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Article type : Original Article

A controlled randomised clinical trial to assess post-operative analgesia after thiopental-isoflurane anaesthesia or total intravenous anaesthesia with alfaxalone in dogs.

Running Title: No analgesia from alfaxalone TIVA in dogs

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ABSTRACT

Alfaxalone, a synthetic neuroactive steroid, has been attributed with properties including sedation, anaesthesia and analgesia. The clinical relevance of any analgesic properties of alfaxalone has not been demonstrated. This study was a prospective, blinded, randomised, negative control clinical trial in 65 healthy dogs presented for ovariohysterectomy. Anaesthesia was induced and maintained; for Group 1 (TIVA) dogs (n=30) with intravenous alfaxalone alone and for Group 2 dogs (n=35) with thiopental followed by isoflurane in 100% oxygen inhalation. After ovariohysterectomy, quantitative

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jvp.12740

measures of pain or nociception were recorded at 15 min intervals for 4 h using three independent scoring systems; a composite measure pain scale (CMPS), von Frey threshold testing and measures of fentanyl rescue analgesia. The mean CMPS scores of Group 2 (THIO/ISO) dogs remained higher than Group 1 (TIVA) dogs from 15 to 135 min post-surgery but this difference was not statistically significant. There were no significant differences between groups in the proportions of dogs requiring rescue fentanyl analgesia, the total fentanyl dose used or the time to first fentanyl dose. Frey threshold testing was found to be unsuitable for measurement of pain in this experimental model. When administered as total intravenous anaesthesia, alfaxalone did not provide analgesia in the post-operative period.

Key Words: alfaxalone, anaesthesia, analgesia, dog, thiopental, TIVA

INTRODUCTION

Alfaxalone is a progesterone derivative which acts as a synthetic neuroactive steroid. Neuroactive steroids influence a variety of behavioural and neuroendocrine processes (Lambert, Belelli et al., 1995; Belelli & Lambert, 2005; Dubrovsky, 2005). Demonstrated activities of neurosteroids include sedation, general anaesthesia and analgesia. The analgesic properties of neurosteroids have been attributed to a positive allosteric potentiation of ligand-gated gamma-amino butyric acid type A ($GABA_A$) receptors and to inhibition of voltage-gated T-type Ca2+ channels (Goodchild, Guo et al., 2000; Todorovic, Pathirathna et al., 2004; Pathirathna, Todorovic et al., 2005). Modifications to structure alter activity, so not all neurosteroids exhibit all activities (Maitra & Reynolds, 1998; Visser, Gladdines et al., 2002). Alfaxalone has been demonstrated to alleviate thermal and mechanical hyperalgesia in rats (Gilron & Coderre, 1996; Pathirathna, Todorovic et al., 2005). However, some experimental studies with alfaxalone in laboratory animals, in cats and in dogs have failed to demonstrate analgesic properties (Nadeson & Goodchild, 2000; Murison & Taboada, 2010; Bennett, Salla et al., 2017). Whether alfaxalone provides analgesia for dogs in clinical settings has not been evaluated.

Alfaxalone is formulated in 2-hydroxypropyl beta cyclodextrin for use as a veterinary anaesthetic for cats and dogs (Alfaxan®, Jurox Pty. Ltd, Rutherford, NSW, Australia). The formulation is labelled for the induction and maintenance of general anaesthesia by intravenous bolus injection and intravenous infusion. The properties and use of this formulation in the dog were initially described elsewhere (Ferre, Pasloske et al., 2006; Muir, Lerche et al., 2008; Pasloske, Sauer et al., 2009).

The barbiturate intravenous anaesthetic thiopental is not thought to have any analgesic effects (Ilkiw, 2002; Ilkiw, 2002). Isoflurane is a volatile anaesthetic agent used for maintenance of general anaesthesia in dogs, similarly without analgesic effects (Cheng, Yeh et al., 2008).

The routine veterinary surgical procedure of ovariohysterectomy in dogs is an example of painful surgery. Administration of an analgesic agent or agents prior to the surgical insult is thought to pre-emptively improve analgesic outcomes. Effective pre-emptive analgesia is thought to require both the establishment of an effective degree of analgesia before injury and the continuation of this analgesia into the post-injury period (Ong, Lirk et al., 2005).

Although multimodal analgesia is recommended, it is also known that with an increasing number of agents that are co-administered, the probability of adverse drug reactions increases (Fattinger, Roos et al., 2000). Therefore, it would be ideal if the anaesthetic agent used for total intravenous anaesthesia (TIVA) also contributed to analgesia.

Several methods have been developed to quantify the amount of pain or nociception perceived by dogs. Ordinal scales such as the Colorado State University (Hellyer & Gaynor, 1998) and University of Melbourne (Firth & Haldane, 1999) pain scales are based on both behavioural and physiological characteristics. These ordinal scales can be difficult to use experimentally because the effect-distance between ordinal steps is either not known or not equal, precluding reliable interpretation of statistical evaluation. A modification of the Glasgow Pain Scale (Holton, Reid et al., 2001) created an interval level composite measure pain scale (CMPS) where the intervals are thought to be of similar size. Differences observed between the CMPS scores of orthopaedic and of soft tissue surgery, medical and control groups enabled the CMPS to discriminate between levels of pain among treatment groups (Morton, Reid et al., 2005).

In order to quantify mechanical sensitivity, von Frey developed a testing system based on a series of calibrated filaments that bend with the application of a known force (von Frey, 1922). In dogs, von Frey filaments have been used to assess mechanical nociceptive thresholds adjacent to a surgical incision on the common digital pad (Duque, Valadao et al., 2004).

Interventions in the post-operative period can be used to achieve desired levels of analgesia. Such "rescue" analgesia not only fulfils clinical and ethical obligations but offers a quantitative measure of pain. The proportion of subjects that require rescue analgesia, total analgesic consumption and time to first analgesic administration have each been used to compare the analgesic effects of earlier interventions (Lloyd, Derry et al., 2009; Morgaz, Navarrete et al., 2013). The opioid agonist fentanyl has a rapid onset of action and a short duration of action and therefore is a suitable agent for rescue

analgesia where quantification of the analgesic consumption is desired (Sano, Nishimura et al., 2006; Lamont & Mathews, 2007).

This study aimed to test the hypothesis that the neuroactive steroid alfaxalone when administered to dogs in a TIVA protocol would provide analgesia in the post-surgical period. A secondary aim was to compare three quantitative pain measurement techniques for the clinical assessment of post-operative pain in dogs.

MATERIALS AND METHODS

This study was conducted with the approval of the University of Melbourne Animal Ethics Committee (Approval Number 16148) and the care and use of the animals conformed to national guidelines. The study conformed to the principals of Good Clinical Practice (Anonymous, 2001).

Experimental design

This study enrolled 65 healthy assorted breed dogs aged from 6 months to 8 years, in a prospective, negative controlled, randomised, investigator-masked clinical trial. The study was conducted between January and June 2007 in Melbourne, Australia. Dogs were eligible for enrolment if they were presented at an animal welfare shelter for ovariohysterectomy and were deemed suitable for the surgery by the attending veterinarian. Further inclusion criteria included; age (6 months to 8 years), weight (4 to 25 kg), American Society of Anaesthesiologists (ASA) category I status (American Society of Anesthesiologists 1963). The ASA category scoring was based both on history and clinical examination. Greyhounds and other sight-hounds were excluded because of the prolonged action of thiopental in these breeds (Sams, Muir et al., 1985). The surgery duration was timed and inclusion was restricted to surgeries which were between 10 and 30 minutes in duration.

Dogs were randomly allocated without restriction to an anaesthetic protocol group using tables generated by one of the authors (ET) with the random function of Microsoft® Excel (Microsoft Corp., Redmond WA). The investigator author (PMB) was blinded to both treatment groups and sequence and was not present during induction of anaesthesia or performance of the surgery. After completion of the surgical procedure the animal was disconnected from any anaesthetic delivery and monitoring devices and moved to a separate recovery room, where the investigator was located, for post-operative monitoring.

Thirty to sixty minutes prior to the induction of anaesthesia the study dogs were pre-medicated subcutaneously with 0.2 mg/kg of acepromazine (ACP 2; Delvet Pty Ltd, Seven Hills, Australia). This

dose was chosen because no other pre-medication was used. In addition, this dose is within the approved labelled dose for acepromazine in the dog. Furthermore, acepromazine's effect on cardiovascular parameters in healthy dogs at this dose have been demonstrated as minimal and also to lack a dose response between 0.05 mg/kg and 1.1 mg/kg (Coulter, Whelan et al., 1981; Whittem, Pasloske et al., 2006). Immediately prior to induction two peripheral veins of each subject were catheterised.

Group 1 dogs (n=30) were anaesthetised with 2 mg/kg alfaxalone intravenously over 60 sec (Alfaxan®; Jurox Pty Ltd, Rutherford, Australia) and maintained with a constant rate infusion of alfaxalone diluted with normal saline (Group 1, TIVA). The dilution rate was such that the administration of fluids approximated 2.0 mL/kg/h. The initial 12 cases received a TIVA rate of 7 mg alfaxalone/kg/h. This rate was increased to 11 mg/kg/h for subsequent dogs to maintain a deeper level of anaesthesia.

Groups 2 dogs (n=35) were anaesthetised with 5% thiopental at a calculated dose rate of 12.5 mg/kg administered by intravenous boluses, to effect (VR Thiobarb Powder; Jurox Pty Ltd, Rutherford, Australia) and anaesthesia was maintained with a vaporiser setting of ~2% isoflurane in oxygen (I.S.O.; Veterinary Companies of Australia Pty Ltd, Artarmon, Australia) (Group 2, Thio/ISO). These dogs also received a normal saline infusion at 2.0 mL/kg/h to simulate the fluid administration of the Group 1 (TIVA) dogs.

Ovariohysterectomy was performed by experienced veterinarians using a standard midline open laparotomy approach. Routine anaesthetic monitoring parameters were recorded at 5 min intervals intra-operatively.

Assessment of pain

Pain was assessed pre-operatively for baseline determination and then following ovariohysterectomy. Endotracheal extubation was initiated at first coughing or gagging response, then the quantitative measures of pain were recorded at 15 min intervals for 4 h; CMPS, mechanical sensitivity testing and titrated fentanyl rescue analgesia.

The CMPS assessment of acute pain in dogs used six of the seven behavioural categories; posture, comfort, vocalisation, attention to wound, demeanour, and response to touch to give a total CMPS score. Assessment of mobility was excluded from the CMPS in this study because of the differing effects of the anaesthetic agents. Total CMPS scores for each time point were obtained by summation of the values assigned to each of the six behavioural categories (Morton, Reid et al.,

2005). The maximum possible CMPS score was 11.53. A cut-off score of 2.3 out of 11.53 was chosen by consensus in consultation with the Animal Welfare Officer, as the intervention point for rescue analgesia.

Von Frey threshold testing was conducted using a series of 13 Touchtest™ Sensory Evaluators (Stoelting Co., Wood Dale, Illinois, USA) with logarithmically incremental stiffness ranging from 0.6 to 180 grams. Von Frey hairs of increasing force were sequentially applied to the testing sites: carpal pad; hind paw; wound-perpendicular; wound-stroking. If a response was obtained to the smallest filament the threshold was assigned a value of 3.61 and if no response was observed to any filament a threshold value of 6.45 was assigned. Von Frey thresholds within the testing range were determined using two different methods. In the first method, the logarithmic value of the filament one increment smaller than the first filament to which the dog responded was assigned as the threshold. A second threshold value was computed using the up-down method of Dixon as previously described (Chaplan, Bach et al., 1994).

Rescue analgesia was available to all dogs. Fentanyl (Fentanyl citrate; AstraZeneca Pty Ltd, North Ryde, Australia) at 1 μg/kg was administered as an intravenous bolus if the CMPS score was greater than 2.3 at any time in the post-operative monitoring period (Lamont & Mathews, 2007). For three dogs the investigator deemed the level of analgesia achieved by repeat boluses of 1 ug/kg bolus of fentanyl to be inadequate on ethical grounds and for the remainder of the monitoring period for these cases the fentanyl bolus dose was increased to 2 ug/kg. The pain score remained above the cut-off value for rescue analgesia despite using intravenous fentanyl bolus dosing for two dogs. Postoperative monitoring was terminated early for these cases and upon exit from the study the dogs were administered meloxicam subcutaneously at 0.2 mg/kg (Metacam Anti-inflammatory Injectable for Dogs and Cats, Boehringer Ingelheim Animal Health Australia Pty. Ltd.). The time from extubation to first dose of rescue analgesia was regarded as the primary outcome measure.

Other monitoring and secondary outcomes

Baseline rectal temperature was measured pre-surgery and mean rectal temperature at base line was the same for both groups (38.4 C). Rectal temperature was also recorded during recovery. Standard anaesthetic monitoring included heart and respiratory rates and oxygen saturation (SpO2), and reflexes responses.

Onset of anaesthesia was defined as the time of endotracheal intubation, and termination of anaesthesia was defined as the time of endotracheal extubation.

Sedation scoring used the ordinal assessment scale evaluating posture and alertness to give a numerical score from 0 (fully alert) to 5 (very sedate) as previously described (Hardie, Hansen et al., 1997). All dogs were assessed for sedation for four hours post extubation, at which time they received meloxicam subcutaneously at 0.2 mg/kg and were returned to the care of the shelter using their standard operating procedures.

Statistics

An initial sample size was estimated at 55 dogs per group, calculated to allow detection of a between-group effect difference of 1 based on known variance in the CMPS (Morton, Reid et al., 2005), with 80% power and alpha error of 0.05.

Statistical analyses were performed using GraphPad Prism Version 5.00 for Windows (GraphPad Software Inc., San Diego, California, USA) unless indicated otherwise. Results are presented as mean ± standard error; two-tailed P values ≤ 0.05 were regarded as statistically significant.

Visual appraisal of 'box and whisker' and 'scatter dot' plots was used to assess the distribution of data. Student's t tests were used for data of Gaussian distribution and Mann-Whitney tests were used for data not of Gaussian distribution. The P values were calculated using Student's t tests unless otherwise indicated. Fisher's exact test was used to compare proportions for the two treatment groups.

The CMPS scores in the two treatment groups were analysed using repeated measures analysis of variance. Data were assessed for a treatment effect, a time effect and a treatment by time interaction. If a time effect was observed, Dunnett's multiple comparison test was used to compare each post-operative time point with baseline.

Paired data were used to evaluate the response of dogs to fentanyl. Paired Student's t tests were used for CMPS scores. Pre-rescue CMPS scores were compared to scores five minutes after rescue. The exact McNemar's significance probability was used to compare two paired proportions.

Survival curves for the time to first rescue were compared using the Log-rank test and Cox regression using Stata 9.2 for Windows. The Cox proportional hazards regression model was used to estimate a hazard ratio (Cox, 1972). A hazard ratio of one indicates no effect of treatment.

RESULTS

Sample Population

None of the 65 dogs that were enrolled were excluded from the study prior to acquisition of the primary outcome variable. Enrolled dogs were predominantly mixed-breed dogs with the large variation in breeds reflecting the demographics of the dog population in the greater Melbourne area (Table 1). All of the dogs enrolled satisfied ASA Class I criteria based on history and clinical examination and no significant differences were detected between groups for baseline heart rate, respiratory rate, rectal temperature bodyweight or age. The population variables are summarised according to treatment group in Table 2.

Intraoperative monitoring

The thiopental plus isoflurane (Thio/ISO) and TIVA anaesthetic protocols were well tolerated by the majority of dogs. Heart and respiratory rates and SpO2 were acceptable throughout the anaesthetic for all dogs in both groups. After the first 12 TIVA cases had completed the study, the initial TIVA protocol was revised because a high proportion of these dogs required additional boluses to maintain a surgical plane of anaesthesia. The revised TIVA protocol resulted in a more stable plane of anaesthesia.

The number of intra-operative observations per dog varied according to surgery and anaesthetic duration. Recordings made while the dogs were anaesthetised were averaged to give one intra-operative measure per dog. The mean anaesthetised respiratory rate in Group 1 (TIVA) dogs was not significantly different between dosing subgroups (Subgroup-1 = 16 ± 6.0 brpm versus Subgroup-2 = 16 ± 9.7 brpm, $p=0.93$). The mean intra-operative heart rate however, was significantly higher for Group 1 (TIVA) dogs which received the higher dose (Subgroup-1 = 115 ± 14.9 bpm versus Subgroup-2 = 134 ± 17.6 bpm, $p=0.007$). The heart rates (Group 1 = 117 ± 15.8 bpm versus Group 2 = 125 ± 19.1 bpm, $p=0.08$) and respiratory rates (Group 1 = 15 ± 6.5 brpm versus Group 2 = 16 ± 7.9 brpm, $p=0.85$) did not differ statistically between the Groups 1 and 2.

Apnoea was subjectively assessed by study nurses. There were no significant differences in the proportions of dogs experiencing apnoea when comparing sub-TIVA protocols or when comparing treatment groups. There were no reports for either group of adverse effects.

For rectal temperature, a two way analysis of variance showed that there was no effect of treatment group ($p=0.68$), but there was a time effect ($p<0.0001$) and a treatment group by time interaction ($p<0.0001$). Dunnett's multiple comparison tests showed that mean rectal temperature from extubation to 150 minutes were significantly different from baseline ($p<0.05$). All other time points

were not significantly different from baseline, suggesting that rectal temperature had returned to normal 150 minutes after extubation.

von Frey filament scores

At baseline many of the dogs did not respond to any von Frey filament presented, at any of the four testing sites (carpal pad, hind paw, wound-perpendicular, wound-stroking). When a response was observed there was high variability in terms of the filament within the series which was responsible for the response. A few dogs were responsive to the first filament in the series and these were designated as having a threshold below testing range. If a response was not received for any filament in the series the threshold was classed as above testing range. At all of the testing sites except the hind paw, the majority of dogs had baseline thresholds above testing range.

The carpal pad and wound-stroking were the sites least responsive to the filaments. Fifty seven of 65 dogs (88%) had baseline carpal pad von Frey thresholds which were above the testing range and post-operatively no carpal pad thresholds were able to be quantified. Similarly, 58 of 65 dogs (89 %) did not respond to stroking of the proposed incision site pre-operatively and this proportion increased post-operatively. The low frequency of responses at these two sites precluded them from any further analysis.

The hind paw was the most sensitive site at baseline with 52 of 65 dogs (80 %) responsive. The wound perpendicular was the next sensitive with 15 of 65 dogs (23 %) responding at baseline. The responsiveness at both the hind paw and wound-perpendicular had decreased postoperatively with the lowest proportion of responsive dogs for both sites occurring at 30 min. The reduced responsiveness at the hind paw lasted for the duration of monitoring whereas the proportion of dogs responding at the wound-perpendicular had returned to baseline level at 120 min. At 240 min 18 of 65 dogs (28 %) were responsive to wound-perpendicular testing, a higher percentage than at any other time.

An inverse relationship between sedation and von Frey filaments responsiveness was suggested by increasing proportions of responsive dogs with increasing time from extubation.

CMPS Pain Scores

The majority of dogs had baseline CMPS scores of 0.08 or 0.87 and the maximum pre-operative CMPS score was 2.25. Temperament was attributed as a principal determinant of CMPS score at baseline.

The mean CMPS scores are presented in Figure 1. Post-operative pain scores were higher than baseline values and tended to increase with time. At extubation the mean CMPS score for Groups 1 and 2 dogs was similar but by 15 minutes the mean pain score of Group 2 (THIO/ISO) dogs had exceeded that of Group 1 (TIVA) dogs. The mean CMPS scores of Group 2 (THIO/ISO) dogs remained higher than the Group 1 (TIVA) dogs for 135 min. From 135 to 240 min there was increased variation within groups and no obvious trend between groups. A two-way repeated-measures analysis of variance on CMPS scores showed there was no significant effect of treatment ($p=0.84$) nor a treatment by time interaction ($p=0.90$), although the effect of time was significant ($p<0.0001$). Dunnett's multiple comparison test showed that every time point post-operatively was significantly different from baseline ($p<0.05$).

The maximum CMPS score achieved by individual dogs was potentially reduced by rescue fentanyl administration however this was still considered a worthwhile comparison between treatment groups.

Maximum post-operative CMPS scores were compared for dogs in the two sub-TIVA groups. The mean maximum post-operative CMPS score recorded for sub-TIVA1 was 2.60 ± 0.24 and the mean maximum post-operative CMPS score for sub-TIVA2 was 2.54 ± 0.16. These scores were not significantly different ($p=0.81$) and the sub-groups were combined for further comparisons with the Group 2 (THIO/ISO) dogs.

The maximum post-operative CMPS score recorded for a Group 2 (THIO/ISO) dog was 5.55, while the maximum post-operative CMPS score reached by a Group 1 (TIVA) dog was 4.29. The maximum post-operative CMPS scores for individual dogs in each group are shown in Figure 2. The Group 2 (THIO/ISO) dogs had a mean of 2.49 ± 0.18 ($n=35$) and the mean for Group 1 (TIVA) dogs was 2.58 ± 0.12 ($n=30$). There was no significant difference in the mean maximum postoperative CMPS scores of the two treatment groups ($p=0.67$).

The mean of CMPS for shivering dogs (1.69 ± 0.072) was significantly different from non-shivering dogs (1.26 ± 0.068) ($p< 0.001$). There was no significant difference between post-operative body temperatures by group.

Rescue analgesia

Intravenous bolus dosing with fentanyl provided rapid and effective analgesia which was successfully titrated to effect.

Five of 11 (45 %) sub-TIVA1 dogs required rescue analgesia and 9 of 16 (56 %) sub-TIVA2 dogs required rescue analgesia. There was no evidence of a significant difference in the proportions of dogs requiring rescue treatment for the two sub-TIVA protocols (p=0.70). The mean total rescue dose for sub-TIVA1 dogs was 1.0 ± 0.6 ug/kg (n=10) while sub-TIVA2 dogs received a mean total dose of 2.1 ± 0.6 ug/kg (n=16). The mean total rescue doses were not significantly different (p=0.23).

There were no significant differences in the survival curves of time to first rescue for sub-TIVA1 and sub-TIVA2 dogs (Log-rank test, P=0.64). The hazard ratio derived from the Cox proportional hazards model was 1.3 (95% CI 0.4 to 3.9, P=0.64) when comparing sub-TIVA2 with sub-TIVA1. There was no evidence that the two survival curves were not proportional to each other (Test for proportional hazards, P=0.42). The time to first rescue for sub-TIVA1 dogs was 91 ± 45 minutes (n=5) and the time to first rescue for sub-TIVA2 dogs was 93 ± 17 minutes (n=9). For rescued sub-TIVA dogs there was no significant difference between protocols in the time to first rescue (p=0.45). Statistical analyses showed no significant difference in the level of post-operative pain experienced by sub-TIVA1 and sub-TIVA2 dogs, so these groups were pooled for comparisons between the two primary treatment groups.

Thirty one of 65 dogs (48 %) included in the study required rescue analgesia: 15 of 35 (43 %) Group 2 (THIO/ISO) dogs and 16 of 30 (53 %) Group 1 (TIVA) dogs required rescue analgesia. These proportions were not significantly different (p = 0.46). The largest total dose of rescue analgesic given to any Group 2 (THIO/ISO) dog was 21 ug/kg compared to 8 ug/kg for any Group 1 (TIVA) dog. Five dogs in Group 2 (THIO/ISO) required more rescue than the maximum Group 1 (TIVA) dog. The mean total dose of rescue administered to Group 2 (THIO/ISO) dogs was 3.0 ± 1.0 ug/kg (n=34) and for Group 1 (TIVA) dogs was 1.7 ± 0.4 μg/kg (n=29). There was no significant difference between groups in the total amount of rescue administered (p=0.92). When only considering the dogs which required rescue however, there was a significant difference in the total dose of rescue administered between groups (p=0.02): the mean total dose for rescued Group 2 (THIO/ISO) dogs was 7.7 ± 1.7 μg/kg (n=15) and for rescued Group 1 (TIVA) dogs was 3.3 ± 0.6 μg/kg (n=16).

There did not appear to be any systematic pattern to the time to first rescue administration with some dogs requiring analgesia immediately after extubation and some not requiring analgesia at all during the four hours of post-operative monitoring. There was no obvious time when the risk of requiring rescue was especially high for either of the groups, although from 30 to 120 min Group 2 (THIO/ISO) dogs were at slightly more risk than Group 1 (TIVA) dogs. There were no significant differences in the survival curves of time to first rescue of the groups (Log-rank test, P=0.54) (see Figure 3). The hazard ratio derived from the Cox proportional hazards model was 1.2 (95% CI 0.6 to

2.5, P=0.54) when comparing groups. There was no evidence that the two survival curves were not proportional to each other (Test for proportional hazards, P=0.22). Given that a dog needed rescue, the time to first rescue for Group 2 (THIO/ISO) dogs was 82 ± 17 min and for Group 1 (TIVA) dogs was 107 ± 17 min. For dogs given rescue, there was no significant difference detectable between groups in the time to first rescue (p=0.30).

In summary, the dogs anaesthetised with thiopental plus isoflurane versus those anaesthetised with alfaxalone TIVA experienced post-operative pain which was not statistically different in degree.

General

The relationship between sedation score and CMPS was examined: both the mean CMPS and the individual within-dog CMPS changed by -0.003 for every one unit increase in sedation score. However, these were not statistically significant associations (p = 0.89 and p = 0.86, respectively).

Shivering was found to strongly correlate with pain scores. Heart rate and respiratory rate were also statistically correlated with pain scores, but these two associations were not perceived to be clinically important because of the small dimension of the changes, i.e. insensitivity.

Alfaxalone using TIVA was effective as an anaesthetic; characterised by a stable plane of anaesthesia and a smooth, rapid and uneventful recovery. Although dogs in the Group 1 (TIVA) were maintained for the duration of surgery on a continuous intravenous infusion, the speed of anaesthetic recovery in these dogs was not delayed relative to the inhaled anaesthetic Group 2 (THIO/ISO) dogs.

DISCUSSION

Mechanical testing using manual von Frey filaments was unable to yield reliable results in these dogs because some responded with unpredictable variability while others did not respond at all. This finding may reflect inter-animal differences in breed, age, sex, previous conditioning or the instinctive masking of behavioural changes which may complicate the assessment of pain in animals.

The challenge of assessing post-operative pain in dogs is aided by valid, reliable and reproducible behavioural pain scales and an understanding of the parameters which consistently indicate pain. The composite measure pain scale (CMPS) used in this study was an effective pain assessment tool which was sensitive to both surgery and analgesic administration. The sensitivity of the CMPS to surgical pain was demonstrated by significant differences in pain scores before and after surgery. Significant reductions in CMPS scores after the administration of the rescue fentanyl validated the sensitivity of the CMPS to analgesic treatment.

The finding that sedation scores were not associated with CMPS between groups or within individuals suggests that evaluation of CMPS scores for the groups was not confounded by sedation.

Fentanyl proved to be an ideal rescue analgesic agent; its rapid onset of effective analgesia combined with a short duration of action ensured that pain was ethically managed while enabling titration of analgesia on an individual basis. In this study the use of rescue analgesia served as the end point for quantitative pain scoring because it affected all of the other pain measurements. The groups did not differ statistically in pain score, perhaps because the group sizes may have been too small to provide sufficient power to identify real differences or because of the large inter-dog variability and the parallel group study design.

The enrolled number of dogs represented 65/110 (59%) of the target sample population based on the *a priori* power calculation. For pragmatic reasons this study was terminated prior to enrolling the targeted number of dogs. The power calculation was made based on the ordinal CMPS scoring system, since *a priori* information about variance of this primary outcome measure was available. While it is possible that enrolment of more animals might have resulted in identification of a statistically significant difference between groups, the effect-size of the difference between means of groups in this study was only 0.09 i.e., 2.58 (Group 1, TIVA) minus 2.49 (Group 2, THIO/ISO). Even if it were statistically significant, we contend that this effect size is clinically irrelevant. Our contention is supported by the analysis of the secondary outcome, the percent of dogs administered fentanyl for rescue analgesia versus time from extubation (figure 3). This analysis showed that the Group 1 (TIVA) dogs required less rescue analgesia than Group 2 (THIO/ISO) during the first 2 post-operative hours but crossed over with Group 2 (THIO/ISO) and required more in hours 2 to 4 post operatively. The potential for alfaxalone to provide analgesia has been in dispute; Gilron (Gilron & Coderre, 1996) and Nadeson (Nadeson & Goodchild, 2000) used different laboratory animal models of analgesia and achieved contrasting results. In the cat alfaxalone was not demonstrated to produce detectable pre-emptive analgesia (Murison & Taboada, 2010). Our study also failed to demonstrate from intravenous alfaxalone a discernible analgesic effect of a clinically relevant dimension, in the dog.

The possibility of a relationship between shivering and pain in dogs does not appear to have been previously reported in the literature. Shivering was a reliable indicator of pain in dogs in this experimental setting. The clinical utility of shivering as an indicator of pain may be compromised by the post-anaesthetic return of the shivering reflex and the lowered core body temperature caused by the anaesthesia and surgery (Muir, Lerche et al., 2008). The identified association between

shivering and higher pain scores and the known physiological response of shivering to hypothermia together justify effort to avoid hypothermia during anaesthesia for surgical procedures.

The rescue analgesia protocol used in this study largely alleviated the ethical cost of conducting a negative-controlled clinical trial for analgesia. The trial site was chosen for this study because it used no routine pre-emptive analgesia in its anaesthetic protocol. Therefore, conduct of this study improved animal welfare at the trial site and, further, demonstrated the benefits to both animal welfare and surgical recovery consequent from the use of analgesic drugs. The trial site now routinely includes pre-emptive analgesics in its pre-anaesthetic protocol, as the acceptable minimum standard for surgical procedures at the time of publication.

The neuroactive steroid alfaxalone, when administered as a combination of intravenous bolus and constant rate infusion in a total intravenous anaesthetic protocol, did not provide analgesia in the post-operative period for dogs undergoing ovariohysterectomy.

Acknowledgments

The authors would like to thank Mr. G. Anderson for his assistance with the statistical analysis of the data and Jurox Pty Ltd for the supply of anaesthetic drugs.

Conflicts of Interest

One of the authors (TW) was an employee of Jurox at the time of the in-life parts of this study. No other conflicts of interest exist.

Author Contribution

All authors contributed equally to this study and have read and approved the final manuscript.

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516

517 **Table 1** Number of dogs enrolled according to breed and treatment allocation.

Breed	**Thio/ISO †**	**TIVA ‡**
SMALL BREEDS (≤8 KG)		
Chihuahua	1	0
Fox Terrier	1	0
Jack Russell Terrier	6	7
Maltese	2	1
Pomeranian	2	2
Pomeranian/Australian Terrier	0	1
Poodle	1	0
Pug	0	1
Sheltie/Terrier	0	1
Silky Terrier/Maltese	1	0
Terrier	6	5
Terrier/Kelpie	0	1

LARGE BREEDS (>8KG)		
Australian Bull Terrier/Staffordshire Bull Terrier	1	0
Beagle	0	2
Border Collie	0	2
Boxer	2	0
Boxer/Staffordshire Terrier	1	0
German Coolie	0	1
German Shepherd/Kelpie	0	1
German Shepherd	1	0
Golden Retriever	1	0
Heeler	1	0
Kelpie	1	3
Kelpie/Labrador	1	0
Labrador	2	0
Rhodesian Ridgeback	1	0
Staffordshire Bull Terrier	2	2
Staffordshire Bull Terrier/Jack Russell Terrier	1	0
Total	35	30

† Thiopental and Isoflurane

‡ Total Intravenous Anaesthesia

Table 2 Baseline variables according to treatment group. The number of Group 1 (TIVA)‡ dogs was 30 and Group 2 (Thio/ISO)† dogs was 35.

Baseline variable		Group 1 (TIVA)					Group 2 (Thio/ISO)					
		Mean	SE	SD	Median	Range	Mean	SE	SD	Median	Range	p value
Weight	(kg)	9	0.97	5.31	6.55	3.5 to 21.4	9.6	1.06	6.24	6.2	3.0 to 22.4	0.94 [a]
Age	(years)	2.2	0.3	1.6	2	0.5 to 7.0	1.9	0.2	1.3	1.5	0.5 to 5.0	0.37 [a]
Heart rate	(beats per minute)	125	5.5	30.1	124	84 to 216	123	4.9	28.8	120	76 to 192	0.81
Respiratory rate	(breaths per minute)	26	1.6	9	24	12 to 60	39	5.8	34.5	28	12 to 156	0.30 [a]
Respiratory rate*	(breaths per minute)	26	1.6	9	24	12 to 60	32	3.3	19	28	12 to 96	0.50 [a]
Rectal temperature	(° C)	38.4	0.08	0.45	38.35	37.7 to 39.6	38.4	0.1	0.57	38.4	37.3 to 39.7	0.86
Sedation score		1	0.1	0.3	1	1 to 2	1	0.1	0.5	1	1 to 2	0.01 [a]
CMPS score§		0.73	0.13	0.7	0.87	0.08 to 2.25	0.71	0.12	0.72	0.87	0 to 2.25	0.88

† Thiopental and Isoflurane

‡ Total Intravenous Anaesthesia

§ composite measure pain scale

a p value derived using Mann-Whitney test

* Excluding two panting dogs which were assigned 156 for respiratory rate

FIGURE LEGENDS

Figure 1: Composite measure pain scale score (mean ± SE) by treatment group and time from extubation; Group 1 (TIVA, Total Intravenous Anaesthesia) dogs n= 29 and Group 2 (Thio/ISO, Thiopental and Isoflurane) dogs n= 34. Post-operative monitoring for one dog from each group was terminated early and these were excluded from this graph.

Figure 2: Maximum post-operative composite measure pain scale score according to treatment group; Group 1 (TIVA, Total Intravenous Anaesthesia) dogs n= 30 and Group 2 (Thio/ISO, Thiopental and Isoflurane) dogs n= 35. The maximum post-operative composite measure pain scales scores were not significantly different between groups (p = 0.67). Symbols in the graph represent the following;

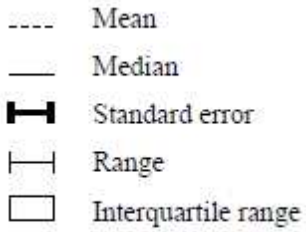

Figure 3: Percent of dogs administered rescue analgesia and standard errors according to treatment group and time from extubation; Group 1 (TIVA, Total Intravenous Anaesthesia) dogs n= 30 and Group 2 (Thio/ISO, Thiopental and Isoflurane) dogs n= 35. There were no significant differences in the survival curves of time to first rescue between groups (Log-rank test, p=0.54).

Figure 1:

Composite measure pain scale score

Figure 2:

Maximum CMPS Score

Figure 3:

Percent rescued

Figure 1:

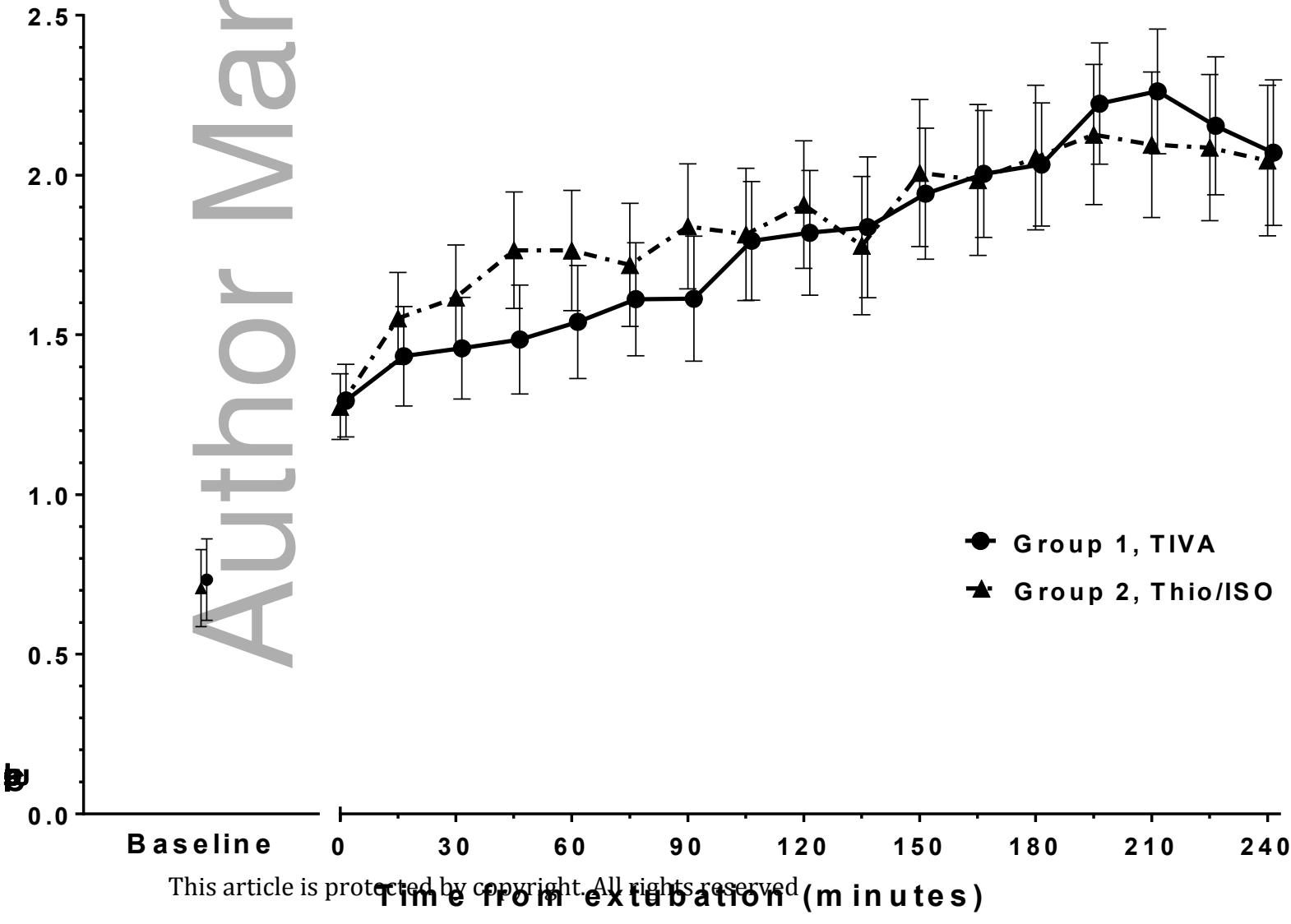

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Figure 2:

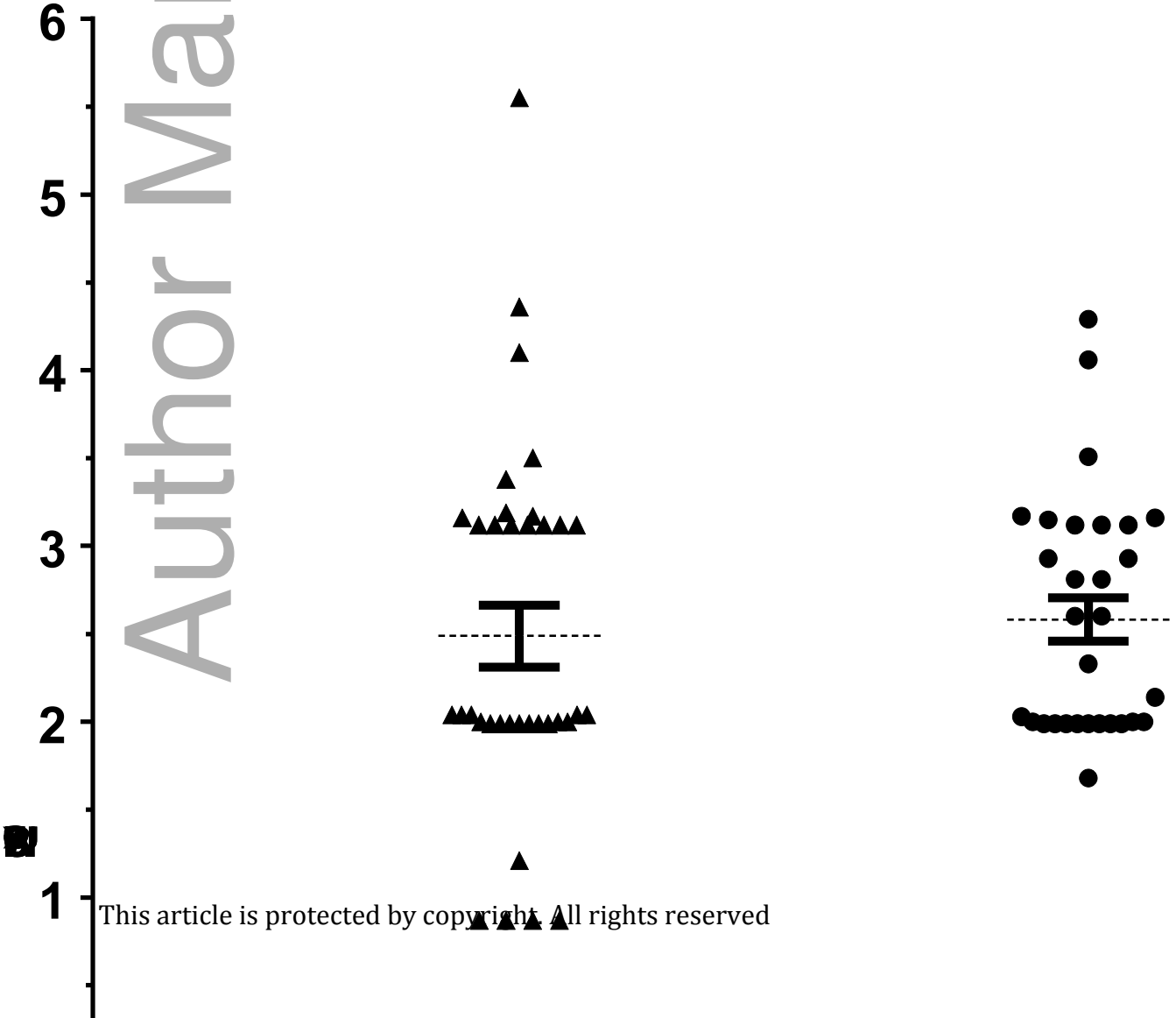
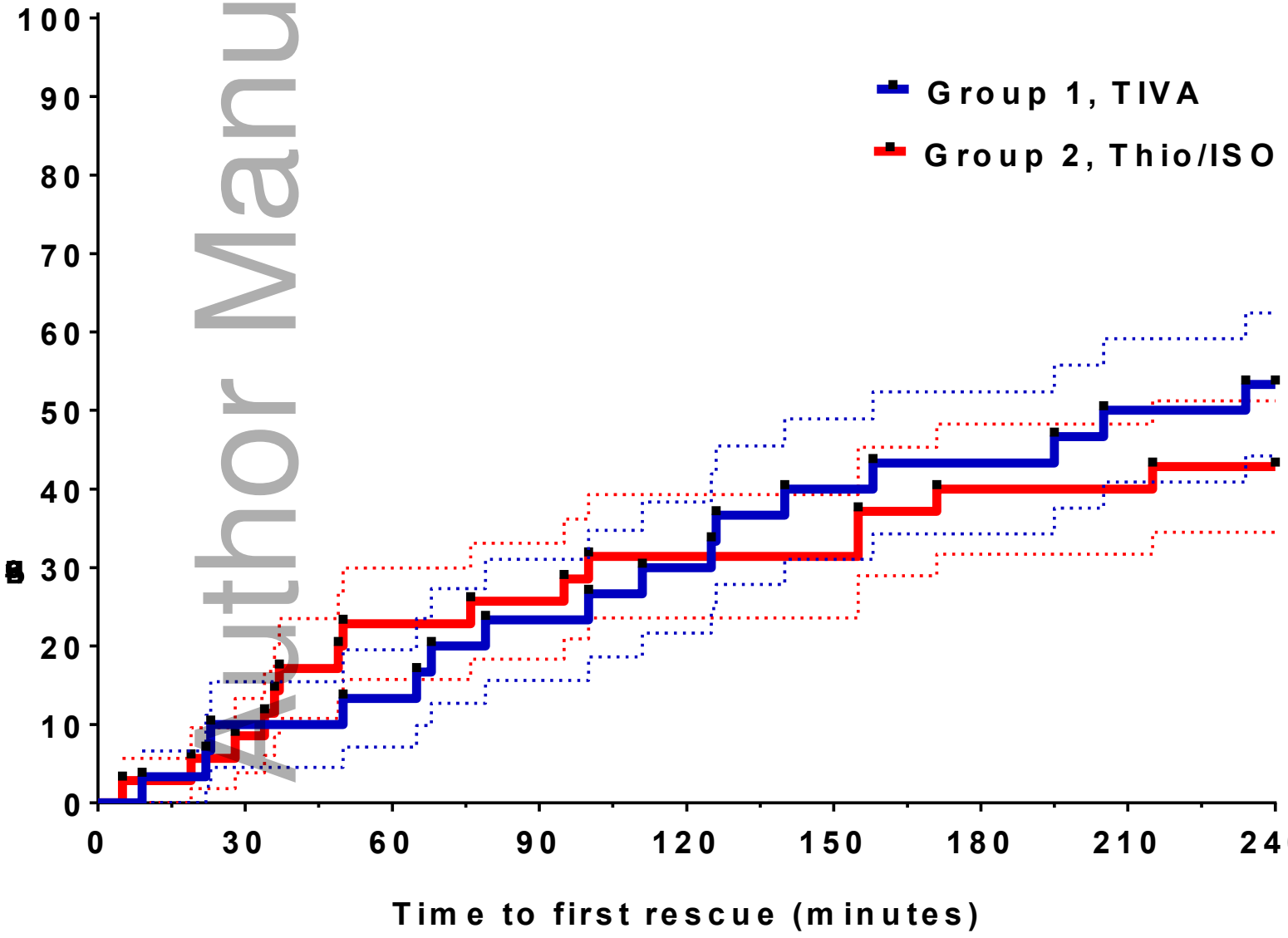


Figure 3:



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