defined	Short, well defined
Soft tissue involvement	Present	Moderate or non-present
Progression	Fast	Slow

Table 1. Radiographic features of aggressive and non-aggressive bone lesions.

CYTOLOGY

Fine-needle aspiration (FNA) and cytology of a malignant

Figure 1a and 1b: Lateral (a) and caudo-cranial (b) radiographic projections of the left thoracic limb in a nine-year-old Labrador retriever with a history of chronic left forelimb lameness. Osteolysis (moth-eaten pattern) in the proximal metaphysis of the humerus can be observed. The margins are ill defined and irregular, with a long transition zone to the proximal diaphysis. An irregular periosteal reaction can be seen associated with moderate inflammation of the adjacent soft tissues and muscular atrophy. There are no signs of pathological fracture.

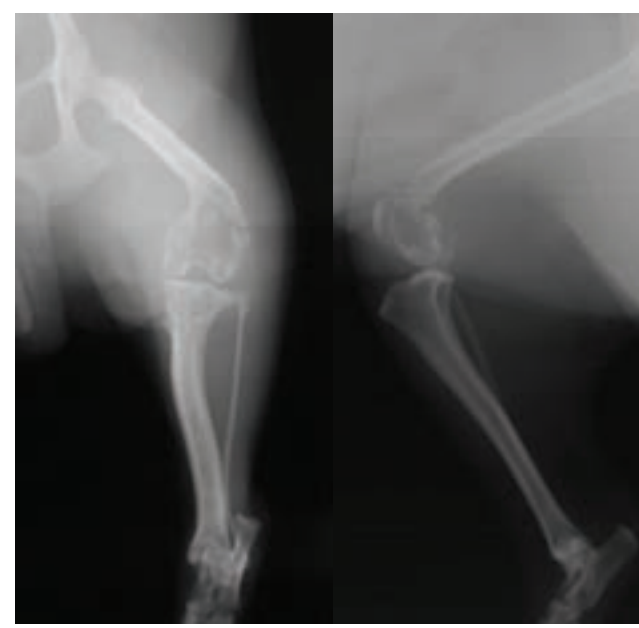

Figure 2a and 2b: Cranio-caudal (a) and lateral (b) radiographic projections of the left pelvic limb in a nine-year-old Jack Russell Terrier with a sudden onset of left hindlimb lameness. Observe the mottled and irregular osteolysis, together with the long transition area in the distal femoral metaphysis. A secondary transverse metaphyseal pathological fracture can also be seen.

bone lesion is a widely used alternative to performing a bone biopsy. Minor cost, faster technique, less invasiveness and quick results (biopsy needs decalcifying time) are advantages that cytology offers when compared to biopsy. Cytological evaluation does not always provide a definitive diagnosis for osteosarcoma but often provides a diagnosis of malignant mesenchymal neoplasia, highly suspicious of osteosarcoma (see Figure 3).

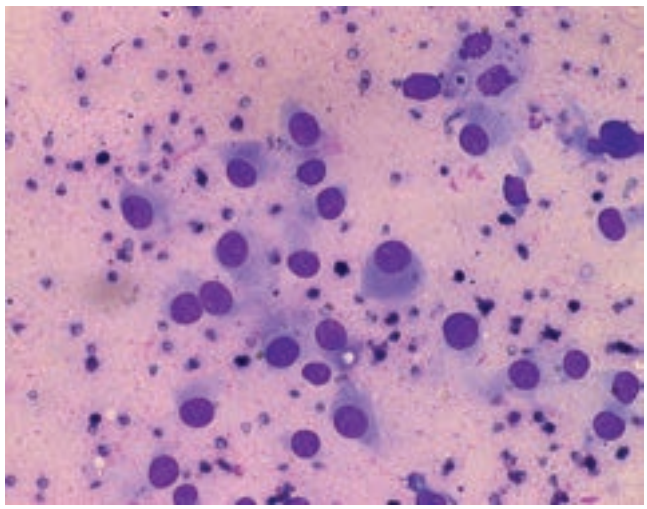

Figure 3: Cytology after fine needle aspiration (FNA) of a malignant lytic lesion compatible with mesenchymal neoplasia and highly suspicious of osteosarcoma. Wright stain (100x). Pleomorphic population of malignant mesenchymal cells with ovoid (osteoblast like appearance) or fusiform morphology and evident anisocytosis. Prominent multiple nucleoli and clumped chromatin can be observed in most cells and also one mitotic figure. Osteoid matrix can be evidenced as eosinophilic material in the background. No inflammatory cells can be observed.

Cytological results, combined with clinical features and radiographic lesion appearance, are often enough for the clinician to discuss treatment options with the client.[1] Ultrasound guided FNAs can sometimes help, especially when there is little soft tissue involvement, to improve diagnostic quality of samples. Ultrasound can identify a lytic area in the cortical bone, through which a 20G hypodermic needle is placed into the lesion and a sample obtained. Bone FNA and cytology agreed with incisional and excisional biopsies in 71% of the cases regarding identification of a primary process (inflammation, non-neoplastic proliferative process or neoplasia); and for lesions with a cytological diagnosis of neoplasia, cytology and histopathology agreed in 92% of the cases.[27] Another study found that ultrasound-guided FNA of osteosarcoma lesions had a specificity of 100% and sensitivity of 97%.[28] One recent report also supports the use of cytology with alkaline phosphatase (ALP) staining for the diagnosis of OSA in dogs. For dogs with OSA in this study, the cytological diagnosis was accurate in 85% of FNA and 95% of core aspiration cytology (CA). CA allowed penetration of cortical bone using a larger needle and an increased diagnostic accuracy. This report also showed a 100% sensitivity for OSA when ALP staining was used.[30]

BIOPSY

As previously mentioned, the diagnosis of OSA can be reached by putting together clinical signs, radiographic findings and bone lesion cytology results. In atypical cases and/or when the lesion is not in a common location, then biopsy might be indicated. However, if surgery (amputation or limb-sparing) is performed, histopathologic evaluation of the specimen should be performed to confirm the diagnosis of OSA (see Figure 4).

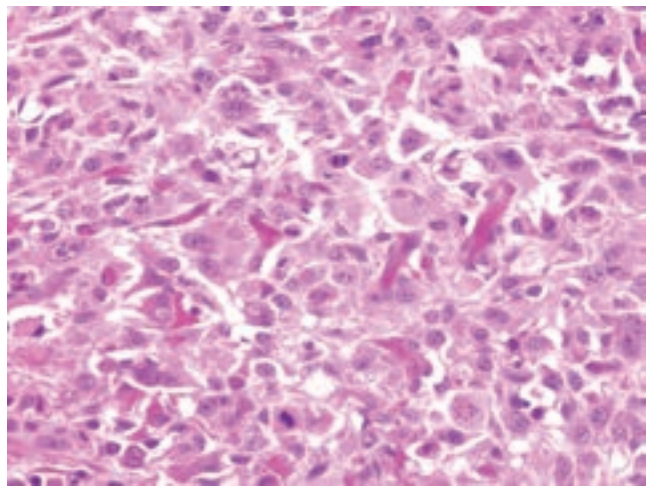

Figure 4: Histopathology of a dog with osteosarcoma. Hematoxilin-eosin stain (40x). Fusiform cells with nuclear atypia and osteoid deposition can be observed.

A tissue sample can be obtained by close, open or excisional biopsy. The majority of bone biopsies are performed by close technique using either a Michele trephine or a Jamshidi needle.[31] Michele Trephine is an instrument that provides a larger sample and has been associated with 93% diagnostic accuracy. The disadvantages of this technique include increased likelihood of post-biopsy fracture compared with other techniques.[31,32] The use of a Jamshidi needle is considered less invasive. Multiple samples should be obtained due to the smaller sample size and the risk of fracture with this device is unlikely. In 92% of cases, a correct diagnosis of tumour versus non-tumour is

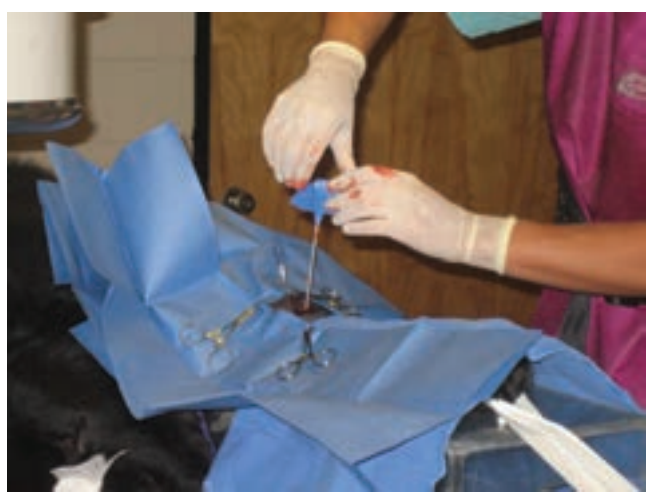

Figure 5: Bone biopsy performed with a Jamshidi needle. This technique must be performed under aseptic conditions.

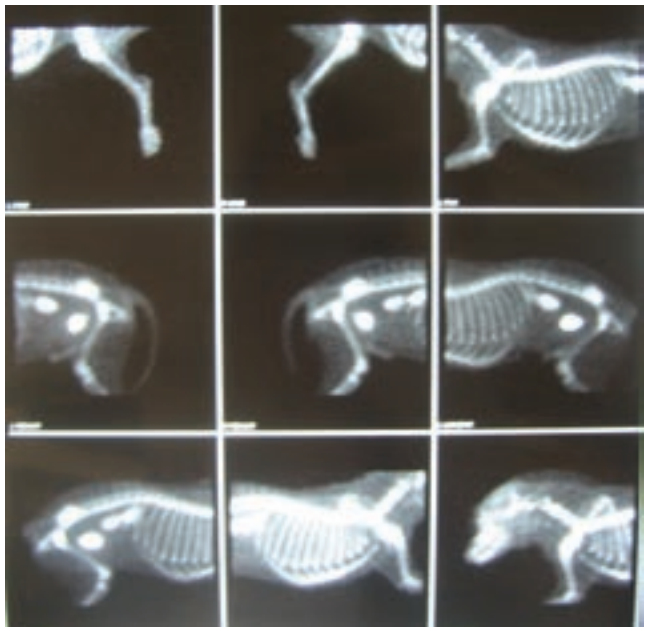

Figure 6a: Scintigraphy showing areas with accumulation of tecnetium-99m and (b) Gammagraphy room.

achieved.[31] This accuracy can be lower in atypical, small and/or lesions with limited bone destruction (see Figure 5). Bone biopsy carries the risk of exacerbating lameness and increasing risk of pathologic fracture during or after the procedure.[32] The risk of taking a non-diagnostic sample is also possible, which would require repeating the procedure or moving to a complete surgical resection. Choosing accurately the biopsy site is important. Samples of soft tissue lesions usually are taken from the periphery of the lesion, avoiding the centre because necrotic tissue is frequently found in this area. However, when biopsying bone, it is the centre of the lesion where the sample should be taken, avoiding the periphery as this would often result in a misdiagnosis of reactive bone, making samples non-representative or non-diagnostic.[32,33] Fluoroscopy, if available, is a tool that can aid to guide the sampling procedure. Before the biopsy is taken, it is important to know the treatment options that will be discussed once diagnosis is confirmed. The biopsy tract is considered contaminated with tumour cells and should be removed in the final surgery. This is particularly important if the option is limb-sparing surgery.[33]

PATIENT ASSESSMENT AND STAGING

It is essential to perform a complete physical examination of the patient to detect evidence of metastasis and/or to detect concomitant orthopaedic or neurological conditions. Complete blood work with urinalysis including bone alkaline phosphatase levels should be performed to determine the general status of the patient.

Due to the high rate of metastasis, staging is always performed when osteosarcoma is suspected or has been confirmed, as finding evidence of gross metastasis will affect the expected survival time. Metastasis is present in nearly all patients by the time of diagnosis as microscopic disease in most cases, and gross metastasis is identified in around 15% of cases. The most common sites of metastasis are lungs (10%) following by bone (7.8%) and lymph nodes (4.4%). [2,33]

Regional lymph nodes should be palpated, and fine needle cytology performed from any palpable regional node: axillar and prescapular lymph nodes for forelimb lesions, and popliteal and inguinal nodes for hind limb. Patients with affected lymph nodes have shorter survival times after surgery and chemotherapy.[34] Evaluation of pulmonary metastatic disease can be performed with radiographs or computed tomography (CT). Nuclear scintigraphy is recommended when secondary or synchronous primary bone lesions are suspected.[33]

COMPUTED TOMOGRAPHY (CT)

CT is a very useful diagnostic tool due to its versatility, availability and speed of image acquisition. It can be helpful for both local and distant staging. It provides high-quality bone images and higher sensitivity for the detection of pulmonary nodules (from 1mm) than radiography (from 7-9mm).[35] One recent report showed that metastatic bone lesions are better assessed by nuclear scintigraphy, although in the absence of this technique, CT is very effective as an adjunctive diagnostic modality.[36]

ABDOMINAL ULTRASONOGRAPHY

Abdominal ultrasonography as a part of the staging process is unlikely to reveal metastases from OSA and may not be a recommended routine staging tool; however, in certain dogs, such as those with palpable abdominal masses, abdominal ultrasonography may reveal abnormalities that may influence treatment decisions. In a recent study of 107 dogs with OSA, metastatic lesions during abdominal ultrasonography were detected in three of them (2.5%). The affected organs were kidney, liver and iliac lymph node. [37]

NUCLEAR SCINTIGRAPHY

This modality is recommended if a metastatic bone lesion is suspected. Nuclear imaging involves the use of an intravenous injection of technetium-99m labelled with a phosphate analogue, such as methyl diphosphate or HDP.

Visualisation of 99mTc-HDP is achieved because of the incorporation of the radiolabelled compound into areas of actively metabolizing bone ('hot spots'). This modality provides high sensitivity, although specificity is low because it can not differentiate between a bone tumour, infection, fracture or another orthopaedic condition.[38] However, in conjunction with other modalities, it is possible to assess the probability of bone metastasis in areas where 99mTc-HDP is incorporated[38] (see Figures 6a and 6b).

TREATMENT

Patient status, stage and owner's motivation will affect the chosen treatment options, affecting survival times in patients with OSA (see Table 2).

PALLIATIVE TREATMENT

PAIN MANAGEMENT

In dogs with OSA, an effective analgesic plan must be made including frequent pain assessment and evaluation of quality of life from the moment of diagnosis. In those dogs where surgery is not an option, painkillers should be administered during the complete course of disease. A multimodal analgesic plan is recommended. Patients undergoing surgery shortly may receive a combination of NSAID'S and opioids, such as tramadol or a fentanyl transdermal patch, as this can be effective temporarily[39] (see Table 3).

When patients are hospitalised, intravenous opioids can be administered. When high levels of pain are anticipated, such as those animals with pathologic fractures, loco-regional anaesthesia techniques can be considered, such as an epidural catheter or a plexus block.

On the other hand, when the client declines surgery as a local treatment, pain management must be the clinician's goal. Bone destruction causes excruciating pain, as it has been reported in human medicine, and in this situation a combination of NSAIDs and opioids is unlikely to be 100% effective. The intensity of pain must be monitored with serial and frequent orthopaedic examinations and pain assessments. In this scenario, hypo fractionated or palliative radiation therapy would be a fast and effective method to control pain. This therapy works directly on the osteoclasts, decreasing the inflammation associated with the tumour and the release of chemical pain mediators, at the same time as slowing down the progression of the disease. Usually a total dose of 32Gy (one 8Gy weekly session for four weeks) combined with oral analgesics is recommended. However, one or two 8Gy doses (one weekly session) are usually enough to detect pain decrease and/or lameness improvement. NSAIDs, opioids, gabapentin and/or amantadine might be administered, ideally, in combination with bisphosphonates to increase bone density. If this protocol does not effectively control the pain, other drugs may be considered, such as paracetamol-codeine or fentanyl transdermal patches.[39]

The most common adverse effects of these drugs include gastrointestinal effects. Patients should be monitored periodically in order to adjust the analgesic protocol.

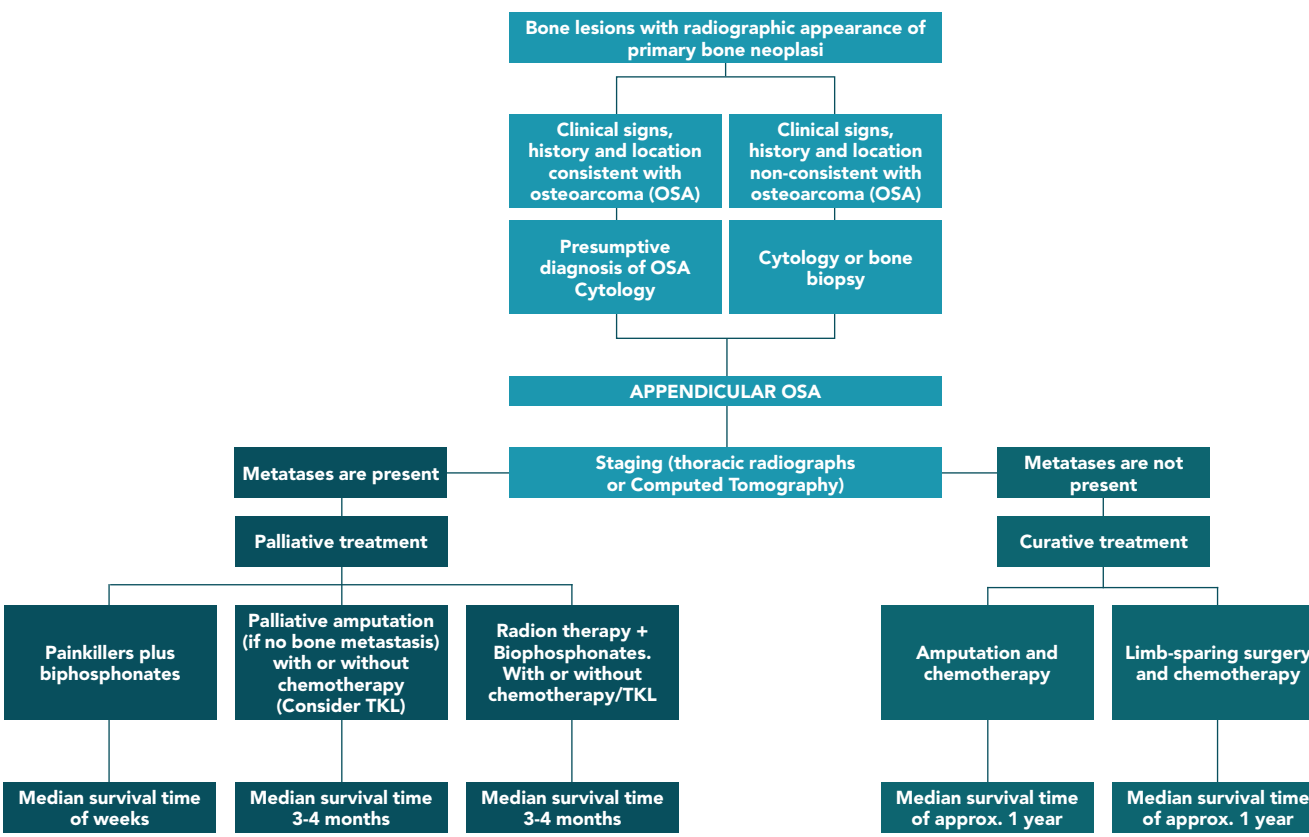

Table 2. Flow chart for diagnosis, treatment and prognosis of canine appendicular osteosarcoma.
***Quality of life often not acceptable or acceptable for short period.**
****Metastases observable with diagnostic imaging.**

Type	Drug	Dosage
NSAIDs	Robenacoxib Carprophen Meloxicam Piroxicam Deracoxib Aspirin	1-2mg/kg PO q24h 4mg/kg PO q24h o 2mg/kg PO q 12h 1.1mg/kg PO q24 h 1.3mg/kg PO q48 h 1-2mg/kg PO q24 h 10mg/kg PO q12h
Opioids	Morphine Methadone Fentanyl Fentanyl patch	0.2-0.5mg/kg IV q4h 0.2-0.5mg/kg IV q4h 1-5microgr/kg/h (CRI) Cats and small dogs (<10kg): 25µg/hr Dogs (10-20kg): 50µg/hr Dogs (20-30kg): 75µg/hr Dogs (>30kg): 100µg/hr
Opioid derivatives	Tramadol	2-4mg/kg PO q8-12h
Tricyclic antide-pressants	Amitriptyline	1-2mg/kg PO q12h
Anticonvulsants	Gabapentin	2-10mg/kg q12h
Antagonist NMDA	Amantadine	3-5mg/kg q24h

Table 3. Analgesic drugs and dosages for pain palliation in dogs with OSA.

BISPHOSPHONATES

Bisphosphonates are osteoclast inhibitors; they decrease the rate of bone resorption, increasing bone densitywhen used in the palliative treatment of osteosarcoma in dogs. There is poor evidence of the analgesic effect of bisphosphonates. Palliative effects have been observed in 30% of the patients where these were used as the sole therapy, and bone density was increased by 18%.[40] Pamidronate is the recommended bisphosphonate in veterinary medicine. Its dosage has been reported between 1.0 and 2.0mg/kg, as an intravenous infusion in combination with saline 0.9% during 20 minutes to an hour, once a month.[40]

Poor bioavailability of bisphosphonates has been described in the dog. Alendronate is the one most commonly used and it is administered in dogs at a dose of 10mg/dog once daily in the mornings, 30 minutes before any meal. Adverse effects of bisphosphonates are uncommon, with gastrointestinal ones described. The *in vitro* effect of pamidronate on the viability of canine osteosarcoma cells has been investigated. The results of this study indicate that pamidronate can inhibit growth of osteosarcoma cell cultures but this effect is unknown *in vivo*. This study suggests that administration of pamidronate could have a potential role in the adjuvant therapy of canine osteosarcoma.[41] However, recently it has been reported that administration of pamidronate in combination with carboplatin after limb amputation does not improve outcome.[42]

RADIATION THERAPY

Hypo-fractionated or palliative radiation therapy (RT) protocols as previously mentioned are considered the most effective treatment modality for the management of osteolytic bone pain in dogs diagnosed with OSA.[1] At the same time this treatment modality can delay progression of the local disease when surgery is not an option (owner declines surgery or the patient is not a good candidate). Most commonly used protocols consist of two to four-weekly fractions of 8-9Gy performed under general anaesthesia. Studies have shown that the alleviation of bone cancer pain was achieved in 50-92% of patients within two to 14 days, and the median time of disease control reported was 53 to 130 days.[43-45] High accumulative dosages of 57Gy obtained with hyperfractionated radiation protocols, or 70Gy with intraoperative extracorporeal radiation, combined with chemotherapeutic agents have shown to increase progression free intervals to 209-274 days.[2,44,46,47] Adverse late effects are not common with these protocols and median survival time described is 122-313 days with hypofractionated protocols, or 209-598 days with hyper fractionated or intraoperative protocols.[45,48] Radiation therapy in general, provides shorter progression free intervals for local disease and limited management of bone pain in comparison with surgery[49] but still being a more effective option than medical therapy for dogs with OSA that do not undergo surgical treatment.

SURGICAL TREATMENT

Surgical intervention with amputation or limb-sparing surgery, is the recommended treatment for OSA as it involves local excision of the primary tumour.

AMPUTATION

The goal of surgical treatment is complete excision of the primary tumour, achieving local control and removing the source of pain to improve the patient´s quality of life. Secondarily, removal of the primary tumour may limit further development of metastasis. The most common surgical approach to appendicular OSA is amputation of the affected limb. Numerous techniques for thoracic limb amputation have been described, but forequarter amputation (including the scapula) is most commonly performed. This technique ensures a radical or wide surgical margin, and provides best cosmetic results, by avoiding scapular muscle atrophy[50] (see Figure 7). Coxofemoral disarticulation is the preferred pelvic limb amputation technique because it is most likely to achieve

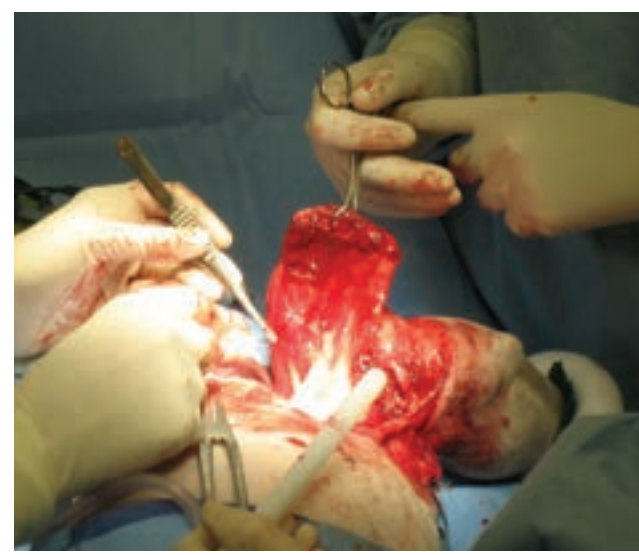

Figure 7: Right forequarter amputation, including the scapula. This bone is being held by a Backhaus clamp.

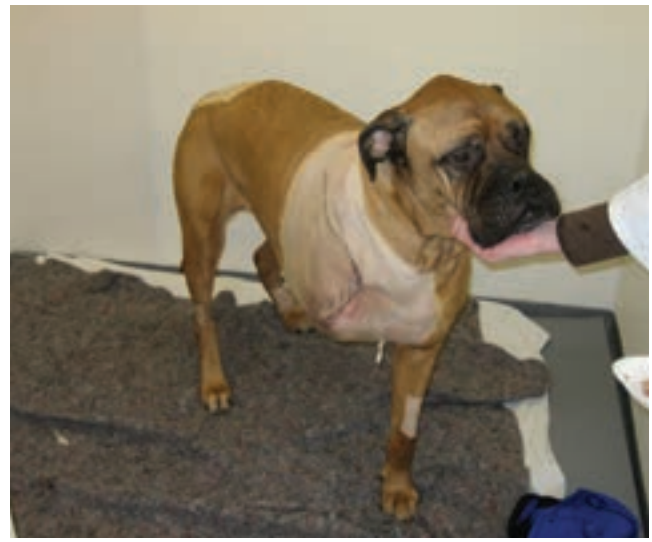

Figure 8a and 8b: Limb amputation is generally well tolerated, even in large and giant breed dogs. Patient on postoperative day 5 (a) and postoperative day 1 (b).

wide or radical surgical margins. For tumours located in the proximal femur, limb amputation with either an en block acetabulectomy or hemipelvectomy is preferred to achieve wide surgical excision.[1,50]

In most cases, a significant reduction in weight bearing occurs in the affected limb before surgery, so the patient generally adjusts easily to walking on three legs postoperatively. The median time expected for dogs to adapt following limb amputation is four weeks and client satisfaction is usually high.[51] Most patients are able to ambulate within 24 hours following the procedure[50,51] (see Figures 8a and 8b).

A thorough orthopaedic examination should be performed before amputation is considered. Rehabilitation is highly recommended postoperatively, especially in overweight dogs or those with osteoarthritis. It is important to mention that if osteoarthritis is significantly affecting the patient's mobility preoperatively, then amputation could be contraindicated.

Major complications associated with limb amputation, such as intraoperative air emboli, inadvertent penetration of the thoracic cavity during forequarter amputation, infection or phantom limb syndrome, are uncommon. Mild complications include intraoperative haemorrhage and postoperative seroma.[52] Behavioural changes have been noted in a third of dogs following amputation, including increased fear, aggression, anxiety and reduced dominance.[53] The authors routinely prescribe gabapentin postoperatively (in combination with NSAIDs and tramadol) to potentially prevent and/or decrease possible phantom limb pain.

Scapular OSA is uncommon. This condition can be treated by forequarter limb amputation or scapulectomy (subtotal or partial) with preservation of the limb. A partial scapulectomy involves removal of the proximal aspect of the scapula, with preservation of the acromion, the acromial head of the deltoideus muscle, and the distal portion of the infraspinatus and supraspinatus muscles. This would preserve more shoulder stability in comparison with a subtotal scapulectomy. A subtotal scapulectomy consists in removing most of the scapula while preserving the glenohumeral joint. Candidates for this surgery are patients with only proximal scapula affected, tumour not extending into surrounding soft tissues, glenohumeral joint not affected and when 2-3cm clean margins distal to the tumour are feasible.[1,50] After partial and subtotal scapulectomy, ambulation is not completely normal but it is pain-free and lameness is mechanical in origin.[1,53]

LIMB-SPARING SURGERY

Limb-sparing surgery aims to remove the primary bone tumour with preservation of the limb. The primary indication for limb-sparing surgery is when it is anticipated that the patient will not ambulate well if the limb was amputated. Dogs with osteosarcoma of the distal radius are the most amenable to limb sparing procedures. Limb function is preserved in 80% of dogs but other complications such as infection (30-50%), implant failure (20-40%) and local recurrence (15-25%) are common.[1,2,50] However, postoperative infection after limb-sparing surgery has been associated with longer survival times.[54] It is hypothesised that the immune system response against infection increases the capacity to recognise and eliminate tumoral cells, increasing survival times.

This surgical procedure is indicated when there is a concomitant neurological or orthopaedic condition, if there has been a previous amputation of another limb, and when owners decline amputation. Clients must be aware that postoperative care of these patients can be challenging especially due to the high risk of complications, the cost of the procedure and the postoperative management of their pet.[1,50] To increase the success rate of this procedure, candidates should fulfil some criteria: tumours should be well defined with minimal extension into adjacent soft tissues, there should not be evidence of pathologic fracture, and involvement of the bone should be less than 50% of its length.[50] The best candidates are those with the tumour located in the distal radius or ulna because pancarpal

arthrodesis has a better outcome.
There are several limb-sparing surgery techniques currently available. Selection of a particular technique will depend on the surgeon's preference and expertise, and the equipment available. These techniques are: cortical allograft, pasteurised autograft, bone transport osteogenesis, intraoperative extracorporal radiation, endoprothesis, vascular ulnar transposition and stereotactic radiosurgery. Nowadays, endoprothesis is the technique most commonly performed by oncological surgeons.[1,50] Except for extracorporal intraoperative radiation and stereotactic radiosurgery techniques, the others techniques include distal radius resection and arthrodesis of the carpal joint.

EXCISION OF THE DISTAL RADIUS

Planned limb-sparing surgical procedures may need to be converted into an amputation intraoperatively, and it is prudent to prepare the patient and surgical field accordingly; therefore it is recommended the whole limb is surgically prepared from the shoulder joint to the level of the digits. If a biopsy has been previously performed, the biopsy site must be excised en bloc.[50] The distal radius is excised with a minimum margin of 3cm from the proximal extent of the tumour. The medial ulna cortex is sectioned sagitally with the distal radius. But, if the tumour extends into the ulna, this is also excised with a bone saw proximal to the level of tumour. A study performed on cadaveric limbs demonstrated that there is no biomechanical advantage by preserving the ulna when an allograft or an endoprosthesis is used for limb-sparing surgery.[55] However, further in vivo studies should be performed. A bone marrow sample from the proximal bone segment is taken for histopathology in order to assess this margin end, and/or proximal margins of the sectioned piece should be inked and submitted for histopathological assessment.[1]

CORTICAL ALLOGRAFT

Cortical allograft was the first limb-sparing technique performed. It was described in 1989 and it has been modified over the years in order to reduce its potential complications. This technique uses a fresh frozen cortical allograft from an in-house or commercial bone bank. The allograft is sectioned to fit the space created by the excision of the radius, and it is stabilised by a dynamic compression plate or a locking plate. Once alignment of the limb and position of the bone plate and allograft are satisfactory, the screws are placed and the medullary cavity of the allograft is filled with antibiotic-impregnated bone cement. Disadvantages of this technique include a high infection rate (approximately 50%), risk of implant failure, and that it requires maintenance of a bone bank or purchase of frozen allograft. This led to development of other limb-sparing surgery techniques.[1,50]

ENDOPROSTHESIS

This technique involves the application of a limb-sparing plate, which will stabilise a 316L stainless steel endoprosthesis that is commercially available in two sizes: 98 or 122mm. It has been reported that shorter endoprothesis and the use of locking screws decrease the risk of implant failure.[55] Primary advantages of the endoprosthesis include availability of the implant and increased simplicity of surgery as an allograft does not need to be prepared. In comparison with the allograft technique, there is no apparent advantage as far as the incidence of complications, such as infection or implant failure, is concerned.[1,50]

PASTEURISED AUTOGRAFT

This technique was developed as an alternative to the difficulties associated with creating and maintaining a bone bank for the cortical allograft limb-sparing technique. The distal radial segment is excised and cleared of surrounding soft tissues, and tumour cells are eliminated by pasteurisation in sterile saline at 65°C for 40 minutes. Once the bone specimen is processed, the procedure is the same as the cortical allograft but the cavity is not filled by cement.[1] Complications are similar to the previously described although the risk of implant failure can be higher because the medullary cavity is not filled. An advantage found with this technique is that the autograft has better fitting in the defect.[1]

ULNAR AUTOGRAFT

The ipsilateral distal ulna can be used as a vascular rollover graft to replace the distal radial defect after tumour excision.[50,56] An advantage of this technique is that the vascularised autograft has a higher potential for healing and hypertrophy of the graft, which decreases the risk of future implant failure as well as infection. A disadvantage is that it will likely cause some limb shortening but with no significant clinical relevance.[56]

BONE TRANSPORT OSTEOGENESIS (BTO)

BTO is a technique resulting in gradual replacement of the excised distal radius with regenerated bone via a process called distraction osteogenesis.[50] Gradually, the new regenerated bone remodels into lamellar bone.[1]
The affected distal radius is excised and an osteotomy is also performed in the proximal bone segment, creating a transport segment. Distraction is performed using a five-ring external circular fixator around three days after surgery (latency period). The transport segment is moved across the gap at a rate of 1mm total per day, divided over two to four distractions daily, stimulating the formation of new bone behind it. When the bone segment is within 5mm of the radiocarpal bone, a cancellous bone graft is inserted into the docking site (mean time was 205 days).[1,57]
The newly formed bone is highly vascularised and it is therefore highly resistant to infection. In addition, once it has remodelled and healed, it does not require permanent internal fixation. The intensive labour required from both the owner and the surgeon is the major disadvantage. The client must commit to adequately perform the distraction two to four times daily, and to have frequent recheck radiographs, which is labour-intensive and expensive.[1,50,57]

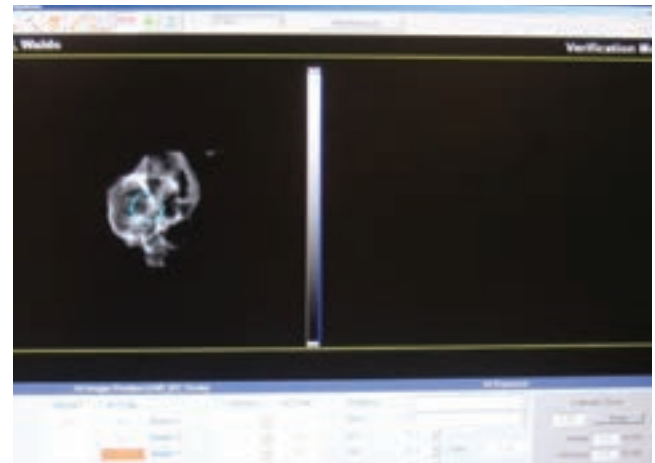

Figure 9a and 9b: Stereotactic radiosurgery: (a) Patient positioned with an oral attachment in order to perform advanced imaging (CT). (b) Computed tomography of the area to be irradiated.

Other BTO techniques have been described, such as double site BTO and transverse BTO, which offer shorter distraction times.[50]

INTRAOPERATIVE EXTRACORPOREAL RADIATION

This technique requires a single dose of 70Gy delivered to the tumoral bone segment previously osteotomised and isolated from the limb by rotating it cranially out of the antebrachium.

The biological effect of this protocol is equivalent to two to four times the same dosage in fractionated protocols of external radiation.[46] The main advantage of this technique over surgical limb-sparing, is that it preserves limb function when the tumour is affecting anatomic sites that otherwise would not be amenable to surgical limb-sparing techniques (eg. proximal humerus).[2] Complications are similar to the ones previously described. Selection criteria is required for these patients: candidates should have less than 50% of the bone affected and with good soft tissue coverage in order to improve the revascularisation.[50,46]

STEREOTACTIC RADIOSURGERY

Conventional RT is based on the use of fractioned protocols aiming to minimise damage to the surrounding soft tissues. Conversely, stereotactic radiosurgery (SRS) offers the ability to deliver high radiation doses (30Gy) to the tumour, with relative sparing of the surrounding normal tissues by use of image guidance (CT) and a sharp drop off in dose intensity[2,58] (see Figures 9a and 9b).

The main advantage of this technique is an intense biological effect on the tumour.[58] However the availability of this technique is limited to some referral centers in the US, and there is the risk of bone fracture after treatment.

Other methods and sites for limb-sparing surgery are ulnectomy – in tumours in the ulna – and the intraosseous transcutaneous amputation prosthesis (ITAP –partial amputation with exoprosthesis) described in 2010 in four dogs with distal limb tumours. The prosthesis consisted of two units: the endoprosthesis, an intraosseous transcutaneous amputation prosthesis, and the exoprosthesis, which is the weight-bearing attachment. This technique is still under study and the devices have been modified to increase bone integrity and decrease potential complications such as implant failure and infections.[59]

SURGERY OF GROSS METASTATIC DISEASE

After surgery (limb-sparing or amputation) and chemotherapy, eventually dogs can develop pulmonary metastasis. In order to maximise the probability of long survival periods, this procedure is considered only if the patient fits the following criteria: the primary tumour is in complete remission, preferably for a long progression-free interval (>300 days); one or two nodules only visible on plain thoracic radiographs; cancer only found in the lung (negative bone scan); and perhaps long doubling time (>30 days) with no new visible lesions within this time.[2,60]

CHEMOTHERAPY

Chemotherapy plays a role in the adjuvant treatment of canine appendicular osteosarcoma, once disease local

Chemotherapeutic	Protocol	Progression free interval	Median Survival time 1 & 2 year survival rate
Carboplatin	300mg/m² q 21 days for 4-6 cycles	137-256 days	277-307 days 36-37% 1 year 19-22% 2 years
Doxorubicin	30mg/m² q 14 days for 5 cycles	Not reported	366 days 35% 1 year 9% 2 years
Doxorubicin & Carboplatin (alternating)	Carboplatin 300mg/m² on day 1 and doxorubicin 30mg/m² on day 21; alternating at intervals of 21 days for three cycles.	227 days	320 days 48% 1 year 18% 2 years

Table 4. Outcome with common chemotherapeutic protocols for canine appendicular OSA.

control has been achieved, with the goal of delaying progression or development of metastatic disease. It has been demonstrated to significantly increase post-surgical survival times.[49] The most common chemotherapy drugs used in the past 25 years are cisplatin, carboplatin and doxorubicin (see Table 4).

Currently, cisplatin is not recommended because of its potential nephrotoxicity and high prevalence of emesis. Median survival times post-amputation followed by adjuvant chemotherapy range between 104 and 413 days, without significant difference between carboplatin and doxorubicin as single agents, with one year survival rates of 34-50%.[2,62-64] The two-year survival rate with carboplatin is (17%), higher than for doxorubicin (10%). Starting chemotherapy perioperatively, before surgery or three weeks after surgery does not seem to have an impact in outcome.[50] The chemotherapeutic protocol must be chosen based on the risk of adverse effects and costs. Doxorubicin has a higher rate of gastrointestinal effects and it has a potential for cumulative cardiac toxicity. Costs may vary depending on different companies but in general, carboplatin is an option with fewer adverse-effects.

CARBOPLATIN

A commonly used protocol with carboplatin involves a dosage of 300mg/m^2 IV q21 days for five to six treatments.[61] The administration is easy and through a slow intravenous bolus. Gastrointestinal toxicity is observed in less than 20% of patients. It is an immunosuppressive drug and the nadir for neutrophils should be monitored 10-14 days after administration. Carboplatin is excreted by the kidney. In patients with decreased glomerular filtration rate, the drug will take longer to be eliminated so these patients may present delayed neutropenia up to 21 days post-administration. A complete blood count should be performed before each treatment. Renal panel and urinalysis should be available at baseline plus at least monitoring of USG every six weeks during treatment. As in humans, dogs with decreased glomerular filtration rate (GFR), should be monitored to tailor the dosage of this chemotherapeutic drug. This can be done using the GFR test that is available in some laboratories including the Royal Veterinary College in London (http://www.rvc.ac.uk/pathology-and-diagnostic-laboratories/therapeutic-drug-monitoring). An empiric reduction of 10-25% and continued blood monitoring is often applied for patients with impaired urine concentration when GFR is not performed; to avoid excessive immunosuppression and be able to adhere to a scheduled regimen.

DOXORUBICIN

Doxorubicin is administered at a dosage of 30mg/m^2 IV q 14 days for five to six treatments.[62] Animals less than 15kg of body weight should receive a dosage of 1mg/kg. Doxorubicin is moderately immunosuppressive and the nadir of neutrophils must be monitored one week post-treatment. This drug is usually affordable and provides similar outcome than carboplatin. The main adverse effect reported is gastrointestinal toxicity, with a prevalence of 40-60%. Prophylactic therapy with gastric protectants (ranitidine or omeprazole) and antiemetics (maropitant or ondansetron) for three to four days post-treatment are recommended. The author also prescribes metronidazol (10mg/kg PO q12h) for those cases developing diarrhoea (usually colitis secondary to doxorubicin associated cytotoxic effect on GI epithelium). Doxorubicin induces cumulative cardiotoxicity in the cardiac myocytes, which rarely becomes clinically relevant below total doses of 180mg/m^2 (equivalent to six doses of doxorubicin at 30mg/m^2). Therefore, patients with normal cardiac function at baseline would rarely develop dilated cardiomyopathy with the set course of five to six treatments. Breeds with cardiac disease predisposition, should be assessed for potential subclinical cardiac disease at baseline and on a case-by-case basis decided if further monitoring is warranted. Alternatively, treatment could consist of carboplatin. Doxorubicin is highly vesicant and administration must be rigorously intravenous with a constant rate infusion over 25 minutes and under constant monitoring. To minimise tisular damage in the potential case of extravasation, the authors dilute the drug to be administered with saline in a 50ml syringe. Administration is performed through an intravenous catheter with a safe system for chemotherapy administration (Phaseal), checking patency several times during the 25-minute administration, which is performed by a trained operator while the dog is restrained by another operator in a quiet, chemotherapy-administration allocated space.

COMBINATION OF CHEMOTHERAPY AGENTS

Alternating combinations of carboplatin and doxorubicin have been described with no significant differences in patient outcome to single agent protocols. Simultaneous administration of these drugs (lower dosage) has shown similar outcomes but higher toxicity.[63,64] Nowadays, protocols with single chemotherapy agents are the preferred option.

TOCERANIB

A group of 23 dogs with OSA pulmonary metastasis, developed after primary tumour resection and postoperative adjuvant chemotherapy were included in a study reporting biological activity of the tyrosine kinase inhibitor toceranib on solid tumours. The drug was administered at a median dose of 2.8mg/kg PO, three times per week (Monday/Wednesday/Friday) and clinical benefit was observed in 11 dogs with metastatic OSA, 10 with stable disease and one with partial response.[65] On the other hand, toceranib and metronomic therapy (ciclophosphamide and piroxicam) did not provide different progression free survivals for canine OSA than using metronomic therapy alone after amputation and four treatments of carboplatin; median progression-free survival of 215 versus 233 days and one-year survival rates of 35% versus 38% respectively for each group.[66]

REFERENCES AVAILABLE ONLINE