Osteosarcoma in dogs

Analysis of patients between 1960 and 2012 in the Netherlands

Drs. W. Verschoor
3156265
January 2013

Supervisor:
Prof. Dr. J. Kirpensteijn
Department of Clinical Sciences of Companion Animals
Faculty of Veterinary Medicine, Utrecht University

Summary

Osteosarcoma is the most common primary bone tumor in dogs and forms metastases to mostly the lungs in early course of the disease. Diagnosis is made by biopsy and histologic evaluation. There are a lot of different treatment protocols with different therapy modalities (e.g. surgery, chemotherapy, radiotherapy, medical management). There are several negative prognostic factors for osteosarcoma. This is a retrospective case study to determine prognostic factors for the survival time and metastasis free interval in dogs with osteosarcoma. 298 dogs presented at different clinics in the Netherlands with histologically confirmed osteosarcoma are used. The individual data of the patients are inserted into a database. The survival time and metastasis free interval of different groups is compared with the Kaplan-Meier curve and logrank test. The significant differences with the Kaplan-Meier analysis are investigated with the cox proportional hazard regression model.

The metastasis free interval is significant negative influenced in the univariate analysis by breed, axial localization, localization in the proximal humerus, leukocytosis and high neutrophil counts, no treatment and marginal resection. Localization in the distal tibia has a significant positive influence on the metastasis free interval in the univariate analysis. In the multivariate analysis only localization in the humerus, neutrophil count and preoperative presence of metastasis significantly influence the metastasis free interval. The survival time is significant negative influenced with the Kaplan-Meier method by localization in the axial skeleton, in the proximal humerus and localization in the middle, high leucocytes and neutrophils, the presence of metastasis and incomplete resection of the osteosarcoma. Localization in the distal radius, perform surgery, perform chemotherapy and the combination of amputation and chemotherapy have a significant positive influence on the survival time by the Kaplan-Meier method. In the cox regression analysis only neutrophil and thrombocyte count significantly influenced the survival time.

Contents

Introduction

Osteosarcoma is a malignant bone tumor mostly with micrometastasis at diagnosis[3,5,14,15,16,17,20,21,26] With surgery and chemotherapy the survival time can be approximately one year with survival rates for 1, 2 and 3 years of respectively 33-68%, 25-26% and 10%.[7,13] There are different prognostic factors for osteosarcoma identified in literature, age at diagnosis, weight, localization, tumor size, serum alkaline phosphatase, histological characteristics, detectable metastases and therapy. I expect I can identify the same prognostic factors in dogs presented to veterinary clinics in the Netherlands. The hypothesis is:

> *The signalment of the dog, diagnostic results and treatment influence the disease free interval and survival time of dogs with osteosarcoma.*

The goal of this research project is to complete a database with dogs diagnosed histologically with osteosarcoma. The patients are diagnosed and treated in the Netherlands at different clinics mostly at the university clinic for companion animals in Utrecht. Also dogs from some other clinics are included. In this database information about the dog, diagnostic tests, therapy and survival is present. This database will be incorporated in a larger international database. The investigators want to predict the best possible treatment and prognosis in individual animals. The veterinarians can give the owners a good evidence based advice about the treatment and prognosis of their dog with osteosarcoma.

Osteosarcoma

Osteosarcoma accounts for 5-6% of all canine neoplasms,[3,8] it is the most common primary bone tumor 80-98% of all bone neoplasms.[1,3,5,8,10,14,15,16,17,21,25,26] Osteosarcoma is highly malignant, local invasive in bone and surrounding soft tissues. This tumor forms metastases in early clinical course,[3,4,5,10,14,15,16,17,20,21,26] at time of diagnosis 90% - 95% of the dogs have already micrometastasis.[18,27] Metastasis occurs by hematogenous spread,[20,25] they can be found in lung, bone, visceral organs, brain, subcutis, skin and all other tissues.[16,17,20] Lymph nodes are involved less commonly, 4.4-9.0%.[20,25]

Osteosarcoma is most frequently (75%) found in the appendicular skeleton,[8,10,17,20] but can also affects the axial skeleton (24%) and rarely soft tissues (1%).[4,8,19,20] Osteosarcoma occurs mostly from metaphysis in distal radius, proximal humerus, distal femur, proximal and distal tibia.[4,8,10,19,20] The most common axial localizations of osteosarcoma are mandibular (27%), maxilla (22%), spine (15%), cranium (14%) and ribs (10%).[10] Osteosarcoma develops more frequently at the forelimbs than the hind limbs,[8,23] it could be related to the fact the forelimbs support more weight (60%) than the hind limbs (40%).[23]

Large and giant dogs, greater than 20 kg weight, are most commonly affected.[1,4,5,6,8,17,20,26] Grey hound, Rottweiler, Great Dane, Saint Bernard, Doberman Pincher, Irish Setter, Golden retriever, German Shepherd, Borzoi, Scottish deerhound and Irish wolfhound have an increased risk for developing osteosarcoma.[6,20,23] The risk for osteosarcoma is significant higher in Irish Wolfhound and Leonberger than in Newfoundland and Labrador retriever. [1] A familial pattern of osteosarcoma is observed in the Saint Bernard, Rottweiler and Scottish Deerhound.[20]

Osteosarcoma is more often seen in male than in female dogs (ratio 1.5: 1).[6,15,20] There is evidence that neutered dogs have twice the risk to develop osteosarcoma.[20,23]

The age of presentation has two peaks, the first small peak around 18-24 months, the second and largest peak round 7 to 10 years.[1,4,5,6,8,20,23] This first peak corresponds with a human peak at late puberty. A possible explanation for this moment is skeletal growth as a possible etiology for osteosarcoma.[1] Dogs with osteosarcoma in ribs tend to be younger 5 – 9 years.

Possible etiology

Exposure to ionizing radiation can induce osteosarcoma in experimental and therapeutic situations. In experimental setting plutonium dioxide aerosols, IV injection with monomeric ^{239}plutonium citrate and ^{241}Americum injection can induce osteosarcoma in beagles. Reports of dogs (3/87) which developed osteosarcoma 1.7 – 5 years after completing radiotherapy are present.[6,20]

Some metallic implants are associated with osteosarcoma development. This can be explained by the direct effect of the implants, instability, corrosion of the implant and infection. It could also be coincidence. Other underlying disease are also meant as cause, like bone infarct, orthopedic surgery and osteochondrosis dissecans.[20] Miniature Schnauzers have a high incidence of dogs developing osteosarcoma secondary to bone infarcts. The pathogenesis of this is unknown.[6]

In Rottweilers gonadal hormone exposure is meant as etiology. Rottweilers neutered before 1 year old

have a higher risk for bone sarcoma. It is independent of adult weight or height.[6]

Genetic alterations are also a possible etiology. Neoplastic cells have growth advantage by altering the regulation of cell proliferation by overexpression of growth factors and their receptors and promoting of angiogenesis.[26] Mutations in the p53 tumor suppressor genes were observed in 40.7% - 47% of the canine osteosarcoma. Expression alterations can lead to high aggressive tumor behavior and high tumor grade. Other genetic mutations meant in canine osteosarcoma are overexpression of erb-B2 gene (coding for human epidermal growth factor receptor 2); deletions and mutations and downregulation of the PTEN tumor suppressor gene. An overexpression of PDGF and expression of PDGF receptor is noticed in canine osteosarcoma. This suggests an autocrine growth stimulating effect in canine osteosarcoma.[6] Matrix metalloproteinases 2 and 9 and ezrin are expressed in osteosarcoma; these proteins can contribute to local progression and metastatic spread.[20]

Clinical presentation

Patients will be presented mostly with minor trauma caused lameness. The lameness is usually progressive, it starts intermittent and mild and becomes persistent and severe. The lameness can also be acute non-weight bearing because of a pathological fracture.[4,5,6,8,20] The lameness is mostly seen in one limb, it rarely affects more limbs.[8] A minor complain is swelling, which is mostly firm and painful by palpation.[4,5,6,8,20] The pain is caused by microfractures and periosteal disruption through bone lysis and tumor expansion. In an advanced situation the patient can be presented with a large swelling, non-weight-bearing lameness, muscle atrophy and cancer cachexia.[4] Axial osteosarcoma can be present a few days to 2 years until the dog will be presented to a veterinarian. The clinical signs of axial osteosarcoma depend of the location of the osteosarcoma, e.g. swelling, anorexia, dysphagia, exophthalmos, dyspnea, epistaxis and paraparesis.[6] A good clinical examination is necessary.[8]

Metastases can be found in bones, lungs, sub cutis, lymph nodes and other visceral organs.[3,24] At presentation less than 5-15% of the dogs have radiographically detectable metastasis in lungs or bones.[3,10,25] Lung metastases can be found in 2/3 of the dogs within 120 days after surgery. In fewer cases metastases were found in the bone.[24] Extraskeletal osteosarcoma tends to spread more to liver and lymph nodes as skeletal osteosarcoma.[6] Pulmonary metastases give signs of respiratory disease, e.g. coughing and dyspnea. Bone metastases mostly develop in the spine and results in (para)paresis.[4] The ultimate cause of death in patients with appendicular osteosarcoma is mostly the development of pulmonary metastases.[3,4] The cause of death in patients with osteosarcoma in the axial skeleton is mostly recurrence of the tumor after removal.[6]

Radiographs of the affected limb and thorax and abdominal ultrasound are necessary for further work up of the patient.[8] In 67/118 dogs alterations in the abdominal ultrasound are noticed. In 3/118 dogs metastases were found in the kidney, liver and iliac lymph node. Two of these dogs didn't have metastases in bone or lungs; the other dog has metastasis in the lung.[25] Regional lymph nodes should be aspirated if enlarged. Patients, whose owner considering chemotherapy, should have a complete blood count, serum chemistry and urine analysis, because of the excretion of the chemotherapeutics by the kidneys and myelosuppression. An electrocardiogram and echocardiogram are necessary due to the

cardiotoxicity of doxorubicin.[8]

Diagnosis

The clinical diagnosis is made on base of the signalment, history and radiographs.[5,13,20]

Diagnostic imaging

The most common changes on radiographs are osteoproductive and osteolytic lesions and soft tissue swelling with calcification.[4,5,6,8,17] The osteosarcoma originates from the medulla and expands to the surroundings. In their expansion they change the cortex, 70% of the osteosarcoma have cortical changes. The 4 cortical changes are erosion, destruction, expansion and punctual lysis. Osteosarcoma rarely affects joints and mostly affects metaphysis. Differential diagnoses for the previous described lesions are primary bone tumor, metastatic bone tumor, systemic mycosis and osteomyelitis. Radiographs are no confession of the diagnosis osteosarcoma and histopathology is required. [4,8] Osteosarcoma in the spine can be diagnosed with myelography, scintigraphy, computed tomography or magnetic resonance imaging.[6]

With different other imaging techniques the size of the osteosarcoma can be measured. The size of the tumor is overestimated with scintigraphy, magnetic resonance imaging and computed tomography compared with histology.[12] Magnetic resonance imaging was the most accurate modality for estimation of tumor size.[6]

Thoracic radiographs are performed for the evaluation of metastases in the lungs.[4,8] Three views are necessary for this purpose, lateral left – right, lateral right – left and ventrodorsal.[4] Metastasis can be seen if greater than 6-8 mm.[8] Only 5-10% of the patients are positive for these metastasis at presentation.[4,6,20] With computed tomography smaller lung lesions can be detected. Bone metastases can be found with radiography and scintigraphy, scintigraphy is more sensitive but has a low specificity. Histological evaluation of the lesion is necessary to prove osteosarcoma metastases.[6]

Laboratory findings

Elevated serum alkaline phosphatase can be found. It is elevated because of the increased osteoblastic activity. Elevated serum alkaline phosphatase can indicate a more aggressive osteosarcoma[22] and is associated with a shortened disease free interval and survival time.[10]
Chemotherapy can give myelosuppression as side effect, it is important to perform a complete blood count before chemotherapy is given. Chemotherapy should be delayed for a couple of days when neutrophil count is < 2.0 10^9 cells/L.

Cytology

Fine needle aspiration (FNA) biopsy for cytology can be used.[20,21] In the biopsy immature mesenchymal cells with intracytoplasmatic or extracellular osteoid are found.[6] The accuracy of FNA for bone lesions is 69-96%. The FNA biopsy has advantages above a histologic biopsy, it is less painful, takes less time to perform and gives a rapid diagnosis. The disadvantages are the risk of hypocellular sample and the difficulty to differentiate between neoplastic and non-neoplastic cells[21] and the different types of bone tumors, fibrosarcoma, chondrosarcoma and osteosarcoma.[21,24] Differentiation between bone tumor and fungal or bacterial osteomyelitis can be made with a FNA biopsy.[6] Differentiation between the different

sarcomas is necessary for the therapy and prognosis. The golden standard for the diagnosis osteosarcoma is a histologic biopsy.[21]

Histology

The definitive diagnosis is made by histological examination of the lesion, a histological biopsy can be performed.[4,5,8,13] The accuracy of a histological biopsy and evaluation for the diagnosis of osteosarcoma is 82-94%.[21] Anesthesia or sedation is required for performing a histological biopsy because it is painful.[6,14] Echo or computed tomographic guidance are used to optimize bone biopsies.[20,21,24] The larger the sample size, the more chance of finding the osteosarcoma. But known disadvantages are the risk of hematoma formation, infection, local seeding of the neoplastic cells and pathologic fracture.[8,21] The biopsy can preformed via closed (Michelle's trephine or Jamshidi needle) or open techniques.[6,8,20] Michelle's trephine has a higher diagnostic accuracy (93.8%), but also a higher risk of a pathologic fracture compared to the Jamshidi needle. A small incision is made with blade eleven. The biopsy device is inserted through this incision and the bone sample can be taken. When a high suspicion of a neoplasm is present, amputation can be used as technique for histologic sampling. Because the differential diagnosis, samples can also be collected for bacterial and fungal culturing. A differentiation between fibrosarcoma, chondrosarcoma and osteosarcoma is possible with a histologic biopsy.[24]

Osteosarcoma is a malignant neoplasm of osteoblasts and form (immature) bone and/or osteoid.[14,19] Canine osteosarcoma is divided on basis of activity and cell type into different subclasses osteoblastic, fibroblastic, chondroblastic, teleangectatic and poor differentiated.[6,8,14,19] Teleangectatic osteosarcoma is composed of a blood filled cyst with a lining of neoplastic osteoblasts.[19] There are also other ways of classification.[19]

Osteosarcoma is an invasive and aggressive neoplasm and causes local skeletal destruction.[5] The aggressiveness can be better assessed with histologic grading. High grade osteosarcoma have a high cell : matrix ratio, poor differentiation, high degree of pleiomorphism, high number of mitoses, vascular invasion of neoplastic cells and high amount of necrosis.[6,14] There is a site dependent aggressiveness between different sites of osteosarcoma. Osteosarcoma in the mandibula tends to metastasize less than osteosarcoma at other sites.[6] High grade neoplasms have a significant shortened survival time and disease free interval.[6,14]

Osteoblasts can be differentiated in cytological and histological samples with an alkaline phosphatase staining. Alkaline phosphatase is a membrane-bound enzyme in most mammalian organs and present on canine osteoblasts. The stain cannot differentiate neoplastic and normal osteoblasts. Therefore it is important to evaluate the sample for malignancy characteristics before this alkaline phosphatase staining. The staining has a sensitivity of 100% and a specificity of 89% for osteosarcoma. In cytology this staining can differentiate between osteosarcoma and other sarcoma. In a histological sample the staining can differentiate osteoblasts and connective tissue.[20,21] Figure 1 shows a slide of an osteosarcoma with the alkaline phosphatase staining.[21]

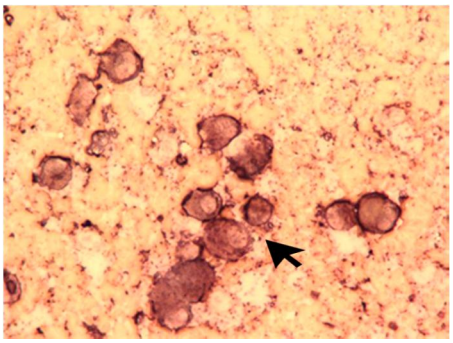

Figure 1 Osteosarcoma slide with the alkaline phosphatase staining[21]

Therapy

There are several ways to treat osteosarcomas, surgery, radiotherapy, chemotherapy, medication and immunotherapy. These therapies can be combined with each other to an optimal multimodal therapy. The combination of therapies can contribute to a significant higher survival time.[5]

Surgery

Local removal of axial osteosarcoma can be performed by resection of the tumor with proper margins.[6] Local removal of appendicular osteosarcoma can be performed by amputation or limb-sparing surgery,[4,6,8,20] surgical intervention prevents a pathologic fracture[20] and eliminates pain.[8,20] Amputation is well tolerated by dogs, even by the large breeds. Contra-indications are obesity and other concurrent neurologic or orthopedic diseases.[4,8,20,29] Forequarter amputation is recommended for osteosarcoma in the forelimbs for a cosmetic and functional result. Amputation of the hind limbs can be performed by osteotomy through the proximal third of the femur, disarticulation in the coxofemoral joint. When the osteosarcoma is present in the proximal femur or the pelvis excision by hemipelvectomy is needed.[4,8,29] Recurrence can occur if amputation is done with improperly soft tissue margins.[4]

Limb-sparing surgery is possible in order to save the dog's leg and remove the osteosarcoma. It results in a functional, pain free limb.[8] It can be used when amputation is not an option due to the owner's preference or neurologic or orthopedic disease.[8,29] Limb-sparing surgery is a more expensive and a more intensive treatment than amputation.[29] The dog should have a solitary bone lesion, which affects less than 50% of the bone.[6,8,29] Good results are reported with limb-sparing surgery of the distal radius,[6,8,20] ulna and proximal humerus. Osteosarcoma around the scapulohumoral, coxofemoral and tarsal joint result in a poor to fair limb function after limb-sparing surgery.[8] Places with limb-sparing surgery, other than the distal radius, can give more complications and poor limb function, but the reported results are inconclusive.[20] Preoperative chemotherapy, radiotherapy or a combination of both can be used to shrink the tumor size. Marginal resection is possible with a smaller tumor size.[29] Most tumor necrosis is reached with radiotherapy or by IA cisplatin.[6] An alternative is intraoperative treatment with open-cell polylactic acid containing cisplatin (OPLA-Pt). The use of OPLA-Pt reduces tumor recurrence.[29]

The resection margins needs to be determined preoperative by computed tomography or magnetic resonance imaging.[8] First resection of the osteosarcoma is performed with margins of at least 3 cm.[8,20] Disarticulation of the adjacent joint is mostly necessary for tumor removal, because osteosarcoma mostly arise from the metaphysis.[29] It is important to excise the biopsy tract in patients with an earlier bone biopsy for confirmation of the diagnosis osteosarcoma.[8] The created defect may be filled with

frozen, sterile, fresh cortical allograft bone or with the neoplastic bone after pasteurization, autoclavation or irradiation and implants.[6,8,20,29] The parts of bone and implants can be fixed with a boneplate and screws. Arthrodesis of the adjacent joint needs to be performed with long dynamic compression plate and screws.[6,8,20,29] With a small osteosarcoma when one-quarter joint replacement is necessary osteoarticular allografts can be used.[29] The leg is bandaged postoperative to allow healing and reduce swelling and automutilation.[8] Limb function returned to good or excellent in 69% of the dogs[8] after 30-60 days postoperative.[29] Local tumor control was accomplished in 79%.[8] Local recurrence of osteosarcoma is seen in 24% to 60% of the patients.[12]

Complications of this technique are infection (31%), recurrence of the neoplasm (24%) and loosening of the implants.[8,20,29] Dogs treated with OPLA-Pt and postoperative IV cisplatin have less recurrence of the osteosarcoma, 15%.[29] The infection risk is increased by a surgical site with a neoplasm with diminished local defenses, decreased blood supply, lack of tissue to cover the implants, implantation of foreign materials, chemotherapy and radiotherapy.[29] Infection can lead to breakdown of the orthopedic construction and severe lameness. The infection rate is 44%. Infection can be controlled by administration of appropriate antibiotics, removal of the infected implants or amputation. Infection gives an improved survival time.[29] When the osteosarcoma reoccurs, a second limb-sparing surgery or amputation can be performed, the survival time isn't different for dogs with no recurrence or with a second surgery.[29] Implant loosening often occurs with infection. Screws can be removed under local anesthesia by incising over the screw head and withdraw them with a screwdriver. Fractured plates can be replaced with a new plate.[29]

Only surgery is palliative, the survival can be extended with adjuvant chemotherapy. No difference is found between amputation and limb-sparing surgery both in combination with chemotherapy in survival time.[8,14,20,29] Chemotherapy is mostly ineffective for radiographic detectable pulmonary metastases. Metastasectomy can be performed with clinical detectable metastases. Therefor the primary tumor needs to be in complete and prolonged remission. Only one or two radiographic detectable metastases in the lungs may be present and no metastases at other locations. Also no other concurrent life-limiting disease may be present. Metastasectomy in suitable patients can contribute to a prolonged survival.[6]

Chemotherapy

Amputation or limb-sparing surgery can be used in combination with chemotherapy to extend the survival.[8,14,20,24] Chemotherapy is the therapy for micrometastases, but it isn't curative.[4] The goal of chemotherapy by dogs with osteosarcoma is elongated life with a good quality and minimal side effects.[4] Different chemotherapy protocols with (a combination of) doxorubicin, cisplatin, carboplatin and lobaplatin are used.[2,8,20,24] Theoretically a combination of two different acting chemotherapeutics will improve the effect on the tumor cells.[2,16] The most optimal protocol and time to start with chemotherapy is unknown. A lot different protocols are reported.[2,4,6,16]

No advantage is proven for early postoperative chemotherapy, so chemotherapy can wait until the patient is recovered from surgery and the early wound healing is finished.[20] Less side effects are seen in dogs which received chemotherapy 10 days after surgery than in dogs 2 days after surgery.[7] Berg advises to start with chemotherapy a week postoperatively.[4] In dogs with osteosarcoma and high alkaline

phosphatase chemotherapy have no - little effect. Dogs with alkaline phosphatase between references benefit from adjuvant chemotherapy.[24] Chemotherapy is less effective against macroscopic metastasis. Surgical removal of pulmonary metastases improved the survival time.[20]

Cisplatin contains platinum and acts as bifunctional alkylating drug.[8] It binds to DNA and produce cross linkages.[4] Cisplatin is excreted by the kidneys[8] and is irreversible and cumulative renal toxic.[4] It is contraindicated by dogs with a poor kidney function. Cisplatin should be given slowly intravenously in a doses of 60 to 70 mg/m^2.[8] The adverse effects of cisplatin are anorexia, vomiting, diarrhea nephrotoxicity and myelosuppression.[2,4,8,24] Vomiting occurs most frequently within 6 hours after administration and can be reduced by giving an antiemetic (e.g. butorphanol). The nephrotoxicity can be diminished by aggressive diuresis with sodium chloride. The urine is 24 to 48 hours after administration polluted and should be treated as chemotherapeutic wasted.

Carboplatin is a cisplatin analog with less nephrotoxiciteit. Carboplatin should be administrated 300 mg/m^2 every 3 weeks. It can be used solely or in combination with doxorubicin.
Like cisplatin, carboplatin is excreted by the kidneys. A good kidney function need to present, but carboplatin does not need diuresis.[8,24]

Doxorubicin is a drug from the class anthracyclines,[8] it inserts in the DNA and affects the DNA, RNA and protein synthesis.[4] It can be given at 30 mg/m^2 every 3 weeks for 5 - 6 treatments. This drug should be given strictly intravenous because of severe tissue ulceration and necrosis when given outside the blood vessels.[4,8] A catheter should be placed for administration. Doxorubicin should be given slowly over 20 minutes to reduce the risk on hypersensitivity and cardiac arrhythmias. Preadministration of diphenhydramine or dexamethasone can also reduce these risks.[8] Doxorubicin gives gastrointestinal side effects (e.g. hemorrhagic colitis), bone marrow suppression and myocardial effects, such as dilated cardiomyopathy.[2,4] For development of cardiomyopathy a cumulative dose of 180-240 mg/m^2 is necessary, but predisposed breeds can develop a cardiomyopathy at a lower dosis.[8,16] In 50% of the dogs threated with doxorubicin a neutropenia of 1000-3000 cells/µl is present, in 9% of the dogs the neutrophils are < 1000 cells/µl. Doxorubicin can give a severe neutropenia and severe gastrointestinal side effects in dogs with MDR-1 gene mutation.[16] Lower doses of doxorubicin gives less severe side effects.[7]

A local technique is available to deliver high doses of chemotherapy at a special location, open-cell polyacetic acid impregnated with cisplatin. This can be implanted at surgery and it slowly releases cisplatin. The local concentration increases a 50-fold in comparison with intravenous injected cisplatin. The tumor cells are a longer time exposed to cisplatin. The peak concentration of cisplatin in blood decrease, which give less side effects.[27] Another local technique is the local infusion of carboplatin subcutaneous. The mean survival time was 365 days, which didn't significantly differ from the survival time with intravenous administration of carboplatin. Also the side effects didn't differ significantly in severity and amount between intravenous and subcutaneous administration of carboplatin.[27]

Ifosfamide is an alkylating agent with non-specific cytotoxicity on the cell cycle. It is an active single agent chemotherapy. Ifosfamide is successfully used in veterinary medicine by different metastatic

tumors, the given doses is 350-375 mg/m^2 every 21 days.[3] Ifosfamide was well tolerated by the patients. The patients were dogs previous treated for osteosarcoma and now developing measurable metastasis. The dogs received ifosfamide treatment, the doses was 375-425 mg/m^2 every 3 weeks. Most dogs developed a neutropenia, one (1/19) dog has a grade IV Neutropenia with sepsis. Some dogs received symptomatic therapy for vomiting and diarrhea. One dog (1/19) developed severe renal problems (azotemia and proteinuria). The response rate was 2/17, 1 dog with a complete response and the other with a partial response. At the other dogs by 2 the disease was stable and by 13 dogs the disease progress despite the ifosfamide treatment. The duration of the treatment was 127 days for partial and complete response and 45 and 158 for stable disease. The median survival was 95 days.[3]

Immunotherapy

Immunotherapy is combined with chemotherapy, the anti-tumor effects are enlarged without increased toxicity. Muramyl dipeptide (MDP) activates macrophages and monocytes to recognize and induce destruction of neoplastic cells.[18,20] Muramyl tripeptide-phosphatidylethanolamine (MTP-PE) is the synthetic derivate of MDP. This MTP-PE can be liposome encapsulated (L-MTP-PE) which elongates the half-life time in the circulation and facilitates the delivery to monocytes and macrophages. This L-MTP-PE activates macrophages and monocytes to destroy chemotherapy resistant cancer cells in vitro. In human treated with L-MTP-PE peripheral fibrosis surrounding lung metastasis was noticed. Also infiltration of tumor with mononuclear cells was recognized, with immunohistochemistry is confirmed these mononuclear cells were activated macrophages. Side effect of this immunotherapy is elevation of the body temperature with 1°C - 2°C.[18]

L-MTP-PE is experimentally used in combination with surgery and chemotherapy (cisplatin and doxorubicin). [18,20] L-MTP-PE is used to treat micrometastasis after surgery. L-MTP-PE is given as monotherapy after amputation for 8 weeks twice a week. The dogs treated with L-MTP-PE have a survival time of 7.3 months compared with 3 months without L-MTP-PE treatment. In another study L-MTP-PE is given in the same dosage scheme after surgery and chemotherapy with cisplatin (4 dosages with 28 days interval). Dogs treated with L-MTP-PE have a survival time of 14,4 months compared with 9.8 months for dogs which receive a placebo. In a study L-MTP-PE and chemotherapy with cisplatin were given after surgery. In this study no significant difference in survival time was found.[18] The combination of L-MTP-PE and doxorubicin gives an induced destruction of osteosarcoma cells by pulmonary alveolar macrophages.[6,20]

Radiation therapy

Palliative radiation is another possible therapy, without surgery.[8,17] Metastases are no contraindication for palliative radiation therapy, as long as the dog doesn't have signs from these metastases. A contraindication for palliative radiation is a pathologic fracture.[8,17] The goal is to diminish the pain and improve the quality of life.[6,8,17] In 74% - 96% of the patients, the pain decreases. The irradiation kills tumor and inflammatory cells. The result of this therapy is more mature bone, with less chance on pathologic fractures by osteosarcoma.[8,17] The optimal dose and timing is unknown. The used protocol is a larger dose given once a week for 2-4 weeks.[17] Other common protocols are 8 Gray every week for 3 weeks or 10 Gray every week for 2 weeks.[8] The radiation field should include the tumor (based on

earlier imaging results) and 2 to 3 cm around the tumor.[8] Improvement in limb function is reached in 1-60 days in 50-93% of the treated dogs.[6,8,17,20] This improvement goes on for 53 – 180 days.[17,20] Side effects are erythema and desquamation, but these are mostly absent to minimal. Most dogs develop a hair color change after radiation therapy.[17]

A combination of palliative radiation and chemotherapy with platinum can help in decreasing the pain and improve the lameness more than only radiation therapy.[17,20] Radiation therapy can also be used in combination with surgery, but intraoperative radiation (70 Gy) gives complications in 69% of the patients. The complications include infection, recurrence, implant failure and fracture.[20]

The use of Samarium (Sm) is described in dogs for treatment of appendicular and axial osteosarcoma. $_{153}$Sm emits β and γ particles, the β particles provide a therapeutic effect. $_{153}$Sm, is bound to ethylene-diamino-tetramethylene-phosphate (EDTMP) and is given intravenously. Metabolic active bone takes it up rapidly. It is given in a bolus of 37 MBq/kg and it may be repeated after 1-19 weeks. Myelosuppression until 4 weeks after given doses is present as main side effect.[6]

Medical Management

Pain medication can be used as amputation is no option. It is important to know all the medication of the dog to prevent drug interactions.[8] NSAIDs are possible for relieving the pain, such as carprofen 2mg/kg BID.[8,17] Owners must be warranted for the side effects as vomiting and dark or bloody feces, if they occur the owner has to stop the medication immediately.[8] Osteosarcoma express COX-2, this can be inhibited through NSAIDs. NSAIDs have antiangiogenic effect and retard the growth of the osteosarcoma. It is debatable or NSAIDS should be used as monotherapy, resection of the osteosarcoma result in a longer survival.[14] When NSAIDS are not tolerated by the patient, prednisone of prednisolone can be used in anti-inflammatory doses (30 mg/m^2). These drugs reduce the inflammation and thereby the pain. The side effects are polyphagia, polydipsia, polyuria and immunosuppression. The owners may not stop this medication at once.[8]

Opioids can also be used for pain relief, they can be given in combination with NSAIDS.[17] Fentanyl patches can be used at home, a patch provides pain relief for approximately three days. The size of the patch depends on the bodyweight. The skin should be shaved and cleaned with alcohol before application of the patch. The owner needs to check the patch, if it's loosen the owner should remove it to prevent ingestion.[8]

Biphosphonates inhibit bone resorption by inhibiting the osteoclast activity and they inhibit tumor growth by inhibiting the proliferation, angiogenesis and inducing apoptosis of the tumor cells.[6,8,28] Bone pain can be attributed to dysregulated bone resorption by osteoclasts.[9] So bisphosphonates can also relieve bone pain.[9,28] Aminobiphosphonates interfere with GTPase, involved in cell signaling, they decrease osteoclast activity and induce apoptosis of osteoclasts. In the research protocol of Fan, there was no statistical difference in pain relief between a treatment protocol with biphosphonates and a placebo.[9]

Prognosis

Variables with negative influence on the prognosis are age at diagnosis, large primary neoplasm, high

body weight (<34-40 kg and > 34-40 kg), increased serum alkaline phosphatase, proteinuria, neoplasm in proximal humerus, rib or scapula, high histological grade, telangiectatic subtype, delayed surgery, incomplete surgical removal of the osteosarcoma, delayed chemotherapy and metastasis (in lymph nodes or other locations).[4,5,6,8,11,14,16,22,24,27] About the age at diagnosis is discussion, some authors indicate this worsen the prognosis with an older dog, [4,8,11] another stated there is no influence[24] and others state a low age at presentation worsen the prognosis.[5,20] Dogs with lymph node metastasis get less chemotherapy, this can explain the worsen prognosis.[11] Tumor localization in radius gives a significant longer disease free interval and survival time (resp. 468 and 596 days), than tumors not in the radius (resp. 187 and 232 days).[2] Localization in the humerus decreases the prognosis.[8] Mandibular osteosarcoma has a better prognosis because of the low metastatic rate compared with appendicular osteosarcoma. Localization more rostral in the appendicular skeleton has a longer survival time and disease free interval. This can be explained because tumors in the rostral part are earlier visible and surgery is easier in more rostral osteosarcoma.[6] Also completion of the total chemotherapy protocol of six doses alternating doxorubicin and carboplatin gives a significant longer disease free interval and survival time (resp. 295 and 507 days) compared with dogs, which didn't finish the complete protocol (resp. 106 and 156 days).[2] Serum alkaline phosphatase was higher in dogs with a mutation in TP53 gene. Dogs with this mutation have a significant shorter survival time.[15] Histology is also an important factor for providing a prognosis. The survival time is significant worsen when more than five mitotic figures in 3 high power fields were seen. Tumor cells in blood vessels give also a worsen prognosis than when no tumor cells are seen in the blood vessels.[24]

The mean survival time for only surgery is 3-6 months.[7,8,18,20,21,24] Survival rates are 10% - 20% for 1 year[7,8,14,20,24] and 2% - 4% for 2 years.[7,20,24] The survival time without infection after limb-sparing surgery is 685 days and 289 days with no infection.[20] The cause of death is mostly the pulmonary metastases.[13] The prognosis increases with adjuvant chemotherapies to survival time of 9-14 months.[2,8,14,18,21,22,24] The median disease free interval for treatment with carboplatin is 257 days and the median survival time is 321 days. The survival times of 1, 2, 3 years for treatment with carboplatin are respectively 35.4-36.8%, 18.7% and 11%.[13,22] The median survival time for treatment with cisplatin is 262-413 days. The 1 year resp. 2 year survival rates for treatment with cisplatin are 20-62% resp. 16-21%.[7,13,18] The survival time for treatment with doxorubicin is 104 days – 366 days The 1 and 2 year survival rates for doxorubicin are respectively 14 – 50% and 10%.[7,13] There are different protocols reported using alternating doxorubicin and cisplatin or carboplatin. The disease free interval is 227-471 days.[6,13,16] The survival times of these protocols are 235-540.[2,6,13] The survival rates for one, two and three years are respectively 33-68%, 25-26% and 10%.[7,13] When chemotherapy was stopped, the survival time worsen significant. The reason for discontinuation could be presence of metastasis, which also gives a significant shorter survival time.[16,24] After only palliative radiation therapy the median survival range from 4.1-10.4 months. Most dogs were euthanized due a diminished quality of life caused by tumor progression or metastases.[17,20]

Materials & Methods

For this 298 dogs with osteosarcoma are used. These animals were presented between 1960 and 2012 to their own vet or to a specialist. The diagnosis osteosarcoma must be confirmed by histology, the dogs without this confirmation are excluded from this study. The included variables are seen in Box 2, the calculated variables are seen in box 3. The information for these variables is collected from the patient files. For the patients before 2002 the paper patient files were used, for the newer patients the digital patient files in Vetware were used. Also patients with missing information are included in the database. When there is no information available about a certain variable, the dog is excluded in that specific analysis. A part of the database was already made for earlier research purposes. This database is used, the missing data and newer patients are included. When follow up is missing, the own veterinarians were called to complete the database. The database was made in IBM SPSS Statistics version 20.

The histologic evaluation was done by a pathologist. The tumors were evaluated by type, only osteosarcomas were included in this study. The osteosarcoma were evaluated by a previous classification system following Kirpensteijn et al. Osteosarcoma was classified in histological subtypes, osteoblastic, chondroblastic, fibroblastic and teleangeiectatic or a combination of these. Matrix of the osteosarcoma was classified as none, cartilage, fibrous, osteoid or a combination thereof. The amount of matrix was evaluated and classified as 0 = none, 1 = small, 2 = moderate or 3 = large. The percentage tumor cells versus matrix and amount of tumor necrosis was evaluated and scored,0 = <25%, 1 = 25-50%, 2 = 50-75%, 3 = > 75%. Percentage tumor cell pleomorphism was evaluated and scored in the following way: 0 = none, 1 = < 25%, 2 = 25-50%, 3 = 50-75% and 4 = > 75%. The amount of multinucleated giant cells was scored as following: 0 = none, 1= minimal number, 2 = moderate number or 3 = large number of multinucleated giant cells. Whirl formation is scored as 0 = none, 1 = minimal, 2 = moderate or 3 = severe whirl formation. The amount of mitosis is evaluated by counting the amount of mitosis in a high powerfield (400 x). The presence of metastasis in lymph nodes and vessels was evaluated as present or not present or unknown, if no vessel or lymph node was included in the sample. Tumor grade was scored as 0 = benign, 1 = low, 2 = medium or 3 = highly malignant.[14]

Continuous data were summarized by mean, standard deviation and 95% confidence interval. Categorical data were summarized by frequency. Proportions are compared with the test for a single proportion, $test_6$. The metastasis free interval, recurrence free interval and survival time were evaluated with the Kaplan-Meier survival curves and compared with the logrank test. These data were summarized by median and standard error of the median. Dogs lost for follow up, living patients and dogs died because of another reason are censored. The cox proportional hazards multivariate analysis is used to compare the statistical significant different factors following the Kaplan-Meier and logrank test. $P < 0,05$ is considered as statistical significant difference between groups. When the variable was unknown the dog is classified in the category 'other'. For the analysis of the blood results the reference range in Table 1 are used to classify the blood results as low (below reference range), normal (inside reference range) and high (above reference range).

$$Test6 = \frac{|p - \pi| - \frac{1}{2n}}{\sqrt{\frac{\pi(1-\pi)}{n}}}$$

Box 2: included variables in the database

Signalement	Sex, Neuter status, Birth day, Breed, Weight
History	Date of presentation, Presenting complains, Location of tumor
Diagnostic tests	Hematocrit, Leukocytes, Neutrophil granulocytes, Monocytes, Lymphocytes, Thrombocytes, Urea, Creatinin, Alanine transaminase, Serum alkaline phosphatase level, Results of Thoracic Radiography, Histological grade, Histological subtype, Pleiophormism, Number of mitoses, Type of tumor matrix, Amount of osteoid, % tumor cells versus matrix, Amount of necrosis, Metastasis present in vessels or lymph nodes, Tumor grade ,Amount of multinucleated giant cells, Whirl forming
Treatment	Preoperative chemotherapy, Type treatment performed, Postoperative infections and complications, Postoperative chemotherapy
Follow up	Date of first treatment, date of death, date of recurrence, date of first metastasis, reason of death.

Box 3: Calculated variables

Age of presentation	Difference between date of birth and date of presentation
Survival time	Difference between date of presentation and date of death
Metastasis free interval	Difference between date of presentation and date of first metastasis
Recurrence free interval	Difference between date of presentation and date of recurrence

Results

Totally 298 dogs were included 68 intact females, 69 neutered females, 121 intact males and 37 neutered males. From 3 dogs the sex was unknown. There was no significant difference between the number of males and females (P = 0.25). Significant more intact dogs were included than neutered dogs (P<0.001). The most prevalent breeds of dogs were the Rottweilers (61) mix-breeds (50), Boxers (18), Bouviers (15), German shepherds (14) Great Danes (12), Doberman pinchers (11) and Leonbergers (11). The mean weight was 38.0 kg ± 12.5 kg, range from 7 to 87 kg. The median age of presentation was 7.5 years ± 3.11, range from 0 to 13 years old.

From not every patient the presenting complain was known. The most common presenting complains were lameness (102/182) and a mass (61/182). 206 osteosarcomas were present in the appendicular skeleton, 75 in the axial skeleton and 15 extraskeletal and the localization is missing by 2 dogs. The most affected sites were distal radius (62/296), proximal humerus (41/296), rib (28/296), distal femur (22/296), proximal tibia (17/296), distal tibia (16/296), extraskeletal (15/296), mandibula (15/296), ulna (13/296) and maxilla (12/296). 136 osteosarcomas occurred at the right side and 119 at the left side. 131 osteosarcomas occurred at the frontlimbs and 73 at the hindlimbs. Osteosarcoma occurred significant more at the front limbs than the hind limbs (P<0.001)

Diagnostic results

The results of the complete blood count en serum chemistry are visible in Table 1.

Table 1: Outcome of laboratory findings

	Number of dogs	Reference range	Mean	Standard Deviation	Between reference range	Below reference range	Above reference range
Hematocrit (%)	77	0.42 – 0.61	.43	0.07	52	25	0
Leucocytes (10^9 cells/L)	77	4.5 – 14.6	12.63	12.89	58	0	19
Lymphocytes (10^9 cells/L)	75	0.8 – 4.7	1.75	1.22	49	13	3
Monocytes (10^9 cells/L)	75	0.0 – 0.9	0.71	0.84	58	-	17
Neutrophils (10^9 cells/L)	75	2.9 – 11.3	9.84	6.29	55	0	20
Thrombocytes (10^9 cells/L)	51	144 – 603	362.5	147.0	44	2	5

Urea (mmol/L)	75	3.0 – 12.5	6.51	8.31	63	8	4
Creatinine (µmol/L)	80	50 - 129	96.4	24.0	73	0	7
AF (IU/L)	74	< 89	152.3	418.4	50	-	24
ALT (IU/L)	25	< 54	35.7	23.1	19	-	6

From 149 dogs thoracic imaging was performed, 121 dogs did not have visible metastasis, 24 dogs had visible metastasis and in 4 dogs the results were inconclusive. In 62 dogs a fine needle aspiration biopsy of the tumor was performed. The results are visible in Table 2. Fine needle aspiration biopsy of the regional lymph node was performed in 9 dogs; in one dog metastasis was present at the lymph nodes. The most common locations of metastases were the lungs and bones; other locations are the skin, subcutis, muscles, abdominal organs, pleura and the eye.

Table 2: Outcome of cytology

Outcome	**Number of dogs**
Non diagnostic	6
Mesenchymal tumor	19
Mesenchymal proliferation	18
Osteoblasts, osteoclasts and fibroblasts	5
Osteosarcoma	14

Material for histologic evaluation was present from 254 dogs. In 190/238 samples metastasis were present in the vessels and in 15/73 samples metastasis in the lymph nodes were present. The subtype was 129/254 osteoblastic, 5/254 chondroblastic, 8/254 fibroblastic, 0/254 teleangiectactic, 28/254 combination of osteoblastic and chondroblastic, 44/254 combination of osteoblastic and fibroblastic, 14/254 combination of osteoblastic and teleangiectacic, 19/254 combination of osteoblastic, chondroblastic and fibroblastic, 4/254 combination of osteoblastic, chondroblastic and teleangiectatic and 3/254 combination of osteoblastic, fibroblastic and teleangiectatic. The matrix was 3/253 none, 0/253 cartilage or fibrous, 165/253 osteoid, 38/253 osteoid and cartilage, 33/253 osteoid and collagen and 14/253 osteoid, collagen and cartilage. The mean number of mitosis in a high power field is 9.7 ± 10.3 mitosis, range from 0 - 65 mitosis. In Table 3 the outcome of the other histological evaluations is

visible.

Table 3: Outcome of histologic evaluation

	0	1	2	3	4
Pleomorphism	4	20	82	125	22
Amount of matrix in tumor	3	92	128	30	
% tumor cells versus matrix	6	27	124	96	
Amount of necrosis	24	89	102	37	
Whirl forming	113	91	33	15	
Multinucleated giant cells	99	94	45	15	
Tumor grade	3	12	41	197	

Therapy

Most owners chose for euthanasia, 115 of the 298 dogs were euthanized. Seventy dogs underwent amputation of the affected leg. 42 owners chose to do nothing, and euthanize the dog at a later time. 30 dogs underwent total resection of the osteosarcoma, 17 dogs had marginal resection of the osteosarcoma. In 5 dogs regional perfusion of the osteosarcoma with cisplatin was performed. Three dogs were only treated with bisphosphonates, one dog received only chemotherapy without surgery and a dog underwent radiotherapy. In three dogs a combination therapy with holmium was used, one dog had a combination of holmium amputation and chemotherapy, but this dog died during the amputation. Another dog received a combination of holmium and marginal resection, the last dogs was given a combination of radiotherapy, holmium and chemotherapy. The treatment was unknown in eleven dogs. After surgery complications were present in 13 dogs and infection in 5 dogs. The main complications were inflammation, leaking of fluid from the wound and swelling. Two dogs died in a few days postoperative. Chemotherapy was performed in 71 dogs. The given chemotherapy is seen in Table 4.

Table 4: Chemotherapy treatment

	Number of patients
Lobaplatin	28
Carboplatin	6

Cisplatin	1
Adriamycin	2
Regional perfusion with Cisplatin	5
Carboplatin & Doxorubicin*	25
Others	4

* When Doxorubicin wasn't available Adriamycin was used

Survival

The metastasis free interval of 145 dogs was known. The median metastasis free interval was 54 ± 8.9 days (95% CI 37 – 71). The 1, 2, 3 year metastasis free rates are respectively 4.8%, 3.4% and 2.7%.
In 26 dogs recurrence of the osteosarcoma was recognized. The median recurrence free interval was 80 ± 32.5 days (95% CI 16 – 144). 50% had no sign of regrowth of the osteosarcoma at 87 days. The 1, 2, 3 year recurrence free rates were respectively 11.5%, 7.7% and 0.0%.
The survival time of 229 dogs was known, the median survival is 89 ± 17.7 days (95% CI 54-124). 52 dogs were censored. The 1, 2, 3 year survival rates were respectively 12.7%, 6.1% and 3.9%.
The median survival time for only surgery was 239 ± 37 days (95% CI 166 - 312 days) and for surgery and chemotherapy the median survival time was 256 ± 57 days (95% CI 144 – 368 days). The median survival time of surgery and a combination of two chemotherapeutical agents was 293 ± 98 days (95% CI 101 - 485 days).

Table 5: Comparison of the metastasis free interval and survival time based on sex and neuterstatus

Sex & neuterstatus	N	MMFI ± SD	P	N	MST ± SD	P
Female Male	66 80	47 ± 7.6 64 ± 11.7	0.60	107 122	94 ± 20.3 78 ± 30.4	0.35
Intact Neutered	88 58	49 ± 13.6 55 ± 21.6	0.55	130 99	68 ± 15.5 119 ± 22.8	0.47
Male intact Male neutered	57 23	66 ± 16.2 49 ± 15.2	0.62	88 34	77 ± 34.8 79 ± 72.4	0.57
Female intact Female neutered	31 35	31 ± 6.7 71 ± 23.1	0.37	42 65	66 ± 18.2 119 ± 19.5	0.22
Female intact Male intact	31 57	31 ± 6.7 66 ± 16.2	0.70	42 88	66 ± 18.2 77 ± 34.8	0.73

Female neutered Male neutered	35 23	71 ± 23.1 49 ± 15.2	0.36	65 34	119 ± 19.5 79 ± 72.4	0.16
Female intact Female neutered Male intact Male neutered	31 35 57 23	31 ± 6.7 71 ± 23.1 66 ± 16.2 49 ± 15.2	0.72	42 65 88 34	66 ± 18.2 119 ± 19.5 77 ± 34.8 79 ± 72.4	0.46

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

Table 6: Comparison of the metastasis free interval and survival time based on age

Age	N	MMFI ± SD	P	N	MST ± SD	P
≤ 5 year > 5 year	33 112	40 ± 16.7 63 ± 9.4	0.12	50 178	43 ± 10.3 99 ± 18.2	0.15
≤ 7.3 year > 7.3 year	72 73	50 ± 13.3 54 ± 14.2	0.92	109 119	83 ± 23.9 81 ± 23.2	0.86

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

Table 7: Comparison of the metastasis free interval and survival time based on breed

Breed	N	MMFI ± SD	P	N	MST ± SD	P
Rottweiler Other breeds	30 116	26 ± 24.1 56 ± 9.9	**0.03**	48 181	89 ± 39.9 93 ± 18.1	0.40
Mix-breeds Other breeds	23 123	85 ± 25.6 50 ± 8.5	0.13	42 187	103 ± 142.5 83 ± 17.4	0.06
Boxer Other breeds	2 144	69 ± 0.0 50 ± 8.3	0.73	5 224	34 ± 15.3 93 ± 18.1	0.17
Bouvier Other breeds	9 137	36 ± 37.3 55 ± 9.0	0.91	11 218	128 ± 63.8 81 ± 17.5	0.97
Rottweiler Mix-breeds Boxer Bouvier Other breeds	30 23 2 9 82	26 ± 24.1 85 ± 25.6 69 ± 0.0 36 ± 37.3 50 ± 9.5	0.21	48 42 5 11 123	89 ± 39.9 103 ± 142.5 34 ± 15.3 128 ± 63.8 79 ± 19.7	0.25

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

Table 8: Comparison of the metastasis free interval and survival time based on localization

Localization	N	MMFI ± SD	P	N	MST ± SD	P
Forelimbs Hindlimbs	64 35	65 ± 11.0 96 ± 21.3	0.52	100 59	115 ± 18.7 140 ± 46.3	0.77
Appendicular Axial	100 35	69 ± 15.0 15 ± 14.2	0.046	160 55	121 ± 20.7 27 ± 9.5	**0.001**
Skeletal Extraskeletal	135 11	56 ± 8.9 36 ± 15.4	0.45	215 14	93 ± 18.1 28 ± 20.6	0.99
Distal radius Other locations	34 112	66 ± 25.5 50 ± 9.7	0.83	54 175	155 ± 61.3 66 ± 17.8	**0.023**
Proximal humerus Other locations	15 131	35 ± 30.9 64 ± 13.7	**0.014**	24 205	22 ± 28.2 99 ± 18.5	**0.040**
Distal femur Other locations	11 135	44 ± 30.2 55 ± 8.9	0.30	18 211	81 ± 80.6 89 ± 17.3	0.56
Proximal tibia Other locations	8 138	83 ± 31.8 50 ± 8.8	0.72	14 215	147 ± 107.0 81 ± 19.3	0.16
Distal tibia Other locations	12 134	167 ± 32.9 47 ± 6.6	**0.027**	14 215	188 ± 14.1 81 ± 14.7	0.34
Middle Left Right	3 61 63	2 ± 1.6 49 ± 12.3 55 ± 19.8	0.78	7 92 106	1 ± 1.3 68 ± 14.6 26,5 ± 47.1	**0.003**

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

Table 9: Comparison of the metastasis free interval and survival time based on weight

Weight	N	MMFI ± SD	P	N	MST	P
≤ 20 kg > 20 kg	6 134	36 ± 15.3 64 ± 12.3	0.92	13 201	54 ± 49.7 99 ± 17.6	0.71
≤ 34 kg > 34 kg	46 94	54 ± 16.4 65 ± 15.0	0.62	77 137	81 ± 23.3 115 ± 17.7	0.69
≤ 38 kg > 38 kg	65 75	49 ± 13.8 66 ± 15.4	0.70	111 103	83 ± 26.5 119 ± 20.1	0.35

≤ 40 kg > 40 kg	83 57	54 ± 17.1 65 ± 11.9	0.25	130 84	99 ± 26.2 115 ± 24.9	0.15

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

Table 10: Comparison of the metastasis free interval and survival time based on presenting complain

Presenting complain	N	MMFI ± SD	P	N	MST ± SD	P
Lameness Mass Other	58 44 44	65 ± 20.9 31 ± 17.6 63 ± 10.0	0.59	100 57 72	81 ± 18.5 40 ± 17.1 121 ± 39.8	**0.042**

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

Table 11: Comparison of the metastasis free interval and survival time based on the blood results

Blood results	N	MMFI ± SD	P	N	MST ± SD	P
Low hematocrit Normal hematocrit	17 37	71 ± 7.5 84 ± 14.6	0.17	25 51	67 ± 29.6 210 ± 71.6	**0.020**
Normal leucocytes High Leucocytes	43 11	89 ± 12.6 44 ± 22.0	**0.027**	58 18	210 ± 62.2 44 ± 22.2	**0.007**
Normal neutrophils High neutrophils	42 12	89 ± 12.4 44 ± 11.3	0.050	55 19	199 ± 58.6 64 ± 23.1	**0.01**
Low lymphocytes Normal lymphocytes High lymphocytes	7 45 2	178 ± 162.4 80 ± 12.1 44 ± 0.0	0.80	12 59 3	81 ± 125.6 123 ± 52.2 148 ± 84.9	0.85
Normal monocytes High monocytes	43 11	83 ± 15.1 71 ± 22.0	0.31	58 16	148 ± 76.4 94 ± 28.6	0.18
Low thrombocytes Normal thrombocytes High thrombocytes	1 30 4	66,0 ± 0.0 103 ± 32.2 26 ± 37.5	0.26	2 43 5	34 239 ± 48.4 103 ± 24.1	**0.005**
Low urea Normal urea High urea	5 48 3	54 ± 28.5 84 ± 13.3 55 ± 44.9	0.19	8 63 4	54 ± 20.5 143 ± 73.3 56 ± 71.0	0.64
Normal creatinin High creatinin	53 4	84 ± 14.6 55 ± 33.0	0.11	72 7	127 ± 59.4 143	0.998

Normal SAF High SAF	37 16	89 ± 11.7 28 ± 15.0	0.08	50 23	148 ± 39.4 78 ± 28.6	0.31
Normal ALT High ALT	17 5	56 ± 26.1 98 ± 76.7	0.87	19 6	96 ± 53.0 143 ± 120.6	0.69

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days); SAF = Serum alkaline phosphatase; ALT = Alanine aminotransferase

Table 12: Comparison of the metastasis free interval and survival time based on the presence of metastasis at diagnosis diagnosed by computed tomography, thoracic radiography and/or lymph node cytology

Metastasis	N	MMFI ± SD	P	N	MST ± SD	P
No metastasis Metastasis present	73 19	103 ± 20.1 0 ± 0.0	<0.001	114 25	139 ± 29.3 38 ± 22.3	<0.001

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

Table 13: Comparison of the metastasis free interval and survival time based on histological results

Histological results	N	MMFI ± SD	P	N	MST ± SD	P
Osteoblastic Chondroblastic Fibroblastic Mixed O and C Mixed O and T Mixed O and F Mixed O, F and T Mixed O, C and F Mixed O, C and T	61 2 3 12 6 28 2 7 1	35 ± 14.6 65 ± 37.2 28 ± 18.4 89 ± 27.8 55 ± 6.5	0.38	93 4 7 18 11 40 3 12 2	64 ± 17.7 171 ± 0.0 66 ± 111.4 79 ± 46.4 132 ± 44.2 274 ± 218.0 121 ± 58.9	0.61
No pleiomorphism < 25% pleiomorphism 25-50% pleiomorphism 50 -75% pleiomorphism > 75% pleiomorphism	2 8 40 60 11	 46 ± 34.6 31 ± 37.9 50 ± 16.1 115 ± 55.6	0.21	4 14 57 95 19	 12 ± 30.9 96 ± 40.6 93 ± 30.4 78 ± 35.4	0.98

None Osteoid Osteoid and cartilage Osteoid and collagen Osteoid, cartilage and collagen	2 80 14 20 5	 42 ± 11.7 65 ± 57.1 63 ± 23.5 335 ± 210.3	0.36	3 124 24 30 8	 66 ± 19.1 33 ± 25.1 155 ± 30.4 81 ± 241.0	0.42
No matrix small amount matrix moderate amount matrix large amount matrix	2 43 60 16	 50 ± 15.7 48 ± 19.4 66 ± 65.0	0.71	3 65 96 25	 83 ± 53.2 81 ± 14.2 67 ± 59.8	0.77
% tumorcells vs matrix < 25% 25-50% 50-75% >75%	 3 11 62 45	 8 ± 6.5 66 ± 33.0 70 ± 30.5 46 ± 6.0	0.70	 5 17 98 69	 33 ± 23.0 62 ± 21.3 115 ± 30.0 68 ± 23.3	0.34
Amount of necrosis < 25% Amount of necrosis 25-50% Amount of necrosis 50-75% Amount of necrosis > 75%	10 47 50 13	42 ± 13.4 69 ± 22.6 41 ± 20.6 64 ± 41.3	0.66	20 75 72 21	228 ± 105.5 119 ± 35.1 62 ± 25.5 54 ± 42.5	0.050
No metastasis present in vessels Metastasis present in vessels	23 89	89 ± 42.3 48 ± 13.1	0.19	44 131	274 ± 116.1 64 ± 16.0	**< 0.001**
No metastasis present in lymph node Metastasis present in lymph node	34 8	96 ± 21.1 14 ± 19.8	0.49	52 11	140 ± 22.7 14	0.45
No whirl forming Minimal whirl forming Moderate whirl forming Severe whirl forming	59 38 14 9	44 ± 11.5 63 ± 23.1 89 ± 31.8 84 ± 56.6	0.46	90 61 26 11	81 ± 27.4 77 ± 42.0 89 ± 58.0 127 ± 66.3	0.99
None MNGC Some MNGC Multiple MNGC Abundant MNGC	53 37 23 8	48 ± 20.8 65 ± 26.1 44 ± 32.7 64 ± 67.9	0.51	79 61 38 11	81 ± 25.7 99 ± 42.7 77 ± 33.2 81 ± 42.9	0.67

Histologic grade Benign Low malignant Medium malignant Highly malignant	 2 5 24 90	 89 ± 25.2 42 ± 25.1 49 ± 13.3	0.25	 3 11 38 137	 67 ± 30.8 148 ± 81.7 68 ± 20.4	0.08
< 10 mitosis 10-20 mitosis > 20 mitosis	71 30 20	65 ± 20.1 36 ± 8.9 65 ± 23.5	0.10	115 47 27	121 ± 33.2 39 ± 12.2 147,0 ± 57.5	0.13

O = osteoblastic; C = chondroblastic; F = Fibroblastic; T = telegangiectatic; MNGC = Multinucleated giant cells; N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

Table 14: Comparison of the metastasis free interval and survival time based on the therapy

Therapy	**N**	**MMFI ± SD**	**P**	**N**	**MST ± SD**	**P**
None Surgery Others	21 83 8	15 ± 13.7 115 ± 15.2 46 ± 31.8	**<0.001**	39 112 12	33 ± 18.2 239 ± 37.2 94 ± 26.3	**<0.001**
Marginal resection Total resection Amputation	13 21 49	80 ± 38.3 105 ± 25.2 127 ± 23.9	0.79	15 29 68	81 ± 18.7 266 ± 93.0 274 ± 35.3	**0.014**
No postoperative infection Postoperative infection	61 1	126 ± 22.9	0.28	76 4	212 ± 33.3	0.92
No postoperative complications Postoperative complications	54 9	99 ± 26.9 105 ± 3.0	0.48	68 13	210 ± 35.8 312 ± 143.1	0.32
No chemotherapy Chemotherapy	93 51	34 ± 13.6 87 ± 14.3	0.07	150 70	38 ± 8.9 199 ± 45.3	**<0.001**
One chemotherapeuticum Two chemotherapeutica	33 17	96 ± 19.1 87 ± 29.5	0.37	43 26	183 ± 26.8 293 ± 77.8	0.40
Lobaplatin Carboplatin Carboplatin & Doxorubicin or Adriamycin Others	22 3 16 9	103 ± 28.6 44 ± 2.4 87 ± 42.0 46 ± 29.8	0.14	28 6 25 10	256 ± 72.9 44 ± 65.5 293 ± 87.2 94 ± 16.2	0.09

Surgery with chemotherapy Surgery without chemotherapy	43 20	103 ± 11.8 128 ± 19.8	0.35	60 52	256 ± 57.1 237 ± 60.6	0.24
Only chemotherapy Chemotherapy and surgery Chemotherapy and others	5 43 2	46 ± 46.0 103 ± 11.8	0.06	6 60 3	96 ± 2.1 256 ± 57.1 99	**0.003**

N = amount of dogs; MMFI = Median metastasis free interval (days); MST = median survival time (days)

There was no significant influence of weight, age, sex and neuterstatus on the median metastasis free interval and median survival time based on Table 5, 6 and 9. Rottweilers had a significant shorter median metastasis free interval compared with other breeds based on Table 7. The localization of the osteosarcoma had a significant influence on the median metastasis free interval and median survival time as seen in Table 8. Axial location had a significant shorter median metastasis free interval and median survival time compared with appendicular localization. Localization of the osteosarcoma in the middle gave a significant shorter median survival time than localization left or right. Osteosarcoma in the proximal humerus had a significant shorter median metastasis free interval and median survival time in comparison with other localization. Osteosarcoma in the distal tibia had a significant longer median metastasis free interval in comparison with other locations. Localization in the distal radius gave a significant longer median survival time compared with all other locations. Dogs with a mass as presenting complain had a significant shortened median survival time compared with dogs with other complains, this can be seen in Table 10.

Dogs with a normal hematocrit had a significant longer median survival time than dogs with a low hematocrit. A significant shorter median metastasis free interval was noticed in dogs with high leucocytes in comparison with dogs with normal leucocytes. The median survival time was significant shortened in dogs with high leucocytes and high neutrophils compared with dogs with normal leucocytes and neutrophils. Dogs with low thrombocytes had a significant shorter median survival time compared with dogs with thrombocytes within the reference range. The other blood results did not significantly influence the median metastasis free interval and median survival time as shown in Table 11. As seen in Table 12, patients with detectable metastasis at diagnosis by computed tomography, thoracic imaging or lymph node cytology had a significant shorter median metastasis free interval and median survival time.

The influence of the histologic grading of the osteosarcoma on the median metastasis free interval and median survival time is visible in Table 13. The histologic subtype, tumor cell pleiomorphism, type of matrix, amount of matrix, amount of tumor cells versus matrix, amount of necrosis, whirl formation, amount of multinucleated giant cells, number of mitoses, histological grade and presence of metastasis in lymph node didn't significant influenced the median metastasis free interval and median survival time. The median metastasis free interval was not influenced by the presence of metastasis in the blood vessels. The presence of metastasis in the blood vessels influenced the median survival time significant negatively.

In Table 14 the influence of the therapy is visible. Surgery elongated the median metastasis free interval and median survival time significant, but marginal resection of the osteosarcoma had a significant shorter median metastasis free interval and median survival time compared with total resection or amputation. Chemotherapy elongated also the median survival time compared with no chemotherapy. Chemotherapy in combination with surgery had a significant longer median survival time compared with chemotherapy in combination with other therapies and only chemotherapy. The type of chemotherapy and the use of one or more different chemotherapeutical agents had no significant influence on the median metastasis free interval and median survival time. Postoperative complications and infections did not significantly influence the median metastasis free interval and the median survival time.

The results of the multivariate analysis for metastasis free interval are visible in Table 15. In the multivariate analysis of the metastasis free interval only localization in the humerus, number of leucocytes and presence of preoperative metastasis influenced the metastasis free interval significant.

Table 15: Multivariate analysis of metastasis free interval

	P	Hazard ratio	95% CI
Breed (Rottweiler, others)	0.21	0.59	0.25 – 1.36
Localization (axial, appendicular)	0.05	1.65	0.99 – 2.76
Localization (proximal humerus, others)	**0.029**	0.31	0.11 – 0.89
Localization (distal tibia, others)	0.86	0.87	0.20 – 3.88
Number of leucocytes (normal, high)	**0.001**	4.42	1.79 –10.93
Preoperative presence metastasis (present, not present)	**0.001**	11.64	2.91 – 46.61
Therapy (none, surgery, others)	0.82	0.92	0.44 – 1.91

The results of the multivariate analysis for the survival time are visible in Table 16. In the multivariate analysis of the survival time neutrophil and thrombocyte count influence the survival time significant.

Table 16: Multivariate analysis of survival time

	P	Hazard ratio	95% CI
Localization (axial, appendicular)	0.39	21.94	0.02 – 24296.57
Localization (left, right, middle)	0.93	1.09	0.18 – 6.63

Localization (distal radius, others)	0.68	1.48	0.23 – 9.52
Localization (proximal humerus, others)	0.18	0.00	0.00 – 438.61
Presenting complain (lameness, mass, others)	0.91	0.91	0.17 – 5.00
Preoperative metastasis (present, not present)	0.73	1.71	0.08 – 34.75
Hematoctit (low, normal)	0.28	0.19	0.01 – 3.90
Leucocytes (normal, high)	0.60	0.43	0.02 – 10.54
Neutrophils (normal, high)	**0.02**	937.18	3.57 – 246194.87
Thrombocytes (low, normal, high)	**0.02**	0.01	0.00 – 0.45
Histological presence of metastasis in blood vessels (present, not present)	0.30	2.20	0.49 – 9.89
Therapy (none, surgery, others)	0.982	0.003	0.00 – ∞
Postoperative chemotherapy (performed, not performed)	0.73	0.51	0.01 – 23.26

95% CI = 95% confidence interval

Discussion

This retrospective study has some weak points, one of them are the missing data. Not all the patient files were available or complete available to complete the database. Some documents were not readable. Some dogs were lost for follow up, so the cause of death, survival time, metastasis free interval and recurrence free interval were unknown. The group size is too small to evaluate for some groups. There is no control group, which is another weak point of this retrospective study. The treatment protocols also differ between the dogs, it is the owners choose which treatment they give to their dog. Some owners choose for euthanasia, which decreases the mean survival time. The dogs in this study are from 1960 until 2012, in these years the treatment protocols for osteosarcoma changed. Some dogs were included in other studies for holmium, biphosphonates or lobaplatin treatment.

In this study there was no significant difference in the sex. Kirpensteijn et al. (2008) and Morello reported osteosarcoma more often seen in males than females.[15,20] In this study, significantly more intact dogs were included than neutered dogs. Morello et al. and Rosenberger et al. reported neutered dogs were more often affected by osteosarcoma.[20,23] The most common dog breed in this study was the Rottweiler, this is reported as a common affected breed. Also other breeds with a high risk of osteosarcoma were seen in this study, German Shepherd, Doberman Pincher, Great Dane and Leonberger.

Age, weight, serum alkaline phosphatase doesn't have a significant influence on the survival time in this research, but in literature influence is reported.[5,8,11,14,22,24,27] The numbers of animals with serum biochemistry can be too low to have a statistical significant difference. Also the owner's choice for a treatment can play a role in the survival, when they choose for euthanasia or no treatment. Localization in the proximal humerus is found as negative prognostic influence by the Kaplan-Meier method, as meant in literature.[5,8,11,14,22,24,27] The tumors are seen later and treated at a later moment, which can explain the worsened prognosis. Perhaps tumors in the humerus can be more aggressive, which can be seen with histology.[22] Localization in the (distal) radius gives a significant better prognosis by the Kaplan-Meier method, also found by Bacon et al.[2] Osteosarcoma on this location may be less aggressive as other locations.

In this paper 70% of the osteosarcoma is located in the appendicular skeleton, 25% in the axial skeleton and 5% extraskeletal. This percentage in appendicular skeleton is lower and the percentage in extraskeletal locations is higher than reported by others.[8,10,19,20] The most common appendicular locations are the same in this report as others.[8,10,19,20] The axial localization differs in this research with literature, ribs (37% versus 10%), mandibular (20% versus 27%), maxilla (16% versus 22%), spine (10% versus 15%) and cranium (13% versus 14%). Osteosarcoma occurs significant more at the front limbs than the hind limbs as also reported by Endicott et al. and Rosenberger et al.[8,23]

Boerkamp et al. reports a high alkaline phosphatase as a negative prognostic factor, dogs with a high alkaline phosphatase have a significant shorter survival time.[5] In this research the mean metastasis free interval and mean survival time with a high alkaline phosphatase are shortened compared with normal alkaline phosphatase, but it isn't significant different. A smaller number of animals or because of missing

data can be the explanation.

The metastasis and recurrence free interval is subjective. Thoracic imaging can be done on different times during the treatment and is also the choice of the owners. Metastasis will be detected earlier when a computed tomography is done instead of radiographs. When owners decided not to do thoracic imaging, the detection of metastasis can be delayed. Metastasis can also be found in bones or other visceral organs, so a bone scan and abdominal ultrasound can detect metastasis on other place. These metastases can be missed when only thoracic imaging is performed. Recurrence of the tumor can be detected earlier or later. At some places masses are seen more easily and the recurrence will be reported earlier. In this paper the recurrence free interval isn't evaluated because of the low number of dogs with a known recurrence.

The median survival time for only surgery is 237 days (95 CI 118 - 356 days), which is shorter than mentioned in literature. The median survival time is 256 days (95% CI 144 – 368 days) for surgery and chemotherapy,this is shorter than described in the literature.[2,7,8,14,20,21,22,24] It can be declared by the owners decision to decline the treatment.

In the multivariate analysis only a small group of patients is included because of the missing data. This analysis uses multiple variables instead of one in the Kaplan-Meier method. So the results from the multivariate analysis differ from the Kaplan-Meier method. The metastasis free interval is only significant influenced by localization in the humerus, in accordance with Boerman et al. (2012)[5], neutrophil count and preoperative presence of metastasis with the multivariate analysis. The survival time is only significant influenced by neutrophil and thrombocyte count. In the mean survival multivariate analysis some specific treatment groups, which gives significant differences in the Kaplan-Meier univariate analysis, are not investigated, because no patients would be left to perform the analysis.

Further research should be done to determine prognostic negative or positive factors for osteosarcoma. A larger group of patient is needed with a good documentation of the investigated factors, so a more detailed multivariate analysis can be performed. Low thrombocytes is described as a negative prognostic factor, but the group is very small for this conclusion, further research is necessary. Also other therapies like immunotherapy and biphosphonates needs to be further investigated. In this project a few patients were treated with bisphosphonates (incorporated in the group others), but not enough for significant statistical conclusions. With these results veterinarians can give the owners a better advice about the prognosis and therapy of their dog based on signalment and diagnostic results. Based on the different prognoses for the different therapies owners can choose the best fitting therapy for their own circumstances.

Conclusion

The metastasis free interval is significant negative influenced in the univariate analysis by breed, axial localization, localization in the proximal humerus, leukocytosis and high neutrophil counts, no treatment and marginal resection. Localization in the distal tibia has a significant positive influence on the metastasis free interval in the univariate analysis. In the multivariate analysis only localization in the humerus, neutrophil count and preoperative presence of metastasis significantly influence the metastasis free interval.

The survival time is significant negative influenced with the Kaplan-Meier method by localization in the axial skeleton, in the proximal humerus and localization in the middle, high leucocytes and neutrophils, the presence of metastasis and incomplete resection of the osteosarcoma. Localization in the distal radius, perform surgery, perform chemotherapy and the combination of amputation and chemotherapy have a significant positive influence on the survival time by the Kaplan-Meier method. In the cox regression analysis only neutrophil and thrombocyte count significantly influenced the survival time.

References

1. ANFINSEN, K.P., GROTMOL, T., BRULAND, O.S., JONASDOTTIR, T.J. (2011) Breed-specific incidence rates of canine primary bone tumors – A population based survey of dogs in Norway. *The Canadian Journal of veterinary Research* **75**: 209-215.
2. BACON, N.J., EHRHART, N.P., DERNELL, W.S., LAFFERTY, M., WITHROW, S.J. (2008) Use of alternating administration of carboplatin and doxorubicin in dogs with microscopic metastases after amputation for appendicular osteosarcoma: 50 cases (1999-2006). *Journal of the American Veterinary Medical Association* **232**: 1504-1510.
3. BATSCHINKSI, K., DERVISIS, N.G., KITCHELL, B.E. (2012) Evaluation of ifosfamide salvage therapy for metastatic canine osteosarcoma. *Veterinary and Comparative Oncology* (Early online published)
4. BERG, J. (1996) Canine Osteosarcoma: Amputation and Chemotherapy. *Veterinary Clinics of North America: Small Animal Practice* **26**: 111-121.
5. BOERMAN, I, SELVARAJAH, G.T., NIELEN, M., KIRPENSTEIN, J. (2012) Prognostic factors in canine appendicular osteosarcoma – a meta-analysis. *BMC Veterinary Research* **8**: (Early online published)
6. CHUN, R., LORIMIER, L.P. de, (2003) Update on the biology and management of canine osteosarcoma. *Veterinary Clinics of North America: Small Animal Practice* **33**: 491-516.
7. DEREGIS C.J., MOORE, A.S., RAND, W.M., BERG, J. (2003) Cisplatin and Doxorubicin Toxicosis in Dogs with Osteosarcoma. *Journal of Veterinary Internal Medicine* **17**: 668-673.
8. ENDICOTT, M. (2003) Principles of Treatment for Osteosarcoma. *Clinical Techniques in Small Animal Practice* **18**: 110-114.
9. FAN, T.M., CHARNEY, S.C., DE LORIMIER, L.P., GARRETT, L.D., GRIFFON, D.J., GORDON-EVANS, W.J., WYPIJ, J.M. (2009) Double-Blind Placebo Controlled Trial of Adjuvant Pamidronate with Palliative Radiotherapy and Intravenous Doxorubicin for Canine Appendicular Osteosarcoma Bone Pain. *Journal of Veterinary Internal Medicine* **23**: 152-160.
10. Bone Neoplasia **In:** FOSSUM, T.W., HEDLUND, C.S., JOHNSON, A.L., SCHULZ, K.S., SEIM, H.B., WILLARD, M.D., BAHR, A., CARROLL, G.L. (2007) Small animal surgery. 3rd ed. Mosby Elsevier, St. Louis, 1338-1351.
11. HILLERS, K.R., DERNELL, W.S., LAFFERTY, M.H., WITHROW, S.J., LANA, S.E. (2005) Incidence and prognostic importance of lymph node metastases in dogs with appendicular osteosarcoma: 228 cases (1986-2003). *Journal of the American Veterinary Medical Association* **8**: 1364-1367.
12. KARNIK, K.S., SAMII, V.F., WEISBRODE, S.E., LONDON, C.A., GREEN, E.M. () Accuracy of computed tomography in determining lesion size in canine appendicular osteosarcoma. *Veterinary Radiology & Ultrasound* **53**: 273-279.
13. KENT, M.S., STROM, A., LONDON, C.A., SEGUIN, B. (2004) Alternating Carboplatin and Doxorubicin as Adjunctive Chemotherapy to Amputation or Limb-Sparing Surgery in the Treatment of Appendicular Osteosarcoma in Dogs. *Journal of Veterinary Internal Medicine* **18**: 540-544.
14. KIRPENSTEIJN, J., KIK, M., RUTTEMAN, G.R., TESKE, E. (2002) Prognostic Significance of a New Histologic Grading System for Canine Osteosarcoma. *Veterinary Pathology* **39**: 240-246.
15. KIRPENSTEIJN, J., KIK, M., TESKE, E., RUTTEMAN, G.R. (2008) TP53 Gene Mutations in Canine Osteosarcoma. *Veterinary Surgery* **37**: 454-460.
16. LANE, A.E., BLACK, M.L., WYATT, K.M. (2012) Toxicity and efficacy of a novel doxorubicin and carboplatin chemotherapy protocol for the treatment of canine appendicular osteosarcoma following limb amputation. *Australian Veterinary Journal* **90**: 69-74.
17. MAYER, M.N., GRIER, C.K. (2006) Palliative radiation therapy for canine osteosarcoma. *The Canadian Veterinary Journal* **47**: 707-709.
18. MACEWEN, E.G., KURZMAN, I.D. (1996) Canine Osteosarcoma: Amputation and

Chemoimmunotherapy. *Veterinary Clinics of North America: Small Animal Practice* **26**: 123-133.
19. Primary Neoplasms of Bone **In:** MCGAVIN, M.D., ZACHARY, J.F. (2007) Pathologic basis of veterinary disease. 4th ed. Mosby Elsevier, St. Louis, 1086-1091.
20. MORELLO, E. MARTANO, M., BURACCO, P. (2011) Biology, diagnosis and treatment of canine appendicular osteosarcoma: Similarities and differences with human osteosarcoma. *The Veterinary Journal* **189**: 268-277.
21. NEIHAUS, S.A., LOCKE, J.E., BARGER, A.M., BORST, L.B., GORING, R.L. (2011) A Novel Method of Core Aspirate Cytology Compared to Fine-Needle Aspiration for Diagnosing Canine Osteosarcoma. *American Animal Hospital Association* **47**: 317-323.
22. PHILLIPS, B., POWERS, B.E., DERNELL, W.S., STRAW, R.C., KHANNA, C., HOGGE, G.S.,VAIL, D.M. (2009) Use of Single-Agent Carboplatin as Adjuvant or Neoadjuvant Therapy in Conjunction With Amputation for Appendicular Osteosarcoma in Dogs. *Journal of the American Animal Hospital Association* **45**: 33-38.
23. ROSENBERGER, J.A., PABLO, N.V., CRAWFORD, P.C. (2007) Prevalence of and intrinsic risk factors for appendicular osteosarcoma in dogs: 179 cases (1996-2005). *Journal of the American Veterinary Medical Association* **7**: 1076-1080.
24. SAAM, D.E., LIPTAK, J.M., STALKER, M.J., CHUN, R. (2011) Predictors of outcome in dogs treated with adjuvant carboplatin for appendicular osteosarcoma: 65 cases (1996-2006). *Journal of the American Veterinary Medical Association* **2**: 195-206.
25. SACORNRATTANA, O., DERVISIS, N.G., MCNIEL, E.A., (2012) Abdominal ultrasonographic findings at diagnosis of osteosarcoma in dogs and association with treatment outcome. Veterinary and Comparative Oncology (Early online published)
26. SELVARAJAH, G.T., VERHEIJE, M.H., KIK, M., SLOB, A., ROTTIER, P.J.M., MOL, J.A., KIRPENSTEIJN, J. (2012) Expression of epidermal growth factor receptor in canine osteosarcoma: Association with clinicopathological parameters and prognosis. The Veterinary Journal 193: 412-419.
27. SIMCOCK, J.O., WITHERS, S.S., PRPICH, C.Y., KUNTZ, C.A., RUTLAND, B.E. (2012) Evaluation of a single subcutaneous infusion of carboplatin as adjuvant chemotherapy for dogs with osteosarcoma: 17 cases (2006-2010). Journal of the American Veterinary Medical Association 5: 608-614.
28. SPUGNINI, E.P., VINCENZI, B., CARUSO, G., BALDI, A., CITRO, G., SANTINI, D., TONINI, G., (2009) Zoledronic acid for the treatment of appendicular osteosarcoma in a dog. *Journal of Small Animal Practice* **50**: 44-46.
29. STRAW, R.C., WITHROW, S.J. (1996) Limb-sparing surgery versus amputation for dogs with bone tumors. *Veterinary Clinics of North America: Small Animal Practice* **26**: 135-143.