

## Which dogs with appendicular osteosarcoma benefit most from chemotherapy after surgery? Results from an individual patient data meta-analysis



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### ABSTRACT

Osteosarcoma (OS) is a malignant tumor of mesenchymal origin that produces osteoid. Given that the prognosis can vary considerably between dogs, we aimed to explore whether treatment could be tailored towards patient subgroups, characterized by their predicted risk of mortality. For the current study, a subset of five nonrandomized studies (400 subjects of whom 88 were dead at 5 months follow-up) was used from a previously published 20 study individual patient data meta-analysis. Missing data was dependent on observed variables and was imputed to correct for this dependency. Based on a previously published multivariable prognostic model, the 5-month mortality risk was predicted. Subsequently, in surgically treated dogs, using a logistic regression model with a random intercept for a study indicator, we explored whether chemotherapy effectiveness depended on predicted 5-month mortality risk. After adjustment for potential confounders the main effect of any chemotherapy was 0.48 (odds ratio) (95%CI 0.30; 0.78). Testing for chemotherapy by predicted 5-month mortality risk interaction revealed that the effects of any chemotherapy decreased with increasing predicted risk; interaction OR 3.41 (1.07; 10.84). Results from individually comparing carboplatin, cisplatin, doxorubicin and doxorubicin combination therapy to no chemotherapy, were similar in magnitude and direction. These results indicate that the main treatment effects of chemotherapy do not necessarily apply to all patients.

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### 1. Introduction

Osteosarcoma (OS) is a malignant tumor of mesenchymal origin that produces osteoid. In dogs, OS most frequently occurs in large and giant breeds (Cooley and Waters, 1997; McNeill et al., 2007; Norrdin et al., 1989; Ru et al., 1998; Spodnick et al., 1992). Dogs that are treated with amputation have a median survival time of

five months, with the majority succumbing to metastatic disease (Brodey and Abt, 1976; Straw and Withrow, 1996). Clinical studies have shown that on average survival in OS dogs can be extended by administering chemotherapy (Bailey et al., 2003; Chun et al., 2005, 2000; Straw et al., 1991; Vail et al., 2002).

After performing an aggregated meta-analysis (Boerman et al., 2012), a prognostic model for mortality in surgically treated canine osteosarcoma patients was developed using a 20 study individual patient data meta-analysis (IPDMA) (Schmidt et al., 2013). Such a prognostic model can be used to predict a dog's risk of early mortality (Moons et al., 2012). This offers the possibility to identify subgroups of dogs according to their baseline prognosis and target treatment to patients most likely to benefit. This can poten-

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tially prevent dogs from unnecessarily receiving treatment, which is relevant in terms of both costs and quality of life.

In the current paper, using a five study subset of our previously published IPDMA (Schmidt et al., 2013), chemotherapy effects were individualized by determining whether dogs with a different 5-month mortality predicted risk, reacted differently to chemotherapy treatment. Specifically, using an adapted version of the previously published prediction model (Schmidt et al., 2013), we first predicted a dog's 5-month mortality risk based on age, weight, gender neuter status, serum alkaline phosphatase (SALP) level, breed, and tumor location at time of surgery. Subsequently we evaluated what the effect was of "any chemotherapy" compared to no chemotherapy on the 5-month mortality incidence and if this effect differed between dogs with different predicted 5-month mortality risks. These estimates were compared for consistency to compound specific estimates for carboplatin, cisplatin, doxorubicin and doxorubicin combination therapy (the available groups of chemotherapy).

## 2. Materials and methods

The effects of the different chemotherapeutics compared to no chemotherapy were determined using individual patient data (IPD). These IPD were used previously in an IPD meta-analysis (IPDMA) combining data of 20 studies to determine prognostic factors for early mortality in dogs with osteosarcoma (Schmidt et al., 2013). A detailed description of the data accrual can be found in the original publication (a review protocol is unavailable). Briefly, studies were collected via the Veterinary Society of Surgical Oncology (VSSO). In January 2012, a call for collaboration was sent out to VSSO members and other veterinary oncologic researchers. Data was 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. All dogs in these studies were diagnosed with osteosarcoma. For the present analysis, dogs were excluded if they did not receive surgery; due to euthanasia ( $n = 197$ ), who received limb-sparing surgery ( $n = 41$ ), who received an infrequently used chemotherapeutic protocol ( $n = 13$ ) or who received radiation therapy ( $n = 11$ ). Note that the exclusion of the 41 dogs (collected in 3 studies) treated with limb-sparing surgery is contrary to the original publication, and given the small number does not markedly influence our results. Additionally, the study by Sottnik et al. (2010) only collected data on metastasis, not mortality, and was excluded. Data was available from 1295 dogs collected in 16 studies.

To answer our present questions, does chemotherapy effectiveness differ between dogs with different predicted 5-month mortality risk, we used the 1295 dogs to construct a logistic regression prediction model; predicting mortality at 5 months. Subsequently, from these 1295 dogs (16 studies), studies were selected that included at least five dogs on no chemotherapy and at least five dogs treated with one of the interventions of interest (i.e., carboplatin, cisplatin, doxorubicin or doxorubicin combination therapy). Five nonrandomized studies fulfilled this criterion; of these 5 studies, three were previously published (Amsellem et al., 2014; Kirpensteijn et al., 2002; Kow et al., 2008), the two unpublished studies, by Maritato and Bacon, were based on routine healthcare records. After excluding dogs that received lobaplatin chemotherapy ( $n = 27$ ) 400 subjects remained. Regrettably, none of these 5 studies randomly allocated chemotherapy hence chemotherapy associations are likely confounded; an issue that will be addressed later. We will first briefly describe how the logistic regression prediction model was derived (using the 1295 dogs). Second, we describe in detail how the predicted 5-month mortality risk was

calculated for each individual dog, resulting in an individualized prediction. Third, we explain how individualized chemotherapy effect estimates were derived (based on the 400 dogs). Finally, a number of sensitivity analyses are discussed. Note that this study focused on 5-month mortality, because this is regarded as a clinically relevant endpoint (Brodey and Abt, 1976; Spodnick et al., 1992; Straw et al., 1991), however we are not aware of any biological rationale other than that it reflects the median survival time after amputation (without further treatment).

### 2.1. Data analysis: prediction model

Instead of using the Cox's proportional hazards prediction model described in Schmidt et al. (2013), the current analysis uses a logistic regression model with random intercept for study. The reasons for switching to a "simpler" logistic regression model were twofold. First, the logistic model has a time independent intercept (contrary to the baseline hazard in a Cox model) making it easier to introduce our methodology, second, the proportional hazard assumption for the treatment by predicted risk interaction term seemed to be violated, including the null at 1 year (see Manuscript 2 for more detail). The logistic regression prediction model used the previously described 1295 dogs IPDMA and regressed a 5-month mortality indicator on the predictor's gender, neuter status, tumor location (proximal humerus, distal femur or proximal tibia, distal radius, versus other locations), age (years, continuous), weight (kg, continuous), breed (Rottweiler, Golden Retriever, Labrador Retriever, Greyhound, Doberman, mixed breeds, versus other breeds) and serum alkaline phosphatase (SALP, using study specific cut-off values for high and normal SALP levels). Chemotherapy was included as a nuisance variable and was set to zero (no chemotherapy) when predicting the 5-month mortality risk. As in the original publication, all predictors were predefined and no model selection was used (Schmidt et al., 2013). However, linearity of the continuous predictors was assessed by comparing a model (using a likelihood ratio test) with restricted cubic splines (5 knots) to a model forcing linearity. Additionally, restricted cubic spline plots were created to visually inspect linearity. Besides, SALP which was dichotomized, no deviations from linearity were observed (Refer to Table 1 for the derived prediction model based on 1295 dogs with). To prevent overfitting our prediction model (further) no additional model comparisons were performed (Chatfield, 1995). Please see the Appendix for a description of the model performance and Fig. A, a calibration plot comparing predicted versus observed 5-month mortality risk.

In the 1295 dogs about 8% of the data was missing, information on 5-month mortality was missing for 4.2% of the observations and chemotherapy for 2.4% of the observations (see for more details Schmidt et al., 2013). Univariable tests showed that missingness was associated with observed variables (results available from the first author) biasing a complete case analysis (Altman and Bland, 2007; Rubin, 1976). To adjust bias due to missing data, this dependency was taken into account by imputing missing observation using the *aregImpute* algorithm from the *Hmisc* package version 3.13-0 (Harrell and Dupont, 2013). The *aregImpute* algorithm was implemented using 10 burn-in iterations, 100 approximate bootstrap samples and predictive mean matching. To get correct estimates of the standard errors 100 imputed datasets were created (i.e., multiple imputation). Results over all 100 imputed datasets were pooled using Rubin's rules (Little and Rubin, 2002; Marshall et al., 2009).

### 2.2. Data analysis: predicting 5-month mortality

An individual dogs' risk of 5-month mortality, under no chemotherapy, was predicted using the coefficient presented in

**Table 1**  
Multivariable prediction model for 5-month mortality in canines surgically treated for osteosarcoma.

Variables	Odds ratio (95%CI)	Regression coefficients	Standard errors
Intercept		$\hat{\beta}_0 = -1.2379$	0.48
Chemotherapy			
No chemotherapy	Reference	$\hat{\beta}_1 = 0.0000$	
Cisplatin	0.60 (0.31; 1.15)	$\hat{\beta}_2 = -0.5108$	0.33
Lobaplatin, carboplatin	0.59 (0.32; 1.10)	$\hat{\beta}_3 = -0.5276$	0.31
Doxorubicin	0.52 (0.29; 0.95)	$\hat{\beta}_4 = -0.6539$	0.30
Doxorubicin combinations	0.38 (0.21; 0.68)	$\hat{\beta}_5 = -0.9676$	0.30
Age (years)	1.03 (0.97; 1.09)	$\hat{\beta}_6 = 0.0296$	0.03
Weight (kg)	1.02 (1.00; 1.03)	$\hat{\beta}_7 = 0.0198$	0.01
Male gender	0.79 (0.60; 1.05)	$\hat{\beta}_8 = -0.2357$	0.14
Neutered	0.79 (0.54; 1.15)	$\hat{\beta}_9 = -0.2357$	0.19
High SALP	1.45 (1.08; 1.95)	$\hat{\beta}_{10} = 0.3716$	0.15
Breed			
Other breed	Reference	$\hat{\beta}_{11} = 0.0000$	
Rottweiler	0.89 (0.58; 1.35)	$\hat{\beta}_{12} = -0.1165$	0.22
Golden retriever	0.86 (0.53; 1.39)	$\hat{\beta}_{13} = -0.1508$	0.24
Labrador retriever	0.81 (0.48; 1.37)	$\hat{\beta}_{14} = -0.2107$	0.27
Greyhound	1.29 (0.70; 2.37)	$\hat{\beta}_{15} = 0.2546$	0.31
Doberman	1.47 (0.81; 2.69)	$\hat{\beta}_{16} = 0.3853$	0.31
Mixed breed	0.73 (0.49; 1.09)	$\hat{\beta}_{17} = -0.3147$	0.20
Tumor location			
Other	Reference	$\hat{\beta}_{18} = 0.0000$	
Prox. humerus	1.54 (1.05; 2.25)	$\hat{\beta}_{19} = 0.4318$	0.19
Dist. femur or prox. tibia	0.97 (0.65; 1.44)	$\hat{\beta}_{20} = -0.0305$	0.20
Dist. radius	0.69 (0.46; 1.04)	$\hat{\beta}_{21} = -0.3711$	0.21

**Example patient's predicted logit (5-month mortality risk)** =  $-1.2379 + 0.0000 \times \text{chemotherapy (0)} + 0.0296 \times 7.7 \text{ years} + 0.0296 \times 44 \text{ kg} + -0.2357 \times \text{female (0)} + -0.2357 \times \text{neutered (1)} + 0.3716 \times \text{high salp (1)} + -0.0000 \times \text{other breed (0)} + 0.0000 \times \text{other location (0)} = -0.0031$ .

**Example patient's predicted 5-month mortality risk** =  $1/(1 + e^{-(-0.0031)}) = 0.4992$ .

Numbers represent odds ratios with 95% confidence intervals (95%CI). All odds ratios were adjusted for all other presented variables and a random intercept for a study indicator. This multivariable logistic regression model is based on the cox proportional hazard model described in Schmidt et al. (2013) including the same predictors and using 1295 OS dogs of which 295 died within 5 months collected in 16 studies.

Table 1 and by setting the chemotherapy to zero (no chemotherapy):

$$\text{logit}(5\text{-month mortality risk}) = \text{logit}(\hat{p}_i)$$

$$= \hat{\beta}_0 + \hat{\beta}_1 \times \text{chemotherapy (0)} + \sum_{j=6}^J \hat{\beta}_j x_{ij} \quad (1)$$

Here  $\hat{p}_i$  indicates an individuals' predicted risk of being dead at 5 months.  $\hat{\beta}_j$  represent the coefficient for the  $j$ th variable presented in Table 1. Finally,  $x_{ij}$  represents an individuals' value for the  $j$ th variable. Note, that this is equal to calculating the linear predictor conditional on no chemotherapy. Additionally we note that, while Eq. (1) is applied to every individual patient, and conditional on a patient's characteristics the difference in predicted risk can be large, the predicted risk is not a truly individual estimate of a patients' risk of 5-month mortality. Instead, the predicted risk should be interpreted as an average predicted risk for a patient with similar characteristics. For these predictions, the random intercept (from the logistic regression model) was ignored because in clinical practice this prediction model would not be limited to the studies included in our meta-analysis. The predicted logit(5-month mortality risk) can vary from minus to plus infinity, with zero referring to a risk of 50%. This predicted logit(5-month mortality risk) can be transformed to the predicted 5-month mortality risk, bounded between 0 and 1, by the following equation:

$$\hat{p}_i = \frac{1}{1 + e^{-\text{logit}(\hat{p}_i)}} \quad (2)$$

see Table 1 for an example.

Applying Eq. (2) results in an estimate of the predicted risk of 5-month mortality given that the patient did not receive chemotherapy. Note that the predicted logit(5-month mortality

risk) is expected to be linearly related with the outcome. Where appropriate the predicted logit(5-month mortality risk) was transformed to the predicted 5-month mortality risk using Eq. (2); for example when graphing results.

### 2.3. Data analysis: estimating chemotherapy effectiveness

As indicated previously, first the association of "any chemotherapy" compared to no chemotherapy with 5-month mortality was estimated. If this association was significant we determined how the different chemotherapeutics carboplatin, cisplatin, doxorubicin or doxorubicin combination compared to no chemotherapy. These analyses used the previously defined subset of 400 subjects, collected by combining 5 studies (see Appendix Table A). Depending on the comparison, a different subset of these 5 studies was used (see Appendix Tables B through E): for any chemotherapy all studies were used, similarly for doxorubicin combination all studies were used, for carboplatin the study by Kirpensteijn was excluded, for doxorubicin the studies by Kirpensteijn and Amsellum were excluded and finally for the cisplatin comparison only the study by Bacon was used. This selection was based on whether the studies included any dog on the mentioned chemotherapeutic and aims to prevent bias due to study specific influences.

Before determining whether chemotherapy effects differed between dogs with a different predicted logit(5-month mortality risk) we first estimated the main effect of chemotherapy (i.e., a model regressing 5-month mortality on chemotherapy and covariables without an interaction term with chemotherapy). These main effects provide an estimate of the average effect of chemotherapy and were derived using a logistic regression model including a random intercept for study. Specifically, a model was fitted,

regressing 5-month mortality on the chemotherapy variable and a random intercept for study. A second model additionally included gender, neuter status, tumor locations, age, weight and SALP. The third model additionally adjusted for breed. These variables were included in an attempt to adjust for confounding and were selected based on prior knowledge (Hernan et al., 2002). To reduce the risk of residual confounding and prevent the usual multiple testing problem (Bland and Altman, 1995), no model reduction strategy was employed and no differentiation was made between predictors of the outcome and confounders.

After determining the main effects, we explored whether chemotherapy effectiveness depended on predicted logit(5-month mortality risk) by including a chemotherapy by predicted logit(5-month mortality risk) interaction term in the model.

$$\begin{aligned} & \text{logit}(\text{prob}[y_{is} = 1]) \\ &= \hat{\alpha}_{0s} + \hat{\alpha}_1 \times \text{chemotherapy} + \hat{\alpha}_2 \times \text{logit}(\hat{p}_i) + \hat{\alpha}_3 \times \text{chemotherapy} \\ & \times \text{logit}(\hat{p}_i) + \varepsilon_{is} \end{aligned}$$

Here  $y_i$  represent an individual's mortality status at 5-months. Let  $\hat{\alpha}_1$  represent the estimated association of chemotherapy compared to no chemotherapy when all co-variables are zero [i.e., when the logit(5-month mortality risk) = 0],  $\hat{\alpha}_2$  the association of predicted logit(5-month mortality risk) when a patient does not receive chemotherapy, and  $\hat{\alpha}_3$  the association of the chemotherapy by predicted logit(5-month mortality risk) interaction term.  $\hat{\alpha}_3$  indicates how much the association of chemotherapy changes per unit increase or decrease of the predicted logit(5-month mortality risk). In the absence of interaction,  $\hat{\alpha}_3$  becomes zero and can be omitted. Epsilon indicates the amount of residual error per individual  $i$  and per study  $s$ . Also note that because the predicted logit(5-month mortality) variable include the same confounder information (see above), this model also adjust for confounding similar to a diseased risk score (Arbogast et al., 2008). Using these estimates an individualized effect of chemotherapy can be calculated:

$$\widehat{\text{OR}}_i = e^{\hat{\alpha}_1 \times \text{chemotherapy}(1) + \hat{\alpha}_3 \times \text{chemotherapy}(1) \times \text{logit}(\hat{p}_i)} \quad (3)$$

Here OR represent the estimated odds ratio of chemotherapy for the  $i_{\text{th}}$  individual. Note, that  $\text{logit}(\hat{p}_i) = \text{logit}(5\text{-month mortality risk})$  and is calculated using Eq. (1). Also, note that this methodology has been previously applied in human medicine most notably in the SYNTAX trial (Farooq et al., 2013; van Klaveren et al., 2015). Similar to the results from Eq. (1) we underline that, while the treatment effect is calculated for every individual patient it should not be interpreted as a truly individual estimate of chemotherapy. Instead, it reflects a treatment effect taking into account a patient's predicted risk of mortality, which is more individualized than usual treatment effect but certainly not the effect of chemotherapy in an individual.

For the subset of 400 dogs on average 12.6% of the information was missing; specifically, 5-month mortality 9%, chemotherapy 7.5%, tumor location 9.5%, gender 5.3%, neuter status 5.3%, age 6.5%, weight 26.3%, high SALP 57.5% and breed 4.5%, (see Table 2 for an overview). Again missing values were imputed as previously described.

#### 2.4. Data analysis: sensitivity analyses

In the following section we describe a few sensitivity analyses evaluating the appropriateness of assumptions made.

Throughout a logistic regression model was used including a random intercept for study. Such a model assumes that the random intercept can sufficiently be described by a normal distribution. To evaluate this assumption a regular logistic regression model was used including study as a categorical factor, which does not assume

any parametric distribution; results did not differ (see Appendix Table F).

In all 5 studies included, chemotherapy was not allocated randomly. Therefore it is possible that dogs did not receive chemotherapy because of a worse prognosis, which would overestimate any beneficial effects of chemotherapy. To explore this, all analyses were repeated using the subset of patients that survived the first month (30 days). While, this analysis potentially decreases bias, precision was decreased because 22% of the events occurred in the first month.

Previously, we implicitly assumed that the association of chemotherapy by predicted logit(5-month mortality risk) interaction term with the outcome was linear. The appropriateness of this assumption was evaluated by comparing a model with a restricted cubic spline (with five knots) for the interaction term to a model without splines, using a likelihood ratio test. Additionally, a model was compared that categorized the predicted logit(5-month mortality risk) in quintiles. No significant deviations from linearity were observed.

All tests were applied using a significance level of 0.05, estimates are presented as odds ratios (OR) with 95% confidence intervals (95%CI). Analyses were carried out using the R statistical package for windows version 3.0.2 (R Development Core Team, 2013), the lme4 package version 1.1-7 for random effect models (Bates et al., 2012), and the metafor package (Viechtbauer, 2010). R codes can be found in Appendix 2, a PRISMA checklist is included in Appendix 3.

### 3. Results

Baseline characteristics of the 406 included dogs are presented in Table 2, after surgical amputation 227 received additional chemotherapy and 143 dogs did not, of these 87 were dead after 5 months. Information on chemotherapy was missing for 30 subjects and 5-month mortality for 36 subjects. In general, dogs not receiving chemotherapy were older, weighed less, were more often female, neutered and had high SALP. The range of the predicted logit(5-month mortality risk) was  $-1.91$  to  $1.03$ . Baseline characteristics for the other comparisons (carboplatin, cisplatin, doxorubicin and doxorubicin combination) are presented in Appendix Tables B through E.

The crude main effect estimates of “any chemotherapy” versus no chemotherapy on 5-month mortality was OR 0.43 (95%CI 0.27; 0.70). After adjustment for potential confounders the OR was 0.48 (95%CI 0.29; 0.78), for details refer to Table 3. Results for the other comparisons were similar; with the possible exception of the cisplatin effect, which was non-significant (Table 3).

Testing for chemotherapy by predicted logit(5-month mortality risk) interaction revealed that the effects of any chemotherapy (compared to no chemotherapy) decreased with increasing predicted logit(5-month mortality risk, Table 4); interaction OR 3.41 (95%CI 1.07; 10.84)  $P$ -value = 0.04. Fig. 1 depicts how the OR of chemotherapy changes with predicted logit(5-month mortality risk) and 5-month mortality risk (i.e., on the risk scale) and shows that dogs' with a predicted 5-month mortality risk of approximately 0.43 or less benefit from chemotherapy. For dogs' at a higher predicted risk, the effectiveness chemotherapy is uncertain because the 95% confidence interval includes the 1. Results for the other chemotherapy compounds and studies are presented in Figs. 2 and 3. While the compound and study specific results are not significant they agree well with the results shown in Fig. 1 indicating uncertainty in the effectiveness of chemotherapy in dogs with higher predicted 5-month mortality risk.

The results of the sensitivity analysis of excluding those subjects who died within the first month are depicted in Table 4. The main



**Table 2**  
Baseline characteristics of 400 canines with osteosarcoma stratified by treatment status.

Variables	No chemotherapy <i>N</i> = 143	Any chemotherapy <i>N</i> = 227	Number of missings <i>N</i>
5-Month mortality <i>N</i> (%)	44 (33%)	44 (20%)	36
Chemotherapy			30
No chemotherapy	143 (100%)	0 (0%)	
Cisplatin	0 (0%)	37 (16%)	
Lobaplatin, carboplatin	0 (0%)	45 (20%)	
Doxorubicin	0 (0%)	77 (34%)	
Doxorubicin combinations	0 (0%)	68 (30%)	
Age (years) mean (sd)	8.97 (2.98)	8.68 (2.72)	26
Weight (kg) mean (sd)	32.20 (15.77)	34.40 (15.43)	105
Male gender <i>N</i> (%)	75 (52%)	127 (56%)	21
Neutered <i>N</i> (%)	109 (76%)	195 (87%)	21
High SALP <i>N</i> (%)	30 (58%)	54 (47%)	230
Breed			18
Other breed <i>N</i> (%)	62 (43%)	94 (41%)	
Rottweiler <i>N</i> (%)	13 (9%)	26 (11%)	
Golden retriever <i>N</i> (%)	7 (5%)	22 (10%)	
Labrador retriever <i>N</i> (%)	13 (9%)	11 (5%)	
Greyhound <i>N</i> (%)	5 (3%)	16 (7%)	
Doberman <i>N</i> (%)	6 (4%)	6 (3%)	
Mixed breed <i>N</i> (%)	37 (26%)	52 (23%)	
Tumor location			38
Other <i>N</i> (%)	73 (54%)	82 (38%)	
Prox. humerus <i>N</i> (%)	19 (14%)	41 (19%)	
Dist. femur or prox. tibia <i>N</i> (%)	24 (18%)	48 (22%)	
Dist. radius <i>N</i> (%)	18 (13%)	46 (21%)	

Data was available on 400 subjects, because 30 had missing information on chemotherapy the columns add up to 370. Serum alkaline phosphatase (SALP); *N* equals the number of subjects, sd equals the standard deviation. These dogs were originally included in studies by Amsellum, Bacon, Kirpensteijn, Kow and Maritato (co-authors of the current IPDMA).

**Table 3**  
Treatment effect estimates of different chemotherapeutics compared to no chemotherapy on 5-month mortality in dogs surgically treated for osteosarcoma.

	Any chemotherapy	Carboplatin	Cisplatin	Doxorubicin	Doxorubicin combination
Crude model					
Treatment effect	0.43 (0.27; 0.70)	0.30 (0.11; 0.80)	0.66 (0.26; 1.67)	0.34 (0.16; 0.72)	0.43 (0.22; 0.86)
Adjusted model 1					
Treatment effect	0.45 (0.27; 0.76)	0.27 (0.09; 0.84)	0.59 (0.19; 1.84)	0.34 (0.16; 0.72)	0.42 (0.20; 0.89)
Adjusted model 2					
Treatment effect	0.48 (0.29; 0.78)	0.32 (0.11; 0.88)	0.72 (0.28; 1.88)	0.38 (0.19; 0.76)	0.45 (0.22; 0.89)
Interaction model					
Treatment effect	0.81 (0.41; 1.62)	0.73 (0.12; 4.53)	1.34 (0.33; 5.37)	0.74 (0.26; 2.09)	0.66 (0.26; 1.72)
Interaction effect	3.41 (1.07; 10.84)	4.06 (0.28; 59.07)	3.95 (0.42; 36.91)	6.46 (0.89; 46.66)	2.49 (0.50; 12.43)

Results presented as odds ratios (ORs) and 95% confidence intervals (95%) with no chemotherapy as the reference group, based on 5 studies, including 400 dogs of whom 88 died (for 1 deceased dog chemotherapy treatment was not recorded). For the number of subjects and events of the chemotherapy specific estimates see Table 2 and Appendix Tables. The crude model refers to a model regressing a 5 month mortality indicator on a chemotherapy factor, Adjusted model 1 additionally includes variables for age, weight, gender, neuter status, SALP, and tumor location, Adjusted model 3 additionally corrects for breed, Interaction model, refers to a model with a chemotherapy by predicted logit(5-month mortality) product term. All models included a random intercept for study; note that a random intercept was not needed for the carboplatin comparison, which included the single study of Bacon. Besides exploring non-linearity of associations, no model selection was performed and confounders were included based on prior knowledge.

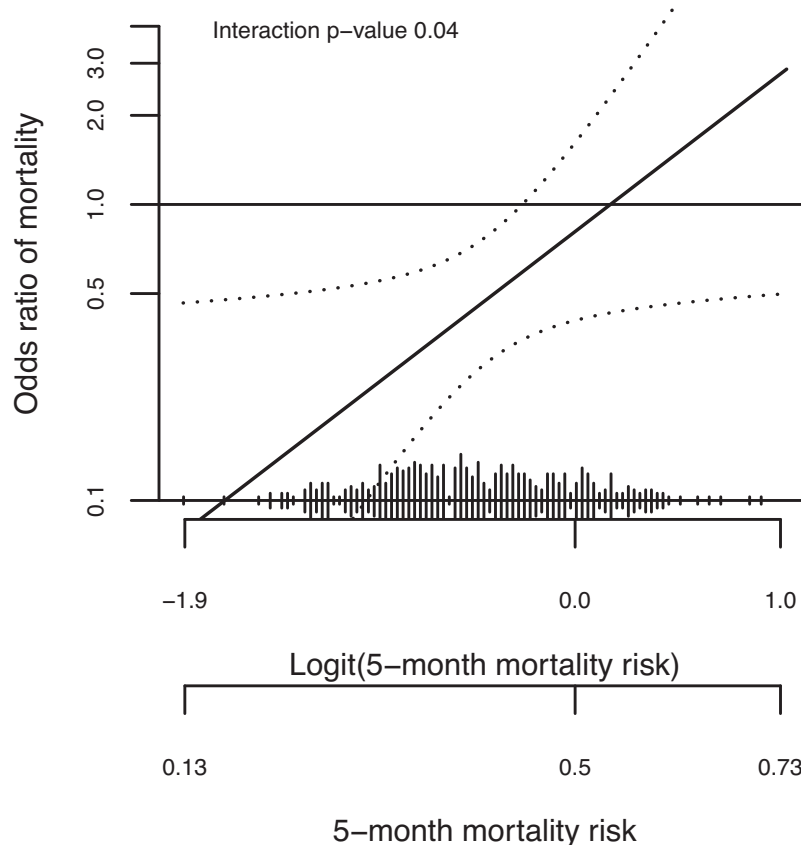
**Table 4**  
Sensitivity analysis including patients surviving the first month. Treatment effect estimates of any chemotherapeutics compared to no chemotherapy on 5-month mortality in dogs surgically treated for osteosarcoma.

	Crude model	Adjusted model 1	Adjusted model 2	Interaction model
Treatment effect	0.68 (0.40; 1.16)	0.74 (0.42; 1.31)	0.81 (0.47; 1.39)	1.13 (0.54; 2.35)
Interaction effect				2.44 (0.66; 8.97)

Results presented as odds ratios (ORs) and 95% confidence intervals (95%), based on 5 studies, including 340 dogs surviving the first month of follow-up of whom 69 were dead at 5 months follow-up. The crude model refers to a model regressing a 5 month mortality indicator on a chemotherapy factor, Adjusted model 1 additionally includes variables for age, weight, gender, neuter status, SALP, and tumor location, Adjusted model 3 additionally corrects for breed, Interaction model, refers to a model with a chemotherapy by predicted logit(5-month mortality) product term. All models included a random intercept for study membership.

effect estimates were closer to the null than in the entire sample; OR of “any chemotherapy” 0.81 (95%CI 0.47; 1.39). Interaction effects were also closer to the 1: OR 2.44 (95%CI 0.66; 8.97). However, the magnitude and direction of these interaction effects were in

agreement with those estimated using the entire sample. Similar and consistent results were found for the individual comparisons (data not shown).



**Fig. 1.** Estimated effect of any chemotherapy compared to no chemotherapy on 5-month mortality in surgically treated dogs with osteosarcoma. Figure shows the odds ratio (OR) of any chemotherapy treatment (solid line) with 95% confidence intervals (dotted lines) for dogs with different predicted risks of 5-month mortality. The horizontal solid line indicates a neutral OR of 1.00. At the bottom a histogram is given, corresponding to the patient frequencies of the x-axis measurement. Results based on 5 studies, including 400 dogs of whom 88 died (for 1 deceased dog chemotherapy treatment was not recorded).

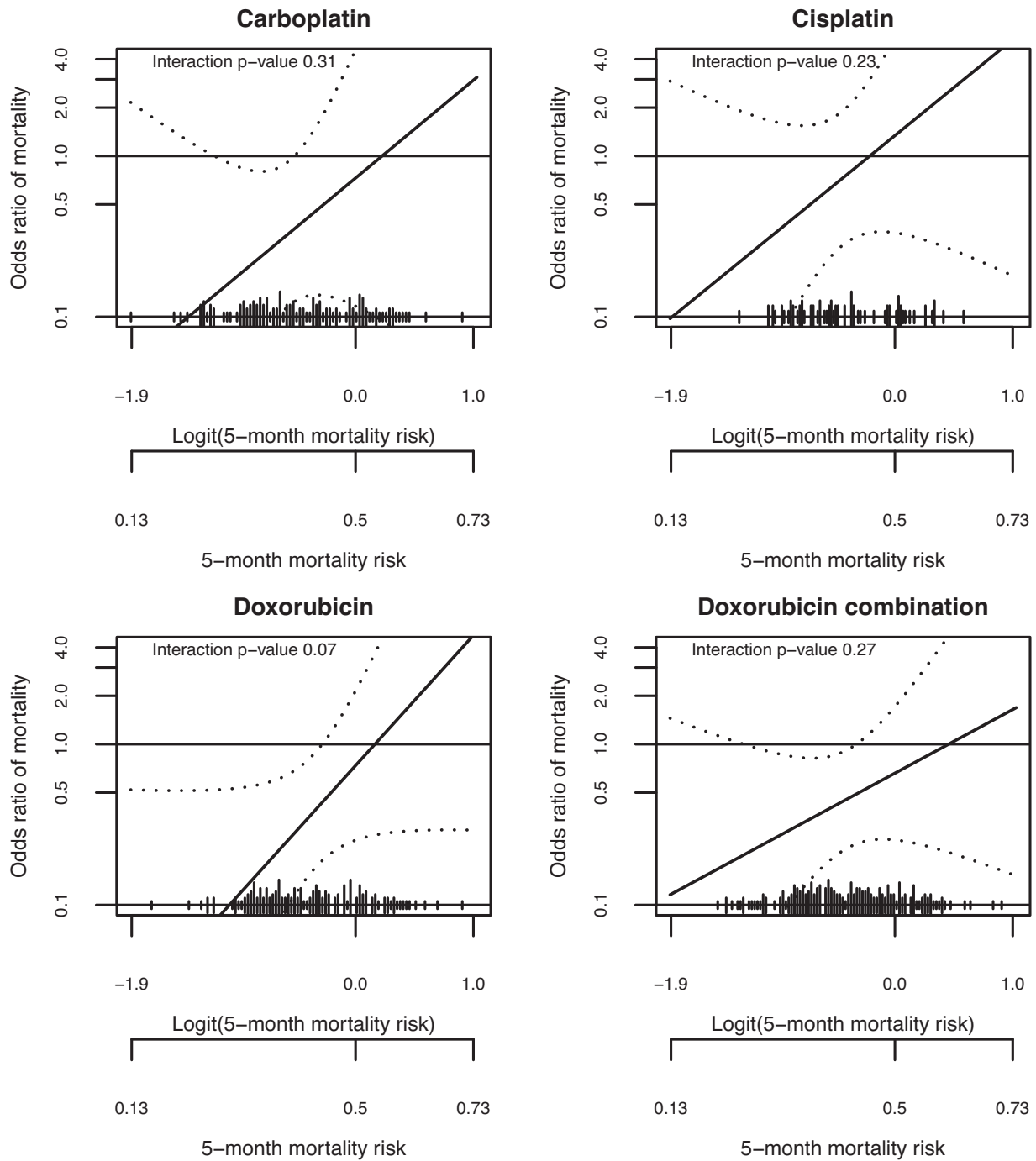
#### 4. Discussion

This study showed that dogs with osteosarcoma and a relatively low predicted 5-month mortality risk (<0.43) benefited more from “any chemotherapy” compared to no chemotherapy than those with a higher predicted risk. Results from individually comparing the chemotherapeutics, carboplatin, cisplatin, doxorubicin or doxorubicin combination therapy, to no chemotherapy were similar in direction and magnitude, indicating consistency, however these compound specific interaction terms did not attain significance.

Previous clinical studies showed that the effect of chemotherapy might be modified by other factors. One of the clearest examples of this in dogs is the synergistic effect between immunotherapy and chemotherapy (MacEwen and Kurzman, 1996; Vail et al., 1995). To the best of our knowledge, our study is the first to explore whether chemotherapy effects vary according to predicted baseline mortality risk (using multiple variables).

The current study has some limitations. First, only data from nonrandomized studies were available. Therefore, chemotherapy effect estimates could be biased due to unobserved and residual confounding. To explore this, a sensitivity analysis was performed, including dogs who survived the first month. Results in this subgroup showed estimates closer to an OR of 1. Interaction effects remained in the same direction and of the same magnitude as the interaction effects using the entire sample, implying consistency. A second issue is that the prediction model was derived including the subset of studies, which were used to test for the presence of an interaction. However, a recent simulation study showed that such an internally developed model only deviated

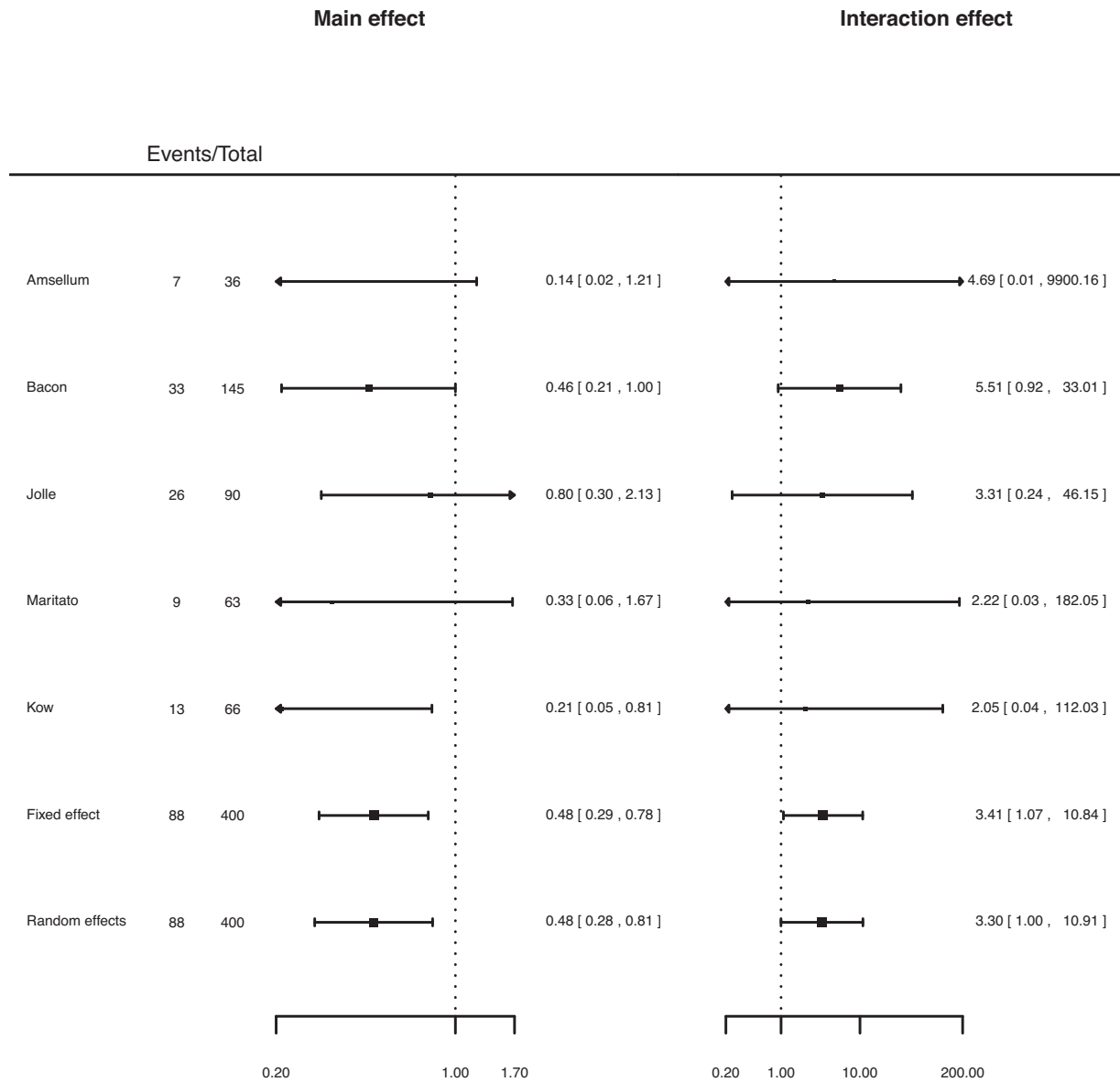
slightly from externally derived models (Burke et al., 2014). To some, including non-significant predictors may seem erroneous. Perhaps surprisingly, numerous studies have shown that focussing on significant predictors results in an overfitted model, which does not generalize well to other settings (Steyerberg, 2009; Steyerberg et al., 1999, 2011, 2010). To remedy this, it has been suggested to use prior knowledge to select relevant predictors, which we have implemented here. More importantly, we note that the discriminatory ability of the prediction model was limited (c-statistic 0.63). However, calibration (i.e., how well the predicted risk matched the observed risk) was very reasonable. For the current purpose, predicting individualized chemotherapy effects, good calibration is perhaps more relevant. Another limitation is that a number of observations were missing. Instead of focussing on complete cases we tried to address this problem using multiple imputation. While imputing missing data is likely to decrease bias it possible that results were nevertheless still biased, due to missingness being related to unmeasured variables. We did not perform a comparison with a complete case analysis (dropping missing values), because our analyses showed missing data to be associated with observed variables invalidating a complete case analysis. Furthermore, too many subjects ( $n = 231$ ; 58%) would be excluded to allow a proper (multivariable) analysis. Similarly, due to the small sample size available and the coinciding modest power, we did not adjust for multiple testing. Finally, by ignoring the uncertainty in the predicted logit(5-month mortality) the variance in the interaction effect is underestimated. To correct for this, the entire two stage process was bootstrapped 2000 times (accounting for clustering by study), repeated 100 times for each imputed dataset, refitting



**Fig. 2.** Estimated effects of four different chemotherapeutics compared to no chemotherapy on 5-month mortality in surgically treated dogs with osteosarcoma. Figure shows the odds ratio (OR) of carboplatin, cisplatin, doxorubicin or doxorubicin combination therapy compared to no chemotherapy (solid line) with 95% confidence intervals (dotted lines) for dogs with different predicted risks of 5-month mortality. The horizontal solid line indicates a neutral OR of 1.00. At the bottom of each graph a histogram is given, corresponding to the patient frequencies of the x-axis measurement. Results based on 5 studies, including 400 dogs of whom 88 died (for 1 deceased dog chemotherapy treatment was not recorded). The per compound comparisons sample size (event/total), was 56/180 for cisplatin, 55/220 for doxorubicin, 57/211 for doxorubicin combination, and 18/122, note that, depending on the studies used, the same no chemotherapy group was used in every comparison and results are thus dependent.

the prediction model and the interaction model, and calculating the covariance of the beta coefficients. This resulted in an interaction effect OR 3.41 (95%CI 0.94; 12.43), compared to the naïve estimate of OR 3.41 (95%CI 1.07; 10.84). Due to the small sample size (per study, e.g., the smallest study included 37 patients), it is likely that some bootstrapped samples contained few exposed subjects or mortality events, resulting in a skewed bootstrapped distribution erroneously inflating the estimated covariance. Due to the

imputation, it was not possible to instead use the bootstrap to estimate a confidence interval, which is known to perform better if the distribution is skewed (Carpenter and Bithell, 2000). Given this, it is probably, best to view the corrected estimated as conservative. To give more insight in our discussed shortcomings, we replicated our findings using an independent validation study (see Manuscript 2 jointly submitted). Despite successful replication of our results one main and very important shortcoming remains, both the current



**Fig. 3.** Forest plot of the estimated effect of any chemotherapy compared to no chemotherapy on 5-month mortality in surgically treated dogs with osteosarcoma. The left panel shows the main effects of chemotherapy from a model without an interaction term with logit(5-month mortality), the right panel shows the per study interaction effect of chemotherapy and logit(5-month mortality). Effects are depicted as odds ratio (OR) with 95% confidence interval, pooled across studies using fixed effects (random intercept only) and random effects (random intercept and random slope) logistic regression models, and adjusted for potential confounders. The interaction effects are per unit increase in the predicted logit(5-month mortality risk). Heterogeneity, measured as the tau-squared, was 0.01 95%CI (-0.30; 0.33) for the main effect and 0.01 95%CI (-0.19; 0.19) for the interaction effect. Please note these dogs were originally included in studies by Amsellum, Bacon, [Jolle] Kirpenstijn, Kow and Maritato (co-authors of the current IPDMA).

IPDMA and the validation study used nonrandomized data, and therefore we stress that our results need replication using independent (historical) RCT data. If our result can be confirmed using independent RCT data it would also be sensible to include prediction intervals (Sofroniou and Hutcheson, 2002) with the predicted point estimates, reflecting the prediction uncertainty. For this purpose our estimated covariance matrix can be requested with the lead author.

Such a follow-up study may also want to explore adverse events, such as quality of life, additional healthcare costs, or immunological related variables such as leukocytes. Unfortunately, this was not systematically measured by the studies included in this IPDMA. Additionally, we note that some researchers prefer absolute effect measure such as risk differences over odds ratios. Presumably, the risk difference is preferred because, when the outcome incidence is low, the odds ratio can be large while the risk difference is small. For example, if the incidence in an unexposed group of subjects is



0.05 and 0.01 in the exposed the odds ratio is 5.21 while the risk difference is only 0.04. However, 5-month mortality in OS dogs is very common (24% in our sample) making this distinction less relevant. Nevertheless, the risk difference can be calculated from the equations presented. Finally, we want to underline that the here described approach has two major advantages compared to “regular” one-variable at a time subgroups analysis. First, patients and their baseline risk of mortality are rarely defined by only one characteristics, our approach correctly takes this into account, albeit that subgroup indicators not related to the risk of mortality are excluded (Kent and Hayward, 2007). Second, and dependent on its implementation, our approach can greatly reduce the type 1 error rate (i.e., number of false positives) due to reducing the number of interaction tests to 1 (Sun et al., 2011).

## 5. Conclusions

In conclusion, surgically treated dogs with osteosarcoma which have a relatively low predicted risk of 5-month mortality might benefit most from additional chemotherapy.

## Conflict of interest

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

## Author contributions

AFS, RHHG and MN 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, PA, NB, KK, KM 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 were neither previously published. An abstract containing this work was presented at the 2014 International Evidence-Based Veterinary Medicine Network Conference.

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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.2015.10.016>.

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