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Plasma Cytokeratin 18 and fecal Alpha-1 Antitrypsin concentrations in dogs with osteosarcoma receiving carboplatin chemotherapy

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Abstract

Gastrointestinal (GI) toxicosis is a common side effect of cytotoxic chemotherapy treatment in humans and dogs. Measurement of cytokeratin 18 (CK18), an intracellular structural protein released during epithelial apoptosis, and Alpha1-Antitrypsin (A1AT) in faeces provides a mechanism for evaluating damage to the intestinal mucosa secondary to cytotoxic chemotherapy. Our goal was to evaluate the clinical utility of plasma CK18 and faecal A1-AT levels as non-invasive biomarkers of cytotoxic chemotherapy induced GI toxicity. We conducted a prospective cohort study in dogs ($N = 10$) with osteosarcoma undergoing amputation followed by carboplatin chemotherapy. We hypothesized that plasma CK18 and faecal A1-AT levels would increase following carboplatin administration due to drug-induced GI epithelial damage/apoptosis, and that plasma CK18 and faecal A1-AT levels would correlate with severity of GI toxicity. Mean baseline plasma CK18 concentration was variable amongst patients; however, CK18 concentration prior to carboplatin chemotherapy treatment was not significantly different from CK18 levels after treatment. There was significant intra and inter-patient variability in mean faecal A1-AT levels at baseline. Mean A1-AT concentration did not change significantly from day 0 to day 21. Gastrointestinal toxicity was minimal; therefore, we were unable to determine the association of plasma CK18 and faecal A1-AT concentrations with development of GI toxicosis. In this study population, plasma CK18 and faecal A1-AT concentration were not clinically useful biomarkers for the detection of GI toxicosis secondary to carboplatin administration. Further prospective evaluation of CK18 and A1-AT as biomarkers of drug-induced GI toxicity is warranted in a larger cohort of dogs receiving cytotoxic chemotherapy.

AVMA clinical trial registration number: AAHSD004827.

KEYWORDS
chemotherapy-associated gastrointestinal toxicity, cytotoxic chemotherapy, gastrointestinal tract, neoplasia

Abbreviations: A1AT, alpha 1 antitrypsin; CK18, cytokeratin 18.

1 | INTRODUCTION

Gastrointestinal (GI) toxicosis is a frequently reported adverse event (AE) associated with cytotoxic chemotherapy in humans and dogs (Biller et al., 2016; Melichar & Zezulova, 2011). The effect of chemotherapy on rapidly dividing epithelial cells can lead to widespread inflammation and ulceration of GI mucosal membranes, commonly referred to as GI mucositis (Melichar & Zezulova, 2011; Sonis, 2004). Mucositis is reported in up to 40% of human patients undergoing standard dose cytotoxic chemotherapy profoundly impacting quality of life and willingness to continue treatment (Gibson & Keefe, 2006) Similarly, chemotherapy-induced toxicosis is reported in 78% of dogs treated with cytotoxic chemotherapy with 3%–5% of patients requiring hospitalization (Bronden et al., 2003; Thamm & Vail, 2007). Severe GI toxicosis can lead to chemotherapy treatment delays, dose reductions and/or discontinuation of treatment which can translate to compromised disease control and decreased survival (Foote, 1998; McCullough, 2017).

Cytokeratins are structural proteins expressed in epithelial cells which serve multiple homeostatic and stress-triggered mechanical functions including the maintenance of structural integrity and scaffolding, regulation of cell-signalling processes and protection from apoptosis (Barak et al., 2004; Jacob et al., 2018) Cytokeratin 18 (CK18) is a member of the cytokeratin family expressed in epithelial cell populations in the gastrointestinal tract, liver, lungs, kidneys, pancreas and mammary glands (Barak et al., 2004; Greystoke et al., 2008; Oshima et al., 1996). During epithelial cell apoptosis, caspases cleave CK18, releasing caspase-cleaved CK18 fragments (ccCK18) into the bloodstream. In contrast, intact CK18 is released primarily from cells undergoing necrosis and can be differentiated from ccCK18 via enzyme-linked immunosorbent assays that recognize CK18-containing backbone epitopes (termed M65) or its apoptosis-associated fragments (termed M30; Greystoke et al., 2008; Ku et al., 2016; Yilmaz, 2009). Based on these findings, the measurement of circulating caspase-cleaved cytokeratin fragments has been suggested as a novel method to assess the intensity of epithelial-specific cellular apoptosis.

Studies aimed at identifying biomarkers of epithelial toxicosis following doxorubicin administration in humans found that serum CK18 concentrations increased significantly in patients that experienced high grade GI toxicosis (Gibb et al., 2013; Greystoke et al., 2011). In veterinary patients, CK18 has been evaluated as a systemic marker of epithelial damage in healthy dogs following antimicrobial administration and in dogs with mast cell tumours treated with toceranib phosphate, a tyrosine kinase inhibitor (Jugan et al., 2018; Kovac et al., 2018). While alterations in plasma CK18 levels in healthy dogs receiving antimicrobial drugs were observed and suggest an effect on gastrointestinal epithelium, CK18 levels did not correlate with the severity of GI toxicosis in dogs treated with toceranib. The clinical utility of CK18 as a biomarker of GI toxicosis secondary to cytotoxic chemotherapy in dogs is unknown.

Intestinal permeability is a sequela to chemotherapy-induced GI mucositis following disruption of epithelial tight-junction integrity, intestinal crypt injury and villous atrophy (Carneiro-Filho et al., 2004; Keefe et al., 1997). Markers of intestinal permeability might serve as sensitive indicators to detect intestinal epithelial damage associated with the development of cytotoxic chemotherapy GI mucositis. Alpha-1 antitrypsin (A1AT) is a proteinase inhibitor produced by hepatocytes that is normally present in plasma, interstitial fluid, and lymph fluid. A1AT is similar in size to albumin; however, in contrast to albumin, which degrades following transmucosal loss into the GI lumen, A1AT is resistant to proteolytic degradation in the feces (Cummins et al., 2017; Murphy et al., 2003) Increased faecal A1AT levels are present in dogs with protein-losing enteropathy, parallel a decrease in serum A1AT levels, and correlate with severity of gastrointestinal lesions (Heilmann et al., 2016) This supports the potential use of faecal A1AT as a biomarker of gastrointestinal protein loss and permeability associated with GI mucositis. Clinically, the development of non-invasive biomarkers would allow institution of supportive medications prior to development of symptoms of gastrointestinal toxicity secondary to chemotherapy administration, which could significantly improve patient quality of life during chemotherapy treatment.

The objective of this study was to evaluate markers of gastrointestinal epithelial damage and permeability in dogs receiving carboplatin chemotherapy for treatment of appendicular osteosarcoma by measuring plasma CK18 and faecal A1AT levels. We hypothesized that plasma CK18 and faecal A1AT levels will increase following a single dose of carboplatin chemotherapy and that this will correlate with the severity of chemotherapy-induced gastrointestinal toxicosis.

2 | MATERIALS AND METHODS

2.1 | Study design

Client-owned dogs were enrolled in a prospective cohort study from October 2018 until October 2019 at The Ohio State University Veterinary Medical Center after meeting all inclusion and exclusion criteria and obtaining informed client consent. The clinical trial was approved by The Ohio State University Veterinary Medical Center Institutional Animal Care and Use Committee (IACUC). . To be eligible for enrolment, dogs must have had prior surgical limb amputation of an appendicular osteosarcoma confirmed with histopathology and undergo adjuvant treatment with at least one dose of carboplatin chemotherapy. Additional inclusion criteria included age ≥ 1-year of age, weight ≥ 5 kg, no evidence of metastatic disease, adequate organ function as indicated by standard laboratory tests and performance status of 0 or 1 (according to the modified Eastern Comparative Oncology Group [ECOG] performance score) (Veterinary Cooperative Oncology Group, 2016). All dogs were required to have a complete blood count (CBC, serum biochemistry profile, urinalysis, and thoracic radiographs prior to enrolment. Dogs were not eligible for enrolment if they were pregnant or lactating, receiving medications within one week of the study starting, receiving

nutraceutical supplements, fed a raw meat diet, or if there was a history of signs of GI disease within 30 days of time of enrolment or other serious systemic disease.

2.2 | Study schedule

Signed informed consent was obtained from all owners before study entry. Prior to enrolment, all dogs underwent a complete physical examination and had a CBC, biochemistry panel, and thoracic radiographs performed. Abdominal ultrasound was performed if deemed necessary by the attending clinician but was not required for enrolment in the study. All dogs underwent surgical amputation of the affected limb two weeks prior to the start of the study (considered day −14). Two weeks following amputation, all study patients were scheduled for administration of the first dose of carboplatin chemotherapy (considered day 0). At that visit, a physical exam, body weight, CBC, biochemistry profile, urine specific gravity and urine dipstick were performed and 6 ml of blood was collected for plasma CK18 measurements. Three naturally voided stool samples were collected in pre-measured faecal collection tubes from up to 3 days prior to the day 0 visit for analysis of faecal A1AT concentrations. At the day 0 visit, surgical incisions were evaluated by the Surgical Oncology Service to ensure adequate tissue healing prior to chemotherapy administration and continuation in the study.

All dogs were required to return for recheck evaluations at study days 7 and 21. At those visits, a CBC was performed and whole blood was collected for CK18 measurement. At Day 21, three naturally voided stool samples were collected in pre-measured faecal collection tubes from up to 3 days prior to the visit for analysis of faecal A1AT concentrations. At the day 21 visit, dogs received their second dose of carboplatin chemotherapy if deemed suitable by their attending clinician and were considered off study. All study samples were collected prior to the administration of the second dose of chemotherapy. Throughout the study, owners completed a daily diary documenting any signs of potential gastrointestinal toxicosis, including diarrhoea, vomiting, reduced appetite, anorexia or nausea. Concomitant medication and AEs were recorded and prospectively graded based on the Veterinary Comparative Oncology Group Common Terminology Criteria for Adverse Events v1.1 (Veterinary Cooperative Oncology Group, 2016). If abnormal clinical signs occurred, owners were asked to both quantify and characterize the abnormalities. The owner diary was collected at days 0, 7 and 21. Additionally, owners completed a quality of life assessment form at each visit. An overview of the study schedule is provided in Figure 1.

2.3 | Treatment

Carboplatin was prescribed at days 0 and 21 at a dose of 300 mg/m^2 and administered via closed system transfer device. All dogs received an intravenous injection of maropitant (Cerenia) at 1 mg/kg IV with chemotherapy, and were discharged with oral maropitant (Cerenia) at 2 mg/kg PO for 4 days following treatment. Owners were also provided with ondansetron 0.5 mg/kg PO and metronidazole 10 mg/kg PO to use only as directed by attending clinician. No other medications, probiotics or nutraceuticals were administered during the study period.

2.4 | Sample collection and processing

Six millilitres of blood were sampled from the jugular vein, placed into an EDTA tube, and placed on ice until processing for CK18 measurement. Blood samples were processed within 30 min of collection. Samples were centrifuged for 15 min at 1,000 × *g* (or 3,000 rpm) at 2–8°C. Plasma was aliquoted into 3–4 cryovials and stored at −80°C until analysis. Three stool samples from naturally voided bowel movements were collected at day 0 and day 21 for A1AT measurement. These samples were collected in pre-measured faecal collection tubes and were stored in owner's freezers and brought to The Ohio State University Veterinary Medical Center for submission on provided ice packs. Once collected from owners, they were stored in a −20°C freezer until all study samples were collected. In preparation

Study Schedule	Study Day																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Carboplatin Treatment (300 mg/m2 IV)	X							X														X
Complete Blood Count	X							X														X
Biochemistry Profile	X																					
Plasma CK18 Measurement	X							X														X
Fecal A1AT Measurement	X																					X
Owner Diary Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Owner QOL Assessment	X							X														X

FIGURE 1 Study schedule demonstrating procedures at day 0, 7, and 21. Boxes marked "X" indicates procedure done on that day. QOL, quality of life

TABLE 1 Patient Demographics

Variable	
Age (median)	7 years (1–11)
Weight (median)	30.4 kg (28.5 – 62.6)
Sex	SF (4) CM (6)
OSA Location	Distal radius (3) Distal femur (2) Proximal tibia (1) Mid-diaphyseal radius (1) Proximal radius (1) Mid-diaphyseal femur (1) Metacarpus (1)

Abbreviations: OSA, Osteosarcoma; SF, spayed female; CM, castrated male.

for the ELISA, 0.3 g faecal matter from each individual faecal sample was uniformly resuspended in 1 ml 1X PBS, and then centrifuged for 15 min at 1,000 × *g* (or 3,000 rpm) at 2 – 8°C as per the manufacturer's instructions. The supernatant was then removed and stored at −20°C until analysis.

2.5 | Biomarker ELISAs

Plasma CK18 concentrations were measured using a canine-specific enzyme-linked immunosorbent assay (ELISA) (ABClonal, Woburn, MA, USA) according to manufacturer's instructions. The absorbance reading of each well at 450 nM was measured with a microplate reader (Spectra Max; Molecular Devices, Sunnyvale, CA, USA). A standard curve was generated for each plate using the provided standard solutions and all readings were performed in duplicate or triplicate for each standard or patient sample, respectively. The intra-assay and inter-assay CVs were 6.8% and 8.6%, respectively. Faecal A1AT concentrations were measured by use of a canine-specific ELISA (Creative Diagnostics, Shirley, NJ, USA) according to manufacturer's instructions. The absorbance reading of each well at 450 nM was measured with a microplate reader (Spectra Max) and recorded. The intra-assay and inter-assay CVs were 8.9% and 11.4%, respectively. A standard curve was generated for each plate using the provided standard solutions and all readings were performed in duplicate or triplicate for each standard or patient sample, respectively.

2.6 | Statistical analysis

Statistical analyses were performed using commercially available software (GraphPad Prism version 8.3.0 Software, Inc, La Jolla, CA, USA). Descriptive statistics were calculated and reported for age, sex, body weight and other clinical variables. Abnormalities identified during treatment are summarized and reported. Continuous variables were tested for normality using the Shapiro–Wilk test. All data were non-normally distributed. Wilcoxon signed-rank test and Friedman's test with Dunn's multiple comparisons test were used to compare data at different time points.

2.7 | Cell line validation statement

Cell lines were not utilized in this study. As such, cell line verification assays were not performed.

3 | RESULTS

Ten dogs met all inclusion and exclusion criteria and were included in the study (Table 1). Six dogs were castrated males and four were spayed females. Median age was 7 years (range, 1–11 years). Median body weight was 30.4 kg (range, 28.5–62.6 kg). Breeds included six mixed breed dogs, two Boxers, one Great Dane and one Greyhound.

Oral post-operative medications given between days −14 and 0 included cephalexin (4 dogs), carprofen (9 dogs), grapiprant (1 dog), gabapentin (10 dogs), trazodone (9 dogs) and sotalol (1 dog). One dog received aminocaproic acid for 2 days post-operatively. One dog received oral metronidazole between days 14 and 13 for diarrhoea. One dog received Apoquel throughout the study period. All dogs were given an injection of maropitant concurrently with their carboplatin chemotherapy treatment, and were sent home with prophylactic oral maropitant given on days 1–4.

Carboplatin treatment was overall well-tolerated in this study population and all reported adverse events were considered low grade (Table 2). Clinical complaints reported between day 0 and Day 7 included two episodes of grade 1 diarrhoea, two episodes of grade 1 nausea, four episodes of grade 1 hyporexia and anorexia, and two episodes of grade 1 lethargy. One dog developed a grade 2 thrombocytopenia following treatment. Clinical complaints reported between day 7 and day 21 included one episode of grade 1 vomiting and grade 1 diarrhoea in one dog, and four episodes of grade 1 hyporexia and anorexia. One dog developed a grade 1 neutropenia at day 21 post-carboplatin treatment.

Only one dog required additional gastrointestinal supportive medications after treatment with carboplatin. This dog received one dose of metronidazole on study day 5 during an episode of diarrhoea, and two doses of ondansetron during an episode of anorexia on study days 3–4. This dog had plasma CK18 concentrations of 4.343 ng/ml, 8.096 ng/ml and 4.375 ng/ml at days 0, 7 and 21, respectively, and faecal A1AT concentrations of 21.028 ng/ml, and 7.682 ng/ml, at days 0 and 21, respectively.

Data for CK18 and A1AT concentrations at each time point are presented in Figures 2 and 3, respectively. Friedman's test of differences among repeated measures of total CK18 concentrations was not statistically significant ($p = .23$). Post hoc testing with Dunn's multiple comparisons test did not reveal any significant differences between individual time points. Friedman's test of differences among repeated measures of CK18 percent change was not statistically significant ($p = .44$). Post hoc testing with Dunn's multiple comparisons

TABLE 2 Adverse events in dogs receiving carboplatin

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal	78% of all AEs			
Diarrhea	3			
Vomiting	1			
Nausea	2			
Anorexia	8			
Constitutional	11% of all AEs			
Lethargy	2			
Hematologic	11% of all AEs			
Thrombocytopenia		1		
Neutropenia	1			
Total	17	1		

Abbreviation: AE, adverse event.

test did not reveal any significant differences between individual time points. Wilcoxon signed-rank test among repeated measures of AIAT concentrations and was not statistically significant ($p = .13$).

4 | DISCUSSION

In this study, we investigated plasma CK18 and faecal A1AT concentrations as potential biomarkers of gastrointestinal toxicosis secondary to carboplatin administration in dogs with appendicular osteosarcoma. In this study population, carboplatin chemotherapy was overall well-tolerated and we observed no difference in CK18 or A1AT levels prior to or following treatment with carboplatin. Furthermore, neither CK18 nor A1AT concentrations predicted the development of signs of GI toxicosis in dogs receiving carboplatin chemotherapy. This could be explained, in part, because clinically observed GI toxicities in this patient population were relatively mild with the most commonly reported AE being grade 1 anorexia and the most severe AE being a grade 1 vomiting and diarrhoea. The low incidence of high grade GI AEs could have limited our ability to detect significant differences in plasma CK18 and faecal A1AT between dogs developing mild versus. severe GI toxicosis.

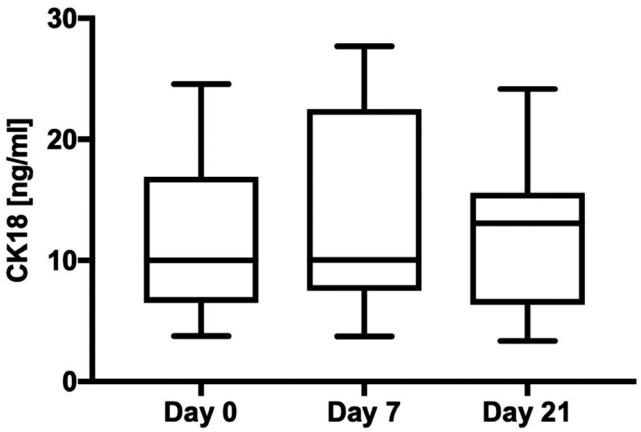

FIGURE 2 Box and whisker plot illustrating serum cytokeratin 18 (CK18) concentrations measured in ng/ml in dogs receiving carboplatin at day 0 (baseline and carboplatin #1), day 7, and day 21 (carboplatin #2). The boxes represent the 25th and 75th percentiles and the central lines represent the mean values. The whiskers represent the 10th and 90th percentiles. No statistically significant difference between the groups was observed

Chemotherapy-induced gastrointestinal mucositis consists of four phases, beginning with an acute inflammatory/vascular phase, progressing to an epithelial phase with epithelial hypoproliferation, followed by an ulcerative phase, and ending with a healing phase (Kornblau et al., 2000; Melichar & Zezulova, 2011). Morphologic changes that occur during GI mucositis include flattening of the intestinal villi, infiltration of lamina propria by inflammatory cell populations, reduction of epithelial cell mitotic activity, and subsequent apoptosis and necrosis of epithelial cells resulting in increased crypt exposure and increased intestinal mucosal permeability (Melichar & Zezulova, 2011) Clinical manifestations of GI mucositis include diarrhoea, which occurs due to imbalanced secretory and absorptive activity, altered GI motility, enhanced pro-inflammatory cytokine signalling from damaged epithelium and infiltrating inflammatory cells, and increased osmolality of intestinal contents (Melichar & Zezulova, 2011; Kornblau et al., 2000) While some patients with GI mucositis appear subclinical, a subset of patients can suffer severe consequences including bacterial translocation and potentially, sepsis (McCullough, 2017) Cytotoxic chemotherapy drugs, including platinum drugs such as carboplatin, directly damage the GI epithelium resulting in mucositis and clinical signs of GI toxicosis.

We chose to evaluate serum CK18 and faecal A1AT levels as markers of chemotherapy-induced GI toxicosis in dogs with osteosarcoma receiving cytotoxic chemotherapy because these dogs are typically an otherwise healthy patient population, thus minimizing any potential confounding factors affecting GI health. Additionally, CK18 expression is largely restricted to epithelial cell populations and is rarely reported in sarcomas; therefore, osteosarcoma patients would not be expected to have altered levels of circulating CK18 due to their disease (Greystoke et al., 2012). A previous study of owner perceptions of pet quality of life during carboplatin treatment reported that 57% of dogs experienced adverse side effects (Bowles et al., 2010). Similarly, 5/10 dogs in this study developed adverse events, however 4/10 of these were very mild adverse events, and only a single dog developed clinical signs which were deemed severe enough by the owner to warrant intervention. While the incidence of adverse GI events in our study population was low, the severity of observed signs is consistent with prior reports indicating that carboplatin is relatively well-tolerated in dogs and that clinical signs of GI toxicosis are generally mild and self-limiting with this drug (Bowles et al., 2010). In a 2014 study by Selmic et al., adverse events were reported in 62.3% of dogs receiving 4–6 doses of carboplatin for treatment of osteosarcoma; 39.8% of these patients experienced grade 1 adverse events and 18.3% had grade 2 adverse events. Higher-grade (grade 3 or 4) adverse events were observed in only 4.3% of patients

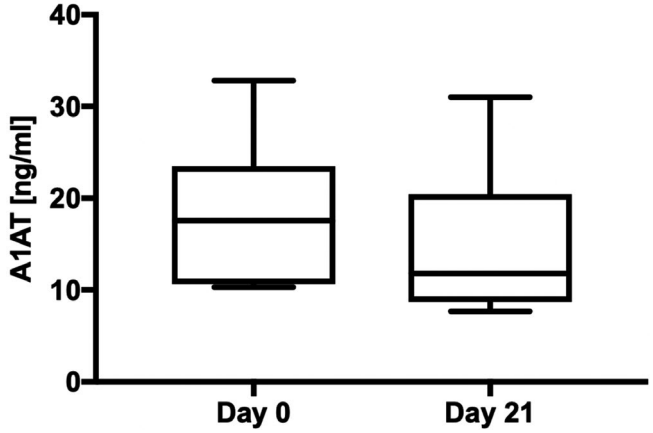

FIGURE 3 Fecal alpha-1 antitrypsin (A1AT) concentrations measured in ng/ml at day 0 and day 21 in dogs receiving carboplatin. The boxes represent the 25th and 75th percentiles and the central lines represent the mean values. The whiskers represent the 10th and 90th percentiles. No statistically significant difference between the groups was observed

(Selmic et al., 2014). It is therefore possible that the mild nature of observed gastrointestinal signs in our study population may explain the lack of robust changes in the biomarkers evaluated. However, the relatively low incidence of high grade GI adverse events associated with carboplatin administration may limit the utility of CK18 as a biomarker for carboplatin-induced GI toxicosis in dogs. Interestingly, the dog experiencing the most severe GI clinical signs had the largest increase in CK18 levels following chemotherapy administration as compared to baseline values; however, the significance of this finding is unclear. Future studies involving a larger population of dogs receiving carboplatin and/or other cytotoxic chemotherapy drugs experiencing more severe GI side effects is required to better assess the clinical utility of CK18 as a biomarker of GI toxicosis secondary to cytotoxic chemotherapy administration.

In humans, biomarkers that predict early chemotherapy-induced GI toxicosis are used to alter treatment protocols in such a way that patient toxicity is minimized while ensuring that chemotherapeutic dose escalation is maximized throughout treatment. This is important as human cancer patients requiring a change to the intended treatment plan experience poorer outcomes, with reductions in response, progression free survival, and overall survival. Circulating CK18 has been shown to be a useful biomarker to predict GI toxicosis secondary to cytotoxic chemotherapy administration in humans. Greystoke et al. found that CK18 levels peaked at day 3 following chemotherapy treatment and that CK18 levels were highest in patients experiencing grade 3 or higher GI AEs (Greystoke et al., 2011). In the current study, we evaluated CK18 concentrations 7 days and 3 weeks post-chemotherapy administration as these collection points coincided with routinely scheduled hospital visits for osteosarcoma patients undergoing systemic chemotherapy treatment. Therefore, it is possible that the collection points assessed in this study did not accurately capture the peak in CK18 levels. Future investigations should be conducted in a population of patients that include additional collection points 3–5 days post-chemotherapy administration to better assess peaks in circulating CK18 concentrations. Alternatively, our lack of significant changes in CK18 levels may be due to poor intrinsic sensitivity of CK18 as a biomarker for apoptosis of the intestinal epithelial cells secondary to cytotoxic chemotherapy drug administration in dogs. Research on CK18 as a biomarker for GI disease is limited to a small number of human and veterinary studies; therefore, more sensitive and accurate biomarkers in the setting of chemotherapy-induced GI toxicosis may exist that warrant investigation (Greystoke et al., 2011; Jugan et al., 2018; Kovac et al., 2018).

Faecal A1AT has been evaluated as a marker of GI protein loss and permeability in dogs with protein-losing enteropathy (PLE). Levels of faecal A1AT are increased in dogs with PLE and A1AT concentrations correlate with the severity of lacteal dilation and intestinal crypt disease (Heilmann et al., 2016) This is the first veterinary study investigating faecal A1AT as a biomarker of cytotoxic chemotherapy-induced GI toxicosis. Morphologic changes to the intestinal crypts are observed following cytotoxic chemotherapy administration, resulting in increased intestinal permeability. However, the degree of GI mucosal damage that results in protein loss into the GI lumen may represent a very severe manifestation of chemotherapy toxicity. We did not observe significant changes in faecal A1AT concentrations after carboplatin administration; however, this may be explained by the relatively low incidence and severity of GI toxicosis in the study population. Additionally, feasibility of the study limited the ability to measure A1AT at multiple time points following carboplatin administration. This study only measured A1AT at 3 weeks following chemotherapy administration, and as such, this collection point may be insufficient to detect changes in faecal A1AT levels that would correlate with GI mucosal damage severe enough to result in clinically detectable protein loss in the faeces. In the current study, we evaluated faecal A1AT as a non-invasive biomarker of intestinal permeability; however, additional GI markers have been studied in setting of chemotherapy-induced GI mucositis in humans. These include measurements of non-metabolized sugars such as lactulose and citrulline, a non-protein amino acid produced by enterocytes, which can be used as a biomarker of enterocyte mass (Crenn et al., 2003; Herbers et al., 2010; Melichar & Zezulova, 2011). Citrulline levels have been investigated in dogs with parvovirus enteritis and were found to be significantly decreased in affected dogs compared to normal dogs; however, this has not been evaluated in the setting of GI mucositis secondary to cytotoxic chemotherapy administration in veterinary medicine (Dahan et al., 2016). Faecal A1AT and/or other biomarkers of intestinal permeability could represent a promising area of study in veterinary oncology for the early detection of antineoplastic drug-induced GI toxicosis.

5 | CONCLUSIONS

While we failed to detect any significant correlation between serum CK18 or faecal A1AT concentrations and the development of signs

of GI toxicosis in this study, our ability to draw conclusions is limited by the low incidence of high-grade GI toxicosis observed in this small patient population. As the only dog developing any significant GI toxicity had the largest increase in CK18 and A1AT, it is possible that these biomarkers could have utility in the setting of higher-grade GI AEs. Further prospective studies evaluating CK18 and A1AT as biomarkers of antineoplastic drug-induced GI toxicity is warranted in a larger cohort of dogs receiving cytotoxic chemotherapy.

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AUTHOR CONTRIBUTION

Kathryn Taikowski: Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing-original draft; Writing-review & editing. **Adam Rudinsky:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing. **Darian Louke:** Data curation; Resources; Software; Supervision; Validation. **Emma Warry:** Conceptualization; Funding acquisition; Methodology; Supervision. **Joelle Fenger:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Writing-review & editing.

PEER REVIEW

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REFERENCES

Barak, V., Goike, H., Panaretakis, K. W., & Einarsson, R. (2004). Clinical utility of cytokeratins as tumor markers. *Clinical Biochemistry*, *37*(7), 529–540. https://doi.org/10.1016/j.clinbiochem.2004.05.009

Biller, B., Berg, J., Garrett, L., Ruslander, D., Wearing, R., Abbott, B., Patel, M., Smith, D., & Bryan, C. (2016). 2016 AAHA oncology guidelines for dogs and cats*. *Journal of the American Animal Hospital Association*, *52*(4), 181–204. https://doi.org/10.5326/JAAHA-MS-6570

Bowles, D. B., Robson, M. C., & Walker, L. et al (2010). Owner's perception of carboplatin in conjunction with other palliative treatments for cancer therapy. *Journal of Small Animal Practice*, *51*, 104–112. https://doi.org/10.1111/j.1748-5827.2009.00891.x

Bronden, L. B., Rutteman, G. R., Flagstad, A., & Teske, E. (2003). Study of dog and cat owners' perceptions of medical treatment for cancer. *The Veterinary Record*, *152*, 77–80. https://doi.org/10.1136/vr.152.3.77

Carneiro-Filho, B. A, Lima, I. P. F., Araujo, D. H., Cavalcante, M. C., Carvalho, G. H. P., Brito, G. A. C., Lima, V., Monteiro, S. M. N., Santos, F. N., Ribeiro, R. A., & Lima, A. A. M. (2004). Intestinal barrier function and secretion in methotrexate-induced rat intestinal mucositis. *Digestive Diseases and Sciences*, *49*, 65–72. https://doi.org/10.1023/B:DDAS.0000011604.45531.2c

Crenn, P., Vahedi, K., Lavergne-Slove, A., Cynober, L., Matuchansky, C., & Messing, B. (2003). Plasma citrulline: a marker of enterocyte mass in villous atrophy-associated small bowel disease. *Gastroenterology*, *124*(5), 1210–1219. https://doi.org/10.1016/S0016-5085(03)00170-7

Cummins, G., Yung, D. E., Cox, B. F., Koulaouzidis, A., Desmulliez, M. P. Y., & Cochran, S. (2017). Luminally expressed gastrointestinal biomarkers. *Expert Review of Gastroenterology & Hepatology*, *11*(12), 1119–1134. https://doi.org/10.1080/17474124.2017.1373017

Dahan, J. M., Giron, C., Concordet, D., & Dossin, O. (2016). Circadian and postprandial variation in plasma citrulline concentration in healthy dogs. *American Journal of Veterinary Research*, *77*(3), 288–293. https://doi.org/10.2460/ajvr.77.3.288

Foote, M. (1998). The importance of planned dose of chemotherapy on time: do we need to change our clinical practice? *The Oncologist*, *3*, 365–368. https://doi.org/10.1634/theoncologist.3-5-365

Gibb, A., Greystoke, A., Ranson, M., Linton, K., Neeson, S., Hampson, G., Illidge, T., Smith, E., Dive, C., Pettitt, A., Lister, A., Johnson, P., & Radford, J. (2013). A study to investigate dose escalation of doxorubicin in ABVD chemotherapy for Hodgkin lymphoma incorporating biomarkers of response and toxicity. *British Journal of Cancer*, *109*(10), 2560–2565. https://doi.org/10.1038/bjc.2013.605

Gibson, R. J., & Keefe, D. M. (2006). Cancer chemotherapy-induced diarrhoea and constipation: Mechanisms of damage and prevention strategies. *Supportive Care in Cancer*, *14*, 890–900. https://doi.org/10.1007/s00520-006-0040-y

Greystoke, A., Cummings, J., Ward, T., Simpson, K., Renehan, A., Butt, F., Moore, D., Gietema, J., Blackhall, F., Ranson, M., Hughes, A., & Dive, C. (2008). Optimisation of circulating biomarkers of cell death for routine clinical use. *Annals of Oncology*, *19*(5), 990–995. https://doi.org/10.1093/annonc/mdn014

Greystoke, A., Dean, E., Saunders, M. P., Cummings, J., Hughes, A., Ranson, M., Dive, C., & Renehan, A. G. (2012). Multi-level evidence that circulating CK18 is a biomarker of tumour burden in colorectal cancer. *British Journal of Cancer*, *107*(9), 1518–1524. https://doi.org/10.1038/bjc.2012.416

Greystoke, A., O'Connor, J. P. B., Linton, K., Taylor, M. B., Cummings, J., Ward, T., Maders, F., Hughes, A., Ranson, M., Illidge, T. M., Radford, J., & Dive, C. (2011). Assessment of circulating biomarkers for potential pharmacodynamic utility in patients with lymphoma. *British Journal of Cancer*, *104*(4), 719–725. https://doi.org/10.1038/sj.bjc.6606082

Heilmann, R. M., Parnell, N. K., Grutzner, N., Mansell, J., Berghoff, N., Schellenberg, S., Reusch, C. E., Suchodolski, J. S., & Steiner, J. M. (2016). Serum and fecal canine alpha1-proteinase inhibitor concentrations reflect the severity of intestinal crypt abscesses and/or lacteal dilation in dogs. *The Veterinary Journal*, *207*, 131–139.

Herbers, A. H., Feuth, T., Donnelly, J. P., & Blijlevens, N. M. (2010). Citrulline-based assessment score: First choice for measuring and

monitoring intestinal failure after high-dose chemotherapy. *Annals of Oncology*, *21*, 1706–1711. https://doi.org/10.1093/annonc/mdp596

Jacob, J. T., Coulombe, P. A., Kwan, R., & Omary, M. B. (2018). Types I and II keratin intermediate filaments. *Cold Spring Harbor Perspectives in Biology*, *10*(4), a018275. https://doi.org/10.1101/cshperspect.a018275.

Jugan, M. C., Rudinsky, A. J., Gordon, A., Kramer, D. L., Daniels, J. B., Paliy, O., Boyaka, P., & Gilor, C. (2018). Effects of oral Akkermansia muciniphila supplementation in healthy dogs following antimicrobial administration. *American Journal of Veterinary Research*, *79*, 884–892.

Keefe, D. M. K., Cummins, A. G., Dale, B. M., Kotasek, D., Robb, T. A., & Sage, R. E. (1997). Effect of high-dose chemotherapy on intestinal permeability in humans. *Clinical Science (Lond)*, *92*, 385–389. https://doi.org/10.1042/cs0920385

Kornblau, S., Benson, A. B., Catalano, R., Champlin, R. E., Engelking, C., Field, M., Ippoliti, C., Lazarus, H. M., Mitchell, E., Rubin, J., Stiff, P. J., Vokes, E., & Wadler, S. (2000). Management of cancer treatment–related diarrhea. *Journal of Pain and Symptom Management*, *19*(2), 118–129

Kovac, R. L., Ballash, G., Fenger, J., London, C., & Warry, E. (2018). Plasma cytokeratin-18 concentrations as noninvasive biomarker of early gastrointestinal toxicosis in dogs receiving toceranib. *Journal of Veterinary Internal Medicine*, *32*(6), 2061–2068. https://doi.org/10.1111/jvim.15326

Ku, N.-O., Strnad, P., Bantel, H., & Omary, M. B. (2016). Keratins: Biomarkers and modulators of apoptotic and necrotic cell death in the liver. *Hepatology*, *64*(3), 966–976. https://doi.org/10.1002/hep.28493

McCullough, R. W. (2017). US oncology-wide incidence, duration, costs and deaths from chemoradiation mucositis and antimucositis therapy benefits. *Future Oncology*, *13*, 2823–2852. https://doi.org/10.2217/fon-2017-0418

Melichar, B., & Zezulova, M. (2011). The significance of altered gastrointestinal permeability in cancer patients. *Current Opinion in Supportive and Palliative Care*, *5*, 47–54. https://doi.org/10.1097/SPC.0b013e328343a043

Murphy, K. F., German, A. J., Ruaux, C. G., Steiner, J. M., Williams, D. A., & Hall, E. J. (2003). Fecal alpha1-proteinase inhibitor concentration in dogs receiving long-term nonsteroidal anti-inflammatory drug therapy. *Veterinary Clinical Pathology*, *32*, 136–139.

Veterinary Cooperative Oncology Group. (2016). Veterinary cooperative oncology group - common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. *Veterinary and Comparative Oncology*, *14*, 417–446.

Oshima, R. G., Baribault, H., & Caulin, C. (1996). Oncogenic regulation and function of keratins 8 and 18. *Cancer and Metastasis Reviews*, *15*, 445–471. https://doi.org/10.1007/BF00054012

Selmic, L. E., Burton, J. H., Thamm, D. H., Withrow, S. J., & Lana, S. E. (2014). Comparison of carboplatin and doxorubicin-based chemotherapy protocols in 470 dogs after amputation for treatment of appendicular osteosarcoma. *Journal of Veterinary Internal Medicine*, *28*, 554–563. https://doi.org/10.1111/jvim.12313

Sonis, S. T. (2004). Pathobiology of mucositis. *Seminars in Oncology Nursing*, *20*, 11–15. https://doi.org/10.1053/j.soncn.2003.10.003

Thamm, D. H., & Vail, D. M. (2007). Aftershocks of cancer chemotherapy: Managing adverse effects. *Journal of the American Animal Hospital Association*, *43*, 1–7. https://doi.org/10.5326/0430001

Yilmaz, Y. (2009). Systematic review: Caspase-cleaved fragments of cytokeratin 18 - the promises and challenges of a biomarker for chronic liver disease. *Alimentary Pharmacology & Therapeutics*, *30*, 1103–1109. https://doi.org/10.1111/j.1365-2036.2009.04148.x

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