

RESEARCH ARTICLE

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Prognostic factors in canine appendicular osteosarcoma – a meta-analysis

Ilse Boerman¹, Gayathri T Selvarajah², Mirjam Nielen³ and Jolle Kirpensteijn^{1*}

Abstract

Background: Appendicular osteosarcoma is the most common malignant primary canine bone tumor. When treated by amputation or tumor removal alone, median survival times (MST) do not exceed 5 months, with the majority of dogs suffering from metastatic disease. This period can be extended with adequate local intervention and adjuvant chemotherapy, which has become common practice. Several prognostic factors have been reported in many different studies, e.g. age, breed, weight, sex, neuter status, location of tumor, serum alkaline phosphatase (SALP), bone alkaline phosphatase (BALP), infection, percentage of bone length affected, histological grade or histological subtype of tumor. Most of these factors are, however, only reported as confounding factors in larger studies. Insight in truly significant prognostic factors at time of diagnosis may contribute to tailoring adjuvant therapy for individual dogs suffering from osteosarcoma. The objective of this study was to systematically review the prognostic factors that are described for canine appendicular osteosarcoma and validate their scientific importance.

Results: A literature review was performed on selected studies and eligible data were extracted. Meta-analyses were done for two of the three selected possible prognostic factors (SALP and location), looking at both survival time (ST) and disease free interval (DFI). The third factor (age) was studied in a qualitative manner. Both elevated SALP level and the (proximal) humerus as location of the primary tumor are significant negative prognostic factors for both ST and DFI in dogs with appendicular osteosarcoma. Increasing age was associated with shorter ST and DFI, however, was not statistically significant because information of this factor was available in only a limited number of papers.

Conclusions: Elevated SALP and proximal humeral location are significant negative prognosticators for canine osteosarcoma.

Keywords: Bone tumor, Dog, Survival, Disease-free interval, Prognostic factors

Background

Osteosarcoma (OS) is a malignant tumor of mesenchymal origin that produces osteoid. OS accounts for approximately 85 % of all primary canine bone tumors and is almost exclusively observed in large or giant breeds [1-5]. There is anecdotal evidence suggesting that males are more predisposed. The median age of onset of clinical signs ranges from 8 to 10 years [5,6], although it also occurs in younger dogs [7].

Dogs are often presented with a history of lameness or in some cases with a pathologic fracture of the affected bone. Predilection sites are the weight-bearing regions of the long bones (humerus, femur, radius, tibia and ulna) [8] with approximately 25 % of tumors arising in the axial skeleton including the flat bones of the skull, ribs, vertebrae, sternum, and pelvis [9,10]. OS is an aggressive and invasive neoplasm that causes local skeletal destruction and resulting in radiographic evidence of both osteoproliferative and osteolytic lesions. They are highly metastatic, predominantly to the lungs with a lower frequency of spread to distant bones, regional lymph nodes [11] and other soft tissues [12,13]. A clinical diagnosis is made following assessment of case signalment and history and based on the radiographic appearance of the

* Correspondence: j.kirpensteijn@uu.nl

¹Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, Utrecht 3584CM, The Netherlands

Full list of author information is available at the end of the article

lesion. The definitive diagnosis is currently obtained by histological examination with tumor classification based on the formation of osteoid matrix with osteoblastic, fibroblastic, chondroblastic, telangiectic and combined subtypes [14,15]. There can be considerable variation in the histological appearance both between and within individual neoplasms, however, rendering histological parameters less reproducible between studies.

Dogs with OS that were treated by amputation alone have poor overall survival outcome: median overall survival times are typically less than five months, with the majority suffering from metastatic disease [5,16,17]. Over the years, advances in disease management, including 'limb-sparing' surgical and radioablative methods used only to selectively eradicate tumors located in the distal radius, ulna and tibia have been described [18,19]. Adjuvant therapy, such as multimodal chemotherapy regimes, treatment with bisphosphonates or immune modulators and palliative radiation, can also be provided and are reported to improve clinical or survival outcome [20-23]. Combination of therapy modalities and drugs may contribute significantly to survival statistics but randomized, double-blinded studies have not been performed routinely.

There are many well-documented prognostic indicators for canine OS, and the majority of these are similar to those reported from large retrospective studies on human OS, including tumor location, histologic grade, certain biomarkers, the use of chemotherapy, etc. [24-27]. The accurate segregation of canine patients into distinct prognostic subgroups based on such indicators is essential in the tailoring of appropriate treatment. However, the required information is not always completely available in the existing literature and studies report sometimes conflicting results. In this paper, we perform a meta-analysis to estimate the combined effect size over a number of studies of a selection of well-described prognosticators.

The aims of the current study were to systematically review the prognostic factors that are described for canine appendicular osteosarcoma and validate their scientific importance. After validation, a meta-analysis was performed on serum alkaline phosphatase (SALP), tumor location and age at diagnosis to study the association of these factors with survival time (ST) and disease free interval (DFI).

Materials and methods

Search strategy and quality assessment

A search was performed using Medline (PubMed) and Google Scholar search for all eligible studies performed between January 1970 and May 2011. The following search strategies were applied: ["canine", "osteosarcoma" and "prognosis"] and ["canine", "osteosarcoma" and

"survival"]. Reports were selected and carefully reviewed that included canine stage <2B appendicular osteosarcoma cases [18]. Additionally, all manuscripts were evaluated for methodological quality according to a standardized questionnaire adapted from Bramer et al. (2009) including variables such as institute where the study was performed, year of publication, number of cases reported (at least 5), whether the study was randomized or not, study design, recorded survival end points, univariate (uva) or multivariate analysis (mva), use of control groups, listing of research period, completeness of follow up information, method of follow up examination, listing if cases were lost to follow up, definition of prognostic factors, listing of therapy modalities and listing of confounding factors. Two independent reviewers (JK, GS) performed study selection, assessment of methodological quality and data extraction. A third reviewer (IB) resolved disagreements, if necessary.

Statistical analysis

Studies fulfilling one or two types of quality criteria were selected for meta-analysis. First, only randomized double-blind studies with multivariate data analysis were selected. After that, non-randomized, prospective studies were added to evaluate if additional significant factors could be determined. Studies with similar dog populations, outcome variables, prognostic factors, follow-up information, and adjustment for confounding variables were combined. Adjusted relative risks were pooled with a random effects model and assessed for statistical heterogeneity by Chi-square analysis. After establishment of heterogeneity, the source was determined by meta-analysis.

All meta-analyses were performed using commercial statistical software (Comprehensive meta-analysis V2, ©2006 Biostat, Inc., Englewood, NJ, USA), which provides a table with relative risk for each study and a forest plot. Both binary and continuous data were reported in the selected papers. It was impossible to combine both types of data in the same analysis; therefore for each variable separate sub analyses were performed on the effect measures hazard ratio (HR), median survival time (MST) and median disease free interval (MDFI). The groups compared for the variables SALP and location were as follows: elevated SALP versus SALP within reference range at time of diagnosis; (proximal) humerus compared to other locations in the appendicular skeleton. For age it was not possible to make two groups with the available data; factor data were analysed in a qualitative manner.

Some studies reported univariate data analysis while others reported multivariate data analysis, meaning that a studied factor was corrected for therapy or other confounding factors analysed. Where possible, data of multivariate analysis were used. Where necessary, a (set of) paper(s) was excluded to see if the overall effect changed.

For all meta-analyses performed in this study the random-effects model was used, assuming that the true study effect varies across studies and the observed study effects reflect both this variation and random variation. Selected studies that did not present the data in sufficient detail were qualitatively summarized where necessary.

Results

Inclusion of papers

Through the searches, 821 papers were selected for review of which 55 met the criteria to be included in the study presented here (Additional file 1). No disagreements needed to be resolved by the third reviewer. Of the 55 studies, 16 were multicenter, and 39 monocenter, of which the majority came from Colorado State University (17), Utrecht University (5), University of Wisconsin-Madison and Tufts University (each 4). One study contained two independent studies and was split in two (Kurzman et al., 1995). One study was published in the 1970s, 5 in the 1980s, 14 in the 1990s and 35 in 2000–2011. 14 Studies were considered randomized, 27 studies were prospective, and 29 retrospective. Of all the studies, only 5 were placebo controlled. The number of cases varied from 11 to 303, with a mean of 61 ± 8 . Outcome variables included survival time in 51, disease free interval in 32, metastasis free interval (MFI) in 13, and recurrence free interval (RFI) in 6 studies. Univariate analyses were performed in all studies varying from Kaplan Meier (KM) survival curves in 34, KM combined with (Cox) regression analysis in 20, and one study mentioned an ANOVA. Multivariate analysis was performed with a multivariate cox proportional hazard analysis in 18 and some other multivariate test in 7 reports. Historic control groups are common ($n=19$) and some studies did not use a control group at all. The time of evaluation after the procedure and methodology varied too but most studies evaluated the dogs every 2–3 months using pulmonary radiography ($n=37$); 13 studies did not include any information about follow up techniques. Most studies failed to include how long these dogs needed to be followed up ($n=39$) or how many cases were lost to follow up ($n=33$). An abundance of treatments were described, of which amputation combined with some kind of chemotherapy was most common.

Significant factors

Many significant factors are described in the 55 papers selected, e.g. age, breed, weight, sex, neuter status, location of tumor, SALP, bone alkaline phosphatase (BAP), infection, percentage of bone length affected, histological grade or histological subtype of tumor. The effect of SALP level, location (proximal humerus) and age at diagnosis were reported most often in the 55 selected papers and these factors were subjected to meta-analyses.

Thirteen papers were included in the meta-analysis on SALP and seven papers in the meta-analysis on (proximal) humerus; some studies reported more than one variable. For age it turned out to be difficult to find sufficient studies with comparable selections of age categories to run meta-analysis on, therefore in the results a qualitative summation is given for all relevant age data found. Six papers reported the effect of age as confounding factor using HR, MST or MDFI, three papers did not give an estimate of the effect size, but only reported a p-value (Additional file 1: Table S1).

Serum alkaline phosphatase

Survival time

For the meta-analysis for SALP on survival time using hazard ratios, data from seven studies were available. Six of these studies used univariate analysis and one used multivariate analysis. All studies combined, the meta-analysis (Figure 1) on hazard ratios showed that dogs with an elevated SALP level at time of diagnosis have shorter survival times compared with dogs with a SALP level within reference range, with a hazard ratio of 1.62 (95 % CI: 1.21–2.17). Leaving the one study with the multivariate analysis (Phillips et al., 2009) out, the hazard ratio dropped to 1.44 (95 % CI: 1.11–1.87). The sub-analysis with median survival times (also seven studies) showed that dogs with elevated SALP overall lived 156 days shorter (MST: -156) compared with dogs with SALP within reference range (95 % CI: -209 to -104) (Figure 2). When selecting only the studies with multivariate or univariate analysis the difference became -132 days (95 % CI: -252 to -11) and -186 days (95 % CI: -248 to -124), respectively.

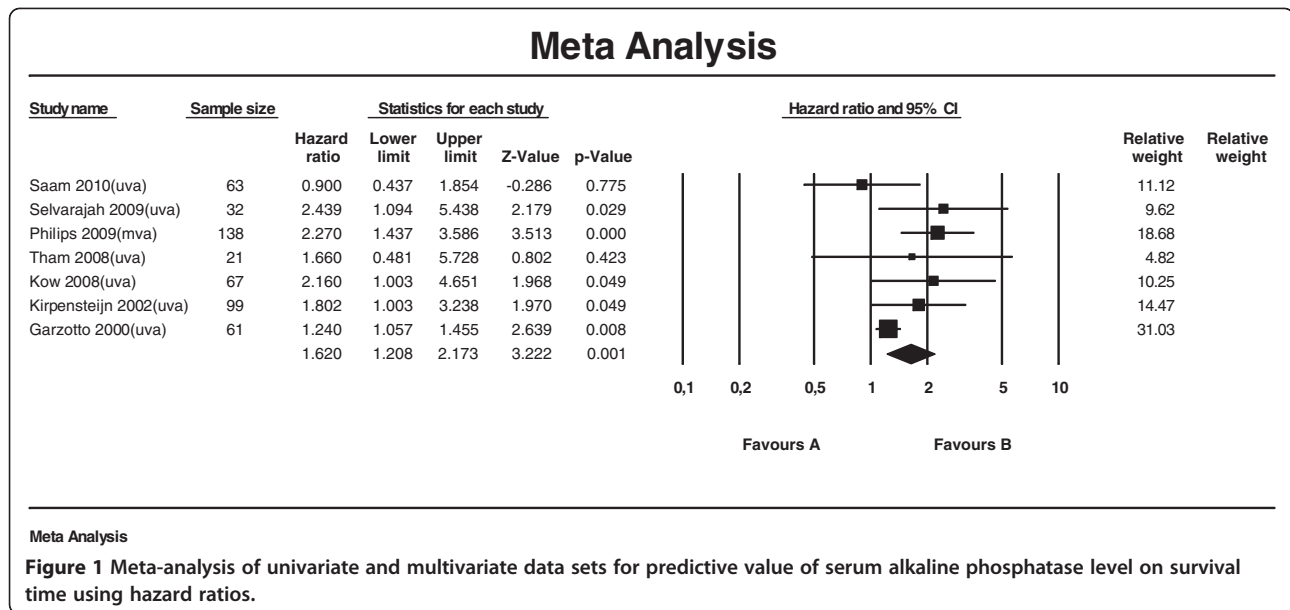
Disease free interval

For the meta-analysis on disease-free interval ten papers were used, of which the majority was also used for the meta-analysis on survival time. The effect direction of elevated SALP on DFI was comparable to that on ST. Figure 3 shows an overall HR of 1.96 (95 % CI: 1.50–2.56) for dogs with an elevated SALP level at time of diagnosis. The two multivariate analyses (Sottnik et al., 2010 and Phillips et al., 2009) together give a HR of 2.33 (95 % CI: 1.60–3.39) for elevated SALP level. Combining only the univariate analyses, the HR dropped to 1.64 (95 % CI: 1.12–2.40). The difference in MDFI (here only univariate analyses were available) was -123 days (95 % CI: -166 to -79) (Figure 4).

Location - (proximal) humerus

Survival time

Four papers were used for the meta-analysis on ST for location. Of these papers, one reported the humerus as a whole as reference (Kow et al., 2008), the other three

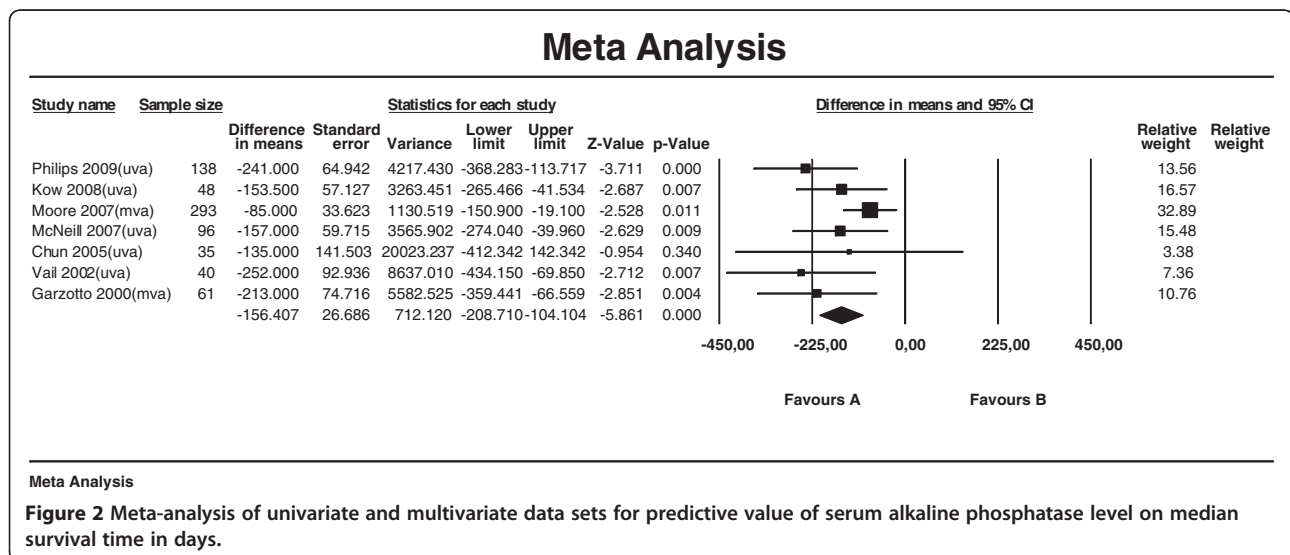


reported the proximal humerus in particular. Analysis on all studies combined (Figure 5) showed that dogs with the primary tumor located in their (proximal) humerus had shorter survival times than dogs with the primary tumor located elsewhere in their appendicular skeleton (HR 1.86; 95 % CI: 1.34–2.57). We also performed subset analyses on three subgroups (mva, uva and proximal humerus) and found no large differences in effect between the subgroups; estimates for HR varied between 1.82 and 2.21. The sub analysis on MST (Figure 6, all manuscripts reporting uva) showed that for (proximal) humerus the MST is 132 days shorter compared with other locations (95 % CI: -211 to -52). Approximately the same difference in MST (-131 days)

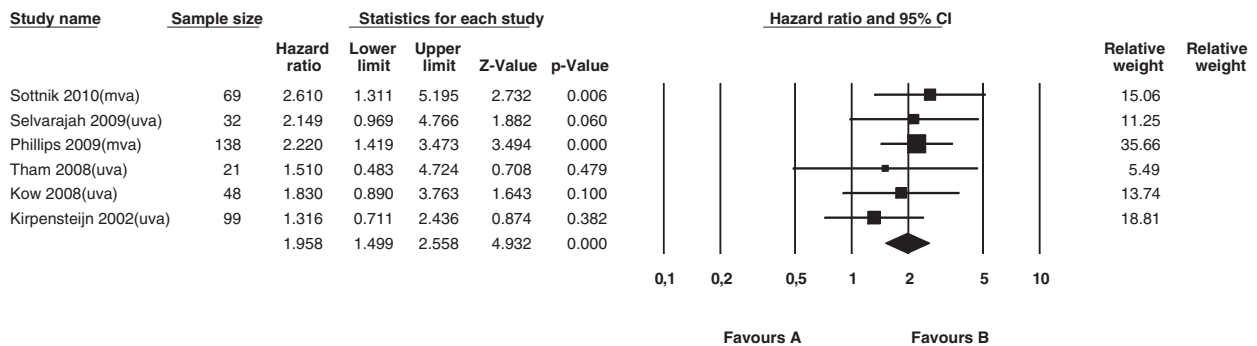
applied for the sub analysis leaving out the proximal humerus.

Disease Free Interval

For the meta-analysis on DFI for location, data from six papers were used, three of which were also used for the analyses on ST. The effect of (proximal) humerus as location on DFI was comparable with the effect on ST. Dogs with primary tumors located in the (proximal) humerus had shorter DFIs compared with other locations (HR 2.53; 95 % CI: 1.34–4.77) (Figure 7). The effect of the proximal humerus in particular was comparable (HR 2.57; 95 % CI: 0.90–7.35). Performing the subanalysis on five papers reporting MDFI (only uva) the overall



Meta Analysis



Meta Analysis

Figure 3 Meta-analysis of univariate and multivariate data sets for predictive value of serum alkaline phosphatase level on median disease free interval using hazard ratios.

difference in MDFI was -110 days (95 % CI: -148 to -73) (Figure 8). Subset analyses on proximal humerus in particular and humerus as a whole showed MDFIs of respectively -91 and -167 days.

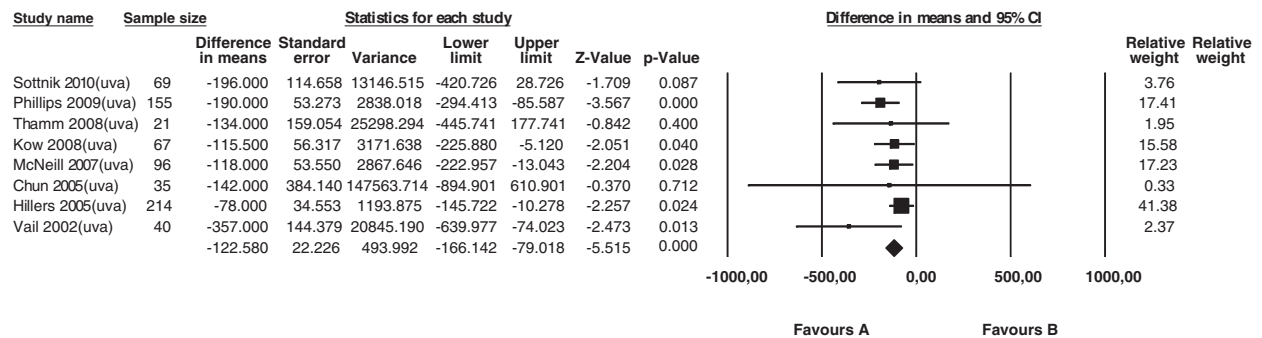
Age

The papers analysing age as confounding factor all reported different age categories and used different effect outcomes. As it did not occur that more than two studies used both the same outcome type and the same age categories, it appeared impossible to run a meta-analysis on more than two studies. Therefore these data are summarized in a qualitative manner in Table 1, 2 and 3.

Survival time

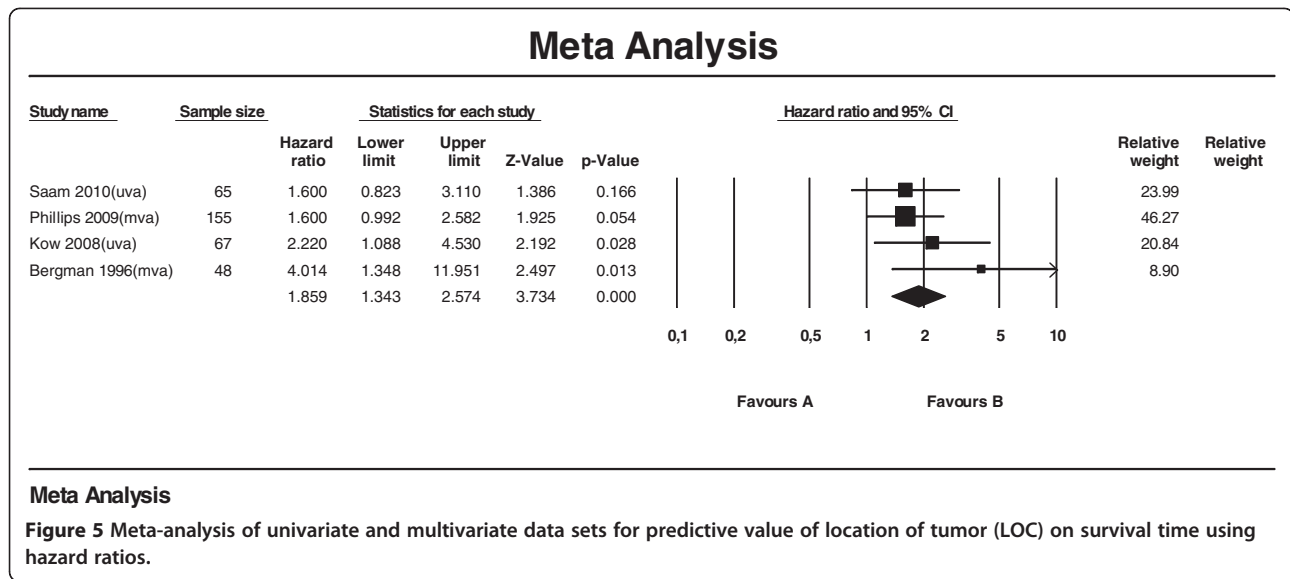
The influence of age on ST reported in the eight papers used is shown in Table 1 and 2. According to Saam et al. (2011) and Phillips et al. (2009), dogs under the age of 5 showed longer MSTs compared with dogs over the age of 5. This was comparable to the study of Liptak, et al. (2006), which reported that older dogs had a significantly shorter MST. In the study of Miller et al. (2009), dogs older than 6 years showed a slightly increased HR (but with insignificant p-value) compared with younger dogs. The study of Kent et al. (2004) reported that the age under the mean age in that study was not prognostic for survival. According to Spodnick et al. (1992) dogs in the age between 7 and 10 years showed a significantly

Meta Analysis



Meta Analysis

Figure 4 Meta-analysis of univariate and multivariate data sets for predictive value of serum alkaline phosphatase level on median disease free interval (in days).



longer MST than dogs under 7 or over 10 years old. Concerning the continuous survival data; in the paper of Selvarajah et al. (2009) age overall did not significantly influence ST. Moore et al. (2007) reported that dogs have significantly worse survival rates as age increases. Overall, most studies performed in the last decennium showed worse survival rates for dogs with increasing age compared with younger dogs.

Disease free interval

Contradictory data were observed for DFI (Table 3). According to the study of Sottnik et al. (2010), dogs older than 8 years showed significantly longer MDFIs compared with younger dogs. Phillips et al. (2009) on the other hand reported that dogs older than 5 showed a significantly increased HR for MDFI and significantly shorter MDFIs compared with younger dogs. Miller et al. (2009) also reported an increased HR for dogs

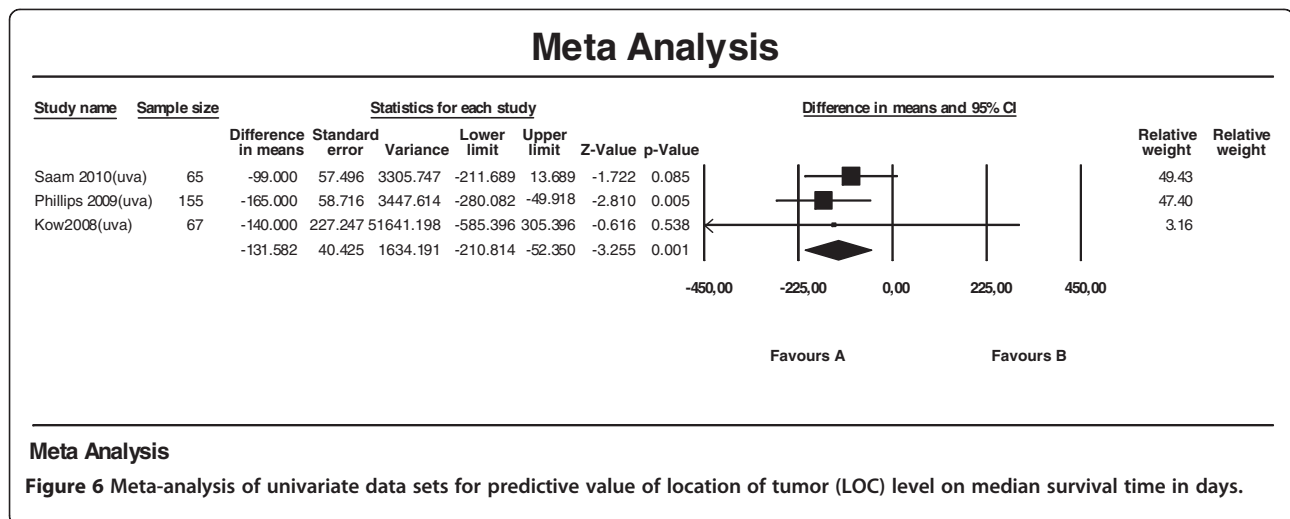
older than 6 years, however, not significantly. Last, in the study of Kent et al. (2004) age under the mean age was prognostic for DFI.

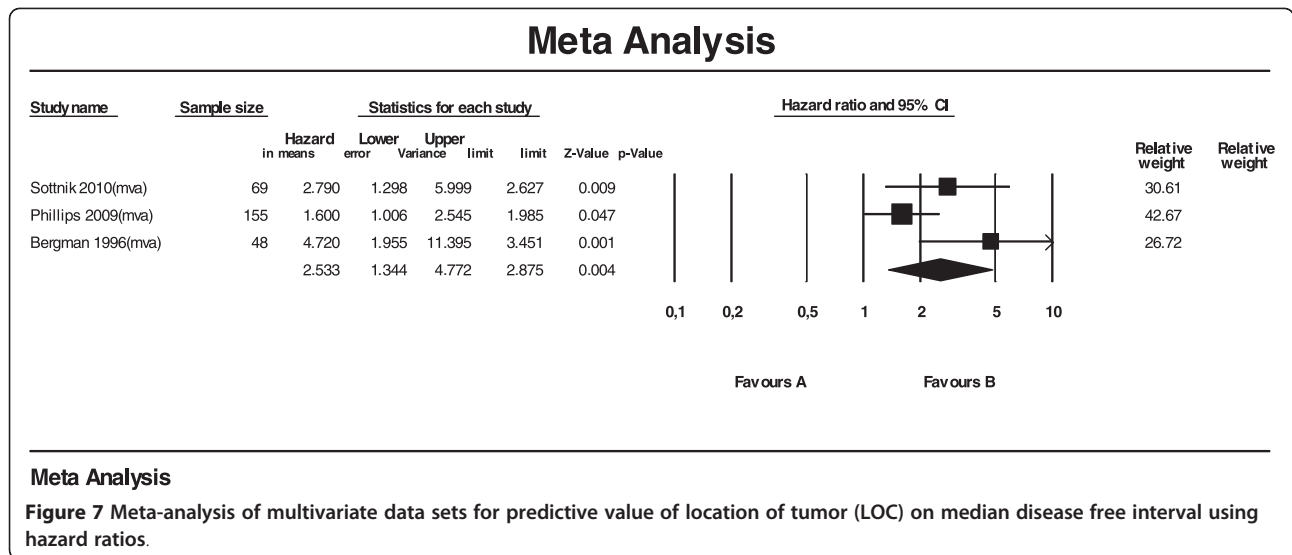
Discussion

This is the first paper published in veterinary literature that compares outcomes in companion animal osteosarcoma using meta-analysis confirming the significance of three prognostic variables, including serum alkaline phosphatase, location and age in this devastating tumor.

Serum ALP is a strong prognosticator

SALP is most likely the strongest prognostic indicator for ST and DFI of canine OS and confirmed expectations in the veterinary literature. Bone alkaline phosphatase is a bone turnover marker measured in serum. It reflects the increased turnover associated with bone destruction or aging and various conditions affecting bone





metabolism [28], such as osteosarcoma. The prognostic significance of serum total alkaline phosphatase activity for human beings with osteosarcoma has been recognized for over 30 years [29]. In most veterinary studies, the total SALP is measured. Total SALP is easily quantifiable and a routine diagnostic test in most veterinary laboratories. Total SALP, however, consists of several other isoenzymes, of which those derived from liver (LALP) and bone (BALP) represent the majority in normal dogs. A corticosteroid-induced isoenzyme (CALP) may be present in dogs with hyperadrenocorticism and dogs receiving exogenous corticosteroids [30]. The prognostic value of total SALP for human osteosarcoma is limited by its lack of specificity for tumor tissue [31,32]. The enzyme's activity may be increased by exogenous corticosteroid administration or hyperadrenocorticism, which may cause increases in CALP, or by cholestasis

which may cause increases in LALP [31]. Measuring the total SALP might therefore not always give reliable outcomes. Measuring the bone ALP (BALP) seems more logical, since in dogs with osteosarcoma, BALP is expected and proven to be elevated and more discriminant than the other isoenzymes [33]. Serum BALP activity was a direct reflection of osteoblastic activity [34] in people. Additionally, the reference range for SALP in dogs differs among various age groups [33,35] and breeds [36], which could be another factor that may influence study results. The overall meta-analysis done in this study for median ST showed a stronger significant effect on HR than the analysis on only MVA or UVA studies. This could be caused by confounding variables that may be associated with SALP, including breed variability, underlying disease processes and differences in detection methods.

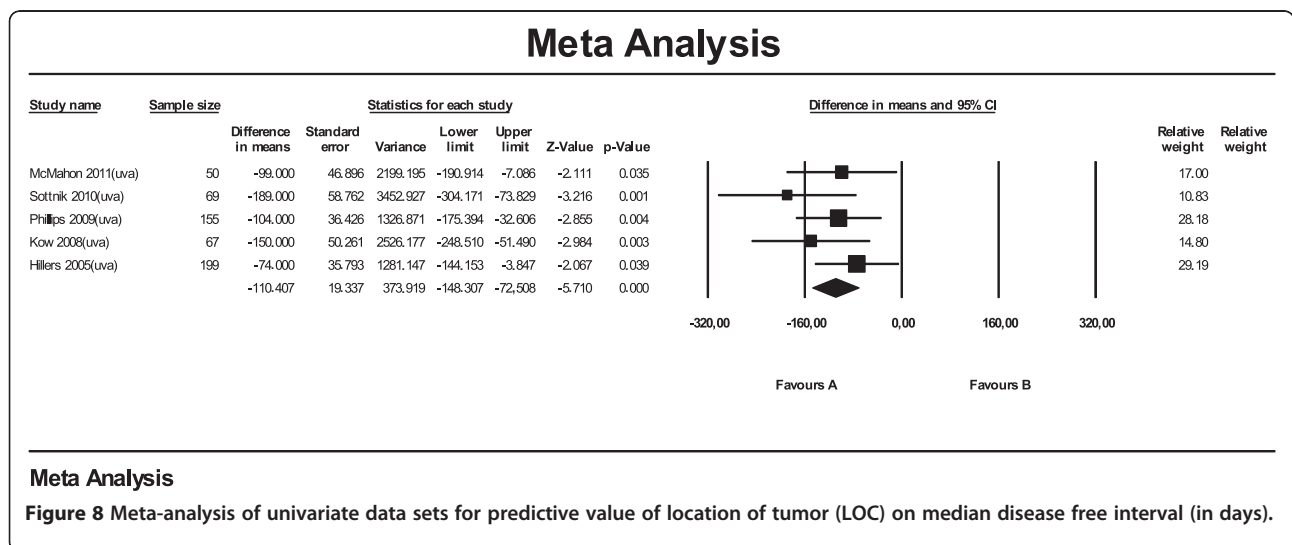


Table 1 Survival data on age of dogs with osteosarcoma coded as binary data

Study (UVA/MVA)	N	Studied age categories (years)	MST (days)	HR (95 % CI)	p-value
Saam 2010 (UVA)	12	<=5	1414	0.49 (0.3-1.1)	0.08
		53	>5		
Phillips 2009 (UVA)	23	<5	1263		0.034
		132	>5		
Miller 2009 (UVA)	3	<6		(reference)	0.89
		73	6-10	1.09 (0.21-5.68)	
		7	>10	1.14 (0.28-4.71)	
Liptak 2006 (MVA)	20	Older*		2.06 (1.03-4.14)	0.042
Kent 2004 (UVA)	32	<mean age**			0.156
Spodnick 1992 (MVA)	162	7-10*** vs. <7 and >10			<0.01

UVA = univariate analysis; MVA = multivariate analysis; N = number; MST = median survival time; HR = hazard ration; reference = the reference group with which the others are compared.
 *MST for older dogs was significantly decreased (median age 8.3 years, range 4.5-11 years).
 **Only median age was mentioned in article (8.8 years, range 2.2-16.5 years)
 ***Longer survival time.
 * MST for older dogs was significantly decreased (median age 8.3 years, range 4.5-11).
 ** Median age 8.8 years, range 2.2-16.5.
 *** Longer ST.

Proximal humeral location is associated with poor survival

The most common human OS sites are the femur (42 %), tibia (19 %), and humerus (10 %) [37]. The proximal humerus, despite the fact that it is not the most common site, was associated with poorer (metastasis-free) survival [38,39] in man. In this study, the (proximal) humerus was also shown to be the location with a worse prognostic outcome for canine OS. One of the reasons is the fact that tumors in this location may be observed at a later stage compared to other locations that are more clinically obvious.

The location with the best prognosis may also be of clinical relevance for both canine and human OS,

Table 2 Survival data on age of dogs with osteosarcoma coded as continuous data

Study (UVA/MVA)	N	Continuous variable	MST (days)	HR (95 % CI)	p-value
Selvarajah 2009 (UVA)	32	Overall		1.137 (0.96-1.35)	0.147
Selvarajah 2009 (MVA)	32	Overall	204	1.18 (0.92-1.52)	0.202
Moore 2007 (MVA)	303	Increasing age	244*		0.004

* MST was recalculated from months (8) to days by multiplying with 30.5.

Table 3 Disease free interval data on age of dogs with osteosarcoma coded as binary data

Study (UVA/MVA)	N	Studied age categories (years)	Median DFI (days)	HR (95% CI)	P-value
Sottnik 2010 (UVA)	39	<=8	204		0.188
		30	>8		
Sottnik 2010 (MVA)	69	>8 vs. <=8		0.42 (0.21-0.85)	0.016
Phillips 2009 (UVA)	23	<5	1035		0.022
		132	>5		
Phillips 2009 (MVA)	155	>5 vs. <5		2.1 (1.04-4.25)	0.038
Miller 2009 (UVA)	3	<6		(reference)	0.54
		73	6-10	1.59 (0.33-7.71)	
		7	>10	1.21 (0.3-4.96)	
Kent 2004 (UVA)	32	<mean			0.049*

UVA = univariate analysis; MVA = multivariate analysis; N = number; DFI = disease free interval; HR = hazard ration; reference = the reference group with which the others are compared.
 * DFI in this paper was stated as 'progression free survival'.

however for canine OS this information was often not available. In human OS, survival of patients with primary malignant bone tumor of distal lower extremity seems to be better than that of other sites [40]. This is comparable to canine OS: in several canine studies, the radius is cited as the location with the best prognosis for ST and DFI [2,41-43].

Age may be prognostic in canine osteosarcoma

In human OS, a primary osteosarcoma in older patients showed a poorer prognosis [38,44]. A predisposing factor in middle-aged to elderly people is Paget's disease, however. One of the most serious complications of Paget's disease is a significant increase in the incidence of osteosarcoma [45]. Despite of the limited amount of canine OS data available for age analyses, it seemed that increasing age is an important prognostic factor for dogs with appendicular OS. Survival time may not be the most sensitive variable, however, because it can be confounded by various other medical problems that arise in older dogs with malignant bone cancer. Additional studies comparing age categories need to be conducted using more elaborate meta-analyses.

The role of adjuvant chemotherapy

Over the years, various compounds have been used in adjuvant chemotherapy protocols against canine OSA including cisplatin, carboplatin and doxorubicin. These have been used in single and in multi-agent regimes and

at varying dosage and treatment interval and an obvious survival advantage of dogs receiving chemotherapy was present compared to (historic) controls [46-49]. No obvious differences in survival were observed when these treatments were compared with pre- or post-operative chemotherapy [50] and no differences in the DFI was reported for dogs treated using single- or multi-agent chemotherapeutic regimens. The prolonged, intense use of chemotherapy is often not a valid option due to adverse side effects compromising any clinical benefits and decreasing client compliance [51]. To date, even aggressive adjunctive therapy has proven ineffective in restricting all growth of metastases. Additionally, a small number of cases of canine OS that do not receive adjuvant chemotherapy do not succumb to metastatic disease once the primary tumor has been removed [52]. This finding suggests that genetic composition of both the host and tumor may be contributing to differences in the metastatic potential. In human OS, the prognosis has increased from 20 % 5-year survival in 1970 to 60 % nowadays because of the various chemotherapeutic protocols [53]. The histological response to preoperative chemotherapy was an important clinical predictor of the result of operative treatment of human osteosarcoma and was similarly important in one study by Powers et al [54]. This indicator should be used to identify patients who are at high risk for metastasis; as such patients may be candidates for more intensive or novel therapy protocols [42]. This important prognostic factor in human OS may also be a very interesting factor to further investigate in the canine OS but very few studies have reported evaluation of this variable making meta-analysis currently impossible. Meta-analyses for this factor and for different (types of) chemotherapy may be a valuable next step to see which therapies give the most optimal results for survival in canine appendicular osteosarcoma.

New insights, limitations and recommendations for prognostic studies in canine OSA

Often scientific papers only report data when they are found significant. Non-significant results are many times only cited as 'non significant,' yet the statistical data are left out. To be able to perform a meta-analysis taking into account all relevant data both significant and non-significant outcomes are essential. Leaving out data may result in an inadequate outcome. Unfortunately, not all available papers could be used for the meta-analyses in this study, since results were not always reported in sufficient detail whereby essential data were missing (e.g. sample size, confidence interval, HR or p-value).

For future reference, data should be coded in a manner that allows comparison of various studies with comparable objectives. For instance, we observed that in some studies data were coded as continuous variables, in

other studies they are coded as binary data. Therefore, the estimates of the effect size are difficult to compare. When specific factors are coded >1 , one would expect them to be a negative prognostic factor. Once coded <0 it would be a protective factor. This was sometimes mixed up. For example, in the study of Phillips (2009) elevated SALP was cited as 'negative prognostic factor,' yet the corresponding HR was 0.44. Since on the other hand following uva analysis both ST and MST for dogs with elevated SALP was shorter than for dogs with SALP within reference range, it seemed that the HR should be $1/0.44$ [55]. The paper of Sottnik, et al (2010) was not completely clear whether the HR of 0.59 corresponded to the age category ≤ 8 (which was the correspondent category in the study or otherwise not stated clearly in the relevant Table) or ≥ 8 years [56]. For the meta-analysis in the current study the HR was linked to the category ≥ 8 years, since in the mva analysis the same category also showed a HR <1 (0.42) and the MDFIs were also longer for that specific category. Yet the range of the DFI (uva) in the category ≤ 8 was extremely large which could mean that the data are not completely reliable. All in all, the systematic coding of risk factors in a consequent way would be extremely helpful for future analyses.

Although many studies are performed, on account of above-mentioned reasons it remains difficult or even impossible to compare these individual studies and significantly prove that possible prognostic factors are really prognostic. Only a few studies could be used for the meta-analyses and of these studies it needs to be said that we stretched to the limit the fact that the studies itself were comparable. This is a common and unavoidable fact for diseases that are relatively infrequent, such as canine OS. Researchers should therefore be stimulated to work together in the OS field, which is not only interesting from a research point of view but also in in the One Health approach of this comparable disease between man and dog.

Conclusions

Both elevated SALP level and the (proximal) humerus as location of the primary tumor are significant negative prognostic factors for both ST and DFI in dogs with appendicular osteosarcoma. Increasing age was associated with shorter ST and DFI, however, was not statistically significant because information of this factor was available in only a limited number of papers. Multicenter, well-designed research efforts are necessary to confirm the message that should be relayed to clients and patients to allow them to make an evidence-based decision in the treatment of their animal or child with this type of malignant bone tumor. Multicenter studies are only possible when researchers use the same variable definitions and show all relevant results.

Additional file

Additional file 1: Papers (55) that met the criteria for inclusion in this study [57-107]. Table S1. Studies selected for meta-analysis.

Abbreviations

OS: Osteosarcoma; SALP: Serum alkaline phosphatase; BAP: Bone alkaline phosphatase; (M)ST: (Median) Survival Time; (M)DFI: (Median) Disease Free Interval; MFI: Metastasis free interval; RFI: Recurrence free interval; HR: Hazard ratio; UVA: Univariate; MVA: Multivariate.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

IB carried out the manuscript search, databasing the various studies, performing the meta-analysis and drafted the manuscript. GTS helped with the manuscript search and reviewed the selected candidate manuscripts. MN supervised the meta-analysis. JK conceived the idea of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Author details

¹Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, Utrecht 3584CM, The Netherlands. ²Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia. ³Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 107, Utrecht 3584CL, The Netherlands.

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