Comparison of alfaxalone and propofol on haematological and serum biochemical variables in cats undergoing radiotherapy with sevoflurane maintenance

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Q7 RESEARCH PAPER

Q1 Comparison of alfaxalone and propofol on haematological and serum biochemical variables in cats Q6 undergoing radiotherapy with sevoflurane maintenance

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Abstract

Objective To evaluate effects of repeated alfaxalone or propofol administration on haematological and serum biochemical variables in cats undergoing radiotherapy.

Study design Prospective, block-randomized, clinical trial.

Animals A group of 39 client-owned cats.

Methods After butorphanol (0.2 mg kg^{-1}) and midazolam (0.1 mg kg^{-1}) sedation, cats were randomly assigned to be administered either alfaxalone or propofol for induction of anaesthesia and sevoflurane maintenance. Cats were anaesthetized daily with the same induction agent for 10–12 days. Complete blood counts, reticulocytes, Heinz body score and serum biochemistry were performed before the first treatment (T1), at T6, T10 and 3 weeks after the final treatment (T21). Cumulative induction agent dose for each cat at each time point was evaluated for an effect on Heinz body score. Data are shown as mean ± standard deviation; $p < 0.05$.

Results At baseline there were no significant differences in signalment or blood variables between groups. A significant decrease in haematocrit of 2.3% ± 0.77 ($p = 0.02$) between T1-T6 and T1-T10 [mean 4.1% (±0.78, $p < 0.0001$)] was detected, with a significant increase in haematocrit of 2.1% ± 0.80 ($p = 0.046$) between T6-T21 and 4.0% ± 0.8 ($p < 0.001$) between T10-T21. Heinz body score significantly increased by 1.86 ± 0.616 ($p = 0.013$) between T1-T10. In the propofol group, reticulocytes increased significantly between T1-T6 [mean 23,090 μL^{-1} ± 7670 ($p = 0.02$)] and T1-T10 [mean 27,440 μL^{-1} ± 7990 ($p = 0.007$)]. Mean cumulative dose at T10 was 19.65 mg kg^{-1} ± 5.3 and 43.4 mg kg^{-1} ± 14.4 for alfaxalone and propofol, respectively, with no significant effect on Heinz body formation at any time point.

Conclusions and Clinical relevance Haematocrit decreased in both groups with recovery after 3 weeks. Repeated alfaxalone and propofol administration was not associated with marked haematological or serum biochemistry changes.

Keywords anaemia, anaesthetic, feline, Heinz bodies, repeated induction agent.

Introduction

Certain therapeutic interventions in cats require repeated short-duration anaesthesia. The anaesthetic agent propofol, a non-barbiturate alkyl phenol dissolved in soybean oil is a suitable drug for daily wound management or procedures such as radiation therapy (RT). Propofol leads to smooth anaesthetic induction, rapid recovery and carries a relatively low risk profile (Matthews et al. 2004; Pascoe et al. 2006; Bley et al. 2007). However, propofol increases Heinz body formation in cats after consecutive anaesthetic episodes or above a certain cumulative dose (Andress et al. 1995; Matthews et al. 2004; Baetge et al. 2020). Heinz bodies are microscopically visible clumps of denatured (oxidized) haemoglobin that indicate oxidative damage to erythrocytes. They are found in healthy cats, but are often present in chronic disease states or neoplasia and may contribute to clinically relevant anaemia (Christopher 1989; Christopher et al. 1990; Stokol 2017).

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While significant changes in haematological variables after repeated propofol anaesthesia in healthy cats are not always observed (Andress et al. 1995; Matthews et al. 2004), haematocrit and haemoglobin decreased in cats undergoing repeated propofol anaesthesia for RT (Bley et al. 2007). Albeit not deemed clinically relevant, the decrease in haematocrit was significantly different from baseline, with a mean decrease of 8.9% ± 6.9% [± standard deviation (SD)] after six and 9.0% ± 6.2% after 12 anaesthetic episodes (Bley et al. 2007). However, if observed Heinz body formation shortens red blood cell survival or results in acute haemolysis, this could exacerbate anaemia in cats already compromised by illness or injury (Whittem et al. 2008; Childress 2012).

Alfaxalone (3α-hydroxy-5α-pregnane-11,20-dione), a synthetic neuroactive steroid, formulated in a new water-soluble preparation with 2-hydroxypropyl-β-cyclodextrin, provides an alternative to the existing intravenous anaesthetic (IV) options for use in healthy cats (Warne et al. 2015). Unpremedicated or premedicated cats anaesthetized with alfaxalone for short procedures in experimental or clinical settings showed a lesser degree of hypoxaemia compared with propofol, while cardiovascular and other ventilatory variables remained within acceptable ranges (Whittem et al. 2008; Muir et al. 2009; Taboada & Murison 2010; Mathis et al. 2012; Campagna et al. 2015; Tamura et al. 2021). Recovery was relatively smooth, albeit with more episodes of paddling, trembling and ataxia compared with recovery after propofol administration (Mathis et al. 2012; Tamura et al. 2021). While the pharmacokinetic, pharmacodynamic and physiological variables relevant to anaesthesia were explored at clinical and supraclinical doses of alfaxalone (Whittem et al. 2008; Muir et al. 2009; Warne et al. 2015; Pypendop et al. 2018), neither changes in haematological nor biochemical variables after repeated use have been described.

The aim of this study was to compare the effect of alfaxalone and propofol on haematological or serum biochemistry variables in cats undergoing consecutive anaesthesia. We hypothesized that cats anaesthetized with alfaxalone as the induction agent for repeated, daily sessions of RT would show a decrease in haematocrit and haemoglobin. Furthermore, we wanted to quantify and compare the degree of Heinz body formation over time.

Material and methods

Study design

This was a prospective, single-centre, block-randomized, controlled, parallel-group study conducted at the Division of Radiation Oncology of the Vetsuisse Faculty, University of Zurich, Switzerland. We used computer-generated block randomization without stratification, with block sizes of 4 (Vickers 2006). Owners and investigators were blinded to group allocation until enrolment was completed. CONSORT and ARRIVE guidelines were used for preparation of this manuscript.

Subjects: eligibility Criteria

Eligible participants were client-owned cats that presented with various tumours for definitive-intent RT. The cats were enrolled by either the first, second or last author after obtaining written owners' consent and reported under a protocol approved by the Animal Ethics Council of the Canton of Zurich, Switzerland (Permit Number: ZH058/17). All breeds, sexes, weights, and ages were included. A Veterinary Co-operative Oncology Group Performance Status Level grade 0 (normal activity) and American Society of Anesthesiologists status score of I-II was mandatory (Veterinary Cooperative Oncology Group 2016; Portier & Ida 2018). Adequate haematological and serum biochemical values according to the Veterinary Co-operative Oncology Group Common Terminology Criteria for Adverse Events version 1.1 were also mandatory for eligibility (Veterinary Cooperative Oncology Group 2016): haematocrit $\geq$ 25%, haemoglobin > 8 g dL^{-1}, adequate renal function [blood urea nitrogen (BUN) $\leq$ 1.5x, creatinine $\leq$ 1.5x the upper limit of baseline] and hepatic values [alanine aminotransferase (ALT) $\leq$ 1.25x, alkaline phosphatase (AlkP) $\leq$ 2.5x upper limit of normal]. Age, weight, sex, tumour type, radiation protocol, and total radiation dose [recalculated as equivalent dose in 2 Gy fractions (EQD2) to render the biological effect of different fractionation schedules comparable] were documented.

Anaesthetic protocol and blood sampling

Before anaesthesia, food was withheld for at least 8 hours. A 22 gauge catheter (B. Braun Vet Care GmbH, Germany) was placed aseptically into a cephalic vein, the area of insertion was checked daily for redness, tenderness and pain. The catheter was changed every 48−72 hours and removed during the weekend. At 5 minutes prior to anaesthetic induction, all cats were sedated with IV butorphanol 0.2 mg kg^{-1} (butorphanolum 4 mg mL^{-1}, Morphasol-4; Dr. E Graeub AG, Switzerland) and preoxygenated by mask. After 5 minutes, midazolam 0.1 mg kg^{-1} IV (midazolamum 5 mg mL^{-1}; Roche Pharma AG, Switzerland) was administered. General anaesthesia was immediately induced IV with either propofol (propofolum 500 mg 50 mL^{-1}, Propofol 1% MCT Fresenius; Fresenius Kabi AG, Switzerland) ($Group_{Propofol}$) or alfaxalone (alfaxalone 10 mg mL^{-1}, Alfaxan; Jurox, UK) ($Group_{Alfaxalone}$) (first 1 mg kg^{-1} over 15 seconds, then at 1 mg $kg^{-1}minute^{-1}$) to effect and the trachea was intubated. Lidocaine spray (lidocaine hydrochloride, Intubeaze 20 mg mL^{-1}; Dechra, The Netherlands) was used on the cats' larynxes prior to endotracheal intubation.

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After intubation, general anaesthesia was maintained with sevoflurane (Sevorane 250 mL; AbbVie AG, Switzerland) mixed in oxygen and air, with flow rates of 0.4−0.5 L minute^{-1} of each to maintain inspired oxygen concentration at 60 ± 5%. Cats were allowed to breathe spontaneously. If apnoea of > 30 seconds was detected, peripheral oxygen saturation (SpO_2) was < 90% or the expired partial pressure of carbon dioxide ($P_{E'}CO_2$) was > 55 mmHg (7.33 kPa), cats were manually ventilated for 1 minute at 4−6 breaths minute^{-1}. If spontaneous ventilation, an increase in SpO_2 or normocapnia did not return, mechanical ventilation (respiratory frequency at 4−6 breaths minute^{-1}) with a pressure-controlled respirator (Aespire View; Datex-Ohmeda, Inc., WI, USA) using a peak inspiratory pressure 8−10 H_2O was initiated until the end of inhalation anaesthesia or restart of spontaneous ventilation.

Monitoring of anaesthesia (Carescape Monitor B450; GE Healthcare, Finland) included: measurement of heart rate and SpO_2 from a pulse oximeter attached to the tongue; respiratory rate, $P_{E'}CO_2$, end-tidal sevoflurane concentration ($F_{E'}$Sevo). Mean (MAP), systolic and diastolic arterial blood pressures were measured using oscillometry every 2 minutes (HDO; S+B MedVet GmbH, Germany) with an appropriately sized cuff. If MAP decreased to < 60 mmHg (8.0 kPa), a bolus of 3 mL kg^{-1} Ringer's solution (Dr. G. Bichsel AG, Switzerland) was administered IV over 10 minutes, then blood pressure was re-evaluated. Spontaneous breathing or manual or respirator-aided breathing was documented. During anaesthesia and until able to stand unaided, the cats received Ringer's solution at a rate of 3 mL kg^{-1} hour^{-1} IV with an infusion pump (Volumed; Arcomed AG, Switzerland) (Davis et al. 2013; Grubb et al. 2020). Body temperature was monitored before and at the end of RT and every 15 minutes thereafter during recovery. Warming mattresses (different types) were used to keep the cats normothermic.

Pre-induction sedation level was graded 5 minutes after butorphanol administration with a score from 0 to 4 (Steagall et al. 2009). The total volume and concentration of alfaxalone or propofol administered, duration of anaesthesia (from induction until sevoflurane inhalation anaesthesia was stopped) and time from induction of anaesthesia until extubation, lifting of the head and until unaided standing, were recorded.

During the recovery phase, paddling, trembling, opisthotonos, regurgitation/vomiting, sneezing, gaging or any other abnormalities were recorded as supplementary data as previously described (Mathis et al. 2012).

For primary outcome measures, either a blood sample from the freshly placed venous catheter or a jugular venous blood sample was collected from each cat for haematological (complete blood count and blood smear examination) and serum biochemical analyses at four time points: T1 = before induction of first anaesthesia, T6 = before the induction of the sixth anaesthesia, T10 = before the induction of the tenth anaesthesia and T21 = at 3 weeks after the last anaesthetic episode. Prior to each anaesthetic, two blood samples of 0.5 mL each were collected and stored in an ethylenediaminetetraacetic acid (EDTA) and a lithium-heparin tube, respectively. For each blood sample stored in lithium-heparin, measurement of bilirubin, glucose, BUN, creatinine, total protein, albumin, globulin, cholesterol, triglycerides, AlkP, lipase, aspartate aminotransferase, alanine transaminase (ALT), creatine kinase, sodium, potassium, chloride, calcium and phosphate concentrations was performed with a Cobas C501 module (Roche Diagnostics, Switzerland). For each blood sample stored in EDTA, haematocrit, haemoglobin concentration, white blood cell count and reticulocyte count were determined using an automated analyser (Sysmex-XT 2000iV; Sysmex, Japan), which was validated for use in cats (Weissenbacher et al. 2011). Heinz bodies were identified as previously described (Bley et al. 2007) by examination of blood smears stained with supravital new methylene blue. In addition, the total number of Heinz bodies in each smear was classified using a semi-quantitative grading system as: 0, none; 1, few; 2, moderate; 3, many Heinz bodies. The blood smears were assessed by experienced laboratory technicians who were unaware of the group allocation or anaesthetic protocol of each cat. Urinalysis obtained by cystocentesis was performed at two time points: T1 and T21.

Exclusion criteria

Cats were withdrawn from the study if there was a haematological toxicity grade ≥ 2 (decrease in haematocrit to < 20% or a decrease in haemoglobin to < 6.5 g dL^{-1} or both, or elevated serum biochemistry variables) and/or the owners withdrew consent at any time point during the study. In case of exclusion due to haematological toxicity, adequate measures were implemented according to the attending clinician.

Statistical analysis

An *a priori* power analysis was performed with R statistical program (version 3.6.3, R Foundation for Statical Computing, Austria, www.r-project.org) to identify an adequate number of animals. For differences in haematocrit, sample size estimate was based on a conservative (i.e., large) SD of 3% (= population variability, = σ = sigma) from prior findings (Bley et al. 2007). To detect a difference in mean differences of haematocrit of 2% (effect size, δ = delta) at the time point T1 in a two-way analysis of variance with interaction, a two-sided significance level of 0.05 (α) and a power of 1-β = 0.80 with equal allocation to two arms and a dropout rate of 10% would require 18 animals in each arm of the trial, that is, 36 in total.

Data were coded in Excel (Microsoft Excel for Mac 2011, Version 14.3.2, Microsoft Corporation, WA, USA) and analysed with R statistical program (version 4.0.3). Data are presented as mean ± SD or median (interquartile range).

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Shapiro–Wilk testing was performed to assess normality for baseline variables (T1 data). A two-sample *t* test was performed for paired observations of data with normal distribution and a Wilcoxon test was used for variables without normal distribution. A multivariable linear mixed model with time as covariate was performed to compare blood variables between different time points and groups using the R package lme4 (Bates et al. 2015). Other variables such as age, tumour type, sex and weight were included in the analysis. Non-significant variables were removed in a backward selection procedure leaving only significant variables in the final model. Heinz body scores and cumulative induction agent dose were examined using an ordinal mixed model. Individual animals were used as repeated measures, that is, multiple measures of the same blood variables were taken on the same subject over the whole time period. For all analyses, a value of $p < 0.05$ was considered significant.

Results

Animals: numbers and characteristics

A total of 42 cats were prospectively enrolled in the study between July 2017 and April 2020. Of the 42 cats, three were excluded because they failed to fulfil the inclusion criteria on the first day of sampling (haematocrit < 25%, despite previous examination being within the acceptable range). Overall, 39 cats were analysed, 20 in the propofol and 19 in the alfaxalone group (Fig. 1). In the alfaxalone group, two cats were censored (removed from statistical analysis) after time point T6 and excluded from further analysis because they were not intubated after their fifth and sixth anaesthetics, respectively, to minimize further irritation in the radiation field (laryngeal lymphoma). No other cats were excluded during the study.

The baseline demographic characteristics of all cats (signalment, haematological variables) are shown in Table 1. There was no significant difference between the two groups regarding signalment (age, sex, weight, breed) and radiation dose (EQD2) ($p > 0.05$). Significantly more cats were administered concurrent prednisolone in the propofol group ($p = 0.02$). Coincidentally, all seven cats with brain tumours were in the propofol group and were administered prednisolone for their neurologic condition. There was no significant difference between baseline blood variables haematocrit, haemoglobin, reticulocytes, Heinz body score, BUN, creatinine, ALT or AlkP (Table 1). In total, 16 of 39 cats had a haematocrit less than our laboratory's lower reference range of 28% at baseline but still above the 25% as stated in inclusion criteria (six cats in the alfaxalone and 10 cats in the propofol group). Only two cats had a Heinz body score of 0 at baseline, while 24 cats had a score of 1, 10 cats had a score of 2 and three cats had a score of 3. There was no statistically significant difference between groups.

In 35 cats, RT was prescribed for 10 radiation fractions (10 × 4.2 Gy in 26 cats, 10 × 4 Gy in five cats, 10 × 4.4 Gy in four cats) and in four cats for 12 radiation fractions (12 × 4 Gy in three cats, 12 × 3 Gy in one cat). All fractions were executed daily (Monday to Friday). From the 39 cats, 23 (59.0%) were treated for a sinonasal/nasopharyngeal neoplasia ($n = 21$ lymphoma, $n = 1$ sarcoma, $n = 1$ adenocarcinoma), four cats (10.3%) for an injection-site sarcoma, seven (18.0%) cats for a presumed brain tumour ($n = 3$ pituitary macroadenoma, $n = 3$ meningioma, $n = 1$ choroid plexus tumour) and one cat (2.6%) each owing to a thyroid carcinoma, adenocarcinoma of the zygomatic gland, carcinoma of the auditory meatus, lymphoma of the third eyelid, and thymoma.

Mean and median dose of induction agent until successful intubation was 1.95 mg kg^{-1} ± 0.63 and 1.87 mg kg^{-1} (0.78) in $Group_{Alfaxalone}$ and 4.34 mg kg^{-1} ± 1.72 and 3.95 mg kg^{-1} (1.84) in $Group_{Propofol}$, respectively. Mean and median duration of anaesthesia (from induction until cessation of inhalation anaesthesia) was 21.54 minutes ± 6.93 and 21.02 minutes (7.3) in $Group_{Alfaxalone}$ and 19.95 minutes ± 8.20 and 18.0 minutes (9.38) in $Group_{Propofol}$, respectively. Time until extubation, head lift and until unaided standing is described in Table S1, as well as sedation score and total volume of induction agent.

No cat developed anorexia or lethargy leading to interruption of RT. Some cats showed reduced appetite according to the owner or were described to be more tired (sleeping more, less energetic) after the end of treatment. This was attributed to acute radiation toxicity of the nasopharyngeal area owing to irradiation of bilateral mandibular and medial retropharyngeal lymph nodes in cats with sinonasal lymphoma or administration of gabapentin as an analgesic for said radiation toxicity. All cats returned to their normal quality of life 3 weeks after the end of treatment. This was not reported in a standardized fashion and therefore not further evaluated.

Haematological analysis: all cats over time

The overall pairwise differences of haematological and biochemical variables between different time points are shown in Table 2 and Fig. 2.

Blood analysis: comparing the two groups (pairwise) over time

Only the reticulocytes were greater in $Group_{Propofol}$ between T1-T6 (mean 23,090 μl^{-1} ± 7670, $p = 0.02$) and T1-T10 (mean 27,440 μl^{-1} ± 7990, $p = 0.007$). This is shown in Fig. 3. None of the other blood variables (haematocrit, haemoglobin, Heinz bodies, creatinine, BUN, ALT, AlkP) showed a significant difference over time when the groups were compared.

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Cumulative induction agent dose and Heinz bodies

Mean cumulative dose at T10 was 19.7 mg kg^{-1} ± 5.3 for alfaxalone and 43.4 mg kg^{-1} ± 14.4 for propofol. There was no significant effect of cumulative dose on Heinz bodies at any time point ($p > 0.05$). This did not change when additional variables (age, weight, group) were included.

Discussion

Alfaxalone and propofol are frequently used in small animal practice owing to the rapid onset and short duration of action, enabling titration to effect and a rapid recovery with both drugs (Taboada & Murison 2010; Mathis et al. 2012; Sano et al. 2018; Tamura et al. 2021). While propofol is inexpensive, it can induce temporary apnoea (Matthews et al. 2004) and was reported to be associated with Heinz body formation, possibly contributing to anaemia (Andress et al. 1995; Bley et al. 2007). A recent case report suggested a possible cumulative or threshold dose (Baetge et al. 2020). Conversely, alfaxalone is more expensive in most countries but can be stored in the refrigerator up to 1 day, 1 week or 1 month (depending on the manufacturer and country) and causes no

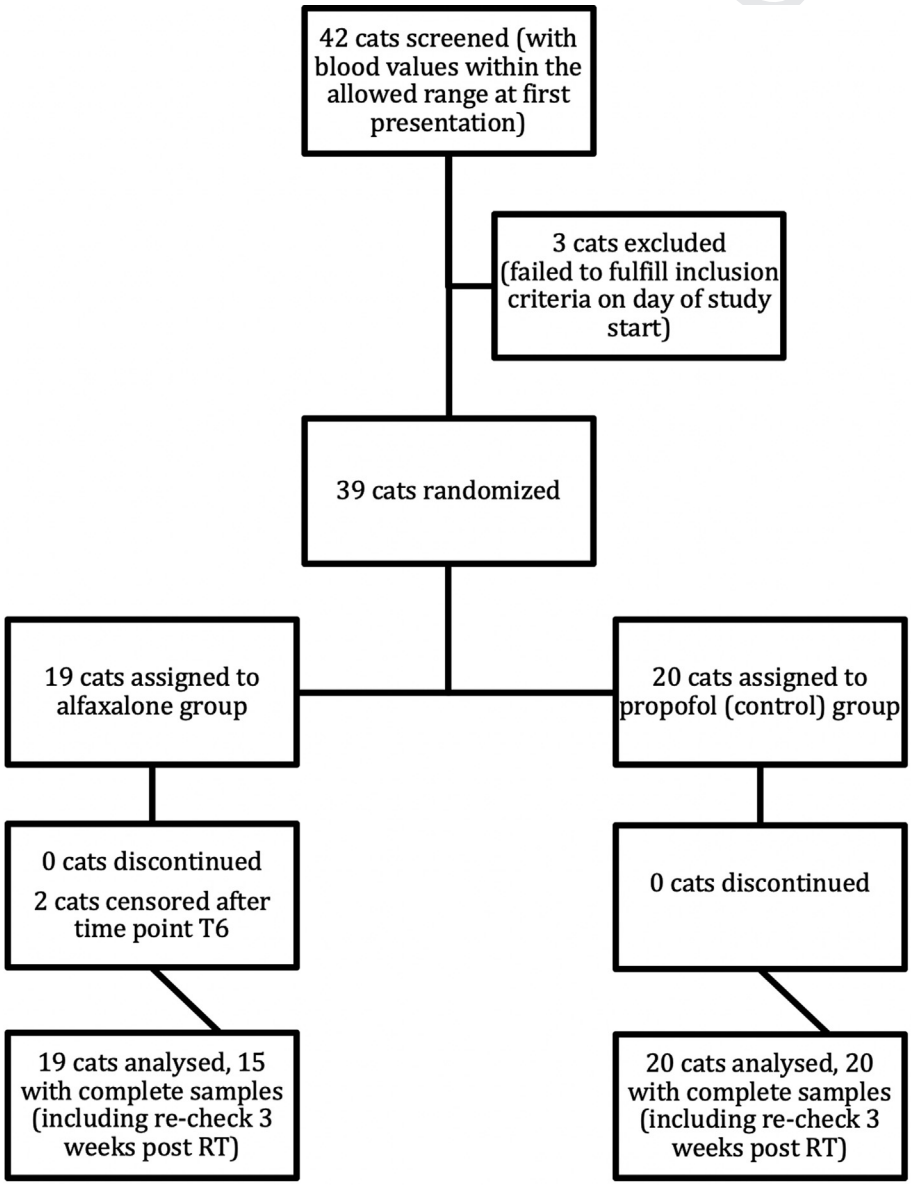

Figure 1 Flow chart of all eligible cats. Overview of cats eligible for inclusion in the study and their distribution between the two different anaesthesia induction agent groups. RT, radiation therapy.

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or only mild ventilatory depression (Whittem et al. 2008; Taboada & Murison 2010; Warne et al. 2015; Tamura et al. 2021). Some studies, however, report increased paddling, trembling and ataxia in recovery (Mathis et al. 2012; Tamura et al. 2021).

While the effects of repetitive propofol administration on haematological variables have been described (Andress et al. 1995; Matthews et al. 2004; Bley et al. 2007), this information is lacking for alfaxalone. Only the pharmacokinetic and haemodynamic effects of alfaxalone after single, prolonged infusion times are reported in cats (Whittem et al. 2008; Muir et al. 2009). Overall, haematocrit and haemoglobin decreased for the duration of RT for both drugs in our cohort. In a previous study, the decrease in haematocrit after 12 anaesthetic episodes was by a mean of 9% for propofol only, and 6.6% for midazolam- and propofol-anaesthetized cats (Bley et al. 2007). The smaller decrease in haematocrit we report could be explained by the use of premedication and consequently the lower individual cumulative doses of propofol. Bley et al. (2007) reported that individual cumulative doses of propofol were 54 mg kg^{-1} when it was combined with midazolam and 76 mg kg^{-1} for the propofol-only group. Also, anaesthesia was maintained with IV bolus administration of propofol, whereas we used sevoflurane after induction.

Table 1 Baseline characteristics of 39 cats that presented for radiation therapy of tumours of different origins using two anaesthesia induction agents (alfaxalone *versus* propofol), in which anaesthesia was maintained with sevoflurane in oxygen and air. Data are presented as mean ± standard deviation (SD) and median [interquartile range (IQR)].

	Total ($n = 39$)	**Group$_{Alfaxalone}$ ($n = 19$)**	**Group$_{Propofol}$ ($n = 20$)**	***p* value**
	Mean ± SD, median (IQR)			
Age (years)	11.25 ± 3.34, 11.71 (5.23)	10.64 ± 3.98, 12.6 (7.10)	11.83 ± 2.56, 11.56 (3.30)	>0.05
Weight (kg)	4.79 ± 1.45, 4.6 (1.25)	5.08 ± 1.87, 4.84 (1.49)	4.84 ± 1.49, 4.5 (1.13)	>0.05
Sex, *n* (%)				>0.05
Female	0	0	0	
Male	0	0	0	
Female spayed	19 (48.7)	10 (52.6)	9 (45.0)	
Male castrated	20 (51.3)	9 (47.4)	11 (55.0)	
Breed (*n*)				>0.05
European Domestic Shorthair	25	9	16	
Other	14	10	4	
Concurrent prednisolone treatment, *n* (%)	28 (71.8)	12 (63.2)	16 (80.0)	0.02*
Radiation dose in EQD2 [Gy]	49.76 ± 2.81, 49.7 (0)	49.43 ± 3.05, 49.7 (0)	50.07 ± 2.61, 49.7 (0.58)	>0.05
Haematocrit (%)	33.92 ± 4.40, 33.0 (6.0)	35.53 ± 4.66, 35.0 (8.0)	32.40 ± 3.63, 32.0 (3.5)	>0.05
Haemoglobin (g dL^{-1})	11.42 ± 1.52, 11.0 (1.8)	11.86 ± 1.84, 11.7 (2.55)	11.0 ± 1.03, 10.85 (1.3)	>0.05
Reticulocytes (μL^{-1})	64,040 ± 54,525, 57,800 (78,300)	71,968 ± 60,039, 58,500 (95,2500)	56,510 ± 49,077, 35,350 (64,975)	>0.05
Heinz bodies, *n* (%)				>0.05
None	2 (5.1)	1 (2.6)	1 (2.6)	
Few	24 (61.5)	12 (30.8)	12 (30.8)	
Intermediate	10 (25.6)	5 (12.8)	5 (12.8)	
Many	3 (7.7)	1 (2.6)	2 (5.1)	
BUN (mmol L^{-1})	8.19 ± 2.01, 8.2 (2.35)	7.7 ± 1.80, 7.4 (1.9)	8.66 ± 2.13, 8.55 (1.63)	>0.05
Creatinine ($\mu mol\ L^{-1}$)	114.5 ± 24.29, 117.0 (24.5)	115.53 ± 24.88, 115.0 (27.5)	113.6 ± 24.32, 117.5 (21.25)	>0.05
ALT (U L^{-1})	42.03 ± 12.18, 40.0 (19.5)	41.79 ± 12.54, 38.0 (18.5)	42.25 ± 12.14, 41.5 (20.0)	>0.05
AP (U L^{-1})	23.95 ± 10.70, 22.0 (15.0)	24.16 ± 11.10, 24.0 (16.5)	23.75 ± 10.58, 21.0 (12.75)	>0.05

ALT, alanine aminotransferase; AP, alkaline phosphatase; BUN, blood urea nitrogen; EQD2, equivalent dose in 2 Gy fractions.
*Significant p value.

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In the present study, both haematocrit and haemoglobin improved by T21. These initial decreases and subsequent increases were paralleled by a decrease in mean reticulocyte count from T1 to T10, albeit with no subsequent increase in reticulocyte count at T21. Comparable to the findings of Bley et al. (2007), the quantity of Heinz bodies increased significantly with both agents during RT. The relationship between propofol or alfaxalone administration and Heinz body formation remains unproven. In contrast to the observation that Heinz body formation may be the result of repeated anaesthetic drug administration, antioxidant properties including protection of erythrocytes against oxidative injury have been attributed to propofol in several species (Murphy et al. 1996; Runzer et al. 2002; Lee & Kim 2012).

A significant decrease in haematocrit, platelets and white blood cell count during RT was described in dogs (Clermont et al. 2012) and termed RT-induced myelosuppression. However, all laboratory values were still within the reference range and the changes were therefore not clinically relevant. RT can cause myelosuppression owing to damage to haematopoietic precursors (Hall & Giaccia 2012a). This may cause mortality at low doses after whole body irradiation in dogs (von Zallinger & Tempel 1998). Myelosuppression may result from inclusion of large areas of bone marrow in the radiation field. However, a dose response relationship was not evaluated (Clermont et al. 2012). We did not document the extent of bone marrow in the treated field, as only a decrease in haematocrit (and not the other cell lines) was seen. However, most cats had head and neck tumours. Furthermore, highly conformal radiation techniques were used, which reduced the radiation dose to neighbouring tissue.

Despite statistical significance, the mean decrease in haematocrit and haemoglobin during the 2 week anaesthetic episodes was rather small with limited clinical relevance in cats with initial normal or near to normal blood values. Furthermore, there were no clinically relevant serum biochemical changes. Hence, according to our findings, both propofol and alfaxalone can be used as induction agents for repetitive short anaesthesia with premedication and subsequent sevoflurane maintenance in cats.

In our study, the Heinz body score increased during the 2 weeks of daily anaesthetics and did not differ significantly between the two groups. Neither could we observe a significant cumulative effect of propofol and Heinz bodies, as others have suggested at a dose between 40 and 60 mg kg^{-1} (Baetge et al. 2020). Only two cats in our study showed a baseline Heinz body score of 0. The incidence of spontaneous Heinz body formation in cats varies between 0 and 96% (Beritic 1965; Christopher 1989; Christopher et al. 1990; Stokol 2017) and is also found in cats with chronic diseases such as diabetes mellitus, hyperthyroidism or—more importantly for our study—in cats with tumours. Cats with lymphoma were specifically mentioned (Christopher 1989; Christopher et al. 1990; Haney et al. 2009) and had a prevalence in our group of 56.4% (22/39). As Heinz body score increased between T1-T10, an influence of anaesthesia or the induction agents seems probable. An earlier study with 37 cats described propofol-induced Heinz body anaemia but found it to be clinically irrelevant (Bley et al. 2007). In contrast, in a small case series, malaise, anorexia or diarrhoea were attributed to Heinz body anaemia on day 5–7 in five/six cats. Signs that disappeared 24–48 hours after propofol administration ceased (Andress et al. 1995). No anorexia or lethargy leading to interruption of radiotherapy were documented in our study and all cats returned to their normal quality of life by T21.

A timeline for resolution of Heinz body anaemia was not reported by Andress et al. (1995) or Bley et al. (2007), while we observed a small, albeit non-significant, decrease between T10 and T21. Anaemia has been associated with poorer outcomes in human patients undergoing RT (Hall & Giaccia

Table 2 Haematological analysis over time of 39 cats that presented for radiation therapy during general anaesthesia maintained with sevoflurane in oxygen and air. Overall pairwise differences between time points (T1, time point day 1 = before induction of first anaesthesia; T6, time point day 6 = before the induction of the sixth anaesthesia; T10, time point day 10 = before the induction of the tenth anaesthesia; T21, time point day 21 = at 3 weeks after the last anaesthetic episode) are shown as mean ± standard deviation. There was no difference over time in creatinine and alanine aminotransferase.

	Decrease	Increase	*p* value
Haematocrit (%)			
T1-T6	2.3 ± 0.77		0.02*
T1-T10	4.1 ± 0.78		<0.0001*
T6-T21		2.1 ± 0.80	0.046*
T10-T21		4.0 ± 0.8	<0.001*
Haemoglobin (g dL^{-1})			
T1-T6	0.7 ± 0.23		0.03*
T1-T10	1.3 ± 0.24		< 0.0001*
T6-T21		0.7 ± 0.24	0.014*
T10-T21		1.3 ± 0.25	< 0.0001*
Reticulocytes (μL^{-1})			
T1-T10	16,908 ± 5390		0.012*
Heinz bodies (*n*, %)			
T1-T6		1.36 ± 0.60	0.101
T1-T10		1.86 ± 0.62	0.013*
T1-T21		0.48 ± 0.59	0.85
T10-T21	1.39 ± 0.61		0.103
BUN (mmol L^{-1})			
T1-T10	1.29 ± 0.29		0.0001*
T1-T21		1.04 ± 0.29	0.0027*
T6-T21		1.68 ± 0.29	0.0001*
T10-T21		2.32 ± 0.29	0.0001*
AlkP (U L^{-1})			
T10-T21		5.08 ± 1.44	0.0035*

AlkP, alkaline phosphatase; BUN, blood urea nitrogen.
*p value significant.

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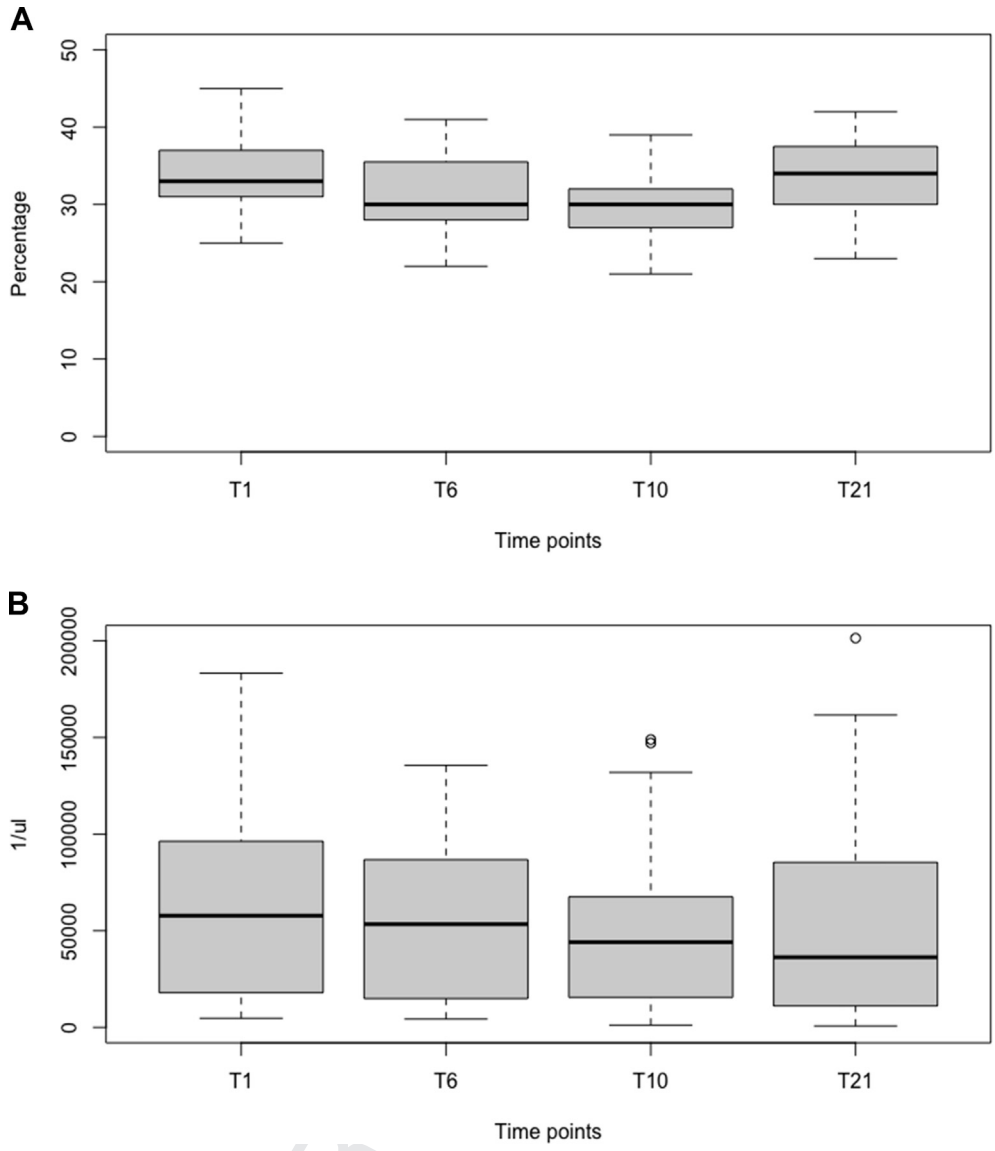

Figure 2 Blood analysis of all cats over time. (a) Box and whisker plots of haematocrit of all cats over time. The y-axis shows the haematocrit (%), the x-axis the different time points, the boxplots show the mean as thick black line and the 75 percentiles as grey box. There was a significant decrease in haematocrit by a mean of 2.3% ± 0.77 ($p = 0.02$) between time points T1 (before induction of first anaesthesia) to T6 (before the induction of the sixth anaesthesia) and by a mean of 4.1% ± 0.78 ($p < 0.0001$) between T1 to T10 (before the induction of the tenth anaesthesia). There was a significant increase in haematocrit by a mean of 2.1% ± 0.80 ($p = 0.046$) between T6 to T21 (at 3 weeks after the last anaesthetic episode) and by a mean of 4.0% ± 0.8 ($p < 0.001$) between T10 to T21. The range is represented by the dashed lines. (b) Box and whisker plot of reticulocyte count of all cats over time. The y-axis shows the reticulocytes (μL^{-1}), the x-axis the different time points, the boxplots show the mean as thick black line and the 75 percentiles as grey box and the empty circles represent outliers. The reticulocyte count of all cats showed a significant decrease by a mean of 16,908 μL^{-1} ± 5390 ($p = 0.012$) between T1 (before induction of first anaesthesia) and T10 (before the induction of the tenth anaesthesia), but no further significant changes.

2012b). In cats irradiated postoperatively for injection-site sarcomas, anaemia may worsen the prognosis and delay wound healing (Mayer et al. 2009; Wright et al. 2014).

Some limitations in our study design could direct future research. 1) Clinical signs associated with Heinz body anaemia such as malaise were not consistently documented and therefore not evaluated. 2) A decrease in haematocrit owing to daily IV fluid therapy with Ringer's solution seems improbable but cannot be excluded. IV fluid therapy was only administered during general anaesthesia and until the cats were able to stand, that is, for a mean duration of 47.43 minutes (Table S1) per day. Furthermore, the recommended infusion

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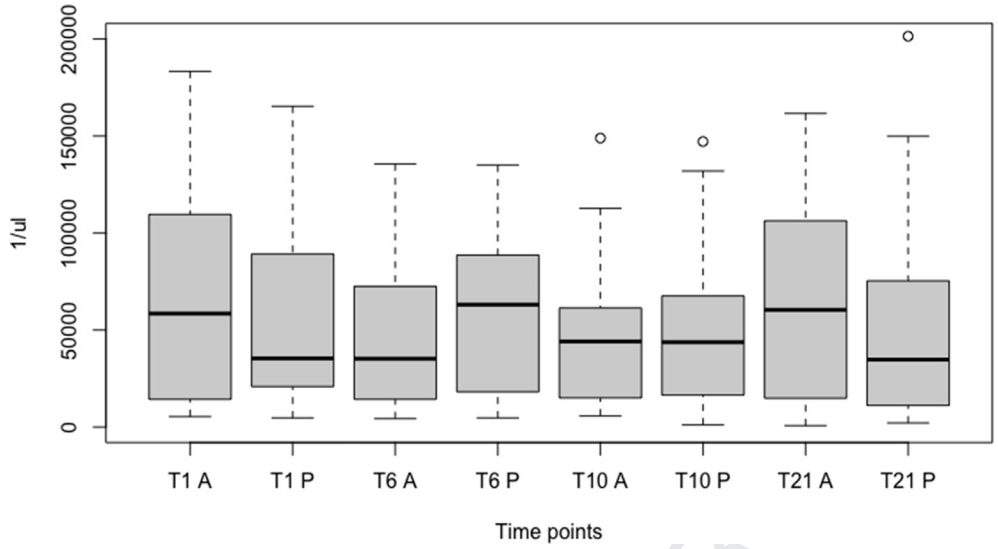

Figure 3 Box and whisker plot of reticulocytes count of the two groups over time. The y-axis shows the reticulocytes (μL^{-1}), and the x-axis the two groups ($Group_{Propofol}$ and $Group_{Alfaxalone}$) at different time points. Differences in blood variables between groups over time showed a significantly higher reticulocyte count in the propofol but not in the alfaxalone group between time points T1 and T6 [mean 23,090 μL^{-1} ± 7670 ($p = 0.02$)] and between T1 and T10 [mean 27,440 μL^{-1} ± 7990 ($p = 0.007$)].

rate of 3 mL kg^{-1} $hour^{-1}$ during anaesthesia is close to the recommended maintenance fluid rate for conscious cats (2−3 mL kg^{-1} $hour^{-1}$) (Davis et al. 2013; Grubb et al. 2020). Possible dilution due to fluid therapy with decreases in haemoglobin concentration and haematocrit values were reported in human patients (Lahsaee et al. 2013; Gokcen et al. 2018). According to our knowledge, there is no information regarding the impact of fluid therapy on haematocrit and haemoglobin in cats. 3) A possible decrease in haematocrit resulting from repeated sevoflurane anaesthesia cannot be excluded. Lower haemoglobin concentrations and haematocrit values were seen in female rats shortly after exposure to isoflurane (Deckardt et al. 2007) as in human patients during sevoflurane and desflurane anaesthesia (Gul et al. 2015). However, further studies to evaluate the impact of repeated inhalation anaesthesia on haematological variables are needed. 4) We performed a semi-quantitative analysis for determination of Heinz bodies. A quantitative analysis could be more accurate. 5) All cats in our cohort were diagnosed with neoplasia, which can lead to anaemia associated with elevated Heinz body scores. Cats with lymphoma were prevalent in our cohort and they have increased Heinz body scores (Christopher 1989). Cats with cancer and concurrent medical treatment (prednisolone), however, represent a more realistic clinical picture than purpose-bred, young, healthy laboratory animals with normal blood values.

Conclusion

While haematological variables changed due to 10−12 daily anaesthetics, neither repetitive administration of alfaxalone nor the control drug led to any significant abnormalities over time and no major complications occurred during recovery. This suggests that alfaxalone can be used for induction of repetitive, short anaesthesia in cats with different tumours.

Open access

All data collected for this study is deposited in the open repository Harvard Dataverse (https://doi.org/10.7910/DVN/TSF4TG).

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Authors' contributions

MK: study design, funding, data collection, statistical analysis, data interpretation, manuscript preparation. CRB and RB: study design, data interpretation, manuscript preparation. FW and BR: data collection, manuscript preparation. VM: study design, data collection, statistical analysis, data interpretation,

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Conflict of interest statement

The authors declare no conflict of interest.

Supporting Information.

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Q4 Table S1. Sedation score, amount of induction agent, duration of anaesthesia and post-anaesthesia reactions of 39 cats that presented for treatment of tumours of different origins using two anaesthesia induction agents (alfaxalone *versus* propofol).

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