Timing of adjuvant chemotherapy after limb amputation and effect on outcome in dogs with appendicular osteosarcoma without distant metastases

(Article begins on next page)

07 January 2022

1 **Timing of adjuvant chemotherapy following limb amputation and effect on outcome in**

2 **dogs with appendicular osteosarcoma without distant metastases**

3

4 Laura Marconato (DVM, Prof.), Paolo Buracco (DVM, Prof.), Gerry A. Polton (VetMB,

5 MSc), Riccardo Finotello (DVM, PhD), Damiano Stefanello (DVM, PhD, Prof.), Ondrej Skor

6 (DVM), Lida Bicanova (DVM), Ombretta Capitani (DVM, Prof.), Franck Floch (DVM),

7 Emanuela Morello (DVM, PhD, Prof.), Maria Teresa Camerino (DVM), Katherine

8 Smallwood (MA VetMB), Silvia Sabattini (DVM, PhD)

9

10

11 From the Department of Veterinary Medical Sciences, University of Bologna, Ozzano

12 dell'Emilia (BO), Italy (LM, OC, SS), the Department of Veterinary Sciences, University of

13 Torino, Grugliasco (Torino), Italy (PB, EM, MTC), North Downs Specialist Referrals,

14 Bletchingley, Surrey, UK (GAP), Department of Small Animal Clinical Science, Institute of

15 Infection, Veterinary and Ecological Science, University of Liverpool, Neston, UK (RF, KS),

16 Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Milano, Italy (DS),

17 Division of Small Animal Internal Medicine, Department of Companion Animals and Horses,

18 University of Veterinary Medicine of Vienna, Vienna, Austria (OS, LB), Oncovet, Villeneuve

19 d'Ascq, France (FF).

20

21 Corresponding Author:

22 Laura Marconato; laura.marconato@unibo.it

23

24

26

27

28 **Abstract**

29 **Objective.** Based on cell kinetics, post-operative adjuvant chemotherapy should

30 eradicate microscopic cancer cells. The appropriate timing of chemotherapy following

31 amputation for osteosarcoma is unknown. We retrospectively examined the time

32 interval (TI) between amputation and chemotherapy initiation in dogs with appendicular

33 osteosarcoma without distant metastases, and whether TI played any role in outcome.

34 Aims of the analysis were to evaluate whether an optimum TI for initiating adjuvant

35 chemotherapy and/or a TI after which treatment benefit became negligible could be

36 identified.

37 **Animals**. 168 client-owned dogs.

38 **Procedures**. Dogs were classified into 8 groups based on whether they received

39 chemotherapy within 3, 5, 7, 10, 15, 20, 30 or >30 days after surgery. Univariate and

40 multivariate analyses were performed to determine the prognostic factors of progression

41 and survival outcomes.

42 **Results**. The median TI between surgery and chemotherapy was 14 days (range, 1-210).

43 Median time to progression of dogs receiving chemotherapy within 5 days [375

44 (95%CI, 162-588)] was significantly longer than that of dogs treated after 5 days [202

45 (95%CI, 146-257)]; $P = 0.005$]. Median overall survival of dogs receiving

46 chemotherapy within 5 days [445 (95%CI, 345-545)] was significantly longer than that

47 of dogs treated after 5 days [239 (95%CI, 186-291)]; $P = 0.003$]. The survival benefit of

48 chemotherapy was lost with a delay of 4 weeks from amputation.

49 **Conclusions and clinical relevance**. The present analysis showed a significant

50 association with survival for earlier delivery of adjuvant chemotherapy. These results

51 suggest that the timing of chemotherapy may be an important prognostic variable.

52

2

53 **Abbreviation list**

54 ALP: alkaline phosphatase

55 CI: confidence interval

56 OS: overall survival

57 TBCT: total body computed tomography

58 TI: time interval

59 TNM tumor node metastasis

60 TTP time to progression

61

62

63 **Introduction**

64

65 Canine appendicular osteosarcoma is an aggressive malignancy with a guarded

66 prognosis. Although therapeutic options continue to evolve, limb amputation or limb

67 sparing followed by systemic chemotherapy remains the only potentially curative option

68 for the disease, yet only 20% of dogs survive >2 years after diagnosis.[1]

69 While only 10% of dogs are diagnosed with clinically evident distant metastasis at

70 admission, approximately 90% have occult micrometastases by the time osteosarcoma

71 is diagnosed.[2,3] Given the rapid growth behavior and metastatic potential of

72 osteosarcoma, adjuvant platinum chemotherapy, with or without alternating

73 doxorubicin, has become the standard of care in dogs undergoing limb amputation, as

74 this has provided a survival benefit in multiple studies.[3]

75 The concept that perioperative delivery of chemotherapy may improve outcome is not

76 new. A murine study suggested that the temporal relationship between surgery and

77 chemotherapy was an important variable in determining outcome in osteosarcoma, with

78 mice treated at the time of surgery experiencing an enhancement of drug effect.[4] The

79   biological rationale behind this phenomenon is most likely due to the recruitment of

80   resting metastatic cells into the cell cycle once the primary tumor has been removed.[5,6]

81   In human osteosarcoma patients, early initiation of chemotherapy following surgery has

82   provided a survival benefit, as data suggest a worse outcome when medical treatment is

83   delayed.[7,8]

84   In dogs there is no clear evidence that TI between surgery and initiation of

85   chemotherapy is associated with better survival. Adjuvant chemotherapy is commonly

86   started two weeks after surgery, to allow maximum patient recovery.[1,9,10] A study

87   comparing survival time for dogs starting chemotherapy 2 and 10 days after surgery

88   suggested no benefit to early initiation of adjuvant therapy.[11] It may be possible that the

89   optimal window for identifying survival benefits was missed in that study.

90   We sought to examine the impact of TI on outcome by a retrospective analysis of a

91   large cohort of dogs with appendicular osteosarcoma. The purposes of the analysis were

92   to evaluate whether an optimal TI could be identified and whether there is a TI after

93   which the benefit of adjuvant chemotherapy is lost. It was hypothesized that early

94   delivery of adjuvant chemotherapy improves outcome.

95

96

97   **Material and methods**

98

99   The current study was designed by the Italian Society of Veterinary Oncology

100   (SIONCOV). Medical data of dogs that underwent limb amputation followed by

101   chemotherapy were retrospectively analyzed. Medical records and reported data were

102   checked and reviewed in each center.

103   Dogs were included if, in accordance with the current TNM staging system, they had a

104   histologically-confirmed appendicular osteosarcoma; had no evidence of distant

105 metastases prior to amputation; had a body weight >15 kg; underwent tumor excision;

106 received at least 2 cycles of adjuvant dose-intense chemotherapy; and had complete

107 follow-up data.

108 Dogs were excluded if the osteosarcoma involved the metacarpus, metatarsus or

109 phalanges due to the presumed better prognosis.[12] Dogs with scapula osteosarcoma

110 were also excluded because of the different surgical procedure (partial or total

111 scapulectomy instead of limb amputation).

112 All owners gave their written informed consent to use of the medical records of their

113 dogs.

114

115 *Staging work-up and follow-up*

116 To be included in the analysis, dogs had to be staged by means of TBCT or 3-view

117 thoracic radiographs and abdominal ultrasound, and cytological and/or

118 histopathological evaluation of the regional lymph node. If available, hematology and

119 serum biochemistry results at admission were also recorded. Routine monitoring for

120 pulmonary metastasis with thoracic radiographs occurred every 2-3 cycles of

121 chemotherapy, unless clinical signs suspicious for metastasis were present, in which

122 case imaging was carried out sooner. Follow-up abdominal ultrasound occurred

123 according to each clinician's and owner's preference. Once chemotherapy was

124 completed, dogs were followed-up every 2-3 months.

125

126 *Statistical analysis*

127 TTP was calculated as the interval between amputation and the date of first-documented

128 disease progression (local recurrence and/ or metastasis); dogs with no disease

129 progression at data-analysis closure or death were censored. OS was calculated as the

130 interval between amputation and death from any cause. Dogs alive at data-analysis

131 closure were censored. Survival plots were generated according to the Kaplan-Meier

132 product limit method and compared with the log-rank test. Survival estimates were

133 presented as medians with the corresponding 95%CI. The influence of potentially

134 prognostic variables, including sex, age (<5 years vs >5 years)[13], weight (median used

135 as cutoff value), symptom duration prior to diagnosis (median used as cutoff value), site

136 of osteosarcoma (proximal humerus vs others), ALP level (normal vs increased),

137 monocyte count (normal vs increased), lymphocyte count (normal vs increased), type of

138 imaging (thoracic radiographs/ TBCT), presence of lymph node metastasis (yes vs no),

139 chemotherapy-related toxicity according to VCOG criteria[14] (yes vs. no and grades 3-4

140 vs. others), surgical complications (none/minor vs major) was investigated with

141 univariable Cox's regression analysis.

142 Minor complications were defined as surgery-related events requiring only minor

143 invasive procedures (such as drainage of wounds infection or seroma, and wound

144 management in case of suture dehiscence or superficial infection), whereas major

145 complications referred to adverse events requiring reoperation or resulting in failure of

146 one or more organ systems.

147 Factors with a P value < 0.1 on univariable analysis were further tested for

148 independence in a multivariable Cox proportional hazard model.

149 TI between amputation and the first day of chemotherapy was analyzed as a categorical

150 variable and as a continuous variable. For the categorical data, dogs were classified into

151 8 groups based on whether they received chemotherapy within 3, 5, 7, 10, 15, 20, 30

152 days or >30 days after surgery. The risk of tumor progression and mortality of the dogs

153 within each group was then compared with the remaining dogs (*e.g.* ≤ 3 days vs. > 3

154 days; ≤ 5 days vs. > 5 days, etc.) with Cox's regression analysis and the TI with the

155 highest significant hazard ratio was selected. For the continuous data, a Mann-Whitney

156 *U* test was applied to assess TI differences between dogs alive 1 year following surgery

157 and those deceased earlier.

158 The distribution of possible prognostic outcome variables between dogs treated within

159 the selected optimal TI and the remaining dogs was compared with Fisher's exact

160 test/χ2test.

161 Analyses were carried out using a commercial software program[a]; the significance level

162 was set at 0.05.

163

164

165 **Results**

166

167 *Demographics*

168 From January 2012 to December 2019, 168 dogs with appendicular osteosarcoma that

169 met the inclusion criteria and that were treated in 9 European oncology centers were

170 included in the database.

171 There were 134 (79.8%) purebred dogs and 34 (20.2%) crossbred dogs. In order of

172 prevalence, Rottweiler (n=27; 16.1%), German shepherd (n=13; 7.7%), boxers (n=11;

173 6.5%), greyhound (n=7; 4.2%), great Dane (n=5; 3%), and Schnauzer (n=5; 3%) were

174 the most commonly represented pure breeds. All other breeds represented less than 3%

175 each. There were 86 (51.2%) male dogs (of which 36 were castrated) and 82 (48.8%)

176 females (of which 52 were spayed). Thirty-four dogs (20.2%) were less than 5 years of

177 age, while 134 (79.8%) were older than 10. Median age was 8 years (range, 1 to 14

178 years) and median weight was 32.2 kg (range, 16.2 to 75 kg).

179 All dogs were lame at the time of presentation; median symptom duration was 28 days

180 (range, 2 to 120 days). Osteosarcoma location was: distal radius (n=44; 26.2%),

181 proximal humerus (n=38; 22.6%), distal femur (n=33; 19.6%), distal tibia (n=22;

182   13.1%), proximal femur (n=13; 7.7%), proximal tibia (n=11; 6.5%), and distal ulna

183   (n=7; 4.2%).

184   Nine diagnostic laboratories provided services to the multiple institutions and veterinary

185   hospitals participating in the study, so several reference intervals were used. Total

186   serum ALP activity was measured in 99 (58.9%) dogs before surgery and was greater

187   than the reference range in 22 (22.2%) dogs. Monocyte and lymphocyte values were

188   recorded for 98 (58.3%) dogs: 11 (11.2%) dogs had monocytosis and 1 (0.6%) had

189   lymphocytosis.

190   None had evidence of intra-thoracic and abdominal metastasis prior to treatment; 89

191   (52.9%) dogs were staged by means of TBCT, whereas 79 (47.1%) underwent 3-view

192   thoracic radiographs and abdominal ultrasound.

193   Cytologic sampling of the regional lymph node was performed in 70 (41.7%) dogs,

194   whereas histopathological evaluation of the excised lymph node was carried out in 98

195   (58.3%) dogs. Overall, 7 (4.2%) dogs had histologically-confirmed regional lymph node

196   metastasis.

197

198   *Treatment and side effects*

199   Osteosarcoma was removed surgically via coxofemoral disarticulation (n=66; 39.3%),

200   forequarter amputation (n= 57; 33.9%), scapulohumeral joint disarticulation (n=32;

201   19%), and hemipelvectomy in conjunction with pelvic limb amputation (n=13; 7.7%).

202   Overall, 45 (26.8%) dogs experienced surgical complications: 38 were classified as

203   minor and 7 as major. Among the minor complications, the following were reported:

204   seroma (n=24), hematoma (n=4), mild wound infection (n=4), edema (n=2), partial

205   suture dehiscence (n=1), tracheitis (n=1), external otitis due to wearing E-collar (n=1),

206   and contralateral muscle injury (n=1). Among the major complications, there were

207   wound dehiscence requiring second surgery (n=2), phantom limb syndrome (n=1),

208   disseminated intravascular coagulopathy (n=1), soft tissue necrosis requiring second

209   surgery (n=1), E. coli infection requiring second surgery (n=1), and life threatening

210   bleeding from the surgery site (n=1). All dogs with surgical complications recovered

211   fully.

212

213   All dogs received intravenous chemotherapy: 107 (63.7%) received single-agent

214   carboplatin (median 4; range, 2 to 6), 12 (7.1%) received single-agent doxorubicin

215   (median, 4; range, 3 to 5), 11 (6.5%) received single-agent cisplatin (median, 3; range 3

216   to 4), 33 (19.6%) received alternating doses of a platinum compound (25 cisplatin, 8

217   carboplatin) and doxorubicin (median, 4; range, 2 to 8), 4 (2.4%) received combined

218   doses of cisplatin and dacarbazine (median, 4; range 4 to 6), and 1 (0.6%) dog received

219   alternating doses of carboplatin and epirubicin (3 cycles each).

220   Carboplatin was given at a median dose of 300 mg/m^2 (range, 210 to 300 mg/m^2),

221   doxorubicin at a median dose of 30 mg/m^2 (range, 15 to 30 mg/m^2), cisplatin at a

222   median dose of 70 mg/m^2 (range, 60 to 70 mg/m^2), dacarbazine was administered at 200

223   mg/m^2 for 5 consecutive days in all dogs, and epirubicin was given at 30 mg/m^2.

224   Toxicity data were available for 156 (92.9%) dogs: among them, 56 (35.8%)

225   experienced adverse events. Overall, there were 40 episodes of bone marrow toxicity

226   (n=27 grade 1, n=6 grade 2, n=5 grade 3, n=2 grade 4), 17 episodes of gastro-intestinal

227   toxicity (n=7 grade 1, n=9 grade 2, n=1 grade 3), 3 episodes of grade 1 lethargy, and 1

228   episode of grade 2 renal toxicity.

229   Overall, 8 (14.3% of cases experiencing side effects, and 5.1% of the total cases) dogs

230   had grade 3-4 toxicity. Two dogs with grade 4 bone marrow toxicity required dose

231   decrease and hospitalization. None dogs required dose delay.

232

233   *Outcome*

234 In the whole study population, 148 dogs died and 20 were still alive at data analysis

235 closure (median follow-up time, 323 days; range, 115 to 1196 days). Among the dogs

236 that died, 135 (91.2%) succumbed to tumor-related causes. The 1-, 2-, and 3-year

237 survival rate were 38%, 12% and 6%, respectively. The median TTP was 230 days

238 (95% CI, 178-282). The median OS was 278 days (95% CI, 226-330).

239

240 *Time interval between surgery and chemotherapy and analysis of prognostic factors*

241 The median TI was 14 days (range, 1 to 210 days). In particular, 40 dogs were treated

242 within the first 3 days from surgery, 52 within 5 days, 61 within 7 days, 69 within 10

243 days, 99 within 15 days, 127 within 20 days, 152 within 30 days and the remaining 16

244 after 30 days.

245 Dogs experiencing no surgical complications received adjuvant chemotherapy at a

246 median of 10 days (range, 1 to 210 days). Dogs experiencing minor surgical

247 complications received adjuvant chemotherapy at a median of 16 days (range, 2 to 49

248 days) versus a median of 28 days (range, 24 to 41 days) for those experiencing major

249 complications. In dogs with major postoperative complications, the administration of

250 adjuvant chemotherapy was significantly delayed ($P < 0.001$).

251 Among dogs with no surgical complications, 10 (8%) had a delay of > 30 days in the

252 start of chemotherapy.

253 Dogs alive at 1 year following surgery received chemotherapy significantly earlier

254 (median, 6 days) than dogs dying before 1 year (median, 14 days; $P = 0.004$).

255 The TI associated with the best survival benefit was 5 days. Median TTP of dogs

256 receiving chemotherapy within 5 days [375 days (95%CI, 162-588)] was longer than

257 that of dogs being treated after 5 days [202 days (95%CI, 146-257)] and this difference

258 was significant ($P = 0.005$; Figure 1; Table 1).

259 Median OS of dogs receiving chemotherapy within 5 days [445 days (95%CI, 345-

260 545)] was longer than that of dogs being treated after 5 days [239 days (95%CI, 187-

261 291)] and this difference was significant (P = 0.003; Figure 2; Table 1). Dogs receiving

262 chemotherapy after 5 days from surgery had a 1.7-fold increase in risk of both tumor

263 progression (95% CI, 1.2-2.4; P = 0.005) and death (95% CI, 1.2-2.5; P = 0.003; Table

264 2).

265 In dogs receiving chemotherapy within 5 days, the 1 year- (58% vs 29%) and 2 year-

266 (20% vs 8%) survival rates were significantly higher (P < 0.001 and P = 0.028,

267 respectively), whereas the 3 year- survival rate (12 vs 4%%) was not significantly

268 different (Table 1).

269 In the 16 dogs receiving chemotherapy greater than 30 days after amputation, median

270 TTP and OS were 136 and 169 days, respectively.

271 A long symptom duration was the only other variable significantly associated with an

272 increased risk of tumor progression, whereas age greater than 5 years was associated

273 with an increased risk of death (Table 2). On multivariable analysis, TI > 5 days and

274 symptom duration > 28 days were significantly associated with an increased risk of

275 tumor progression, while TI was the only variable retaining prognostic significance for

276 overall survival (Tables 3 and 4).

277 When examining all the potential prognostic variables separately among dogs receiving

278 chemotherapy within or after 5 days from surgery, the two groups were well balanced

279 with the exception of weight (lower proportion of dogs > 32 kg in the early

280 chemotherapy group; 36% vs. 57%; P = 0.015), staging (lower proportion of dogs

281 undergoing TBCT in the early chemotherapy group; 28% vs. 64%; P <0.001) and

282 number of subjects experiencing chemotherapy-related toxicity (significantly lower in

283 the early chemotherapy group; 17% vs. 44%; P <0.002) (Table 1).

284

285

Discussion

287

288 While the benefit of adjuvant chemotherapy to dogs with resected osteosarcoma has

289 been documented, optimal timing after limb amputation has never been defined.

290 In the current retrospective study, it was shown that there was a statistically significant

291 association with longer survival for earlier delivery of chemotherapy in the adjuvant

292 setting for dogs with appendicular osteosarcoma without distant metastases at

293 presentation. The clearest statistical difference between patient groups was seen when

294 comparing dogs with TI of 5 days or less with those with a TI of greater than 5 days.

295 The median survival times were 445 days and 239 days, respectively.

296 There are several possible biological reasons behind these findings.

297 The ultimate goal of adjuvant chemotherapy is to decrease the chance of metastatic

298 development by eradicating neoplastic cells after surgery.

299 According to the mathematic model by Goldie and Coldman,[15] drug sensitivity of

300 neoplastic cells is related to their spontaneous mutation rate toward a drug-resistant

301 phenotype; because tumor mutation rate increases over time, a longer TI between

302 surgery and onset of chemotherapy may increase the occurrence of a chemo-resistant

303 phenotype.

304 Also, a long TI may facilitate the proliferation of residual malignant cells. This has been

305 documented by murine studies in which the surgical removal of the primary tumor led

306 to an increase in the number of circulating tumor cells, thereby accelerating the

307 progression of the micrometastatic burden.[6] According to other studies, primary tumor

308 removal accelerates angiogenesis by releasing oncogenic growth factors and is

309 permissive of tumor growth through inducing immunosuppression.[16-20] Due to the

310 above, chemotherapy is believed to be more effective if initiated when the tumor burden

311   is low and when cancer cells are multiplying most rapidly.

312   This being said, it must be noted that while the 1- and 2-year survival rate was

313   significantly higher in those dogs receiving early rather than delayed chemotherapy, the

314   advantage was lost at 3 years. Surviving cases were too few for meaningful

315   comparisons at this point, so the result should be regarded with caution, but if this loss

316   of a differential effect of TI is real, this simply highlights that osteosarcoma remains a

317   largely incurable disease for which new treatment strategies are greatly needed.

318   It has been argued that perioperative chemotherapy-induced toxicity may be maximized

319   due to immunodepression after surgery; therefore, a short TI may cause severe adverse

320   events.[21] In the current study, the initiation of adjuvant chemotherapy within 5 days

321   after surgery was feasible, with no increase in incidence of grade 3 or 4 toxicity.

322   A further unanswered question is whether there is an ideal timing for adjuvant

323   chemotherapy after which treatment benefit decreases.

324   In the current study, dogs receiving chemotherapy after 30 days had a median TTP and

325   OS of 136 and 169 days, respectively. For amputation alone, a median survival time of

326   119-175 days has been previously reported, with the majority of dogs being euthanized

327   because of metastatic disease.[13,22,23] A crude comparison shows that the survival benefit

328   of adjuvant chemotherapy is almost lost with a delay of approximately 4 weeks from

329   amputation, and it is questionable whether systemic treatment is appropriate after this

330   time.

331   That the survival benefit of adjuvant chemotherapy is time-dependent is not new.

332   As stated above, Goldie et al.[24] suggested that the drug sensitivity of a tumor is related

333   to the spontaneous mutation rate toward phenotypic drug resistance, which is a function

334   of time. Furthermore, according to the mathematical model by Harless,[25] the

335   effectiveness of chemotherapy is inversely proportional to the tumor burden that had to

336   be eradicated, which, in turn, is a function of the interval to initiation of chemotherapy

337 after surgery.

338 In people with high-grade appendicular osteosarcoma, neoadjuvant chemotherapy

339 followed by surgery and adjuvant chemotherapy is the most-frequently employed

340 strategy. Neoadjuvant chemotherapy is aimed at treating micrometastatic disease early,

341 thereby reducing the risk of distant metastasis, and provides an indication of tumor

342 responsiveness to chemotherapy.[26]

343 In dogs, chemotherapy did not significantly increase survival time when started

344 preoperatively or intraoperatively;[13,22,23] nevertheless, this has not been addressed in

345 prospective studies and merits further investigation.

346 In multivariate analysis, a long symptom duration was also significantly associated with

347 an increased risk of tumor progression. This was not unexpected, as dogs that had

348 experienced clinical signs for longer were more likely to have had time for

349 micrometastases to establish a niche at distant sites and therefore of to progress sooner.

350

351 Our findings need to be interpreted in the context of the study's strengths and

352 limitations. While every effort was made to reduce any selection bias, due to the

353 retrospective nature of the study some information was not retrieved from the medical

354 records, including ALP, lymphocyte and monocyte count.

355 Dogs were included only if they received at least 2 cycles of chemotherapy as the

356 response to only one cycle would have been difficult to evaluate, given that the first

357 restaging by means of imaging was suggested after 2-3 courses of chemotherapy.

358 In the current study, approximately 50% of dogs were worked-up by means of thoracic

359 radiographs and abdominal ultrasound, which may have potentially underestimated their

360 stage. However, the choice of imaging technique did not appear to affect tumor

361 progression or mortality in our series. Furthermore, when considering the 5-days TI

362 window, only 28% of dogs underwent TBCT versus 64% of dogs receiving

363 chemotherapy at a later timepoint, and this difference was significant. Since the

364 resolution of CT is superior for the detection of metastasis, this observation serves to

365 reinforce the finding of an improved survival following a short TI.

366 Although restaging with thoracic radiographs was recommended every 2-3 cycles of

367 chemotherapy for all dogs, the actual frequency of restaging was variable; the lack of

368 standardization may have overestimated TTP.

369 Also, even though all dogs underwent cytological evaluation of the regional lymph

370 nodes, only 60% of them had a histopathological evaluation, therefore the true

371 prevalence of lymph node metastasis may have been underestimated.

372 Similarly, slides were not reviewed, and histological grade and osteosarcoma subtypes

373 were not available for the majority of dogs, possibly impacting outcome.

374 Why dogs experienced delayed chemotherapy administration was not completely

375 addressed in the current study. Various causes may be responsible for the long TI,

376 including delay in referral, surgical complications, logistical issues, oncologists' and/or

377 owners' choice.

378 Overall, 45 (26.8%) dogs had some form of surgical complications, either minor (n=38)

379 or major (n=7). The observation that 8 of these dogs had a delay in the start of

380 chemotherapy of more than 30 days was not unexpected, because animals with

381 postoperative complications were likely to require more time for recovery, but what it is

382 most surprising is that 8% of the dogs with no reported complications still had a delay

383 of more than 30 days. Although the reasons behind this were not investigated, some

384 hypothesis can be made, including delay to obtain the histopathological diagnosis due to

385 sample decalcifying or delay to refer to an oncologist after diagnosis.

386 We also found that the risk of chemotherapy-related adverse events was not increased to

387 the same extent as the risk of death in the group with delayed start of chemotherapy.

388   Multidisciplinary treatment strategies are needed to reduce postoperative complications

389   and promote timely adjuvant chemotherapy.[27]

390   Various chemotherapy protocols have been used in the current study; however, previous

391   publications have identified similar outcomes regardless of protocols, number of cycles

392   and intertreatment interval.[3,10]

393   Last, the lack of necropsy data and standardized diagnostics to determine cause of death

394   may have affected our outcome data.

395   Despite the potential flaws in the accuracy of the information collected, we do not feel

396   that these shortcomings invalidate the conclusions of the study.

397

398   Our findings support the view that starting adjuvant chemotherapy within 5 days post-

399   surgery is associated with a significant survival benefit in dogs with non-metastatic

400   appendicular osteosarcoma. These results suggest that the timing of chemotherapy

401   initiation is an important variable and that great efforts should be made to minimize

402   post-surgical recovery time. Besides, given the lack of impact on treatment-related

403   toxicity, adjuvant chemotherapy should probably be anticipated, in accordance with

404   patient's condition, at the earliest possible time, following the human model. We hope

405   that future studies will repeat these analyses to verify these results.

406

407

408   **Acknowledgements**

409   The authors would like to thank Adele Barbanera, Paolo Guazzi, and Maurizio Annoni

410   for having contributed with cases.

411

412

413   **Footnotes**

414   a: SPSS Statistics v19, IBM, Armonk, New York

415

416

417 **References**

418   1. Culp WT, Olea-Popelka F, Sefton J, et al. Evaluation of outcome and prognostic
419   factors for dogs living greater than one year after diagnosis of osteosarcoma: 90 cases
420   (1997-2008). *J Am Vet Med Assoc.* 2014;245:1141-1146.

421   2. Withrow SJ, Powers BE, Straw RC, Wilkins RM. Comparative aspects of
422   osteosarcoma. Dog versus man. *Clin Orthop Relat Res.* 1991;270:159-168.

423   3. Selmic LE, Burton JH, Thamm DH, et al. Comparison of carboplatin and doxorubicin-
424   based chemotherapy protocols in 470 dogs after amputation for treatment of
425   appendicular osteosarcoma. *J Vet Intern Med.* 2014;28:554-563.

426   4. Bell RS, Roth YF, Gebhardt MC, et al. Timing of chemotherapy and surgery in a
427   murine osteosarcoma model. *Cancer Res.* 1988;48:5533-5538.

428   5. Fisher B, Gunduz N, Saffer EA. Influence of the interval between primary tumor
429   removal and chemotherapy on kinetics and growth of metastases. *Cancer Res.*
430   1983;43:1488-1492.

431   6. Gunduz N, Fisher B, Saffer EA. Effect of surgical removal on the growth and kinetics
432   of residual tumor. *Cancer Res.* 1979;39:3861-3865.

433   7. Imran H, Enders F, Krailo M, et al. Effect of time to resumption of chemotherapy after
434   definitive surgery on prognosis for non-metastatic osteosarcoma. *J Bone Joint Surg*
435   *Am.* 2009;91:604-612.

436   8. Meyers PA, Heller G, Healey J, et al. Chemotherapy for nonmetastatic osteogenic
437   sarcoma: the Memorial Sloan-Kettering experience. *J Clin Oncol.* 1992;10:5-15.

438   9. Bacon NJ, Ehrhart NP, Dernell WS, et al. Use of alternating administration of
439   carboplatin and doxorubicin in dogs with microscopic metastases after amputation for

440   appendicular osteosarcoma: 50 cases (1999-2006). *J Am Vet Med Assoc.*

441   2008;232:1504-1510.

442   10. Skorupski KA, Uhl JM, Szivek A, et al. Carboplatin versus alternating carboplatin and

443   doxorubicin for the adjuvant treatment of canine appendicular osteosarcoma: a

444   randomized, phase III trial. *Vet Comp Oncol.* 2016;14:81-87.

445   11. Berg J, Gebhardt MC, Rand WM. Effect of timing of postoperative chemotherapy on

446   survival of dogs with osteosarcoma. *Cancer.* 1997;79:1343-1350.

447   12. Gamblin RM, Straw RC, Powers BE, et al. Primary osteosarcoma distal to the

448   antebrachiocarpal and tarsocrural joints in nine dogs (1980-1992). *J Am Anim Hosp*

449   *Assoc* 1995;31:86-91.

450   13. Spodnick GJ, Berg J, Rand WM, et al. Prognosis for dogs with appendicular

451   osteosarcoma treated by amputation alone: 162 cases (1978-1988). *J Am Vet Med*

452   *Assoc.* 1992;200:995-999.

453   14. Veterinary Co-operative Oncology Group. Veterinary Co-operative oncology group-

454   common terminology criteria for adverse events (VCOG-CTCAE) following

455   chemotherapy or biological antineoplastic therapy in dogs and cats v1.0. *Vet Comp*

456   *Oncol* 2004;2:194-213.

457   15. Goldie JH, Coldman AJ. A mathematic model for relating the drug sensitivity of

458   tumors to their spontaneous mutation rate. *Cancer Treat Rep.* 1979;63:1727-1733.

459   16. Eggermont AM, Steller EP, Sugarbaker PH. Laparotomy enhances intraperitoneal

460   tumor growth and abrogates the antitumor effects of interleukin-2 and lymphokine-

461   activated killer cells. *Surgery.* 1987;102:71-78.

462   17. Ono I, Gunji H, Suda K, et al. Evaluation of cytokines in donor site wound fluids.

463   *Scand J Plast Reconstr Surg Hand Surg.* 1994;28:269-273.

464   18. Hensler T, Hecker H, Heeg K, et al. Distinct mechanisms of immunosuppression as a

465   consequence of major surgery. *Infect Immun.* 1997;65:2283-2291.

466   19. Baum M, Demicheli R, Hrushesky W, Retsky M. Does surgery unfavourably perturb

467   the "natural history" of early breast cancer by accelerating the appearance of distant

468   metastases? *Eur J Cancer.* 2005;41:508-515.

469   20. Fisher B, Gunduz N, Coyle J, et al. Presence of a growth-stimulating factor in serum

470   following primary tumor removal in mice. *Cancer Res.* 1989;49:1996-2001.

471   21. Goldman LI, Lowe S, al-Saleem T. Effect of fluorouracil on intestinal anastomoses in

472   the rat. *Arch Surg.* 1969;98:303-304.

473   22. Straw RC, Withrow SJ, Richter SL, et al. Amputation and cisplatin for treatment of

474   canine osteosarcoma. *J Vet Intern Med*. 1991; 5:205-10.

475   23. Berg J, Weinstein MJ, Springfield DS, Rand WM. Results of surgery and doxorubicin

476   chemotherapy in dogs with osteosarcoma. *J Am Vet Med Assoc*. 1995; 206:1555-60.

477   24. Goldie JH, Coldman AJ. A mathematic model for relating the drug sensitivity of

478   tumors to their spontaneous mutation rate. *Cancer Treat Rep* 1979;63:1727–33

479   25. Harless W, Qiu Y. Cancer: a medical emergency. *Med Hypotheses* 2006;67:1054–9.

480   26. Luetke A, Meyers PA, Lewis I, Juergens H. Osteosarcoma treatment - where do we

481   stand? A state of the art review. *Cancer Treat Rev* 2014;40:523–532.

482   27. Hans EC, Pinard C, van Nimwegen SA, et al. Effect of surgical site infection on

483   survival after limb amputation in the curative-intent treatment of canine appendicular

484   osteosarcoma: a Veterinary Society of Surgical Oncology retrospective study. *Vet*

485   *Surg*. 2018;47:E88-E96.

486

Figure legends

Figure 1. Time to progression curves for 168 dogs with appendicular osteosarcoma treated

with adjuvant chemotherapy within or after 5 days from limb amputation. In the early

chemotherapy group, dogs had a significantly longer time to progression (375 vs 202 days; *P*

= 0.005).

Figure 2. Overall survival curves for 168 dogs with appendicular osteosarcoma treated with

adjuvant chemotherapy within or after 5 days from limb amputation. In the early

chemotherapy group, dogs had a significantly longer time to progression (445 vs 239 days; *P*

= 0.003)

Tables

Table 1. Baseline characteristics and survival analysis of 168 dogs with appendicular

osteosarcoma treated with adjuvant chemotherapy within or after 5 days from limb

amputation.

Variables	TI ≤ 5 days (n = 52)	TI > 5 days (n = 116)	P
Sex male female	28 24	58 58	0.646
Age ≤ 5 years > 5 years	14 38	20 96	0.149
Weight[a] ≤ 32 kg > 32 kg	33 19	50 66	0.015*

Symptom duration[a,b]			0.920
≤ 28 days	9	52	
>28 days	9	49	
Tumor site			0.196
proximal humerus	15	23	
other	37	93	
ALP[b]			0.178
normal	14	63	
increased	1	21	
Monocyte[b]			0.683
normal	16	71	
increased	1	10	
Lymphocytes[b]			0.173
normal	16	81	
increased	1	0	
Imaging			<0.001*
TBCT	15	74	
3 RX + ABDO US	37	42	
Regional lymph node metastasis			0.438
no	51	110	
yes	1	6	
Surgical complications			0.100
none/minor	52	109	
major	0	7	
Chemotherapy related toxicity[b]			0.002*
no	38	62	

yes	8	48	
Grades 3 and 4 toxicity[b]			0.437
no	45	103	
yes	1	7	
Median TTP (95% CI)	375 (162-588)	202 (146-257)	0.005*
Median OS (95% CI)	445 (345-545)	239 (187-291)	0.003*
1 year-survival rate	29/50 (58.0%)	31/108 (28.7%)	<0.001*
2 year-survival rate	9/44 (20.4%)	8/107 (7.5%)	0.028*
3 year-survival rate	5/43 (11.6%)	4/106 (3.8%)	0.121

502 Abbreviations: TI, time interval between amputation and chemotherapy; TTP, time to

503 progression; OS, overall survival; 3 RX, 3-view thoracic radiographs; ABDO US,

504 abdominal ultrasound; TBCT, total body computed tomography; CI, confidence

505 interval.

506 * = significant; a = median used as cutoff value; b = data not available for all dogs.

507

508

509

510 Table 2. Univariable Cox regression analysis of variables potentially associated with

511 increased risk of tumour progression and mortality in 168 dogs with appendicular

512 osteosarcoma treated with amputation and adjuvant chemotherapy.

Variables	Tumor progression HR (95% CI)	P	Mortality HR (95% CI)	P
Sex male female[a]	0.9 (0.6-1.2)	0.512	1.0 (0.7-1.4)	0.922
Age ≤ 5 years > 5 years[a]	1.4 (0.9-2.1)	0.136	1.5 (1.0-2.3)	0.050*
Weight[b] ≤ 32 kg > 32 kg[a]	1.0 (0.7-1.4)	0.984	1.1 (0.8-1.5)	0.503
Symptom duration[b,c] ≤ 28 days > 28 days[a]	1.5 (1.0-2.3)	0.033*	1.5 (0.9-2.2)	0.061
Tumor site proximal humerus[a] others	1.3 (0.9-1.9)	1.193	1.3 (0.9-1.9)	0.140
ALP[c] normal increased[a]	1.1 (0.7-1.8)	0.659	1.1 (0.7-1.8)	0.663
Monocyte[c]	1.7 (0.8-3.2)	0.116	1.6 (0.8-	0.149

normal increased[a]			3.2)	
Imaging TBCT 3 RX + ABDO US[a]	1.1 (0.8-1.5)	0.595	1.1 (0.8-1.6)	0.410
Regional lymph node metastasis no yes[a]	0.9 (0.4-2.2)	0.868	1.2 (0.5-2.6)	0.680
Surgical complications[c] none/minor major[a]	1.2 (0.9-1.8)	0.242	1.2 (0.9-1.8)	0.233
Chemotherapy related toxicity[c] no yes[a]	0.8 (0.6-1.2)	0.391	0.9 (0.6-1.2)	0.407
Grades 3 and 4 toxicity[c] no yes[a]	0.6 (0.3-1.2)	0.129	0.5 (0.2-1.1)	0.086
Time interval between amputation and chemotherapy ≤ 5 days > 5 days[a]	1.7 (1.2-2.4)	0.005*	1.7 (1.2-2.5)	0.003*

513 Abbreviations: HR, hazard ratio; CI, confidence interval; 3 RX, 3-view thoracic

514 radiographs; ABDO US, abdominal ultrasound; TBCT, total body computed

515 tomography.

516   * = significant; a = reference category; b = median used as cutoff value; c = data not

517   available for all dogs.

518

519

520

521 Table 3. Multivariable Cox regression analysis of variables potentially associated with

522 increased risk of tumor progression in 168 dogs with appendicular osteosarcoma treated

523 with amputation and adjuvant chemotherapy.

Variable	Tumor progression HR (95% CI)	P
Symptom duration > 28 days	2.8 (1.4-5.4)	0.002*
Time interval between amputation and chemotherapy > 5 days	1.6 (1.0-2.3)	0.027*

524 Abbreviations: HR, hazard ratio; CI, confidence interval.

525 * = significant.

526

527

528

529   Table 4. Multivariable Cox regression analysis of variables potentially associated with

530   increased risk of mortality in 168 dogs with appendicular osteosarcoma treated with

531   amputation and adjuvant chemotherapy.

Variable	Mortality HR (95% CI)	P
Age > 5 years	1.1 (0.5-2.6)	0.721
Symptom duration > 28 days	1.5 (0.9-2.2)	0.069
Time interval between amputation and chemotherapy > 5 days	2.3 (1.2-4.5)	0.011*
Grades 3 and 4 toxicity	1.1 (0.5-2.6)	0.721

532   Abbreviations: HR, hazard ratio; CI, confidence interval.

533   * = significant.

534

535