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# A retrospective study on bone metastasis in dogs with advanced-stage solid cancer

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Objectives: **To review clinical characteristics, treatment, outcome and prognostic factors in dogs with solid cancer-bearing bone metastases.**
Materials and Methods: **Records were reviewed from dogs with histologically-proven solid cancer and bone metastases. Clinicopathologic variables, bone metastases characteristics and skeletal-related events were recorded. Endpoints were time to bone metastases and survival.**

Results: **Fifty dogs were included, 20 of them with synchronous and 30 of them with metachronous bone metastases. In the latter group, median time to diagnosis of bone metastases was 210 days (range, 30 to 1835). Most common primary cancer locations included mammary gland (n=6), spleen (n=5) and tonsil (n=5). Most common histotypes were carcinoma (n=32) and hemangiosarcoma (n=10). Nineteen dogs had multiple bones involvement, with humeri and vertebrae more commonly affected. Twenty-four dogs received antitumoural therapy, five symptomatic treatment and 21 were not treated. Overall median survival after bone metastases diagnosis was 30days (range, 11 to 49); 83% of dogs died because of skeletal-related events. Lack of antitumoural therapy was significantly associated with shorter survival (hazard ratio: 2.7; 95% confidence interval: 1.3 to 5.6) and with in- creased risk of skeletal-related death (hazard ratio: 3.3; 95% confidence interval: 1.4 to 7.4). Dogs with endocrine/neuroendocrine tumours (odds ratio: 8.8; 95% confidence interval: 1.2 to 63.9), without appendicular metastases (odds ratio: 5.1; 95% confidence interval: 1.0 to 25.8), without extra-skeletal metastases (odds ratio: 5.2; 95% confidence interval: 1.1 to 24.5) and receiving anti- tumoural therapy (odds ratio: 14.8; 95% confidence interval: 1.7 to 131.4) had an increased chance of surviving more than 100 days.**

Clinical significance: **Bone metastases in dogs with solid cancers are associated with poor prognosis and a high risk of skeletal-related events. Treatment appears to have an impact on survival.**

## INTRODUCTION

In humans, bones are one of the most common metastatic sites for solid malignancies (Norgaard *et al*. 2010, Li *et al*. 2012). Bone metastases (BM) are extremely aggressive and cause sig- nificant morbidity, resulting in typical skeletal-related events (SREs), including severe bone pain, pathologic

fracture, spi- nal cord or nerve root compression, need for surgery or radia- tion therapy, and malignant hypercalcaemia (Sathiakumar *et al*. 2011, Yong *et al*. 2011, Sathiakumar *et al*. 2012, Sathiakumar *et al*. 2013). In addition, the development of BM is associated with shortened overall survival (OS). Drugs aimed at treating specifically BM, including targeted therapies and zoledronic acid, have become available to human medicine and may provide clinical benefit to patients (Lipton *et al*. 2000, Rosen *et al*. 2004, Schultz *et al*. 2007, Lipton & Balakuma- ran 2012). Only one study on bisphosphonates for palliative treatment of BM in dogs has been published (Fan *et al*. 2005), yet several proved their efficacy in canine patients with primary bone tumours (*i.e*. osteosarcoma) (Spugnini *et al*. 2009, Suva *et al*. 2021).

As anticancer management has improved the OS of dogs with cancer, it is our personal clinical impression that BM has also become an emerging problem in veterinary medicine.

In dogs, BM have most commonly been reported with mam- mary carcinoma (Trost *et al*. 2014, Charney *et al*. 2016), pulmo- nary carcinoma (Trost *et al*. 2014), urothelial carcinoma (Cooley & Waters 1998, Charney *et al*. 2016, Melilli 2020) and prostatic adenocarcinoma (Michalak *et al*. 2021). According to the litera- ture, humerus and vertebrae are most frequently affected (Trost *et al*. 2014, Charney *et al*. 2016).

Currently, there are no clinical studies focusing on BM in dogs with advanced solid tumours. Likewise, it is unknown how therapeutic interventions such as medical treatment, radiotherapy, and surgery could affect the clinical course of dogs with BM and the development of SREs. While prognosis is historically con- sidered poor, published estimates based on older data may not reflect survival trends under recent treatment advances. Survival data based on long follow-up periods are also rarely reported in the literature.

To this aim, we completed a retrospective multi-centric study in dogs with solid cancer-bearing BM to review the clinical characteristics, treatment modalities and potential contributing and prognostic factors.

## MATERIALS AND METHODS

Electronic medical records of five Italian-based referral veteri- nary hospitals or clinics were retrospectively reviewed to find dogs diagnosed with solid cancer and BM. Namely, all consecu- tive cases of BM reported between January 2012 and May 2022 were retrieved by one author for each referral centre. Search terms included “metastasis,” “metastatic,” “bone” and “skeletal.” BM was diagnosed according to various imaging modalities and con- firmed by cytology or histology.

The following clinicopathologic variables were analysed: signalment (breed, age, sex, weight), primary cancer site, his- totype and stage, treatment of primary cancer. Recorded char- acteristics of BM included time interval from primary cancer diagnosis to BM identification, disease extent in dogs with metachronous BM (distant metastasis only or distant metasta- sis and locoregional disease), presence of extraosseous metasta- sis, number of BM, BM sites, patterns on radiography or CT, symptoms at presentation of BM, SREs at the time of develop- ing BM, and treatment for BM (none *versus* symptomatic *versus* antitumoural).

Provided that in human medicine metachronous metastases are defined as metastasis occurring at any time during the follow- up period (from 1 month up to 6 months after having diagnosed the primary cancer, depending on histotype and study design), for the current study metachronous metastasis was arbitrarily defined as BM found at >30 days or more from the time of pri- mary cancer diagnosis.

Dogs with BM diagnosed within 30 days before or after their initial solid tumour diagnosis were consid- ered to have synchronous metastasis.

Morphologic patterns on radiography or CT images were clas- sified into osteolytic, mixed and osteoproliferative/osteosclerotic types.

SREs were defined as pathologic fractures, spinal cord com- pression, malignant hypercalcaemia (which only rarely occurs in dogs with BM), or the requirement for radiation therapy or sur- gery for symptomatic BM (Coleman 1997).

Additional variables that were evaluated, if registered at the time of BM diagnosis, included serum levels of lactate dehydro- genase (LDH), alkaline phosphatase (ALP), calcium and choles- terol. Since interval ranges slightly differed among laboratories, laboratory test results were described as normal, above or below the reference limits provided by each institution.

Dogs were excluded if they had hematologic tumours (including multiple myeloma, plasmacytoma and lymphoma) or osteo- sarcoma. Additionally, dogs were excluded if they had multiple synchronous primaries (*i.e*. two or more different tumour types) recorded within 30 days of the diagnosis of BM.

Survival data were obtained from the medical records, and when necessary, via telephone calls to the owners or referring veterinarians.

Categorical variables were summarised as frequency (percent- age), while numerical variables were summarised as median (range). Non-normality of numerical data was assessed using the Shapiro–Wilk test.

Time to BM (TTBM) was defined as the interval between primary tumour and BM diagnosis.

Survival time was defined from the date of BM diagnosis to the date of death from any cause or the end of data collection. For OS, dogs deceased from any cause were considered as events; for bone metastases-related survival (BMRS), only dogs deceased from causes related to BM were considered as events. Survival estimates were computed as medians and 95% confidence inter- vals (CIs).

Univariable and multi-variable Cox proportional hazards regression analysis was performed to evaluate the influence of potentially prognostic variables on TTBM, including sex, age, weight, primary cancer location, histotype, presence of extra- skeletal metastases, hematologic abnormalities, presence of symptoms, pathologic fractures, number of BM, BM sites, type of imaging used for BM assessment, osteoproliferative pattern at imaging. The influence of the aforementioned variables plus the presence of synchronous BM, administration of antitumoural treatment for BM and administration of bisphosphonates were assessed in univariable and multi-variable analysis for OS and BMRS. The continuous variables age and bodyweight were con- verted to dichotomous variables using the median value as the cut point. Covariates with a significant P-value on univariable tests were included in the multi-variable (adjusted) regression model.

Additionally, univariable binary logistic regression was applied to assess the influence of the listed variables on the risk of devel- oping synchronous metastases and to die within 100days from the diagnosis of BM. The effect size of variables was expressed by odds ratios (Ors) with 95% CIs.

Survival curves were generated with the Kaplan–Meier product limit method and compared between dogs receiving and not receiving antitumoural BM treatment with the log- rank test.

Data were analysed with SPSS v.26 (SPSS, Inc., IBM, Chi- cago, IL, USA). P-values ≤0.05 were considered significant.

## RESULTS

The database search revealed 76 dogs with presumed BM all of which were assessed for eligibility. Twenty-six dogs were excluded due to incomplete clinical records (13), lack of cytological/histo- logic confirmation (12) or because of evidence of multiple pri- mary tumours (one).

In total, 50 bone metastatic dogs met the inclusion criteria. There were 15 (30%) mixed-breed dogs and 35 (70%) pure breed dogs. The most represented pure breeds were Rottweiler (n=4; 11.8%), Labrador retriever (n=4; 11.8%) and boxer (n=3; 8.8%), whereas 20 additional breeds were represented once or twice.

Twenty-five (50.0%) dogs were males (intact, n=17; neutered, n=8) and 25 (50.0%) were females (intact, n=4; spayed, n=21). The median age was 10years (range, 6 to 15), and the median weight was 30.0 kg (range, 3.3 to 51).

At BM diagnosis, 41 (82.0%) dogs were symptomatic, whereas BM was an incidental finding in the remaining 9 (18.0%). The main symptoms were lameness (n=31), pain (n=8), paralysis or paresis (n=3), depression or asthenia (n=3), and rectal tenesmus (n=1).

Primary cancer locations included mammary gland (n=6; 12.0%), spleen (n=5; 10.0%), tonsil (n=5; 10.0%), liver (n=4; 8.0%), prostate (n=4; 8.0%), urethra (n=3; 6.0%), heart (n=3; 6.0%), skin (n=2; 4.0%), anal sac (n=2; 4.0%), salivary gland (n=2; 4.0%), adrenal gland (n=2; 4.0%), lungs (n=2; 4.0%) and

one (2.0%) each of the following: thyroid gland, kidney, urinary bladder, muscle, intestine, oral cavity, retroperitoneum, vagina, carotid glomus and a cancer of unknown primary (MCUP). The most common histotypes were carcinoma (n=32; 64.0%) and hemangiosarcoma (n=10; 20.0%), followed by leiomyosarcoma (n=2; 4.0%), melanoma (n=2; 4.0%), chemodectoma (n=2; 4.0%), histiocytic sarcoma (n=1; 2.0%) and pheochromocytoma (n=1; 2.0%).

BM were synchronous in 20 (40.0%) dogs and metachronous in 30 (60.0%). In the latter, the median TTBM was 210days (range, 30 to 1835).

At BM diagnosis, 36 (72%) dogs had previous or concurrent extra-skeletal metastases. Among them, 13 had nodal metasta- ses, 12 had visceral metastases and 11 had both. In 13 (26.0%) dogs, the skeleton was apparently the only metastatic site. These included carcinoma (n=7), hemangiosarcoma (n=3), chemodec- toma (n=2) and splenic leiomyosarcoma (n=1). In 1 (2%) case, the extra-skeletal involvement could not be assessed.

At the time of BM diagnosis, 31 (62.0%) dogs had solitary BM and 19 (38.0%) had multiple bone metastatic disease, add- ing up to a total of 105 bones as sites of metastasis identified on imaging studies. By considering all affected bones, the most common site was the humerus (n=26; 24.8%), followed by ver- tebrae (n=25; 23.8%), ribs (n=14; 13.3%), pelvis (n=10; 9.6%), femur (n=9; 8.6%), scapula (n=4; 3.8%), radius (n=4, 3.8%), ulna (n=4, 3.8%), tibia (n=4, 3.8%), sternum (n=4, 3.8%) and mandible (n=1, 1.0%).

Overall, lesions involved the axial skeleton alone in 11 (22.0%) dogs, the appendicular skeleton alone in 28 (56.0%) and both in 11 (22.0%) dogs.

By descending order, among the 47 appendicular sites, BM involved the humerus (n=26; 55.3%; n=23 proximal metaphysis, n=3 diaphysis), femur (n=9; 19.2%; n=5 proximal metaphysis, n=3 diaphysis, n=1 distal metaphysis), radius (n=4; 8.5%; n=2 distal metaphysis, n=1 diaphysis, n=1 proximal metaphysis), ulna (n=4; 8.5%; n=2 metaphysis, n=1 proximal metaphysis, n=1 dis- tal metaphysis), tibia (n=4; 8.5%; n=2 distal metaphysis, n=1 diaphysis, n=1 proximal metaphysis).

Imaging technology adopted for the assessment of BM included total body CT (TBCT) in 29 (58.0%) dogs, per- formed under general anaesthesia, and plain radiographs in the remaining 20 (40.0%), whereas in one (2%) case the imaging technique was not recorded. BM was osteolytic in 34 (68.0%) dogs, mixed in 15 (30.0%) and osteoproliferative in one (2.0%). Confirmation of BM was obtained by means of cytology in 31 (62.0%) cases and histopathology in the remaining 19 (38.0%) dogs.

Serum ALP concentration was available for 39 (78.0%) dogs at the time of BM diagnosis. Among them, 13 (33.3%) had an increased level.

Serum cholesterol concentration was available for 35 (70.0%) dogs. Among them, only one (2.9%) had an increased level.

Serum LDH concentration was available for 32 (64.0%) dogs. Among them, six (18.8%) had an increased level.

Ionised calcium level was available for 30 (60.0%) dogs. Among them, only one (3.3%) had an increased level.

Overall, 22 (44.0%) dogs experienced SREs, including patho- logic fracture (n=8), need for RT (n=5), need for surgery (n=4), spinal cord compression (n=4) and malignant hypercalcaemia (n=1).

Regarding BM treatment, 24 (48.0%) dogs received antitu- moural therapy, including chemotherapy (platinum compound with or without 5-fluoruracil) and/or toceranib (Palladia; Zoetis) (n=15; 5 of them also received bisphosphonates), radiation therapy (n=5; 3 of them also received metronomic therapy) and surgery (n=4; 2 of them also received metronomic therapy).

Carboplatin (Carboplatin-Teva; Teva S.r.l.) was adminis- tered IV at the dose of 300mg/$m^2$ every 3weeks, while cispla- tin (Cisplatin-Teva; Teva S.r.l.) was administered IV at the dose of 70mg/$m^2$ every 3weeks in combination with 5-fluorouracil (Fluorouracil-Teva; Teva S.r.l.) administered IV at the dose of 150mg/$m^2$ weekly.

Toceranib was administered orally at the dose of 2.2 to 2.5 mg/kg on a Monday–Wednesday–Friday schedule for as long as deemed effective.

Metronomic therapy consisted of cyclophosphamide (10 mg/$m^2$ daily), thalidomide (4mg/kg daily) and piroxicam (0.3mg/kg daily).

External beam radiation therapy was delivered with a 6 MV linear accelerator using either photons (three-dimensional con- formal radiation therapy, n=2) or electrons (n=3). Dogs were treated with a palliative-intent hypofractionated radiation pro- tocol delivered once, twice or three times weekly over 3 weeks. Total doses ranged from 20.0 to 30.4Gy. Fraction numbers ranged from 3 to 8, and fraction sizes ranged from 3.8 to 8.0Gy.

Five (10.0%) dogs received symptomatic treatment consist- ing of anti-inflammatory drugs (including firocoxib, Previcox – Boehringer; piroxicam, Feldene – Pfizer S.r.l.; carprofen, Cani- dryl – Chanelle Medical; prednisone, Prednicortone – Dechra S.r.l.), tramadol (Tralieve; Dechra), gabapentin (Gabapentin Teva Pharma; Teva S.r.l.) and/or amantadine (Mantadan; Hikma S.p.A.).

Twenty-one (42.0%) dogs received no treatment.

At data analysis closure, 46 (92.0%) dogs had died and 4 (8.0%) were still alive, after a median follow-up of 25 days (range, 10 to 97) following BM development and 389 days (range, 20 to 1932) from primary cancer diagnosis.

Median OS after BM diagnosis was 30days (95% CI, 12 to 48); 38 out of 46 dogs (82.6%) were euthanased because of SREs, including intractable pain (n=28) and/or pathologic frac- ture (n=6) and/or nerve root/ spinal cord compression (n=4).

Nine (19.6%) dogs survived longer than 100days since the development of BM. One-year survival rate from BM develop- ment was 2%.

Of the 29 dogs receiving treatment, either antitumoural or symptomatic, 15 (51.7%) reported clinical benefit, defined as improved quality of life as reported by the owners.

The median OS for dogs receiving antitumoural treatment (81 days; 95% CI, 38 to 124) was significantly longer compared

with those receiving symptomatic treatment (43days; 95% CI, 15 to 71) and untreated dogs (5 days; 95% CI, 1 to 24; P=0.001; Fig 1).

On univariable analysis, none of the investigated variables was significantly associated with TTBM.

Dogs aged ≤10years [hazard ratio (HR): 2.0; 95% CI: 1.1 to 3.7; P=0.028], with non-endocrine/neuroendocrine primary tumours (HR: 3.4; 95% CI: 1.0 to 11.6; P=0.046), with synchronous metastases (HR: 1.9; 95% CI: 1.0 to 3.5; P=0.047), with additional extra-skeletal metastasis (HR: 2.4; 95% CI: 1.1 to 4.9; P=0.021), with proximal humerus involvement (HR: 2.0; 95% CI: 1.1 to 3.7; P=0.031) and not receiving antitumoural therapy for BM (HR: 2.6; 95% CI: 1.4 to 4.8; P=0.003) were significantly associated with a shorter OS post-BM diagnosis. On multi-variable analysis only lack of antitumoural therapy retained prognostic significance (HR: 2.7; 95% CI: 1.3 to 5.6; P=0.009).

Variables significantly associated with an increased risk of skeletal-related death included age ≤10 years (HR: 2.3; 95% CI: 1.2 to 4.5; P=0.013), non-endocrine/neuroendocrine primary tumours (HR: 4.3; 95% CI: 1.0 to 18.7; P=0.049), proximal humerus involvement (HR: 2.1; 95% CI: 1.1 to 4.2; P=0.024), extra-skeletal metastasis (HR: 2.2; 95% CI: 1.1 to 4.8; P=0.049) and lack of antitumoural therapy administration (HR: 2.8; 95% CI: 1.4 to 5.5; P=0.003). On multi-variable analysis only lack of antitumoural therapy retained prognostic significance (HR: 3.3; 95% CI: 1.4 to 7.4; P=0.005).

Dogs with endocrine/neuroendocrine primary tumours [odds ratio (OR): 8.8; 95% CI: 1.2 to 63.9; P=0.032], without appen- dicular metastases (OR: 5.1; 95% CI: 1.0 to 25.8; P=0.048), without extra-skeletal metastases (OR: 5.2; 95% CI: 1.1 to 24.5; P=0.043) and those receiving antitumoural therapy (OR: 14.8; 95% CI: 1.7 to 131.4; P=0.016) had an increased chance of sur- viving more than 100 days from BM.

Dogs with synchronous BM were more likely to be assessed with TBCT (OR: 4.9; 95% CI: 1.3 to 18.4; P=0.018) and to have involvement of multiple bones (OR: 4.1; 95% CI: 1.1 to 15.1; P=0.034), including proximal humerus (OR: 3.7; 95% CI: 1.1 to 12.2; P=0.031).

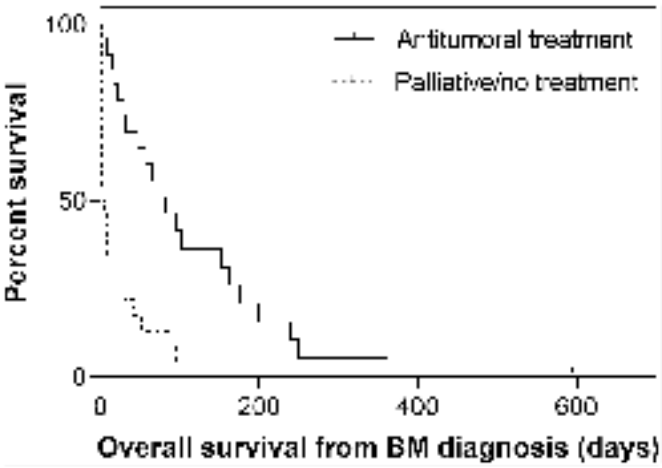


**FIG 1. Kaplan–Meier survival curves for 50 dogs with solid cancer-bearing bone metastases (BM). Dogs receiving antitumoural treatment following BM diagnosis survived significantly longer than dogs receiving no treatment or symptomatic treatment (P=0.001)**

## DISCUSSION

The current clinical study aimed at describing the clinicopatho- logic features and treatment strategies for BM among dogs with solid tumours using electronic medical record data from 5 oncol- ogy practices in Italy. To date, there is no standard treatment for dogs with BM. Thus, the identification of dogs that have a better prognosis could help selecting treatment options.

Due to the high blood flow, the bone appears to provide an attractive environment that allows circulating cancer cells to home, survive and proliferate, ultimately leading to the develop- ment of clinically relevant metastasis. In the current series, the mammary gland was the most common primary tumour loca- tion, occurring in 12% of dogs. This is in line with previous data in both dogs (Trost *et al*. 2014) and humans in which breast and prostate carcinoma, account for the majority of BM (Hage *et al*. 2000, Jiang *et al*. 2020).

Furthermore, for >80% of dogs with mammary tumours in this study, BM represented the first and only distant metastatic site. BM were metachronous in >80% of these dogs and occurred more often as osteolytic lesions involving the appendicular skele- ton. This result reflects the need for advanced diagnostic imaging in the restaging of these patients. In addition to the mammary gland, spleen, tonsils, liver and prostate were responsible for the majority of BM in the current series, up to 36%.

The higher frequency of mammary and other carcinomas in our series supports the seed and soil theory proposed by Paget (1889), according to which specific cancers tend to colo- nise particular sites (Nguyen *et al*. 2009). Indeed, the molecular properties of the malignant cells (the seed) and their reciprocal interactions with the bone microenvironment (the soil) are of great importance in enabling

the metastatic spread of tumours. These properties may vary according to both cell origin (histo- type) and intrinsic characteristics of individual neoplastic clones.

Hemangiosarcomas were the second most frequent tumour type. In this group, concurrent distant extra-skeletal metastases were commonly observed, together with shorter BM latency times, confirming the highly aggressive nature and the ability to reach many organs hematogenously.

In line with the published literature, humerus and vertebrae were the most common affected bones (Trost *et al*. 2014, Char- ney *et al*. 2016). In the current study, there was a higher incidence of long BM (57.2%) than axial skeleton metastasis (20.4%). Another 22.4% of dogs had both, long bone and axial skeleton metastasis. This finding needs to be interpreted cautiously. Axial skeleton metastasis may be asymptomatic and remain undetected if plain radiographs are used as screening diagnostics, thereby underestimating their prevalence.

It is a common belief that metastatic lesions in the appendicu- lar skeleton frequently affect the diaphysis, likely because of the proximity to a nutrient foramen (Ehrhart *et al*. 2020) However, these data are not supported by published studies, but is reported

only in textbooks. In the current series, the distribution pattern of BM showed that 66% of the appendicular lesions were located in the proximal metaphysis, 21% in the diaphysis and 13% in the distal metaphysis.

Several anatomic and biologic reasons might explain the high prevalence of metastatic involvement of bone extremities. The sluggish blood flow in the small and sinusoidal vessels supply- ing the metaphyseal region could promote the entrapment of metastatic cancer cells (Welch *et al*. 2003, Glinsky 2006). More- over, the metaphyseal region makes an ideal microenvironment for cancer cells seeding, due to the trabecular structure of bone, richness of growth factors, cytokines and chemokines (Phadke *et al*. 2006, Shupp *et al*. 2018).

Axial metastasis occurred in 20% of cases. The axial skeleton contains active red marrow, which represents an attractive site for metastatic cancer cells, because of sinusoidal vascular spaces and relatively easy barrier for penetration (Nakamoto *et al*. 2003).

The most common imaging modality for diagnosing BM was TBCT. Most cancers resulted in osteolytic BM, indicating that the balance shifted to more bone destruction than deposition, as shown with the lack of increased activity of ALP in these dogs, which is a marker of osteoblastic activity (Pagani *et al*. 2005). It is believed that osteolysis is due to osteoclastic activity, rather than to a direct effect of the cancer itself (Roodman 2004, Rove & Crawford 2009). Osteolysis puts dogs at risk for pathologic fractures and other subsequent SREs.

Eight dogs were asymptomatic for their BM, and the diagnosis of BM was incidental during the staging work-up of the primary cancer. BM are deep-seated lesions, and obvious symptoms may be absent at the early stage of metastatic spread. Indeed, six of these eight dogs underwent TBCT: it is quite possible that, espe- cially for the axial skeleton, the incidence of BM was underes- timated if staging was performed by radiology and ultrasound only, as already anticipated. As advanced imaging modalities have become widely available, the incidence of BM will probably increase and timely diagnoses will leave more room for the clini- cal management of these patients.

Development of BM was associated with a poor prognosis, as survival time was short (median, 30 days) after they occurred, with 18% of dogs only surviving >100days. SREs may play an important role in the increased mortality risk subsequent to the development of BM.

The multi-variate survival analysis showed that age, weight and lack of antitumour treatment significantly impacted prog- nosis.

The role of age in the prognosis of dogs with BM has not been evaluated before. Based on the current findings, dogs aged ≤10years had a significantly increased risk of tumour-related and SRE-related death, suggesting that in advancing age BM tumour could lose their aggressive nature. The same tendency has been shown in human medicine for breast cancer and lung cancer (Riihimäki *et al*. 2014, Frank *et al*. 2020). Several mecha- nisms related to reduced aggressiveness in elderly patients have been hypothesised, including slower cell proliferation (Holbrook & Ikeyama 2002), reduced tumour-related angiogenesis (Reed *et al*. 2007) and a modified immune system (Purushotham

Higher weight has been advocated as a predisposing factor for appendicular osteosarcoma (Ru *et al*. 1998) and a negative prog- nostic factor in dogs with osteosarcoma of the flat and irregular bones (Hammer *et al*. 1995, Makielski *et al*. 2019). It could be hypothesised that the same mechanical and functional stresses on weight-bearing bones that are thought to contribute to osteosar- coma may also influence the development of BM, although there are no confirmatory studies. Furthermore, an increased weight may also be responsible for a higher probability of SRE in patients with BM, including pathologic fractures as a consequence of a heavy charge on a more fragile bone. Finally, the higher preva- lence of comorbid conditions and more difficult patient handling following the development of BM could play an important role in the increased mortality risk of higher-weighted patients.

Overall, dogs undergoing some forms of antitumoural treat- ment survived significantly longer (median, 81days) than dogs undergoing symptomatic treatment (median, 43days) or no treatment at all (median, 5days). Also, it must be stressed that approximately 50% of treated dogs experienced clinical benefit, whereas the other half still showed pain despite the use of anal- gesic treatment.

In the current study, prognosis after BM diagnosis also depended on the primary cancer type. Dogs with endocrine/ neuroendocrine cancers had an increased chance of surviving more than 100 days from BM development. In the current study, there were five dogs with endocrine/neuroendocrine cancers, and 3 (60%) of them received antitumoural treatment, one received symptomatic therapy and one was not treated. It may be possible that the improved outcome was attributable not so much to the specific histotype as to the implementation of an antitumoural treatment.

The major limitation of the present study was the inherent bias of its retrospective design. Data collection may not have been exhaustive, multiple oncologists and radiologists were involved and different imaging techniques were used, sample size was limited, and there was a marked heterogeneity in treatment, which did not allow us to do a more extensive statistical analysis due to lack of power. Likewise, for many dogs it was not possible to retrieve complete data concerning the initial staging modali- ties and detailed histopathologic variables, including subtype and grade of the primary cancer.

Another limitation is that histologic confirmation of BM was performed in only 19 cases, whereas the others had a cytologic diagnosis. However, histopathologic confirmation of all cases is impractical, and even unethical in dogs being in a poor condi- tion and eligible for symptomatic treatment only. Necropsy data would have also been valuable.

Last, hypercalcaemia identified in one dog may have been due to causes unrelated to BM.

In conclusion, BM foretell a short-term prognosis beyond significantly impairing quality of life. To the best of our knowl- edge, this is the first clinical study to evaluate potential variables

predicting longer survival in dogs with BM from solid can- cers. While dogs with BM have a limited life expectancy, age >10 years, weight <30 kg, and administration of antitumour treatment significantly improved outcome. Further studies are needed to understand the effective role of specific treatments in dogs with BM.

Author contributions

**Chiara Agnoli:** Data curation (equal); supervision (equal); writing – original draft (equal). **Silvia Sabattini:** Data curation (equal); formal analysis (lead); writing – original draft (equal). **Alessandra Ubiali:** Data curation (equal); visualization (equal). **Emiliano Battisti:** Data curation (equal). **Federica Rossi:** Data curation (equal); writing – review and editing (equal). **Alessia Diana:** Data curation (equal); writing – review and editing (equal). **Maria Teresa Camerino:** Data curation (equal); writing – review and editing (equal). **Simone Perfetti:** Data curation (equal); writing – review and editing (equal). **Luca Ciammaichella:** Data cura- tion (equal); writing – review and editing (equal). **Damiano Stefanello:** Data curation (equal); writing – review and editing (equal). **Melissa Papa:** Writing – review and editing (equal). **Riccardo Zaccone:** Writing – review and editing (equal). **Laura Marconato:** Conceptualization (lead); data curation (lead); inves- tigation (lead); methodology (lead); supervision (lead); writing – original draft (lead).

## Conflict of interest

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

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