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Doxorubicin area under the curve is an important predictor of neutropenia in dogs with naturally occurring cancers

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Abstract

Doxorubicin (DOX) area-under-the-curve (AUC) was calculated for 40 dogs with spontaneously occurring cancers using a previously validated limited-sampling approach. All dogs were administered a dose of 30 mg/m^2 by intravenous infusion and serum samples were collected at 5, 45 and 60 minutes post-infusion. DOX and its major metabolite, doxorubicinol (doxol), were quantified in serum samples using high-performance liquid chromatography tandem-mass spectrometry. Wide interpatient variability was observed in the predicted DOX AUC with a coefficient of variation of 34%. A significant relationship was found between DOX AUC and absolute white blood cell count ($P = 0.003$), absolute neutrophil count (ANC; $P = 0.002$) and surviving fraction of neutrophils ($P = 0.03$) approximately 1 week after dosing (nadir). No changes in other hematologic parameters (red blood cells, platelets, lymphocytes, haemoglobin) were found to correlate with DOX AUC. The absolute dose (mg) and the dose per unit body weight (mg/kg) were not significantly correlated with nadir ANC. No relationships were found between maximum serum doxol concentration and myelosuppression. Baseline ANC was also significantly correlated to nadir ANC and a model was constructed using baseline ANC and DOX AUC that significantly described the nadir ANC. These findings demonstrate the important relationship between systemic DOX exposure and degree of neutropenia in dogs, and suggest a potential for individualized, pharmacokinetically-guided DOX dosing in dogs.

Keywords

canine cancer; doxorubicin; limited-sampling model; pharmacokinetic-pharmacodynamic relationship

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

All data can be made available upon reasonable request.

1 | INTRODUCTION

Doxorubicin (DOX), the most commonly used chemotherapeutic agent in veterinary cancer therapy, is characterized by a narrow therapeutic window with large interpatient variability in exposure and toxicity (myelosuppression and gastrointestinal) following administration of equivalent doses. In dogs, DOX is routinely administered at a starting dose of 30 mg/m^2 by an intravenous infusion over 15 to 20 minutes. The use of body surface area (BSA) to calculate dose is common to many chemotherapeutic drugs in veterinary medicine and is based on an initial assumption that BSA correlates better with physiologic processes governing drug activity in the body than patient weight.[1] However, an early pharmacokinetics (PK) study in dogs identified an increased toxicity and increased drug exposure in smaller dogs (<10 kg) when dosed on a BSA basis.[2] This may suggest that there are problems with the current formula used to calculate BSA in dogs, as the shape constant used for the species ignores large differences in shape and conformation among dog breeds.[3] Furthermore, the currently used formula was validated based on the differences in metabolic rate between species, not within animals of the same species that vary in size.[4] Thus, there is a large disparity between the dose of antineoplastic drug administered by BSA and the effect that is achieved, primarily because of the resultant large variability in drug exposure. It is therefore not surprising that reports in human oncology have found that drug exposure (area under the drug concentration-time curve (AUC) or steady-state concentration) more closely correlates with pharmacodynamic (PD) effect than the dose per unit of body surface area or body weight.[5–9] In particular, studies in human subjects administered DOX have demonstrated a lack of correlation between the dose administered and exposure (AUC), but a significant correlation between AUC and bone marrow suppression.[8,9] The same has been found with methotrexate where the AUC following high-dose therapy significantly correlates with the degree of neutropenia, better event-free survival, and overall survival.[7]

The concept of utilizing patient exposure compared with BSA to determine dose has been evaluated for a number of drugs used in human oncology. This method of PK-guided dosing was shown to reduce inter-individual variability in the degree of bone marrow suppression following docetaxel therapy by up to 50%.[10] With respect to fluorouracil (FU) therapy in patients with metastatic colorectal cancer, PK-guided dosing led to significantly improved objective response rates, a trend towards higher survival rates and less severe toxicity than BSA-based dosing even though mean FU doses were higher in the PK-guided group.[11] Thus, there is ample evidence that patient drug exposure correlates better with effect than does the dose administered. However, the use of PK-guided dosing has not been universally adopted in human oncology because the relationship between patient exposure and effect has not been elucidated for all drugs.[12,13] These relationships are even less well understood in veterinary cancer therapy. While there are reports of chemotherapy toxicity having a significant correlation with outcome, particularly in canine lymphoma, there has been no evaluation of the relationship of drug exposure within the same setting.[14–16] In this study, we utilize our previously validated limited sampling method for predicting DOX exposure to describe the PK-PD relationships with regard to DOX-induced myelosuppression in dogs with naturally occurring cancers. This will be an important step towards potential PK-guided dosing of this chemotherapeutic agent in dogs.

2 | MATERIALS AND METHODS

2.1 | Animals

Forty-seven dogs with histologically or cytologically confirmed neoplasia presenting to the Flint Animal Cancer Center at Colorado State University for treatment were eligible to enrol in this study after obtaining informed owner consent. The project protocol was exempt from IACUC approval; project approval was obtained from the Clinical Research Review Board at the Colorado State University Veterinary Teaching Hospital. Patients were required to have laboratory and clinical indices that would allow safe administration of DOX (specifically: total bilirubin not exceeding 1.5× normal; creatinine not exceeding 2× normal; at least 2000 neutrophils/μL; 75 000 platelets/μL and a haematocrit of at least 28%). A modified Eastern Comparative Oncology Group (ECOG) constitutional performance score of 0 to 1 was required for inclusion (0, normal activity; 1, restricted activity [decreased from pre-disease status]; 2, compromised [ambulatory only for vital activities, consistently defecates and urinates in acceptable areas]; 3, disabled [dog needs to be force-fed, is unable to confine urination and defecation to acceptable areas], and 4, dead). Baseline and nadir complete blood counts were obtained for evaluation of hematologic toxicity and its relationship to PK and demographic variables.

2.2 | Pharmacokinetics

All dogs received a standard dose of DOX (30 mg/m^2) by intravenous infusion. Blood samples were obtained prior to infusion and again at five, 45, and 60 minutes post-infusion. These time points represent those that we previously validated to predict $AUC_{0–6\ hour}$, providing an accurate estimation of total DOX exposure in dogs.[17] Three millilitres of blood was collected in a red top tube, placed on ice for 10 to 15 minutes, and centrifuged at 2000×*g* for 15 minutes at room temperature. Serum samples were split into two cryovial tubes and stored at −80°C until analysis. Following determination of serum concentrations, the predicted DOX exposure was calculated using the previously validated limited sampling equation[17]:

$$AUC_{0-6\ hour}\ (nM\ h) = 46.9 + 0.63(C_{5\ minutes}) + 1.96(C_{45\ minutes}) + 6.63(C_{60\ minutes})$$

where $C_{5\ minutes}$, $C_{45\ minutes}$ and $C_{60\ minutes}$ represent the serum DOX concentrations (nM) at 5, 45 and 60 minutes post-infusion, respectively. Maximum doxol ($C_{max,\ doxol}$) data was taken directly from the serum concentration results.

2.3 | DOX and doxol analysis

DOX and its major metabolite doxol were measured in canine serum by liquid chromatography tandem-mass spectrometry. Negative ion electrospray ionization mass spectra were obtained with a 6500 Q-TRAP triple quadrupole mass spectrometer (Applied Biosystems, Inc., Foster City, California) with a turbo ionspray source interfaced to a Shimadzu HPLC system (Columbia, Maryland). Samples were chromatographed with an Xbridge Phenyl, 2.5 μm, 4.6 × 50 mm column (Waters Corporation, Milford, Massachusetts) with a Phenomenex C18 filter frit guard cartridge (Torrance, California). A liquid

chromatography gradient was employed with mobile phase A consisting of 10 mM ammonium formate containing 0.1% formic acid and mobile phase B consisting of acetonitrile (ACN) at 1500 μL/minute. Chromatographic separation was achieved by holding mobile phase B steady at 15% from 0 to 1 minute, increasing linearly from 15% to 80% between 1.0 and 2.5 minutes, holding steady at 80% from 2.5 to 3.0 minutes, decreasing linearly from 80% to 15% between 3.0 and 3.5 minutes and re-calibration at 15% until 4.5 minutes. The sample injection volume was 5 μL and the analysis run time was 4.5 minutes. The mass spectrometer settings were optimized as follows: turbo ion spray temperature, 600°C; ion spray voltage, −4500; source gas 1 and 2, 60 units; curtain (CUR) gas, 20 units; collision (CAD) gas, high. Compound parameters for DOX were optimized as follows: declustering potential (DP), −35 V; entrance potential (EP), −10 V; collision energy (CE), −22 V; collision cell exit potential (CXP), −15 V. Compound parameters for doxol were optimized as follows: DP, −40 V; EP, −10 V; CE,−23 V; CXP, −18 V. Sample concentrations of DOX and doxol were quantified by the internal standard reference method in the multiple reaction monitoring mode with ion transitions *m/z* 542.2 → 395.0 amu for DOX, *m/z* 544.2 → 397.2 amu for doxol and *m/z* 526.2 → 379.2 amu for the internal standard, daunorubicin. Scan times were 50 mseconds, and Q1 and Q3 were both operated in unit resolution mode. Analytical standards of DOX hydrochloride (Selleck Chemicals, Houston, Texas) and doxol citrate (Santa Cruz Biotechnology, Inc., Dallas, Texas) were obtained for generation of calibration curves. Analytical standards ranging from 5 to 2000 ng/mL, quality control (5, 100 and 500 ng/mL) and unknown serum samples were prepared by protein precipitation with ACN containing 1% formic acid. For extraction, 100 μL of standard, quality control or unknown serum sample was added to 1.5 mL polypropylene tubes containing 100 ng/mL of internal standard (daunorubicin) followed by 300 μL ACN with 1% formic acid. Samples were then vortex mixed for 10 minutes, centrifuged at room temperature for 10 minutes at 17 000×*g*, and 100 μL of supernatant was transferred to a fresh 1.5 mL Eppendorf tube containing 100 μL ACN with 1% formic acid. The samples were again vortex mixed and then transferred to HPLC autosampler vials containing polypropylene inserts.

2.4 | Statistical analysis

Based on the strong correlation (92%) between predicted vs actual AUC for DOX in dogs[17] and a reported correlation of 57% between plasma DOX AUC and nadir white blood cell count in human patients,[9] it was assumed that the correlation between limited sampling AUC values and nadir ANC in this study would be between 30% and 78% (based on three-dimensional correlation matrix). A sample number of 44 was found to be sufficient to test the null hypothesis (true correlation of 30%) against the alternative hypothesis (correlation greater than 30%) and give 80% power at the one-sided 5% significance level. Statistical analyses were performed in Prism version 7 (GraphPad Software, La Jolla, California). Normal distribution of data was assessed by D'Agostino & Pearson omnibus K2 normality test. Correlations coefficients for normally distributed data were obtained by two-tailed Pearson correlation. The multiple linear regression model for prediction of nadir ANC was generated using the Data Analysis Tool in Excel. Least squares linear regression analysis was performed on patient demographic and pharmacokinetic data vs baseline and nadir ANC values. In some cases, correlation and linear regression analysis was performed first

for all eligible dogs and then only for those dogs in which nadir values were more likely obtained (ie, removal of dogs with nadir ANC values exceeding baseline values). An unpaired, two-tailed Student's *t* test was used to test for differences in DOX AUC between male and female patients, DOX AUC and nadir ANC between lymphoma and osteosarcoma patients, and DOX AUC in those with and without reported GI toxicity following DOX administration. For all analyses, a *P*-value less than 0.05 was considered significant.

3 | RESULTS

Patient characteristics are presented in Table 1. In all, 47 dogs met inclusion criteria for the study. None of the dogs were known to have a mutation of the *MDR1* gene. Of these, 44 dogs had all three blood samples obtained and were evaluable for serum DOX/doxol concentrations and correlations between demographic characteristics and DOX exposure. There were four dogs for which no post-treatment complete blood counts were obtained, leaving a total of 40 dogs available for analysis of correlations between DOX AUC and PDs response. The patient population had a fairly even male: female ratio (48.9% to 51.1%, respectively) and the majority of patients (79%) presented with lymphoma. The remainder of the patient tumours were osteosarcoma (19%) and one patient with acute lymphoblastic leukaemia (ALL).

The serum DOX/doxol concentrations at the collected time points are depicted in Figure 1A. Following a standard dose of DOX administered as an IV-infusion, there was substantial inter-patient variability in serum DOX concentrations (Figure 1B) with a nearly 10-fold range of concentrations at each time point. This led to large variability in predicted $AUC_{0–6\ hour}$ (mean ± SD, 587.1 ± 201.3 nM hour) with a coefficient of variation (CV) of 34% (Figure 1C). There was no significant correlation between the total mg of DOX administered ($P = 0.293$) or the dose per unit body weight ($P = 0.4703$) and the predicted DOX AUC (Figure 2). Demographic data, such as weight, age and sex were evaluated for correlations with predicted DOX AUC. None of these variables was significantly associated with DOX exposure (Figure 3A,C).

We next evaluated hematologic toxicities following DOX treatment. Complete blood counts obtained at a median of 7 days (range 5–13 days) following DOX administration were evaluated for correlations between the nadir values and DOX exposure, dose administered and demographic variables. There were no significant correlations between DOX AUC and the nadir platelet count ($P = 0.985$), red blood cells ($P = 0.253$), lymphocyte count ($P = 0.512$) or haemoglobin concentration ($P = 0.168$). The maximum serum doxol concentration also had no correlation with nadir blood counts. In addition, the DOX concentration at 5 minutes did not have a significant correlation with nadir ANC. However, as shown in Figure 4, significant correlations were identified between DOX exposure and the nadir white blood cell count ($P = 0.003$) and the absolute neutrophil count ($P = 0.002$). Importantly, neither the total dose (mg) nor the dose per unit body weight (mg/kg) had a significant correlation with nadir ANC ($P = 0.106$), but age of the dog was significantly correlated with nadir ANC when all dogs were evaluated together (Figure 4E). When we next removed dogs with a nadir ANC greater than the baseline ANC, the correlation with age was no longer significant (Figure 4F). The correlation between the predicted DOX AUC and ANC also translated into

a significant correlation between the surviving fraction of neutrophils in the evaluation of all dogs as well as only those with a nadir ANC below baseline ANC (Figure 5). A list of the correlations performed with nadir ANC is presented in Table 2.

Based on the significant correlation between DOX AUC and baseline ANC with the nadir ANC, we next developed a model using both parameters that was capable of significantly describing the nadir ANC ($r^2 = 0.429$, $P < 0.0001$; Figure 6A). The equation that significantly described nadir ANC for all dogs in the study population is:

$$\text{Nadir ANC} = 5.44 + (0.418 \cdot \text{baseline ANC}) - (0.005 \cdot \text{DOX AUC})$$

Although age was found to correlate with nadir ANC for the whole study population, incorporation of this variable into the model did not substantially strengthen the ability to describe nadir ANC nor did it improve the appearance of the residual plots (data not shown). The relationship between the measured and predicted nadir ANC was strengthened when evaluating only dogs with a nadir ANC value lower than baseline ANC value ($r^2 = 0.827$, $P < 0.0001$; Figure 6B) and the equation significantly describing nadir ANC for dogs with reduced neutrophils post-treatment is:

$$\text{Nadir ANC} = 0.816 + (0.603 \cdot \text{baseline ANC}) - (0.002 \cdot \text{DOX AUC})$$

Gastrointestinal toxicity information was obtained from the patient records, and although it was found to be insufficient to accurately grade toxicity, we compared the DOX AUC values for those dogs with any mention of toxicity within 7 days post-treatment against dogs with no mention of gastrointestinal toxicity and found no significant difference in DOX exposure ($P = 0.09$).

When evaluating our patient population for differences between tumour types and DOX PK/PD, we found that predicted DOX AUC was significantly higher in the dogs with OSA than in dogs with LSA or leukaemia (737.6 ± 172.3 nM hour vs 547.3 ± 191.2 nM hour; $P = 0.012$). Dogs with OSA also had significantly lower nadir ANC values ($2.7 \pm 1.6 \times 10^3/\mu L$ vs $6.6 \pm 3.7 \times 10^3/\mu L$ $P < 0.0001$).

4 | DISCUSSION

DOX continues to be widely used in veterinary cancer therapy, and the short- and long-term toxicities have been well described. However, data that describe the relationship between PK and PD parameters are scarce. Here, we demonstrate for the first time that the area under the serum DOX concentration-time curve (AUC), determined with the use of a limited sampling model, is predictive of the reduction in white blood cell and neutrophil count following therapy. Furthermore, we show that there was no correlation between the dose administered and toxicity outcome with regard to myelosuppression in a population of dogs with naturally occurring cancer. Our findings are consistent with an early study of DOX in dogs which suggested that small dogs (<10 kg), dosed by BSA instead of body weight, had higher AUC values and a larger proportion of these dogs developed severe myelosuppression.[2] Our results go a step further by describing the relationship between AUC and myelosuppression.

It is important to note that all dogs in this study were treated at the same dose on an mg/m^2 basis and thus, in our evaluation of the effect of dose on toxicity, we were only able to investigate the total drug administered (mg) or the dose per unit body weight (mg/kg); those were the only dose parameters that differed across our population. We would anticipate that escalating doses beyond 30 mg/m^2 would result in increased toxicity inasmuch as this would also lead to increased drug exposure.

In this patient population, there was large variability in drug exposure following the equivalent dose of 30 mg/m^2 in all dogs. This variability was not explained by patient characteristics, such as weight, age, or sex. This is consistent with studies of DOX administered through different routes in human patients.[8,9] Although there was no correlation between weight and DOX AUC, this study did not evaluate body condition score as a variable patient characteristic, and it has been demonstrated that obesity in human patients may alter DOX PK.[18] It is also possible that this wide variability in PK is, in part, associated with variability in the expression or function of genes across the DOX disposition pathway. DOX is metabolized primarily through reduction to doxol by carbonyl reductase (CRB) enzymes, and polymorphisms of CRB1 and CBR3 in humans have been shown to influence the PK and outcome of DOX therapy.[19,20]

In our evaluation of correlations between PK and patient variables with the nadir ANC, we have reported results from the entire study population as well as a subset of the population that excluded those dogs for which nadir bloodwork appeared have been obtained after their actual nadir. In our study, blood was obtained at a median of 7 days post-DOX, which is commonly reported to be the nadir. However, this can be quite variable and in dogs where the post-treatment neutrophil count exceeded the baseline neutrophil count (in some cases by 200%) it is possible that blood was obtained following the rapid recovery phase from the nadir for that individual. It is also possible that either the nadir was not missed in these dogs and they did not experience a true nadir, or factors other than chemotherapy influenced daily variations in neutrophil counts. Regardless, we also evaluated the subset of dogs with nadir values lower than baseline values separately (n = 29) and found that the associations with DOX exposure were strengthened. Interestingly, the correlation between younger dogs and higher nadir ANC values was no longer significant when only evaluating dogs who were likely sampled closer to their actual nadir (ie, lower than baseline values). However, the median age of the dogs removed for this sub-group analysis (7 years) did not differ substantially from that of the whole population (8 years), nor did the median day post-DOX when blood was obtained (7 days for both). This may explain why inclusion of age into the multiple regression model did not improve the ability to predict nadir ANC. We also identified significant differences in DOX exposure and nadir ANC between dogs with osteosarcoma and lymphoma in our patient population. This difference has not been previously reported, but we caution against making conclusions as to the cause of this difference based only on this study as it was not designed to fully evaluate the physiologic and PK differences between dogs with lymphoma and those with osteosarcoma post-amputation.

Although not a primary focus of the present study, we reviewed patient charts for evidence of gastrointestinal toxicity in our study population. We were unable to correlate patients

having noted GI toxicity with the DOX AUC. Lack of standardized prophylactic therapy for GI toxicity and lack of prospective grading of GI toxicity were limitations in that regard. This relationship may be important in cases where GI toxicity becomes dose-limiting and occurs at exposures below those that cause significant myelosuppression. A future prospective study aimed at the PK-PD relationship between DOX AUC and GI toxicity grade could provide that important information. Another potential limitation to the study was the variability in the timing of nadir bloodwork, although 7 days post DOX was most common. While requiring standardization of timing post DOX for nadir evaluation might seem beneficial, there would be no guarantee that the true nadir for each individual would be identified this way and daily repeated sampling to get the true nadir is not feasible from a clinical standpoint.

In this study, we have described a significant relationship between DOX exposure and neutropenia in dogs with regard to post-treatment white blood cell count, neutrophil count and surviving fraction of neutrophils. In addition, we have developed a model that incorporates baseline neutrophil count and DOX AUC to predict the nadir neutrophil count. The inclusion of baseline ANC is important because it suggests that dogs with higher initial neutrophil counts may better tolerate higher exposure to DOX. When combined with our limited sampling methodology for prediction of DOX AUC, the development of this current model could allow for the initial treatment cycle with DOX to be used to tailor subsequent doses in an effort to maximize efficacy while maintaining tolerable levels of myelosuppression. This would be analogous to previous studies in cats treated with carboplatin where AUC and glomerular filtration rate were used to determine drug doses leading to a predictable degree of myelosuppression.[21,22] Further development of our current model would represent an important step in the individualization of DOX chemotherapy regimens in canine cancer treatment. Future studies should aim to determine whether DOX AUC-based dosing regimens could provide improved efficacy over current BSA-based regimens to validate the pursuit of individualized DOX dosing in dogs.

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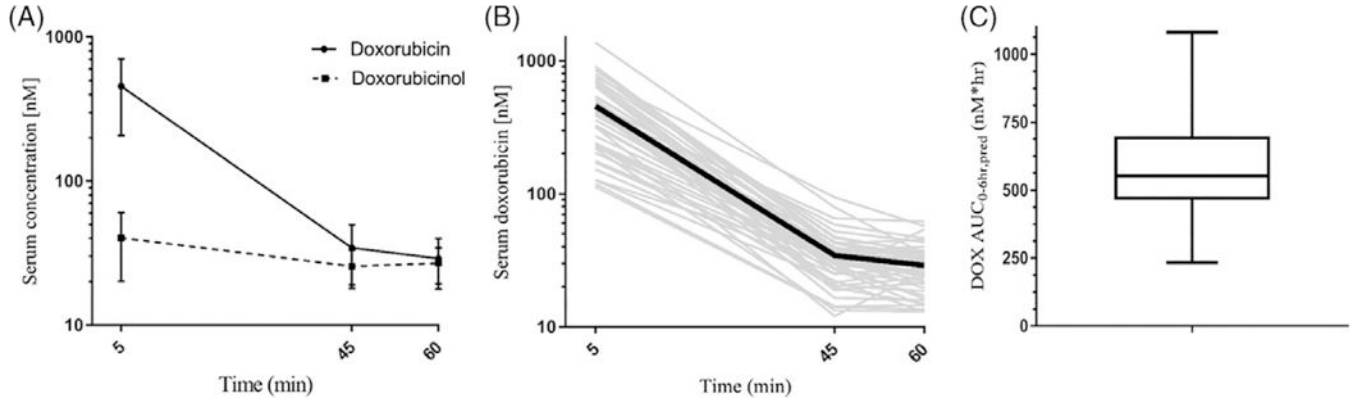

FIGURE 1.

A, Serum doxorubicin (DOX)- and doxorubicinol-time concentration profile for 44 dogs administered a 30 mg/m^2 dose of DOX by intravenous infusion; points represent mean ± SD. B, Individual serum DOX concentration curves for the 44 dogs demonstrating a nearly 10-fold difference in concentration at each time point; dark line represents the population mean. C, Whisker plot of predicted doxorubicin area under the curve (AUC) values in the 44 dogs calculated by a previously validated, limited-sampling model

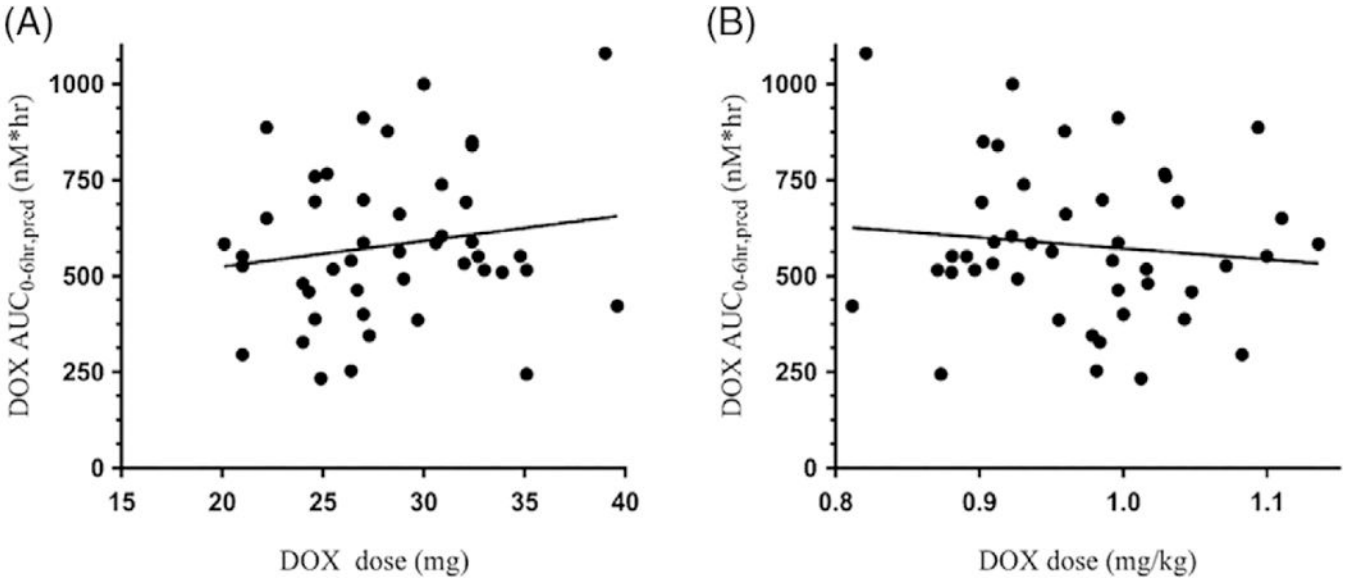

FIGURE 2.

A, Correlation between the total amount of doxorubicin administered and the predicted exposure (area under the curve [AUC]) demonstrating no significant relationship (P = 0.293), and B, correlation between the dose per unit body weight and predicted exposure demonstrating no significant relationship (P = 0.4703). Solid lines represent the least squares linear regression line

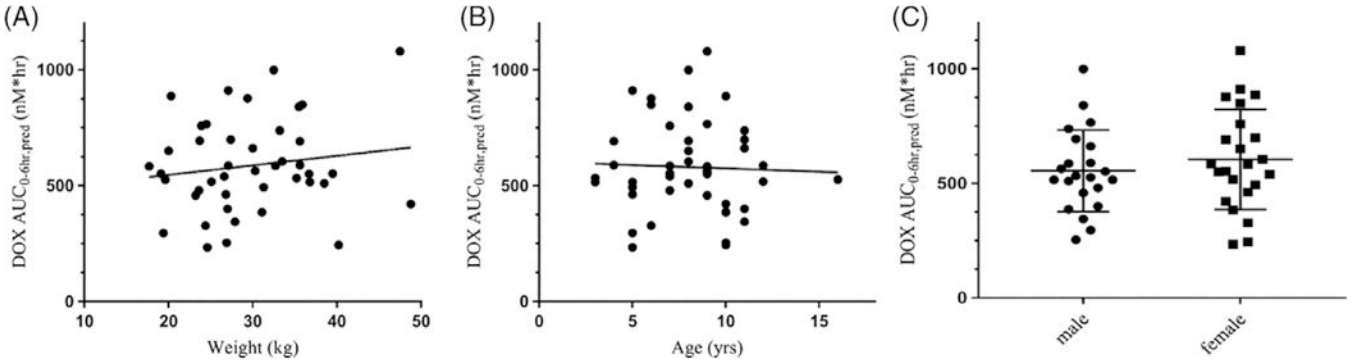

FIGURE 3.

Evaluation of patient demographic variables and predicted DOX exposure shows no significant correlations with A, patient weight ($P = 0.341$) or B, patient age ($P = 0.798$). No difference in DOX exposure was found between male and female dogs (unpaired, two-tailed *t* test; $P = 0.411$). Solid lines in A and B represent the least squares linear regression line

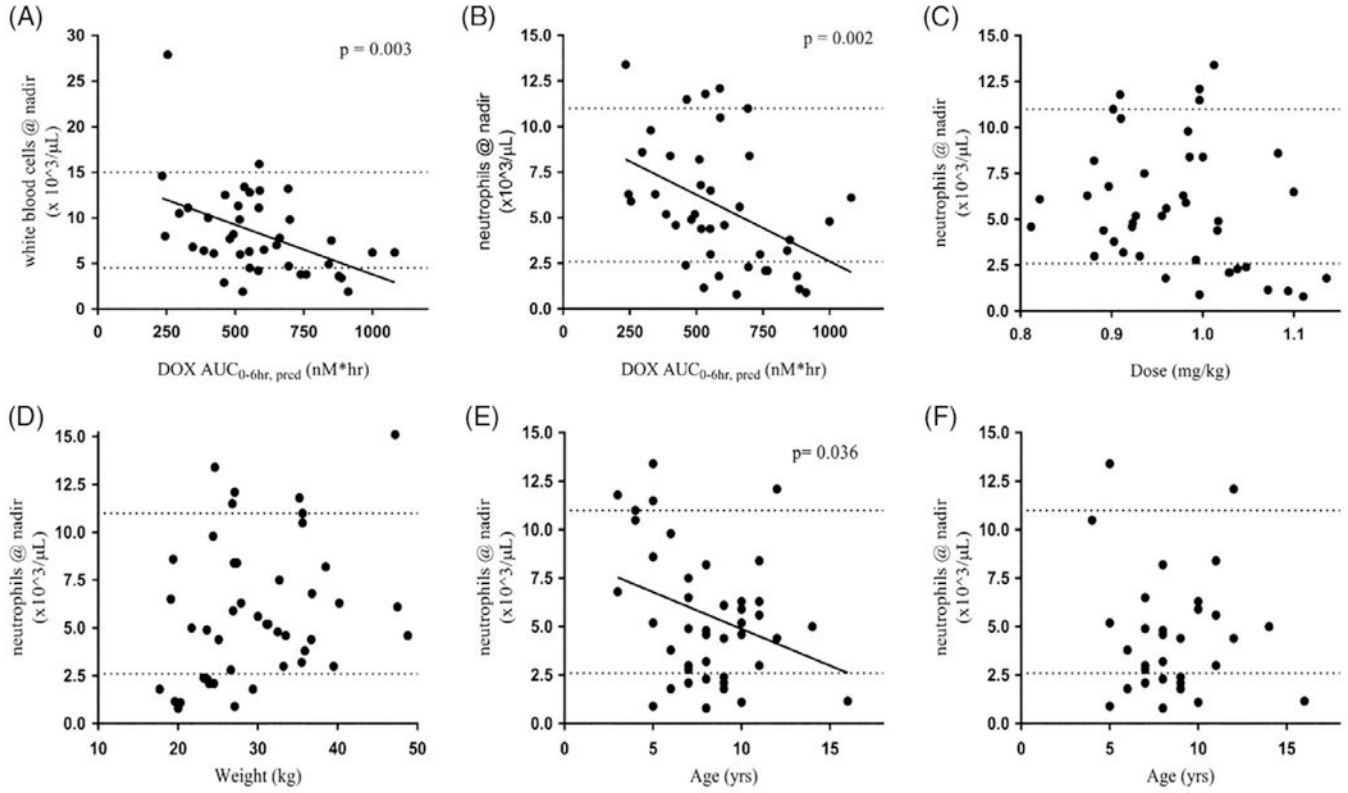

FIGURE 4.

Correlations between pharmacokinetic or demographic variables and myelosuppression. The predicted DOX AUC was significantly correlated with A, white blood cell count at nadir and B, absolute neutrophil count at nadir. C, Dose per unit body weight and D, dog weight were not significantly correlated with nadir neutrophil count ($P = 0.106$ and $P = 0.051$, respectively). E, Age was significantly correlated to nadir neutrophil count when all dogs were evaluated (n = 40), but was not significantly correlated to nadir neutrophil count when the analysis was performed after removal of dogs with increased neutrophils post-treatment F, (n = 29; $P = 0.610$). Solid lines represent the least squares linear regression for variables with significant correlations

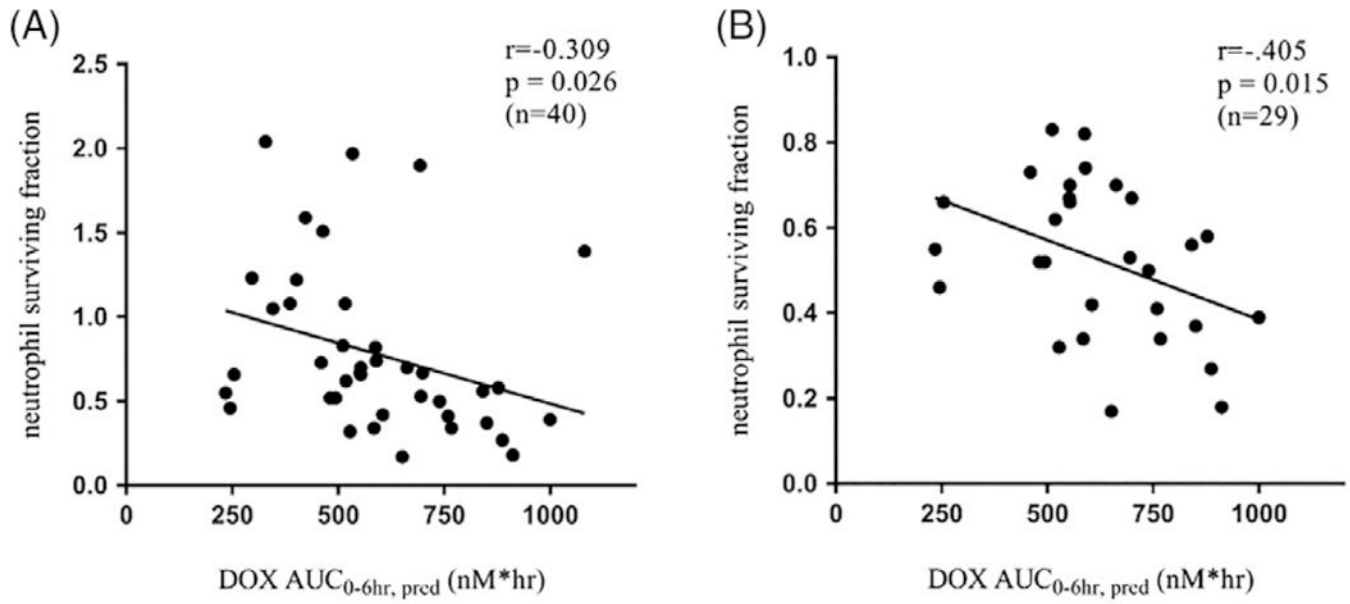

FIGURE 5.

A, Correlation between the predicted DOX exposure and the surviving fraction of neutrophils, calculated by dividing the nadir value by the baseline value, demonstrated a significant association. B, The correlation between predicted DOX exposure and neutrophil surviving fraction was stronger when removing dogs where the nadir was more likely missed (ie, increased neutrophils at nadir leading to surviving fraction greater than 1). Solid lines represent the least squares linear regression

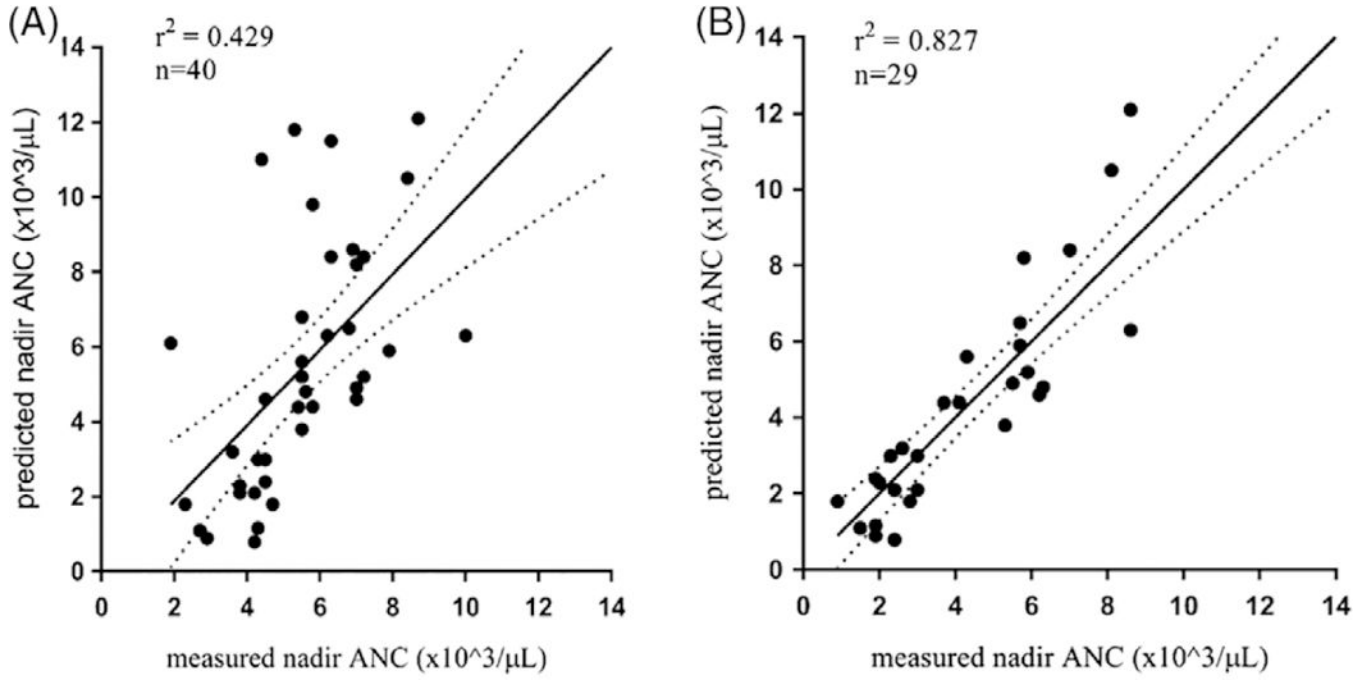

FIGURE 6.

Measured nadir absolute neutrophil count vs the absolution neutrophil count predicted by the regression model that includes baseline neutrophil count and doxorubicin (DOX) AUC for the whole study population (A) and for the subset of dogs with neutrophil reduction post DOX administration (B). The solid line represents the line of best fit and the dashed lines represent the upper and lower bounds of the 95% confidence interval of the best-fit line

TABLE 1

Patient characteristics of dogs enrolled in the study evaluating pharmacokinetic-pharmacodynamic relationships of doxorubicin

Characteristic	Patients (n = 47) No. (%)
Sex	
Male	23 (48.9)
Intact	3 (6.4)
Female	24 (51.1)
Intact	1 (2.1)
Age, years	
Median	8
Range	3–16
Weight, kg	
Median	27.9
Range	17.7–48.8
Tumour histology	
Lymphoma	37 (78.7)
Osteosarcoma	9 (19.1)
Leukaemia	1 (2.1)
Doxorubicin dose, mg	
Median	27.3
Range	20.1–39.6

TABLE 2

Correlations of clinical and pharmacokinetic parameters with nadir absolute neutrophil count in dogs following doxorubicin administration

Parameter	Correlation coefficient (*r*)	*P*-value
Doxorubicin AUC	−0.439	0.002
Baseline neutrophil count	0.414	0.007
Patient age[a]	−0.321	0.037
Patient weight	0.296	0.051
Dose of doxorubicin (mg/kg)	−0.253	0.106
Maximum serum doxorubicinol (nM)	−0.287	0.132

[a]Correlation between age and nadir ANC was not significant when evaluating only those dogs with a nadir ANC lower than the baseline ANC value.