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Prognostic factors of early metastasis and mortality in dogs with appendicular osteosarcoma after receiving surgery: An individual patient data meta-analysis

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Abstract
Recently an aggregated data meta-analysis showed that serum alkaline phosphatase (SALP) and proximal humerus location are predictors for shorter survival in canine osteosarcoma. To identify additional prognostic factors of mortality and metastasis an individual patient data meta-analysis (IPDMA) was conducted. Individual patient data from 20 studies, identified via the VSSO society, were pooled. Univariable and multivariable hazard ratios (HR) for metastasis and mortality were assessed, using stratified Cox models. The study included 1405 dogs who received surgical treatment, of which thè metastasis status was measured in 1155 dogs and mortality status in 1336 dogs ; median survival was 256 days. High versus normal SALP and weight (kg) were associated with an increase in hazard of metastasis [HR 1.34 (95%CI 1.07; 1.68) and HR 1.02 (per kg increase) (95%CI 1.01 ; 1.03)] and for mortality [HR 1.43 (95%CI 1.16; 1.77) and HR 1.02 (95%CI 1.01 ; 1.02)]. Distal radius tumor was associated with a lower hazard of metastasis compared to other locations: HR 0.75 (95%CI 0.58; 0.96). Proximal humerus and distal femur or proximal tibia location were related with an increased mortality: HR 1.53 (95%CI 1.26; 1.84) and HR 1.23 (95%CI 1.01 ; 1.49) compared to other locations. Older age (years) was associated with a higher hazard for mortality [HR 1.06 per year (95%CI 1.03 ; 1.09)] but not for metastasis: HR 1.03 (95%CI 0.99 ; 1.06). These results confirm findings from a recent aggregated data meta-analysis and (in addition) showed that tumor location, SALP, weight were prognostic factors for both mortality and metastasis. Age was a prognostic factor for mortality but not for metastasis.

1. Introduction

Osteosarcoma (OS) is a malignant tumor of mesenchymal origin that produces osteoid. Similarities between human and canine OS are striking and include thè bimodal age distribution, the high incidence of morbidity and mortality, the site of the tumor, histologic features and the response to the various treatment modalities (Withrow and Wilkins, 2010; Rowell et al., 2011). The biggest difference is that OS is much more common in dogs than in people. The majority of canine primary bone tumors can be classified as OS, which predominantly occurs in large and giant breeds (Norrdin et al., 1989; Spodnick et al., 1992; Cooley and Waters, 1997; Ru et al., 1998; McNeill et al., 2007). OS dogs, treated only by amputation, have a median survival time of five months or less, with the majority succumbing to metastatic disease (Brodey and Abt, 1976; Straw et al., 1991; Spodnick et al., 1992). Due to advances in disease management overall survival can be extended to 1 year (Straw et al., 1991). Given the increased treatment options, such as adjuvant chemotherapy, 'limb-sparing' surgery and radio-ablative

methods, it has become even more important to differentiate between dogs with a worse and relatively improved prognosis. Numerous studies have explored the prognostic value of, for example gender, neuter status, age or serum alkaline phosphatase (SALP), but these studies have important limitations. Most notably, the relatively small number of patients included in these studies precludes precise estimation of the prognostic consequences of these factors. A possible solution for this is collecting and pooling reported prognostic associations from individual studies. Recently, Boerman et al. (2012) conducted an aggregated data meta-analysis. This meta-analysis showed that elevated SALP and location of OS in the (proximal) humerus are associated with a shorter disease free survival time. However, as Boerman acknowledges, the included studies did not analyze SALP and tumor location consistently; some explored the univariable association, while other used multivariable methods. Furthermore, other characteristics, for example age, weight and neuter status, could not be analyzed because these were not reported by a sufficient number of studies.

An alternative to pooling the aggregated data is to acquire the individual patient data files. An individual patient data meta-analysis (IPDMA) permits for more uniform analyses with regard to follow-up time, categorization of variables, missing values and analysis methods used (Stewart and Parmar, 1993; Riley et al., 2010). Furthermore, individual patient data allows exploring associations not reported in the original publications. Consequently, such prognostic IPDMAs are powerful tools to identify prognostic factors and subgroups of patients with different prognoses. We conducted an IPDMA in order to estimate the independent prognostic value of gender, neuter status, age, weight, breed, tumor location and SALP in predicting mortality or metastasis in canine OS.

2. Methods

2.1. Inclusion of individual patient data and assessment of data quality

To explore the relations between patient characteristic and (DF) survival we identified studies via the Veterinary Society of Surgical Oncology (VSSO). In January 2012 a call for collaboration was send out to VSSO members and other veterinary researchers. We attempted to include data from as many different researchers and institutes as possible. No a priori sample size calculations were performed.
Data were deemed eligible if baseline patient characteristics of OS dogs and time to event (death or metastasis) were recorded. To reduce the possibility of publication bias (Easterbrook et al., 1991), published and unpublished studies were both eligible. Impossible or unlikely data entries were explored and remaining irregularities were discussed with the original investigators. Data were collected on gender, neuter status, age (years), weight (kg), breed (Rottweiler, Golden Retriever, Labrador Retriever, Greyhound, Doberman, Irish Setter, mixed breeds, and other breeds), tumor location (proximal humerus, distal femur or proximal tibia, distal radius, and other locations), dichotomous SALP (using study specific cut-off values for high and normal SALP levels), surgery (limb-sparing, amputations), chemotherapy (no chemotherapy, cisplatin, lobaplatin or carboplatin, doxorubicin, doxorubicin combinations), and other treatments. To prevent low cell counts we refrained from using finer categories for breed, tumor location and chemotherapy. SALP status (at baseline) was dichotomized to follow clinical practice and because continuous SALP showed a positive linear sloped relationship with the outcomes that stabilized to a flat slope at high SALP values. Patients who did not receive surgery, mostly due to euthanasia (n = 197), who received an infrequently used chemotherapeutic protocol (n = 13), or who received radiation therapy (n = 11) were excluded from all analyses.

2.2. Data analysis

To illustrate how patient characteristics were related to mortality or metastases at the clinically relevant time points of 5 and 12 months (Brodey and Abt, 1976; Straw et al., 1991; Spodnick et al., 1992), we stratified baseline characteristics according to the outcome status (mortality and metastasis) at these points. Univariable associations were estimated using a stratified Cox proportional hazards model (Harrell, 2001). All the models were stratified by study to account for possible differences in baseline hazard. If a variable was missing for a certain patient, that patient was excluded from the univariable analysis (i.e., listwise deletion).
We then performed a multivariable Cox proportional hazards analysis (stratified by study) to assess the independent associations between prognostic factors and outcome. Subjects were censored if they were lost to follow-up or died (censoring for mortality was only applied in models using the metastasis outcome). Associations are given as hazard ratios (HRs) with 95% confidence intervals (95%CI) and p-values using an alpha of 0.05. For categorical variables a p-value for trend was computed and the individual associations were only explored if this overall test was significant (i.e., $p<0.05$). All multivariable models were corrected for chemotherapy status. Variable selection for the multivariable model was based on prior knowledge, no data driven selection method was used (i.e., stepwise selection). The proportional hazard (PH) assumption of the Cox models was checked based on Schoenfield residuals (Harrell, 2001). For the continuous variables weight and age linearity with the outcome was assessed using restricted cubic splines plots (Harrell et al., 1996); relations appeared to be linear. To determine how well the multivariate models discriminate between subjects with a short time to event and subjects with a longer time to event, the c-statistic (i.e., area under the receiver operator characteristic curve) was calculated (Steyerberg et al., 2010). The c-statistic represents the proportion of pairs of subjects where the subject with the longest *observed* time to event also received the longest *predicted* time to event; the c-statistic varies from 0.5 (no discrimination) and 1 (perfect discrimination).

In the multivariable analysis missing values were imputed, across studies, based on the areglmputation algorithm with ten imputations (Sterne et al., 2009; Harrell, 2012a). In each of the ten imputed datasets, a multivariable Cox proportional hazards analyses was conducted and

results were pooled using Rubin's rule (Marshall et al., 2009). The study by Sottnik et al. (2010) (n = 69) did not provide information on time until death. Similarly, studies by Phillips et al. (2009) (n = 156) and Berg et al. (1997) (n = 94) did not record information on time to metastasis. These studies were only used for the analyses they provided data for.

2.3. Sensitivity analysis

Effect estimates of the association between prognostic factors and non-mortality outcomes, such as metastasis, are potentially biased by competing risks. (Lau et al., 2009). In the case of time till metastasis a subject can be censored due to competing risks such as death (informative censoring). In such a case it is obviously wrong to assume that the subjects will get the event somewhere in the future (which is traditionally assumed when censoring). If this informative censoring is systematically related to a specific group (e.g., high SALP) censoring the deceased subjects inflates the cumulative incidence and competing risk occurs. Instead of censoring subjects who die before developing a metastasis a competing risk analysis keeps these subjects in the denominator, decreasing the cumulative incidence. In canine OS, most subjects first experience a metastasis before dying, therefore the impact of competing risk by death is expected to be small. Nevertheless we conducted competing risk analyses to assess how much this impacted our results (Satagopan et al., 2004). We also assessed the impact of missing observations through a sensitivity analysis in which a multivariate analysis was conducted using only those subjects with completely observed data. Additionally, to determine whether including subjects from small studies or unpublished studies biased our results we performed all analyses separately for large (50 or more subjects) and small studies (less than 50 subjects) and also stratified for publication status (i.e., if the study was published or not). To determine how influential the inclusion was of subjects who were not treated with chemotherapy, all analyses were also performed after excluding these patients. Finally, we assessed the impact of grouping lobaplatin and carboplatin in one group by repeated the analyses using separate categories for these chemotherapies.
All analyses were carried out with the R statistical package version 3.0.0 (R Development Core Team, 2013), the survival (Therneau, 2012), the rms (Harrell, 2012b) and the Hmisc (Harrell, 2012a) packages.

3. Results

Data from 20 studies were included in this IPDMA, of which 11 studies were previously published (Kurzman et al., 1995; Berg et al., 1997; Kirpensteijn et al., 2002; Vail et al., 2002; Morello et al., 2003; Moore et al., 2007; Bacon et al., 2008; Kow et al., 2008; Phillips et al., 2009; Sottnik et al., 2010). 19 studies reported solely on large breed dogs, while the unpublished study of Dr. Amsellem included 36 small breed canines. Characteristics of these studies are presented in Table 1. Eighteen studies (1155 patients) provided data on metastasis status and nineteen studies (1336 patients) provided information on mortality.

3.2. *Univariable analysis*

At 5 months, in the 550 dogs without missing data, 153 dogs developed a metastasis (Table 2). High weight (per kg) was related to an increase in metastasis hazard: hazard ratio (HR) 1.02 (95%CI 1.00; 1.03). Compared to the category other tumor locations, the distal radius category was associated with a decrease in hazard: HR 0.40 (95%CI 0.23; 0.68). Elevated baseline SALP was associated with an increased hazard of metastasis: HR2.12 (95%CI 1.52; 2.95); see Appendix I Figure Al for the Kaplan-Meier curves of SALP. Using other breeds as a reference Doberman subjects were related to a higher hazard, while mixed breed subjects were associated with a lower hazard: [HR2.16 (95%CI 1.06; 4.42) and HR 0.49 (95%CI 0.29; 0.84)]. By 1 year of follow-up the associations for metastasis of OS were similar to the results at 5 months (Table 2);

median DF survival was 234 days.

The median survival was 256 days, based on the 598 dogs that had no missing data. At 5 months of follow up, the factors tumor location, breed and SALP at baseline were both univariable related to mortality and the magnitudes of the observed relations were similar to those for metastasis. At 1 year, weight, location, breed and SALP showed similar and significant associations as found for metastasis at 1 year (Appendix I table Al).

3.2. Multivariable analysis

After imputing missing values, 1155 subjects were available for the metastasis outcome (Table 3). By the end of follow-up 765 experienced a metastasis. Weight was associated with an increased hazard [HR 1.02 (per kg increase) (95% CI 1.01; 1.03)], as well as high SALP [HR 1.34 (95%CI 1.07; 1.68)]. Compared to other tumor locations, patients with a distal radius OS were associated with a decreased hazard of metastases: HR 0.75 (95%CI 0.58; 0.96). Furthermore, the proximal humerus location was associated with an increased hazard of metastases, however this association was not significant: HR 1.21 (95%CI 0.96; 1.53). Similarly, breed was no longer significantly associated with metastasis after adjusting for other baseline characteristics.

For the outcome mortality, 1336 dogs were available for analysis, of which 1076 died. The associations between weight and mortality, and SALP and mortality were similar to those found for the outcome metastasis (Table 3). Compared to the category other OS locations, proximal humerus tumors were associated with a higher hazard of mortality: HR 1.53 (95%CI 1.26; 1.84). Similarly, having an OS at the distal femur or proximal tibia was related to an increased hazard: HR 1.23 (95%CI 1.01; 1.49). Finally, older aged subjects were also related to a higher hazard of mortality: HR 1.06 per year (95%CI 1.03; 1.09).

The discriminative performance of the multivariable models was modest: the model for the outcome metastasis had a c-statistic of 0.63, whereas the model for the outcome mortality had a c-statistic of 0.61.

3.3. Sensitivity analysis

Fig. 1 shows the Kaplan-Meier survival curves for the outcome metastasis (with and without accounting for competing risks). Twenty nine (4%) subjects died without experiencing a metastasis event before dying. Consequently, ignoring competing risks had little impact on results: the standard Kaplan-Meier estimates only marginally overestimate the cumulative incidence of metastases when accounting for competing risks.

Additional sensitivity analyses (on the impact of missing observations, size of the included studies, publication status of the included studies, and chemotherapy status of the dogs) did not show material difference compared to the results presented in Table 3. Results of these sensitivity analyses are available upon request.

4. Discussion

In our IPD meta-analysis on prognostic factors for metastasis and mortality among dogs with OS, weight, SALP and tumor location were independently prognostic predictors of mortality as well as metastasis. Age was significantly related with mortality only.

In accordance with the "aggregated data" meta-analysis by Boerman et al. (2012), we found that elevated SALP was associated with a higher hazard of early mortality or metastasis. However, the Boerman study showed somewhat larger and less precise estimates [HR 1.62 (95%CI 1.21; 2.17) for mortality and 1.96 (95%CI 1.50; 2.56) for metastasis] compared to our results: HR 1.43 (95%CI 1.16; 1.77) and HR 1.34 (95%CI 1.07; 1.68). Compared to other tumor sites, proximal humerus and distal femur or proximal tibia OS locations were related to an increased mortality hazard, but not metastasis. This is different from the Boerman study, which concluded that proximal humerus was significantly associated with both mortality and

metastasis [HR 1.86 (95%CI 1.34; 2.57) and 2.53 (95%CI 1.34; 4.77)]. Furthermore, we found that having an OS tumor at the distal radius was associated with a decreased metastasis hazard.

Our IPDMA also showed that independent of breed, high weight increased the hazard of both metastases and mortality. Possibly, this is due to the crude categorisation of the breed variable, with a large "other" category resulting in unexplained variance. Also different from the findings of the Boerman study was that we found age to be significantly related with mortality (increasing the hazard).

In this section we will discuss the limitation and strengths of our study. First, the number of patients with at least one missing observation was high (52% for the metastasis outcome and 57% for mortality). This was predominantly driven by SALP, which was only measured in 9 out of 20 studies. In aggregated meta-analyses, like the one conducted by Boerman et al. (2012), it is difficult to deal with missing data. In the current study we used an IPDMA design, which allows for imputation of missing values. Like all studies with missing observations, it is possible that missingness was dependent not only on measured factors but also on unmeasured factors, thus results may still be biased even though missing data was multiple imputed. However, assuming that at least some of the missing values are dependent on measured factors, imputing missing values would likely decrease bias compared to a complete cases analysis. Second, several sensitivity analyses were performed, all showing similar associations as our main analysis, confirming the robustness of our findings. Third, most studies used 1 or 2 specific chemotherapy regimens, making it difficult to distinguish between chemotherapy effects and other study-specific influences. Thus, while it seems essential to include chemotherapy in modeling the independent prognostic associations between patient characteristics and outcomes, observed associations between chemotherapy and outcomes should not be interpreted causally. Fourth, none of the baseline variables, except chemotherapy (and only in some studies), were randomly allocated. Therefore, it is possible that unmeasured or residual confounding influence our results. Given that it is impossible to randomly allocate baseline characteristics such as gender or age, every study exploring these associations is potentially hampered by the possibility of confounding. Causal interpretation of observed associations might therefore lead to erroneous conclusions. For example, when, contrary to the association reported here, there is no causal relationship between weight and mortality (possible the reported association is due to some unmeasured protective genetic factor that is closely related to lower weight) intervening on weight will have no effect on the outcome. However, in such a situation (no causal relationship of weight) weight will still provide useful information on the baseline risk for the outcome. Thus, the importance of causality of the here reported associations depends on the goal; either to intervene on risk factors or to use those factors for prognostication. Fifth, meta-analyses can be subject to publication bias (i.e., bias due to including published studies only) (Easterbrook et al., 1991). By recruiting data via the VSSO network, about 40% of the included subjects were from unpublished sources, making the potential for publication bias smaller. On the other hand, some researchers did not respond to our requests for collaboration therefore results presented here do not include all possible data and we cannot rule out the possibility that inclusion of more data could change our estimations. Finally, the discriminative ability of all models was modest. Including clinical predictors like grade or type of tumor could potentially increase this discriminative ability. However, in the current study this was impossible due to the large number of studies that did not record data on these variables.

Our present study used relatively new study techniques to combine individual patient data from different sources termed individual patient data meta-analysis (IPDMA). While an IPDMA requires big investments regarding time and resources we believe that the opportunities of using individual patient data compared to the alternative of relying on aggregated data outweigh this burden. An advantage of conducting an IPDMA is that one can explore relations not reported by the original authors. In our case this allowed us to estimate 7 associations while the Boerman aggregated data meta-analysis (Boerman et al., 2012) could only explore 3 associations. Similarly, IPMDA techniques ensure that when one wants to conduct multivariable analysis all estimates are corrected for the same set of variables. Without the same corrections, one has to rely on the reported estimates and as Boerman showed it is likely that every study uses distinct sets of covariables. A third advantage is that one can uniformly recode the data this

can be particularly important if different cut off values or reference categories are used by the original authors (e.g., categorizing age using a cut point of 5 or 7 years). Fourth, IPDMAs also allows one to check model assumption such as linearity or proportional hazard. Lastly by having access to individual patient data one can more easily perform subgroup analysis, sensitivity analysis and apply more refined methods to deal with problems such as missing values or competing risks. However, while we strongly recommend researcher to use IPD techniques in meta-analyses one should remember that the success of any meta-analysis ultimately depends on quality of the original data.

5. Conclusions

In conclusion, the IPDMA design used in this study allowed us to assess prognostic factors in canines with osteosarcoma. We identified weight, SALP, and tumor location as independent prognostic factors of metastasis and mortality, while age was only associated with early mortality. This study design allowed for the application of advanced missing data techniques and multiple sensitivity analyses and showed the necessity to use individual participant data in order to comprehensively assess prognostic factors in this field of research.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Author contributions

AFS, MN, RHHG, JK contributed to the idea and design of the study. AFS and JK approached and coordinated with researchers to collect data. AFS performed the analyses and drafted the manuscript. MN, OHK, AWH, AB, RHHG, the VSSO investigators and JK provided guidance during initial planning of the paper and during critical revision. AFS had full access to all of the data and takes responsibility for the integrity of the data presented.

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Prior postings and presentations

This study and its results have not been previously published, neither has it been presented at conferences.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/ j.prevetmed.2013.08.011.

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Table 1
Characteristics of studies included in the IPDMA for prognostic factors of canine osteosarcoma mortality or metastases[3]

Study	Published?	Design	*N* subjects	*N* mortality events	*N* metastases events	Maximum follow-up in days	Characteristics recorded							
							Age	Weight	Gender	Neutered	SALP	Breed	Location	Chemotherapy
Amsellem	No	NR	36	24	16	2539	√	√	√	√	√	√	√	√
Bacon 1 (Bacon et al., 2008)	Yes	NR	50	44	42	2192	√	√	√	√	√	√	√	√
Bacon 2	No	NR	145	113	113	2570	√	√	√	√	√	√	√	√
Berg (Berg et al., 1997)	Yes	RCT	94	81	NA	1628	√	√	√	√	√	√	√	√
Kirpensteijn (Kirpensteijn et al., 2002)	Yes	NR	134	111	85	2428	√	√	√	√	√	√	√	√
Kow (Kow et al., 2008)	Yes	NR	66	46	36	869	√		√	√		√	√	√
Kurzman 1	No	RCT	60	44	38	825	√	√	√	√		√	√	√
Kurzman 2	No	RCT	36	27	22	1584	√	√	√	√		√	√	√
Kurzman 3 (Kurzman et al., 1995)	Yes	RCT	64	55	54	986	√	√	√	√		√	√	√
Kurzman 4 (Kurzman et al., 1995)	Yes	RCT	25	19	21	1640	√		√	√		√	√	√
Maritato	No	NR	63	23	5	1923	√	√	√	√		√	√	√
Moore (Moore et al., 2007)	Yes	RCT	303	273	221	2109	√	√	√	√	√	√	√	√
Morello 1	No	NR	35	29	23	2209	√		√			√	√	√
Morello 2	No	NR	25	25	18	2023	√		√			√	√	√
Morello 3	No	NR	6	6	4	874	√	√	√			√	√	√
Morello 4	No	NR	5	5	3	1737	√	√	√			√	√	√
Morello 5 (Morello et al., 2003)	Yes	NR	13	13	7	2210	√	√	√			√	√	√
Phillips (Phillips et al., 2009)	Yes	NR	156	126	NA	3463	√	√	√	√	√	√	√	√
Sottnik (Sottnik et al., 2010)	Yes	NR	69	NA	49	1749	√	√	√	√	√	√	√	√
Vail (Vail et al., 2002)	Yes	RCT	20	12	8	730	√	√	√	√	√	√	√	√

[a] *N* subjects = number of observations after excluding patients not receiving surgery, other infrequently occurring chemotherapies or radiation therapy, NA = not recorded, NR = non randomized study, RCT = randomized controlled trial, individual patient data meta-analysis.

Table 2

Baseline characteristics stratified for event status at 5 months and 1 year follow-up for metastases due to osteosarcoma in canines, with univariable hazard ratios (HR) and 95% confidence intervals (95%CI).

Variables	Missing	5 months Event-free $N=872$	Event $N=283$	HR (95%CI) p-value	1 year Event-free $N=568$	Event $N=587$	HR (95%CI) p-value
Mean subjects per study	–	64		–	64		–
Number of subjects without missings N(%)	–	397(46%)	153(54%)	–	241(42%)	309(53%)	–
Number of subjects from published studies N(%)	–	556(64%)	188(66%)	–	337(59%)	407(69%)	–
Follow-up days median (Q1–Q3)	55	148(148–148)	83(62–112)	–	356(172–356)	151(85.5–221)	–
Age (years) mean (sd)	20	8.29(2.66)	8.08(2.64)	1.00 (0.93; 1.06) $p=0.91$	8.33(2.8)	8.15(2.51)	1.00 (0.95;1.05) $p=0.99$
Weight (kg) mean (sd)	200	35.64(12.47)	38.01(15.13)	1.02 (1.00;1.03) $p=0.02$	35.19(13.09)	37.22(13.32)	1.02 (1.01;1.02) $p<0.01$
Male gender N(%)	6	463(53%)	136(48%)	0.84 (0.61;1.15) $p=0.28$	311(55%)	288(49%)	0.82 (0.65;1.03) $p=0.08$
Neutered N(%)	90	674(85%)	217(81%)	0.90 (0.53;1.54) $p=0.71$	439(85%)	452(82%)	0.85 (0.58;1.25) $p=0.40$
High SALP N(%)	525	146(32%)	86(51%)	2.12 (1.52;2.95) $p<0.01$	89(31%)	143(42%)	1.70 (1.34;2.15) $p<0.01$
Breed	9			Overall p-value = 0.01			Overall p-value = 0.01
Other N(%)		293(34%)	150(41%)	Reference	208(37%)	200(34%)	Reference
Rottweiler N(%)		109(13%)	42(15%)	1.20 (0.75;1.90) $p=0.45$	65(12%)	86(15%)	1.48 (1.06;2.09) $p=0.02$
Golden Retriever N(%)		83(10%)	28(10%)	0.69 (0.38;1.27) $p=0.23$	48(9%)	63(11%)	0.89 (0.59;1.33) $p=0.57$
Labrador Retriever N(%)		75(9%)	22(8%)	0.68 (0.36;1.30) $p=0.24$	41(7%)	56(10%)	0.73 (0.47;1.13) $p=0.16$
Greyhound N(%)		44(5%)	17(6%)	1.11 (0.57;2.17) $p=0.76$	32(6%)	29(5%)	1.09 (0.65;1.81) $p=0.75$
Doberman N(%)		39(5%)	18(6%)	2.16 (1.06;4.42) $p=0.03$	24(4%)	33(6%)	1.70 (0.93;3.14) $p=0.09$
Irish Setter N(%)		24(3%)	5(2%)	0.56 (0.14;2.32) $p=0.42$	17(3%)	12(2%)	0.73 (0.29;1.83) $p=0.51$
Mixed N(%)		199(23%)	33(12%)	0.49 (0.29;0.84) $p=0.01$	129(23%)	103(18%)	0.74 (0.53;1.04) $p=0.09$
Tumor location	91			Overall p-value < 0.01			Overall p-value < 0.01
Other N(%)		219(27%)	91(34%)	Reference	151(29%)	159(29%)	Reference
Prox. Humerus N(%)		175(22%)	67(25%)	1.06 (0.69;1.63) $p=0.78$	96(19%)	146(26%)	1.43 (1.05;1.94) $p=0.03$
Dist. Femur or Prox. Tibia N(%)		175(22%)	65(24%)	1.01 (0.65;1.55) $p=0.97$	106(21%)	134(24%)	1.26 (0.92;1.74) $p=0.15$
Dist. Radius N(%)		228(29%)	44(16%)	0.40 (0.23;0.68) $p<0.01$	159(31%)	113(20%)	0.63 (0.44;0.89) $p=0.01$
Chemotherapy	30			Overall p-value = 0.02			Overall p-value = 0.15
No chemo N(%)		104(12%)	47(17%)	Reference	85(16%)	66(11%)	Reference
Cisplatin N(%)		124(15%)	40(14%)	1.14 (0.57;2.30) $p=0.71$	72(13%)	92(16%)	0.92 (0.50;1.66) $p=0.77$
Lobaplatin, carboplatin N(%)		61(7%)	32(11%)	0.67 (0.31;1.46) $p=0.32$	39(7%)	54(9%)	1.02 (0.53;1.94) $p=0.96$
Doxorubicin N(%)		348(41%)	97(35%)	0.47 (0.23;0.95) $p=0.04$	218(40%)	227(39%)	0.61 (0.36;1.04) $p=0.07$
Doxorubicin combinations N(%)		209(25%)	63(23%)	0.30 (0.12;0.73) $p=0.01$	129(24%)	143(25%)	0.59 (0.31;1.14) $p=0.12$

Table 3

Multivariable hazard ratios (HR) with 95% confidence intervals (95%CI) and p-values forthe hazard of metastases or mortality, using the entire follow-up period[3].

Variables	Metastasis HR (95%CI), *p*-value	Mortality HR (95%CI) *p*-value
Number of observations (total dog years at risk)	1155 (621 years)	1336 (1042 years)
Number of events	765	1076
Age (years)	1.03 (0.99;1.06) $p = 0.15$	1.06 (1.03;1.09) $p < 0.01$
Weight (kg)	1.02 (1.01;1.03) $p < 0.01$	1.02 (1.01;1.02) $p < 0.01$
Male gender	0.91 (0.77;1.08) $p = 0.29$	0.95 (0.83;1.09) $p = 0.50$
Neutered	0.90 (0.70;1.15) $p = 0.38$	0.85 (0.70;1.03) $p = 0.09$
High SALP	1.34 (1.07;1.68) $p = 0.01$	1.43 (1.16;1.77) $p < 0.01$
Breed	Overall *p*-value = 0.67	Overall *p*-value = 0.65
Other	Reference	Reference
Rottweiler	1.00 (0.78;1.30) $p = 0.98$	0.98 (0.80;1.21) $p = 0.87$
Golden Retriever	1.09 (0.82;1.45) $p = 0.56$	1.04 (0.83;1.31) $p = 0.73$
Labrador Retriever	1.04 (0.79;1.38) $p = 0.78$	0.89 (0.69;1.15) $p = 0.36$
Greyhound	1.31 (0.91;1.89) $p = 0.15$	1.15 (0.86;1.56) $p = 0.35$
Doberman	1.02 (0.71;1.47) $p = 0.93$	1.10 (0.80;1.51) $p = 0.56$
Irish Setter	0.77 (0.44;1.38) $p = 0.38$	0.91 (0.57;1.45) $p = 0.69$
Mixed	0.92 (0.73;1.15) $p = 0.44$	0.89 (0.73;1.07) $p = 0.22$
Tumor location	Overall *p*-value < 0.01	Overall *p*-value < 0.01
Other	Reference	Reference
Prox. Humerus	1.21 (0.96;1.53) $p = 0.10$	1.53 (1.26;1.84) $p < 0.01$
Dist. Femur or Prox. Tibia	1.10 (0.88;1.39) $p = 0.40$	1.23 (1.01;1.49) $p = 0.04$
Dist. Radius	0.75 (0.58;0.96) $p = 0.02$	0.90 (0.74;1.10) $p = 0.30$
Chemotherapy	Overall *p*-value = 0.28	Overall *p*-value = 0.43
No chemo	Reference	Reference
Cisplatin	1.17 (0.75;1.82) $p = 0.50$	1.04 (0.67;1.60) $p = 0.87$
Lobaplatin, carboplatin	1.27 (0.86;1.87) $p = 0.23$	1.04 (0.72;1.51) $p = 0.85$
Doxorubicin	0.88 (0. 60;1.28) $p = 0.50$	0.98 (0.67;1.46) $p = 0.94$
Doxorubicin combinations	0.91 (0.64;1.31) $p = 0.61$	0.75 (0.53;1.06) $p = 0.10$

[a] Results are based on a model including all variables presented, no stepwise selection was applied.

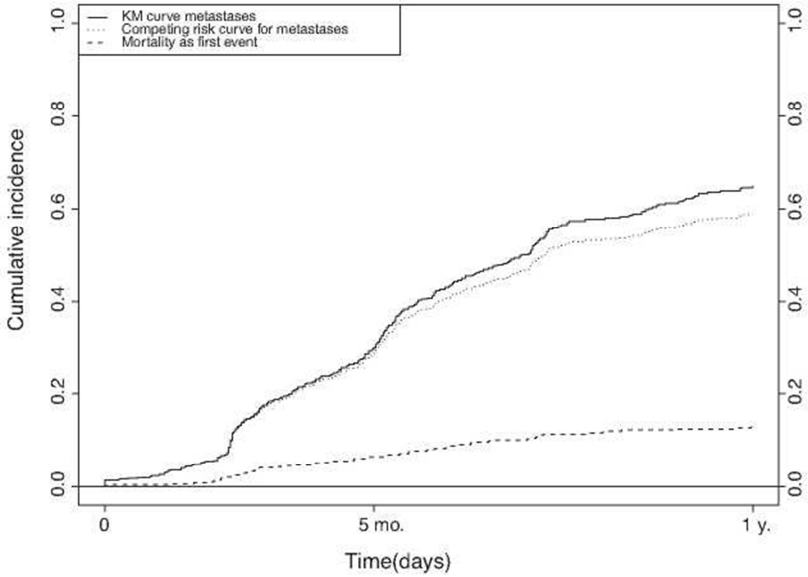

Fig. 1. Kaplan-Meier survival curves for canines with osteosarcoma. Competing risk curve for metastases, with biased Kaplan-Meier curve for metastases and mortality without a metastasis. Results are based on 511 subjects without missings that had data on both mortality and metastases outcome