

1 **Combinations of dexmedetomidine and alfaxalone with butorphanol in cats:**
2 **application of an innovative stepwise optimization method to identify optimal**
3 **clinical doses for intramuscular anaesthesia.**

4 Chiara Adami¹, Tobias Imboden², Annalisa Elena Giovannini³ and Claudia
5 Spadavecchia³

6
7 ¹Department of Clinical Sciences and Services, Anaesthesiology Division, Royal
8 Veterinary College, University of London, Hawkshead Lane, North Mymms AL97TA,
9 Hatfield Herts, UK

10 ²Tierarztpraxis Bachtelwald AG, Chefstrasse 20, 8636 Wald

11 ³Department of Veterinary Clinical Science, Anaesthesiology and Pain Therapy
12 Division, Vetsuisse Faculty of the University of Berne, Länggassstrasse n 124 CH-3012
13 Berne, Switzerland

14
15 **Corresponding author:**

16 Chiara Adami DVM, MRCVS, PhD, Dipl ACVAA, Dipl ECVAA, Department of
17 Clinical Sciences and Services, Anaesthesiology Division, Royal Veterinary College,
18 University of London, Hawkshead Lane, North Mymms, Hatfield Herts, AL97TA, UK

19 Email: cadami@rvc.ac.uk

20 **Abstract**

21 **Objectives** The aim of this study was to optimize dexmedetomidine and alfaxalone
22 dosing, for intramuscular administration with butorphanol, to perform minor surgeries
23 in cats.

24 **Methods** **Initially**, cats were assigned to one of 5 groups, each composed of 6 animals
25 and receiving, in addition to 0.3 mg/kg butorphanol IM, one of the following: A: 0.005
26 mg/kg dexmedetomidine, 2 mg/kg alfaxalone; B: 0.008 mg/kg dexmedetomidine, 1.5
27 mg/kg alfaxalone; C: 0.012 mg/kg dexmedetomidine, 1 mg/kg alfaxalone; D: 0.005
28 mg/kg dexmedetomidine, 1 mg/kg alfaxalone; and E: 0.012 mg/kg dexmedetomidine, 2
29 mg/kg alfaxalone. **Thereafter**, a modified “direct search” method, conducted in a
30 stepwise manner, was used to optimize drugs dosing. The quality of anaesthesia was
31 evaluated on the basis of composite scores (one for anaesthesia and one for recovery),
32 of Visual Analogue Scales, and of propofol requirement to suppress spontaneous
33 movements. The medians or means of these variables were used to rank the treatments:
34 “unsatisfactory” and “promising” combinations were identified to calculate, through the
35 equation first described by Berenbaum in 1990, new dexmedetomidine and alfaxalone
36 doses to be tested in the next step. At each step, 5 combinations (one new plus the best
37 previous four) were tested.

38 **Results** None of the tested combinations resulted in adverse effects. Four steps and 120
39 animals were necessary to identify the optimal drug combination (0.014 mg/kg
40 dexmedetomidine, 2.5 mg/kg alfaxalone and 0.3 mg/kg butorphanol).

41 **Conclusions and relevance** The investigated drug mixture, at the doses found with the
42 optimization method, is suitable for cats undergoing minor clinical procedures.

43

44 **Keywords:** alfaxalone, butorphanol, cats, dexmedetomidine, injectable anaesthesia,
45 optimization method

46

47

48

49

50

51

52

53

54

55

56 **Introduction**

57 In several Swiss veterinary practices, intramuscular injectable anaesthesia is preferred
58 over intravenous and inhalational techniques for feline patients undergoing minor
59 clinical procedures. The rationale for this tendency may be the common belief, among
60 general practitioners, that deep sedation is safer than inhalational anaesthesia. As an
61 alternative explanation, owing to the uncooperative nature of feline patients, intravenous
62 catheterization can be challenging in non-sedated cats and this may prevent
63 veterinarians from attempting physical restraint.

64 To date, several drug combinations have been investigated for feline intramuscular
65 anaesthesia. Commonly used protocols often include an alpha₂-adrenoreceptor agonist
66 to produce reliable sedation, an opioid derivative to provide some analgesia, and
67 ketamine owing to its anaesthetic and analgesic effects.

68 Among alpha₂-adrenoreceptor agonists, dexmedetomidine - the active enantiomer of
69 racemic medetomidine - has recently been licensed for administration to cats in some
70 countries and has gained wide popularity in general practice owing to its convenience,
71 ease of administration and possibility to antagonize its effects. Several investigators
72 found that dexmedetomidine is a reliable sedative in feline patients and produces dose-
73 dependent analgesia and muscle relaxation with acceptable side effects.^{1,2} These features
74 make this compound suitable as sole agent for minor clinical procedures associated with
75 mild nociceptive stimulation.

76 Butorphanol is a synthetic opioid with agonistic activity at κ -opioid receptors and
77 antagonistic effects at μ receptors.³ Because in most countries is not listed among
78 controlled substances, it is very often preferred over more potent opioid derivatives by
79 many practitioners in Europe. After intramuscular administration, butorphanol
80 decreased the thermal nociceptive threshold and produced short lasting and variable
81 analgesia in cats.^{4,5} Its combination with dexmedetomidine resulted in greater sedation
82 and more profound muscle relaxation, but not faster onset of recumbency, than
83 dexmedetomidine alone in experimental cats.⁶

84 Ketamine is a dissociative anaesthetic with high bioavailability and short onset of action
85 after intramuscular administration.⁷ Its use has been widely investigated in feline
86 patients.⁸⁻¹² However, its addition to dexmedetomidine and butorphanol was found to
87 prolong the time to recovery.⁶

88 As an alternative to ketamine, the inclusion of alfaxalone in α_2 -adrenoreceptor
89 agonists-opioids combinations may offer some advantages for cats undergoing minor
90 procedures, namely short duration of the anaesthetic effect, high therapeutic index, and
91 excellent muscle relaxation.¹³ Additionally, clinical studies investigating the effects of
92 subcutaneous alfaxalone prepared in 2-hydroxypropyl-beta cyclodextrin solution seem
93 to indicate a rapid systemic absorption when routes of administration other than
94 intravenous injection are used.^{14,15}

95 On the basis of the current literature, dexmedetomidine-butorphanol-alfaxalone
96 intramuscular combination can be considered a promising anaesthetic technique for cats
97 undergoing minor clinical procedures. The optimal dose combination of these three
98 drugs is unknown and unlikely to be identified by randomized controlled study, due to
99 the numbers of animals required for all possible combinations to be investigated. For
100 this reason, we applied a modified “direct search” model,¹⁶ an innovative optimization
101 method based on a mathematical algorithm, whose main advantage is that a limited
102 number of dose combinations - and therefore a limited number of patients – require
103 investigation.

104 The aim of the current study was to optimize the doses of intramuscular
105 dexmedetomidine, butorphanol and alfaxalone, in terms of reliability and rapid onset of
106 anaesthesia and absence of peri-anaesthetic complications, in domestic cats undergoing
107 minor surgical procedures.

108

109 **Materials and methods**

110 *Study design and ethical approval*

111 This study was designed as an investigator-blind, randomized, prospective clinical trial,
112 and performed with permission of the local Ethics Committee for Animal
113 Experimentation (Canton of Berne, Switzerland, license number: 22197) and written
114 informed owner consent.

115 *Animals*

116 One hundred and twenty client-owned adult cats admitted to the **Veterinary Teaching**
117 **Hospital of the University of Berne** **from January 2013 to January 2015** for minor
118 surgical procedures (wound curettage followed by bandage/wound dressing change, or
119 control radiographic exam followed by external pin removal) were enrolled in the study.
120 Food, but not water was withheld for 12 hours prior to anaesthesia. Cats underwent a
121 routine pre-anaesthetic physical examination in order to assess the health status. In
122 cooperative animals, in which venipuncture and blood sampling could be performed
123 without sedation, basic blood parameters (hematocrit, total proteins and electrolytes and
124 **serum creatinine levels**) were also assessed. Exclusion criteria were pregnancy, systemic
125 diseases, impaired cardiovascular function, elderly (more than **8 years**) and ASA risk
126 classification grade greater than II.
127 The animals were randomly assigned to one of **five** treatment groups. A manual
128 randomization technique was used (drawing group assignment from an opaque
129 envelope).

130 *Treatment groups*

131 Cats were injected intramuscularly (IM) with a mixture of dexmedetomidine
132 (Dexdomitor; Pfizer), alfaxalone (Alfaxan; Vétquinol) and butorphanol (Morphasol;
133 Graeub), mixed in one syringe, at one of the following dose-combinations:

134 • GROUP A: 0.005 mg/kg dexmedetomidine, 2 mg/kg alfaxalone and 0.3 mg/kg
135 butorphanol.

136 • GROUP B: 0.008 mg/kg dexmedetomidine, 1.5 mg/kg alfaxalone and 0.3 mg/kg
137 butorphanol.

138 • GROUP C: 0.012 mg/kg dexmedetomidine, 1 mg/kg alfaxalone and 0.3 mg/kg
139 butorphanol.

140 • GROUP D: 0.005 mg/kg dexmedetomidine, 1 mg/kg alfaxalone and 0.3 mg/kg
141 butorphanol.

142 • GROUP E: 0.012 mg/kg dexmedetomidine, 2 mg/kg alfaxalone and 0.3 mg/kg
143 butorphanol.

144 Each treatment group was composed of 6 cats. The rationale for choosing this number is
145 explained in the Appendix (Supplementary file). The drugs doses for groups A-E were
146 selected on the basis of the following criteria: clinical experience of the study's
147 designer, existing literature and manufacturers' recommendations for the species. In
148 order to avoid iatrogenic muscular lesions, the volume of injectate exceeding 1 ml was
149 equally divided into two injection sites.

150 *Anaesthetic Procedure*

151 Prior to anaesthesia, baseline values for heart rate (HR), respiratory rate (RR) and rectal
152 body temperature were recorded. To evaluate the baseline temperament and behavior,

153 each cat was assigned to one of three categories: tranquil and quiet, stressed or scared,
154 and aggressive.

155 The time to anaesthesia, defined as the minutes elapsing from injection to lateral
156 recumbency, was recorded.

157 The occurrence of undesired effects after IM injection, namely vomitus,
158 hypersalivation, respiratory depression and/or increased muscular tone, was noted. A
159 score ranging from 0 to 4 (Undesired Effects Score) was assigned to each cat, with 0
160 being “none of the listed side effects was observed” and 4 being “all the listed side
161 effects were observed”.

162 Ten minutes after IM injection, a catheter was placed into the right or left cephalic vein,
163 or in a peripheral vein of one hind limb if judged more appropriate with respect to the
164 clinical procedure.

165 The observer evaluated the degree of reaction to IV catheter placement by assigning a
166 score, ranging from 0 to 4, with 0 being “no reaction”, 1 “mild reaction” (attempts to
167 withdraw the limb), 2 “moderate reaction” (vocalization and and/or hissing, movements,
168 one person needed for physical restraint) and 3 “aggressive reaction” (vocalization
169 and/or hissing, attempts to bite, two people required for adequate physical restraint).

170 A score ranging from 0 to 5 (Composite Anaesthesia Score), as described by Biermann
171 and colleagues,¹¹ was assigned every 5 minutes until completion of the clinical
172 procedures. The latter were started as soon as the cats became laterally recumbent.

173 During anaesthesia, the cats were instrumented with a pulse-oximeter (Microcap plus;
174 Oridion), an electrocardiograph (Schiller AT-4; Medical Device Depot) and a Doppler
175 (Model 811B; Parks Medical Electronics) for non-invasive arterial blood pressure
176 measurement. The respiratory rate was determined by visual examination of the thorax.
177 Arterial oxygen saturation, HR, RR and systolic arterial blood pressure (SAP) were
178 manually recorded every 5 minutes.

179 The depth of anaesthesia was assessed on the basis of commonly used clinical
180 descriptors (presence or absence of corneal and palpebral reflexes and degree of
181 muscular relaxation).

182 If major movements, defined as flexion/extension of the limbs and/or of the neck,
183 and/or lifting of the head, were observed during the surgical procedure, propofol
184 (Propofol 1%; Fresenius Kabi) was administered IV in steps of 1.5 mg/kg. The number
185 of propofol boli given to each cat, as well as the time of administration, was recorded.

186 All cats received oxygen supplementation by mask with the flow rate set to deliver 2
187 L/min, and a balanced crystalloids' solution (Ringer-Lactat; Baxter) was administered
188 intravenously (IV) at the rate of 5 ml/kg/h.

189 In the event of bradycardia (HR < 100 bpm) with normo- or hypotension (SAP < 100
190 mmHg) and with or without ventricular escape rhythm, glycopyrrolate (Robinul;
191 Sintetica SA), 0.01 mg/kg, was given IV. In the event of moderate sinus bradycardia
192 (HR 99-70 bpm) accompanied by hypertension (SAP > 150 mmHg), no anticholinergic

193 was given and the rhythm was watched closely to detect any change from the baseline.
194 Finally, if severe bradycardia (HR < 70 bpm), or moderate bradycardia accompanied by
195 hypertension and ventricular escape rhythm, were observed, atipamezole (Antisedan;
196 Provet) was administered IM at five times the dexmedetomidine dose. The duration of
197 the clinical procedure was recorded. At the end of the procedure, a 10 cm Visual
198 Analogue Scale (VAS), with 0 being labeled as worst possible and 10 as best possible,
199 was used for an overall, subjective evaluation of the quality of anaesthesia. Thereafter,
200 unless it had been necessary to antagonize the dexmedetomidine before the completion
201 of the procedure, the cats were injected with IM atipamezole. The time to recovery,
202 defined as the minutes elapsed from atipamezole injection to active interaction, was
203 recorded. The rectal body temperature was measured at the end of anaesthesia.
204 The quality of recovery was assessed by using a Composite Recovery Score, ranging
205 from 0 to 14 and based on the following descriptors: comfort, coordination,
206 vocalization, movement during sternal recumbency, locomotor activity and scratching
207 and grooming, as described by Biermann and others.¹¹ Additionally, a 10 cm VAS, with
208 0 labeled as worst possible recovery and 10 labeled as best possible recovery, was used.
209 All the assessments were performed by the same anaesthetist (C.A.), who also injected
210 the cats and was blind to the treatment.

211 *Optimization Procedure*

212 The optimization procedure used in this study is a modification of the “direct search”
213 method as described by Berenbaum and colleagues¹⁷ and applied by Sveticic in a
214 clinical trial involving human patients.¹⁶ A detailed description of the methods and of
215 the equations used is provided in the Appendix (Supplementary file).

216 The optimization of the drug combination was conducted in a stepwise manner, and the
217 treatment groups tested at each step were defined as a *complex*. The initial 5 drugs
218 combinations tested composed the complex number 1. Within each complex, the best
219 treatment groups in terms of quality of anaesthesia (greatest median Composite
220 Anaesthesia Scores, mean VAS Anaesthesia scores and VAS Recovery scores, and
221 lowest medians of number of propofol boli and Composite Recovery Scores) and
222 minimal side effects (lowest median Undesired Effects Scores) were identified. These
223 groups were defined “promising groups”, whereas the remaining ones were classified as
224 “unsatisfactory groups”. The new complex, to be tested in other 30 cats (6 per each
225 group), was composed of four of the previously tested treatments (all but the worst one)
226 plus a fifth one, generated by applying a mathematical model on the basis of the results
227 of the previous step (Appendix). Data obtained from complex 2 were analyzed as
228 previously described and used to generate a new complex until an optimal drug doses
229 combination was found. For alfaxalone, the maximal dose to be used was fixed at 2.5
230 mg/kg.

231 The optimization procedure was considered completed when the scores obtained from
232 the new drug combination were not better of those of the previously tested treatments,
233 and when these results were consistent for three consecutive steps. The dose searching
234 method was to conclude also in case the mathematical model generated a drug
235 combination which had already been tested in at least one of the previous steps.

236 *Statistics*

237 Commercially available software (NCSS-2007; NCSS and SigmaStat and SigmaPlot 12;
238 Systat Software Inc.) were used. Normality of data was tested with the Shapiro-Wilk
239 tests. Descriptive statistics was used for comparison of treatment groups with respect to
240 the following variables: Composite Anaesthesia Score, VAS Anaesthesia and Recovery
241 scores, Composite Recovery Scores, medians of number of propofol boli, and Undesired
242 Effects Scores. The cardiorespiratory variables (HR, RR and SAP) were analyzed with
243 repeated measures ANOVA, followed by either the Bonferroni or the Tukey-Kramer
244 multiple comparison test. The duration of the clinical procedure, the time to anaesthesia
245 and the time to recovery were analyzed with the Kruskal-Wallis one way ANOVA on
246 ranks, followed by the Dunn's test. One way ANOVA on ranks was used to compare
247 the baseline rectal body temperatures of each group with the values measured at
248 recovery. The Chi-Square test was used to compare the distribution of the two clinical
249 procedures between treatment groups.

250 P values < 0.05 and Z values > 1.96 were considered statistically significant.

251

252 **Results**

253 Data for body weight, age and rectal body temperature were normally distributed.

254 Four complexes, 120 cats and 8 groups, 5 of which included in complex 1 and three new
255 ones (N1, N2 and N3; Table 1), were necessary to establish the optimal drug
256 combination, which was 0.014 mg/kg of dexmedetomidine, 2.5 mg/kg of alfaxalone and
257 0.3 mg/kg of butorphanol.

258 The optimization procedure **concluded** after completion of data collection for complex
259 4, as the fourth new treatment group obtained through the mathematical model was the
260 same as N2, which had been previously tested.

261 The cats had a body weight of 4.2 (\pm 0.8) kg, were aged 4.4 (\pm 1.6) years and were 61
262 males (55 of which castrated) and 59 females (all of which **spayed**). The 8 treatment
263 groups did not differ with respect to type of clinical procedure ($P = 0.66$; Table 2),
264 duration of the latter ($P = 0.17$; Figure 1), time to anaesthesia ($P = 0.79$; Table 3) and
265 time to recovery ($P = 0.29$; Table 3). Hypertension and bradycardia were observed in
266 the 11% and 25% of the cats, respectively. However, any statistically significant
267 difference in HR ($P = 0.76$), RR ($P = 0.32$), SAP ($P = 0.35$) and rectal body temperature
268 ($P = 1.2$) was detected, neither between treatments nor between time points (Figure 2, 3
269 and 4).

270 All 8 drug combinations resulted in a degree of unresponsiveness sufficient to allow for
271 intravenous catheterization ten minutes after injection, so that in all cats physical
272 restraint was unnecessary. None of the cats required administration of glycopyrrolate or
273 atipamezole before the end of the clinical procedure. None of the tested treatments
274 resulted in clinically relevant side effects and anaesthesia and recovery were uneventful
275 for all the cats enrolled in the study. The medians or means of VAS Anaesthesia,
276 Composite Anaesthesia Score, number of propofol boli, Undesired Effects Score, VAS
277 Recovery, and Composite Recovery Score, are summarized in Table 4.

278

279 **Table 1** Doses for intramuscular alfaxalone (A), dexmedetomidine (D) and butorphanol
280 (B), expressed in mg/kg, for 8 treatment groups

GROUP	A	D	B
A	2	0.005	0.3
B	1.5	0.008	0.3
C	1	0.012	0.3
D	1	0.005	0.3
E	2	0.012	0.3
N1	2.5	0.009	0.3
N2	2.5	0.014	0.3
N3	2.5	0.010	0.3

281

282

283 **Table 2** Number of cats per group with one of three types of baseline temperament, undergoing
284 one of two types of minor surgical procedures

GROUP	PROCEDURE		TEMPERAMENT		
	WC (n)	RX (n)	TQ (n)	SS (n)	A(n)
A (n = 18)	8	10	5	8	5
B (n = 6)	2	4	2	3	1
C (n = 24)	12	12	5	13	6
D (n = 12)	8	4	1	8	3
E (n = 24)	13	11	8	10	6
N1 (n = 18)	10	8	8	5	5
N2 (n = 12)	8	4	3	8	1
N3 (n = 6)	4	2	2	3	1
TOTAL (120)	65	55	34	58	28

285

286 WC = wound curettage followed by bandage/dressing change; RX = control RX followed by

287 external pins removal; TC = tranquil and quiet; SS = stressed or scared; A = aggressive.

288

289 **Table 3** Medians and ranges [max-min] of time to anaesthesia, defined as the minutes

290 elapsed from IM injection to lateral recumbency, and time to recovery, defined as the

291 minutes elapsed from atipamezole IM injection to active interaction

GROUP	Time to anaesthesia	Time to recovery
A	6.5 [2-20]	12 [5-35]
B	5 [5-13]	11.5 [2-16]
C	5 [1-20]	7 [1-47]
D	7.5 [2-12]	15 [5-30]
E	5 [1-20]	7 [2-75]
N1	5 [2-20]	18.5 [5-40]
N2	5 [1-11]	9.5 [2-35]

N3	4 [2-8]	15.5 [2-32]
----	---------	-------------

292

293 **Table 4** Variables for complex 1, 2, 3 and 4, presented as either means‡ or medians*.

294 The worst treatment group (to be excluded from the optimization process) is colored in

295 red, while the best final treatments are in bold characters.

Complex	Group	VAS A‡	CAS*	PropB *	UES‡	VAS R*	CRS*	Final score
1	A	8.78	5	0.5	0	7.80	2.5	P
1	B	4.28	3.50	3	0	6.50	3	U
1	C	7.75	4.5	1.5	0	6.10	3	U
1	D	6.15	4	1	0	7.50	2	U
1	E	9.57	5	0	0	8.53	1	P
2	A	9.00	4.5	1	0	7.90	2	U
2	C	7.80	4.5	1	0	8.00	2	U
2	D	7.55	4.5	2	0	7.10	2	U
2	E	9.25	4.5	0	0	9.15	0.5	P
2	N ₁	9.50	5	0	0	9.20	0	P
3	A	8.30	4.5	1	0	7.15	2.5	U
3	C	8.35	4.75	1	0	8.60	2	U
3	E	9.45	5	0.5	0	8.3	2	U
3	N ₁	9.40	5	0.5	0	9.7	1	P
3	N ₂	9.75	5	0	0	9.65	1.5	P
4	C	7.33	4.5	1	0	8.12	2.5	U
4	E	8.97	5	0.5	0	9.12	2	P
4	N₁	9.32	5	0	0	9.37	1.5	P
4	N₂	9.52	5	0	0	9.12	1	P
4	N3	9.18	5	0	0	7.56	3	U

296 VAS A = VAS Anaesthesia; CAS = Composite Anaesthesia Score; PropB = number of
297 propofol boli; UES = Undesired Effects Score; VAS R: VAS Recovery; CRS =
298 Composite Recovery Score; P = promising complex; U = unpromising complex.

299

300

301 **Discussion**

302 The main finding of this study is that the optimal alfaxalone–dexmedetomidine–
303 butorphanol combination established by applying the optimization method resulted in
304 reliable, rapid onset, and uneventful anaesthesia in cats undergoing minor surgical
305 procedures.

306 The modified version of the optimization method as used in this study was feasible,
307 applicable to the clinical setting and compatible with the routine of a busy veterinary
308 hospital.

309 The rationale for using a relatively high number of cats was the premise that, for an
310 objective and unbiased data collection, the investigator needed to be blind to the
311 treatments at each step of the search process. This implied the repetition of the good
312 treatments, so that each complex could be composed of 5 groups. A different study
313 design, with the investigator responsible for data collection completely unaware of the
314 study phases, would have allowed the avoidance of such repetition, making the all
315 process easier and less time consuming.

316 None of the cats had complications. However, mild hypertension and bradycardia were
317 observed in some animals, a finding which limits the use of the anaesthetic protocol to
318 healthy cats.

319 Providing a definition of bradycardia and hypertension in anaesthetized cats is
320 challenging owing to the conflicting results of the currently published work. It was
321 reported that feline patients have considerably lower heart rates when the measurements
322 are taken in home environment compared to the hospital, and that normal values for HR
323 in quiet cats are 132 ± 19 .¹⁸ Considering that under anaesthesia even lower values could
324 be regarded as normal, for this trial bradycardia was defined as HR lower than 100.
325 Regarding the systolic arterial pressure, according to Domanjko and colleagues normal
326 values range from 100 to 150 mmHg in anaesthetized cats.¹⁹ Hence, in the present study
327 values higher than 150 mmHg were considered indicative of hypertension.

328 One limitation of the proposed anaesthetic protocol is that the addition of alfaxalone,
329 which is available on the market only at a concentration of 10 mg/ml, greatly increases
330 the volume of injectate in comparison to a classical dexmedetomidine-ketamine
331 combination. This certainly makes the intramuscular injection an unpleasant experience
332 for feline patients, and implies that two injections are often necessary to split the
333 volume into two anatomic sites. Additionally, a high volume IM injection carries the
334 potential for iatrogenic muscular injuries. In the light of these considerations, it was
335 decided to fix the maximal alfaxalone dose at 2.5 mg/kg (corresponding to a volume of

336 0.25 ml/kg). Nevertheless, none of the cats enrolled in the study showed signs of pain at
337 the injection sites or impairment of the injected limb motor function in the post-
338 anaesthetic period.

339 One important thing to consider when comparing different anaesthetic treatments is that
340 the procedures to be performed in the anaesthetized patients should be similar in terms
341 of duration and degree of nociceptive stimulation. Because of the clinical nature of the
342 current trial, a standardization of the surgical procedure was not possible. After an
343 attentive analysis of the caseload of the Institution in which the study was conducted, it
344 was decided to enroll only cats undergoing one of the two most common minor
345 procedures. Unarguably, the surgical curettage of a skin wound and the removal of a pin
346 from a bone may be different in terms of types and intensity of nociceptive stimulation.
347 However, the even distribution of the types of procedure, and the lack of significant
348 difference in its duration, between treatments, should have prevented our findings from
349 being biased.

350

351 **Conclusions**

352 The modified optimization method as described by Berenbaum was easy to apply and
353 allowed to establish a useful drug combination to be used in clinical patients.

354 Dexmedetomidine-alfaxalone-butorphanol combination, at the doses of 0.014, 2.5 and
355 0.3 mg/kg, respectively, produces good quality injectable anaesthesia, characterized by

356 reliability, rapid onset and lack of complications, in feline patients and can be
357 recommended for minor surgical procedures.

358

359 **Acknowledgements** The authors would like to thank Dr. Laura Züger for her fine
360 contribution to data collection.

361

362 **Conflict of interest** The authors do not have any potential conflicts of interest to
363 declare.

364

365 **References**

366 1 Ansah OB, Raekallio M and Vainio O. **Comparison of three doses of**
367 **dexmedetomidine with medetomidine in cats following intramuscular**
368 **administration.** *J vet Pharmacol Therap* 1998; 21: 380 - 387.

369 2 Granholm M, McKusick B, Westerholm F, et al. **Evaluation of the clinical**
370 **efficacy and safety of dexmedetomidine or medetomidine in cats and their**
371 **reversal with atipamezole.** *Vet Anaesth Analg* 2006; 33:214-223.

372 3 Commiskey S, Fan I, Ho I, et al. **Butorphanol: effects of a prototypical**
373 **agonist-antagonist analgesic on κ -opioid receptors.** *J Pharmacol Sci* 2005;
374 98:109 - 116.

- 375 4 Lascelles B and Robertson S. **Use of thermal threshold response to evaluate**
376 **the antinociceptive effects of butorphanol in cats.** *Am J Vet Res* 2004;
377 65:1085 - 1089.
- 378 5 Johnson J, Robertson S and Pypendop B. **Antinociceptive effects of**
379 **butorphanol, buprenorphine, or both, administered intramuscularly in cats.**
380 *Am J of Vet Res* 2007; 68:699-703.
- 381 6 Selmi A, Mendes G, Lins B, et al. **Evaluation of the sedative and**
382 **cardiorespiratory effects of dexmedetomidine, dexmedetomidine-**
383 **butorphanol, and dexmedetomidine-ketamine in cats.** *J Am Vet Med Assoc*
384 2003; 222:37-41.
- 385 7 Hanna R, Borchard R and Schmidt S. **Pharmacokinetics of ketamine HCl and**
386 **metabolite 1 in the cat: a comparison of i.v., i.m., and rectal administration.**
387 *J Vet Pharmacol Therap* 1988; 11:84-93.
- 388 8 Allen DG, Dyson DH, Pascoe PJ, et al. **Evaluation of a xylazine-ketamine**
389 **hydrochloride combination in the cat.** *Can J Vet Res* 1986; 50:23-26.
- 390 9 Akkerdaas LC, Mioch P, Sap R, et al. **Cardiopulmonary effects of three**
391 **different anaesthesia protocols in cats.** *Vet Q* 2001; 23:182-186.
- 392 10 Belda E, Laredo F, Escobar M, et al. **Anaesthetic and cardiorespiratory**
393 **effects of romifidine/ketamine combinations in cats.** *Vet Anaesth Analg* 2009;
394 36 299-307.

- 395 11 Biermann K, Hungerbühler S, Mischke R, et al. **Sedative, cardiovascular,**
396 **haematologic and biochemical effects of four different drug combinations**
397 **administered intramuscularly in cats.** *Vet Anaesth Analg* 2012; 39:137-150.
- 398 12 Ko J, Austin B, Barletta M, et al. **Evaluation of dexmedetomidine and**
399 **ketamine in combination with various opioids as injectable anesthetic**
400 **combinations for castration in cats.** *J Am Vet Med Assoc* 2011; 239:1453-
401 1461.
- 402 13 Muir W, Lerche P, Wiese A, et al. **The cardiorespiratory and anesthetic**
403 **effects of clinical and supraclinical doses of alfaxalone in cats.** *Vet Anaesth*
404 *Analg* 2009; 36 42-54.
- 405 14. Heit M, Schnell M, Whittam T, et al. **Safety and efficacy of Alfaxane-CD RTU**
406 **administered once to cats subcutaneously at 10 mg/kg.** 22nd American
407 College of Veterinary Internal Medicine (ACVIM) Forum June 2004; 432.
- 408 15. Ramoo S, Bradbury L, Anderson G, et al. **Sedation of hyperthyroid cats with**
409 **subcutaneous administration of a combination of alfaxalone and**
410 **butorphanol.** *Aust Vety J* 2013; 91:131-136.
- 411 16. Svetcic G, Gentilini A, Eichenberger U, et al. **Combinations of morphine with**
412 **ketamine for patient-controlled analgesia - a new optimization method.**
413 *Anesthesiology* 2003; 98:1195 - 1205.

- 414 17. Berenbaum M. **Direct search method in the optimisation of cancer**
415 **chemotherapy regimens.** *Br J Cancer* 1990; 61:101-109.
- 416 18. Abbot JA. **Heart rate and heart rate variability of healthy cats in home and**
417 **hospital environments.** *J Feline Med Surg* 2004; 7:195-202.
- 418 19. Domanjko Petrič A, Petra Z, Jerneja S, et al. **Comparison of high definition**
419 **oscillometric and Doppler ultrasonic devices for measuring blood pressure**
420 **in anaesthetised cats.** *J Feline Med Surg* 2010; 12:731-737.
- 421
- 422
- 423
- 424
- 425
- 426
- 427
- 428
- 429
- 430
- 431
- 432
- 433

434 **Figure legends**

435 **Figure 1**

436 Box and whisker plots of duration of the clinical procedure in 120 anaesthetized cats.

437 Boxes represent the interquartile range (central 50% of values), while the horizontal

438 lines within the boxes are the medians. The upper and lower whiskers represent the

439 upper and lower ranges of values, respectively. The dots represent the outliers.

440 **Figure 2**

441 Box and whisker plots of heart rates (beats per minute) of 120 cats undergoing minor

442 surgical procedures under injectable anaesthesia. Boxes represent the interquartile range

443 (central 50% of values), while the horizontal lines within the boxes are the medians. The

444 upper and lower whiskers represent the upper and lower ranges of values, respectively.

445 The dots represent the outliers.

446 **Figure 3**

447 Box and whisker plots of respiratory rates (breaths per minute) of 120 cats undergoing

448 minor surgical procedures under injectable anaesthesia. Boxes represent the interquartile

449 range (central 50% of values), while the horizontal lines within the boxes are the

450 medians. The upper and lower whiskers represent the upper and lower ranges of values,

451 respectively. The dots represent the outliers.

452 **Figure 4**

453 Box and whisker plots of systolic arterial pressures (mmHg) of 120 cats undergoing
454 minor surgical procedures under injectable anaesthesia. Boxes represent the interquartile
455 range (central 50% of values), while the horizontal lines within the boxes are the
456 medians. The upper and lower whiskers represent the upper and lower ranges of values,
457 respectively. The dots represent the outliers.

458

459