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Effect of administering dexmedetomidine with or without atropine on cardiac troponin I level in isoflurane-anesthetized dogs

Running head: EFFECT OF DEXMEDETOMIDINE ON CTNI

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26 **Abstract**

27 We aimed to determine whether dexmedetomidine administration with or without atropine
28 increases cardiac troponin I (cTnI) level in healthy dogs. We hypothesized that 10 µg/kg
29 dexmedetomidine + atropine increases the cTnI level, whereas 5 µg/kg dexmedetomidine + atropine
30 does not. Eighteen healthy, pet dogs that underwent an orthopedic surgery or ovariohysterectomy
31 were included in this study. The dogs were randomly assigned to atropine (0.02 mg/kg)–
32 dexmedetomidine (10 µg/kg), saline–dexmedetomidine (10 µg/kg), and atropine (0.02 mg/kg)–
33 dexmedetomidine (5 µg/kg) groups. Each dog was premedicated with atropine or saline
34 intramuscularly (IM). After 10 min, they were IM injected with dexmedetomidine (10 or 5 µg/kg)–
35 morphine (0.5 mg/kg)–midazolam (0.2 mg/kg). Following this, anesthesia was induced after 10 min
36 with propofol and maintained with isoflurane in 100% oxygen. The median plasma cTnI level at 6,
37 12 and 24 hr after premedication was significantly higher than that at baseline. The cTnI level in the
38 atropine–dexmedetomidine (10 µg/kg) group was significantly higher than that in the saline–
39 dexmedetomidine (10 µg/kg) and atropine–dexmedetomidine (5 µg/kg) groups at 6 and 12 hr after
40 premedication. The cTnI level returned to normal within 72 hr after premedication in all groups.
41 The administration of atropine in combination with 10 µg/kg dexmedetomidine increased the cTnI
42 level, indicating subclinical myocardial damage.

43

44 **Keywords:** atropine, canine, cardiac troponin I, dexmedetomidine, myocardial injury

45

46 **Introduction**

47 Dexmedetomidine, an α -2 adrenergic receptor agonist, is widely used in veterinary medicine
48 because of its sedative, analgesic, and muscle relaxation properties [8, 16]. Initially,
49 dexmedetomidine causes peripheral vasoconstriction, which results in temporary hypertension,
50 bradycardia, and up to 50% reduction in cardiac output (phase 1). Thereafter, during phase 2, the
51 sympatholytic effect of dexmedetomidine leads to the dilation of the peripheral vessels, while
52 maintaining a lowered heart rate [2, 3, 26]. However, preemptive administration of anticholinergics
53 to prevent bradycardia can cause cardiac arrhythmias, hypertension, and increased myocardial
54 workload, as indicated by previous studies in the field of veterinary medicine [3, 12, 21, 24, 27].
55 The extreme hypertensive effect of dexmedetomidine in combination with atropine premedication
56 or glycopyrrolate treatment has been reported in humans [25, 39]. Therefore, the use of such a
57 combination is controversial.

58 Blood cardiac troponin I (cTnI) level is a highly specific and sensitive marker of myocardial
59 injury, and it has been used to diagnose myocardial damage in humans, dogs, and cats [4, 23]. An
60 imbalance between oxygen supply and demand can lead to perioperative myocardial injury [33, 41].
61 Perioperative myocardial injury is generally characterized by an increase in isolated troponin level
62 after surgery without any signs or symptoms. Mortality at 30 days in patients undergoing
63 noncardiac surgery with increased postoperative cTnI level is observed in at least 3% in humans,
64 and this increased risk of mortality continues for at least 1 year [33]. To the best of our knowledge,
65 only a few studies have evaluated the effects of dexmedetomidine administration along with
66 atropine on the cTnI level in recent years. Hence, in this study, we aimed to determine whether 10
67 or 5 μ g/kg dexmedetomidine along with atropine can increase the cTnI level in clinically healthy
68 dogs. We hypothesized that atropine combined with dexmedetomidine 5 μ g/kg would not increase
69 the cTnI level and could maintain a higher heart rate than dexmedetomidine administration alone.

70

71 **Materials and methods**

72 This study was approved by the Institutional Animal Care and Use Committee of National
73 Chung Hsing University (IACUC No.107-038). Eighteen client-owned dogs were admitted to
74 National Chung Hsing University Veterinary Teaching Hospital from July 2017 to February 2018
75 for ovariohysterectomy (OHE) or orthopedic surgery. Based on the American Society of
76 Anesthesiologists' classification system, these dogs were classified as 1 or 2. The age range of the
77 dogs was 6 months to 5 years. The preoperative electrocardiogram, lactate level, cTnI level,
78 physical examination, complete blood count (CBC), and serum biochemical examination were
79 performed for each dog to fulfil the study enrollment process. None of the dogs had a history,
80 clinical sign, or physical examination findings that indicated a heart disease. A 3-day hospitalization
81 was required for each dog for blood sample collection. Dogs with cardiac disease, renal disease
82 [31], and sepsis were excluded.

83 **Experimental protocol**

84 The dogs were randomly assigned (randomization by lottery) to three groups at the time of
85 premedication (n = 6 per group): 0.02 mg/kg atropine (Atropine Sulfate; Tai Yu, Hsinchu, Taiwan)
86 combined with 10 µg/kg dexmedetomidine (Dexdomitor[®], Zoetis, Inc., Parsippany, NJ, USA)
87 (group AD10), saline combined with 10 µg/kg dexmedetomidine (group SD10), and 0.02 mg/kg
88 atropine combined with 5 µg/kg dexmedetomidine (group AD5). The anesthesiologist was unaware
89 of group assignment until the premedication was administered.

90 Food and water were withheld for 6–8 hr and 2 hr, respectively. A 20 or 22 gauge IV cannula
91 was aseptically placed in the cephalic vein. All dogs rested for 30 min for environmental
92 acclimatization. The baseline heart rates and blood pressures were then measured before
93 premedication (baseline, BL). We referred to Congdon *et al.* [12] to determine the doses of
94 dexmedetomidine and atropine, and Ko *et al.* [21] to decide the timing of administration. Atropine
95 (0.02 mg/kg) (group AD10 and AD5) or saline (0.02 ml/kg, the same volume as atropine) (group

SD10) was injected intramuscularly (IM). After 10 min, dexmedetomidine 10 µg/kg (group AD10 or SD10) or 5 µg/kg (group AD5) combined with 0.5 mg/kg morphine (both groups; Morphine HCl, Food and Drug Administration, Taipei, Taiwan) and 0.2 mg/kg midazolam (both groups; Dormicum[®], Roche, Kaiseraugst, Switzerland), all mixed in the same syringe, was administered IM as premedication. Anesthesia was induced 10 min later with propofol (Fresofol[®] 1%; Fresenius Kabi, Bad Homburg, Germany) injected intravenously (IV) titrated to effect to intubation (T0) and maintained with isoflurane (Terrell Isoflurane USP[®]; Piramal Critical Care Inc., Bethlehem, USA) in 100% oxygen with the end-tidal isoflurane concentration between 1.2% and 1.5%. All dogs were ventilated via intermittent positive pressure ventilation in the pressure-control mode to maintain the end-tidal CO₂ level between 35–45 mmHg. The dogs were in lateral or dorsal recumbency based on the surgery. Noninvasive oscillometric blood pressure was measured in the uncatheterized forelimb above the carpus with Pettrust (Pettrust[®]; BioCare, Taoyuan, Taiwan). If the forelimb was amputated, the oscillometer was placed in the tarsal region. A 22 or 24 gauge IV cannula was aseptically placed in the dorsal pedal artery for invasive blood pressure measurement. During anesthesia, electrocardiographic patterns, end-tidal CO₂, end-tidal isoflurane, tidal volume, heart rate (HR), systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP), esophageal temperature (E-PRESTN and E-CAIOv modules; GE Healthcare, Helsinki, Finland), and pulse oxymetry (Radical-7[®]; Masimo Corp., Irvine, CA, USA) were monitored continuously and recorded every 5 min. Lactated Ringer's solution (Liquor Lactated Ringer's; Chi Sheng Chemical Corp., Hsinchu, Taiwan) was administered at 5 ml/kg/hr after intubation to the end of surgery. Morphine (0.36 mg/kg/hr), lidocaine (1 mg/kg/hr; Xylocaine[®]; Cenexi Inc., Fontenay-sous-Bois, France), and ketamine (0.6 mg/kg/hr; Imalgene[®]; Merial Inc., Lyon Cedex, France) (MLK) constant-rate infusion was given for analgesia in each procedure. If the respiratory rate, HR, and mean blood pressure were $\geq 20\%$ of the baseline values, the dogs were presumed to be experiencing nociception and rescue analgesia with fentanyl (3 µg/kg, Fentanyl; UBI Pharma Inc., Hsinchu, Taiwan) was administered IV. If hypotension (mean blood pressure < 60 mmHg) occurred,

122 IV bolus of Lactated Ringer's solution 3 ml/kg was administered within 5 min, which was repeated
123 if hypotension persisted. A forced-air warming system (Bair Hugger Model 750; 3M Health Care,
124 Saint Paul, MN, USA) was used to keep the dogs warm during the surgery. The dogs were
125 administered 0.2 mg/kg meloxicam (Achefree®; Swiss Pharm. Co. Ltd., Tainan, Taiwan) once
126 subcutaneously (SC) after surgery, followed by 0.1 mg/kg SC once a day. Postoperative pain scores
127 following the Glasgow Composite Measure Pain Scale were assigned by the same person. If the
128 postoperative pain score was $>6/24$ or $5/20$ for non-ambulatory dogs, morphine (0.5 mg/kg) was
129 administered IM as rescue analgesia. In orthopedic surgery, MLK constant-rate infusion was
130 administered as postoperative analgesia.

131 **Cardiac troponin I and lactate**

132 cTnI level was measured using the Abbott i-STAT portable clinical analyzer (i-STAT handheld;
133 Abbott, Ontario, Canada), with a test cartridge, following a two-site ELISA method. The lower limit
134 for detection of cTnI was 0.02 ng/ml. The range of plasma cTnI level in dogs was $< 0.03\text{--}0.07$
135 ng/ml [38]. A cTnI level ≤ 0.07 ng/ml was considered as the normal level in this study. Lactate was
136 measured using a point-of-care device (StatStrip Xpress® Lactate Hospital Meter; Nova
137 Biomedical, Waltham, MA, USA). The normal range for dogs is < 2 mmol/l [6]. Whole blood (0.1
138 ml) was collected from the cephalic or lateral saphenous vein immediately to determine the cTnI
139 and lactate levels at baseline, and 6, 12, 24, 48, and 72 hr after premedication (after
140 dexmedetomidine/morphine/midazolam injection).

141 **Rate pressure product (RPP)**

142 The product of HR and SBP (i.e., RPP) is a useful predictor of myocardial oxygen demand [18,
143 42]. Thus, we utilized this method to estimate myocardial oxygen consumption every 5 min and
144 calculated the RPP from these values. The normal range of RPP was below $12,000$ mmHg $\times$
145 beats/min in humans [29]. In veterinary medicine, the normal range of RPP has not been
146 established. We defined the normal range of RPP as $9,200\text{--}18,500$ mmHg $\times$ beats/min, based on
147 previous studies [12, 20].

148 **Statistical analysis**

149 Continuous data are shown as descriptive statistics, median, and interquartile range (IQR) for
150 each group. Age, weight, propofol dose, fentanyl dose for rescue analgesia in surgery, anesthesia
151 time, rescue times in surgery, and hemodynamic variables were compare among the three groups
152 using Kruskal–Wallis test at baseline (before atropine, BL), induction (T0), and 10 (T10), 20 (T20),
153 30 (T30), 45 (T45), and 60 (T60) min after induction. Post hoc contrast analyses were conducted
154 using Steel’s test for pairwise comparisons when significant group differences were observed. The
155 cTnI and lactate levels were analyzed using the same test to compare the three groups at baseline
156 and 6, 12, 24, 48, and 72 hr after dexmedetomidine/morphine/midazolam administration. The
157 relationship between ECG arrhythmia combined with or without atropine was tested using Fisher’s
158 exact test. The relationship between the type of surgery (OHE or orthopedic), arrhythmia,
159 anesthesia time (>255 min [43]), and cTnI level (>0.07 ng/ml or not) was tested using Fisher’s
160 exact test. Associations between hemodynamic variables were tested using linear regression
161 analyses. In this study, two statistical software were applied for data analysis, including the use of
162 MedCalc version 2.1.4.0 (MedCalc Software®, Mariakerke, Belgium) for regression analysis and
163 Fisher’s exact test, and SAS version 9.4 (SAS institute Inc., Cary, NC, USA) for Kruskal Wallis test
164 and post hoc analysis by Steel’s method. Results with a *P*-value of <0.05 were considered
165 statistically significant.

166

167 **Results**

168 Eighteen dogs, 2 males and 16 females, were included in this study. The breeds included mix
169 breed (n = 15), poodle (n = 1), Shiba (n = 1), and Belgian Shepherd (n = 1). Thirteen dogs
170 undergoing OHE surgery and six dogs undergoing orthopedic surgery (one dog femoral head
171 osteotomy + OHE) were included in this study. One dog (femoral fracture open reduction) in the
172 AD10 group, three (femoral head osteotomy, 2 dogs; forelimb amputation, 1 dog) in the AD5 group,
173 and two (femoral fracture open reduction and forelimb amputation) in the SD10 group underwent
174 orthopedic surgery. Demographic data are summarized in Table 1. There was no significant
175 difference in age, weight, fentanyl dose for rescue analgesia in surgery, and anesthesia time among
176 the three groups. Anesthesia time in only two dogs (285 and 320 min) was more than 250 min in the
177 SD10 group, but their cTnI levels were normal. Propofol dose was significantly higher in the AD5
178 group than that in the SD10 group ($P = 0.0079$). No dog was administered the rescue drug after the
179 surgery.

180 Invasive arterial blood pressure was measured in 4/6, 4/6, and 3/6 dogs in the AD10, SD10,
181 and AD5 groups, respectively. Arterial line placement could not be performed in seven dogs
182 because of vasoconstriction. We measured the blood pressure in all groups using a non-invasive
183 method and compared the values. The HR, SBP, MBP, DBP, and RPP values are summarized in
184 Table 2. Overall, only two dogs developed hypotension in the AD5 group and both were
185 administered an IV bolus of 3 ml/kg LR solution once. The cTnI level in these two dogs was <0.06
186 ng/ml at all time points.

187 There was no significant difference in the HR among the three groups at baseline ($P = 0.7267$).
188 In the SD group, the HR at T0, T45 and T60 was significantly lower than that at baseline ($P =$
189 0.0074 , $P = 0.0142$, $P = 0.0346$, respectively). In the AD10 group, the HR only was lower than that
190 at baseline at T45 ($P = 0.0498$). There was no significant difference in the HR at each observed time
191 point among the three groups (Table 2).

192 No significant difference was observed in the SBP, MBP, and DBP values among the three
193 groups at baseline ($P = 0.9598$, $P = 0.9320$, $P = 0.8488$, respectively). In the AD10 group, the SBP
194 at T10, T45 and T60 was significantly lower than that at baseline ($P = 0.0317$, $P = 0.0158$, $P =$
195 0.0288 , respectively). There were significant differences in the SBP values from T10, T30 to T60 in
196 the SD10 group ($P = 0.00143$, $P = 0.0418$, $P = 0.0023$, $P = 0.0023$, respectively) and T10 to T60 in
197 the AD5 group ($P = 0.0037$, $P = 0.0020$, $P = 0.0018$, $P = 0.0033$, $P = 0.0020$, respectively)
198 compared with the baseline values. There were significant differences in MBP values from T45 to
199 T60 in the SD10 group ($P = 0.0018$, $P = 0.0037$, respectively) and T10 to T60 in the AD5 group (P
200 $= 0.0347$, $P = 0.0066$, $P = 0.0018$, $P = 0.0158$, $P = 0.0033$, respectively) compared with the baseline
201 values. There were differences at T10, T20 and T30 in the SD10 group ($P = 0.0596$, $P = 0.0596$, $P =$
202 0.0547 , respectively) compared with the baselines values. The difference is very close to the
203 significant level statistically by post hoc analysis of Steel's method. DBP was significantly lower in
204 the AD5 group at T30, and T60 than at baseline ($P = 0.0115$, $P = 0.0143$) (Table 2).

205 There was no significant difference in the HR, SBP, MBP, and DBP values among the three
206 groups (Table 2).

207 The cTnI and lactate levels in all three groups are summarized in Table 3. There was no
208 significant difference in the cTnI level after OHE and orthopedic surgery (>0.07 ng/ml) ($P =$
209 1.0000). Furthermore, there was no significant difference in the fentanyl for rescue analgesia during
210 OHE (median, interquartile range: 0, 0–6) and orthopedic surgery (3, 0–9) ($P = 0.3026$). Abnormal
211 ECG recordings such as those indicative of 1–2 degree atrioventricular blocks were found for 3/6,
212 3/6, and 2/6 dogs in the AD10, SD10 and AD5 groups, respectively. No significant difference was
213 found in ECG arrhythmia after atropine administration or increased cTnI level (>0.07 ng/ml) (both
214 $P = 1.0000$). Anesthesia time (>255 min) showed no significant difference with the cTnI level
215 (>0.07 ng/ml) among the 18 dogs.

216 There was no significant difference in the cTnI level among the three groups at baseline ($P =$
217 0.9791). The plasma cTnI level increased in the AD10 (6/6), AD5 (1/6), and SD10 (3/6) groups

218 compared with that at baseline 12 hr after premedication. In three dogs in the AD10 group, the cTnI
219 level reached as high as 0.1–0.25 ng/ml at 6–48 hr after premedication. The plasma cTnI level at 6,
220 12 and 24 hr in the AD10 group was significantly higher than that at baseline ($P = 0.0482$, $P =$
221 0.0022 , $P = 0.0229$, respectively). There was no significant difference in the plasma cTnI level in
222 the SD10 and AD5 groups compared with the baseline values at any time point. The cTnI level
223 returned to the normal levels within 72 hr after premedication in all groups. The plasma cTnI level
224 in the AD10 group was significantly higher than that in the SD10 group at 6 and 12 hr after
225 premedication ($P = 0.0389$, $P = 0.0170$, respectively). And the plasma cTnI level in the AD10 group
226 was significantly higher than that in the AD5 group at 12 hr after premedication ($P = 0.0491$) (Table
227 3).

228 There was no significant difference in the lactate level at baseline and all time points among
229 the three groups (Table 3).

230 There was no significant difference in RPP among the three groups at baseline ($P = 0.6119$).
231 There were significant differences in the RPP values from T30 to T60 in the AD10 group ($P =$
232 0.0038 , $P = 0.0038$, $P = 0.0094$, respectively) and T10 to T60 in the AD5 group ($P = 0.0038$, $P =$
233 0.0094 , $P = 0.0023$, $P = 0.0060$, $P = 0.0060$, respectively) compared with the baseline values. The
234 RPP substantially declined from T0 until T60 in the SD10 group ($P = 0.0018$, $P = 0.0023$, $P =$
235 0.0030 , $P = 0.0023$, $P = 0.0018$, $P = 0.0023$, respectively). There were significant differences in
236 RPP between the AD10 and SD10 groups at T10 ($P = 0.0206$) (Table 2). There were differences
237 between the AD10 and SD10 groups at T20 ($P = 0.0509$) and between the AD10 and AD5 groups at
238 T10 ($P = 0.0509$). The difference is very close to the significant level statistically by post hoc
239 analysis of Steel's method.

240 A significant positive correlation between the cTnI level at 6 hr and RPP T10 ($R^2 = 0.3585$; P
241 $= 0.0087$) and RPP T20 ($R^2 = 0.2488$; $P = 0.0351$) was observed (Figs. 1 and 2). Additionally, a
242 significant positive correlation between the cTnI level at 12 hr and RPP T10 ($R^2 = 0.3657$; $P =$
243 0.0078) was observed (Fig. 3). No significant correlations were detected between the cTnI level and

244 HR, SBP, DBP and MBP.

245

246

247 Discussion

248 On the basis of the results of this study, the IM administration of 0.02 mg/kg atropine along
249 with 10 µg/kg dexmedetomidine can increase the plasma cTnI level, which was not observed with 5
250 µg/kg dexmedetomidine administration in combination with atropine. The cTnI level returned to
251 normal levels within 72 hr.

252 In our study, the cTnI level increased from 6 to 24 hr after premedication, and peaked at 12 hr
253 in the AD10 group, consistent with the results of previous studies [1, 37, 43]; however, a clear
254 threshold of myocardial damage has not been established in dogs. The cTnI level has been reported
255 to mildly increase (<1 ng/ml) in primary cardiac disease and rarely increased to above 1–2 ng/ml
256 [23] in severe congestive heart failure. According to a previous study [23], increased cTnI levels are
257 commonly observed in critically ill dogs and cats with secondary myocardial injury (mild: <1
258 ng/ml, common: >10 ng/ml). For example, increased cTnI level was detected in dogs with gastric
259 dilatation–volvulus syndrome (2.05–24.9 ng/ml) [35] and pyometra (0.3–0.9 ng/ml) [17]. In this
260 study, the highest increase in the cTnI level was in the range of 0.1–0.25 ng/ml at 6–24 hr after
261 premedication in the AD10 group, suggesting that minor myocardial damage had occurred. In
262 addition, a minor increase in the serum cTnI (<1.3 ng/ml) level may occur without histological
263 evidence of myocardial cell injury [7]. The cTnI level returned to the normal range 72 hr after
264 premedication in all dogs.

265 Increased RPP in the AD10 group may be the primary factor to cause an increase in the cTnI
266 level. The association between the cTnI level and RPP has been corroborated by statistical analyses.
267 Dexmedetomidine 10 µg/kg in combination with atropine increased the HR and blood pressure,
268 resulting in increased cTnI level. A previous study [12] reported that when dexmedetomidine (10
269 µg/kg)–atropine (0.02 mg/kg) was administered IM, a peak RPP of $29,500 \pm 1,390$ mmHg
270 beats/min was reached 30 min after injection. These trends are consistent with the outcome of the
271 AD10 group in our study. RPP was higher in the group that received dexmedetomidine with

272 atropine, which increased myocardial oxygen consumption. The peripheral vasoconstrictive effect is
273 predominant after administering a high dose of α_2 -adrenoceptor agonists [32], such as 10 $\mu\text{g/kg}$
274 dexmedetomidine, and anesthetization with isoflurane or propofol [22]. Therefore, the myocardial
275 oxygen consumption in the AD10 group was higher than that in the AD5 group. The increase in the
276 HR increases myocardial oxygen demand and impairs diastolic filling time. HR-induced increase in
277 the cardiac output and initial peripheral vasoconstriction by dexmedetomidine results in this
278 hypertensive effect [39]. The imbalance in myocardial oxygen supply and demand can result in
279 myocardial injury. The severity and duration of myocardial injury may cause reversible or
280 irreversible myocardial ischemia [41]. Perioperative myocardial injury is generally common, silent,
281 asymptomatic, and strongly associated with mortality [33], and it can only be detected by routine
282 troponin monitoring in humans [33]. However, the mortality risk due to subclinical myocardial
283 injury in dogs is currently unknown. Therefore, it seems prudent to avoid RPP increase during
284 surgery.

285 The finding that the baseline HR and blood pressure were high indicated that all dogs were
286 nervous and not acclimated to the induction room; their sympathetic tone was elevated. Surgical
287 stress and direct tissue injury can cause pain, which resulted in increased HR, high blood pressure,
288 increased oxygen consumption, myocardial ischemia, and endocrine response [10]. In our study, the
289 dogs underwent various surgical procedures and experienced different degrees of nociception.
290 Lidocaine and ketamine are widely used in combination with morphine for multimodal analgesia in
291 dogs. All groups received MLK by constant rate infusion to provide pain relief and to avoid these
292 effects. Additionally, opioids such as morphine are known to relieve pain and exhibit
293 cardioprotective effects. These cardioprotective effects occur via the primary δ -opioid receptor
294 immediately after drug administration and reappear 24–48 hr after treatment [36]. Lidocaine
295 infusion increases mitochondrial membrane stability and protects the myocardium from ischemic
296 damage [34]. In addition, lidocaine is commonly used to prevent ventricular arrhythmias [11].
297 Franco *et al.* [13] reported that a ketamine infusion rate of 20 $\mu\text{g/kg/min}$ would not change the cTnI

298 level, HR, MAP, ejection fraction, and cardiac index in dogs. All dogs received MLK by constant
299 rate infusion with a ketamine infusion rate of 10 µg/kg/min in this study. This rate was lower than
300 that in a previous study [13]. It is supposed that the ketamine infusion rate used in our study would
301 not increase the cTnI level. In general, morphine, lidocaine, and ketamine did not affect the cTnI
302 level in this study.

303 To the best of our knowledge, the factors related to increased cTnI level are still not clear.
304 Arrhythmia could be one of the possible causes of myocardial injury in critical noncardiac diseases
305 [23]. Previous studies reported that higher serum cTnI levels were significantly associated with
306 moderate and severe ECG abnormalities (frequent ventricular premature contraction or
307 supraventricular premature contraction (>15/min), couplets, triplets, polymorphic ventricular
308 premature contraction, ventricular tachycardia, and ventricular flutter or fibrillation) in dogs with
309 gastric dilatation–volvulus syndrome [5, 35]. Arrhythmias were observed in all groups in our study,
310 including first and second degree atrioventricular blocks. The first and second degree
311 atrioventricular blocks are somewhat mild ECG abnormalities as reported previously [5, 35]. Thus,
312 these abnormal ECG observations in our study were not severe enough to cause changes in the cTnI
313 level.

314 In addition, increased cTnI levels have been shown to be related to the age of the dogs even
315 without appreciable cardiac disease [30]. Older dogs (>8 years) are more likely to present with
316 increased plasma cTnI level than young dogs [9]. However, in this study, dogs under 5 years of age
317 were enrolled to exclude age-related variables. Meanwhile, a longer operation time (255 min) and
318 the development of hypotension during anesthesia increased the cTnI level to 0.15 ng/ml in a
319 previous study [43]. These observations were not consistent with our results. The anesthesia time
320 was longer in 2 dogs (285 and 320 min) in the SD10 group, but their cTnI levels did not increase.
321 The increase in the cTnI level did not correlate with longer operation time in this study and neither
322 did the two dogs with hypotension exhibit increased cTnI level in our study.

323 This study had some limitations in addition to those mentioned above. First, a small sample
324 size and the use of nonparametric statistics may have contributed to type II error, leading to
325 overlooking of significant differences among the groups. Second, blood pressure was not measured
326 through an invasive procedure via an arterial line in all the dogs. Using a noninvasive blood
327 pressure measurement device might have underestimated the SBP during hypertension [14], and
328 thereby underestimating the RPP. We did not record vital signs immediately after the administration
329 of atropine and other premedication. The interval between baseline and induction was
330 approximately 20 min. After this period, the hemodynamic effects of dexmedetomidine might have
331 progressed to stage 2 (i.e., central hypotensive effect), in which the vascular tone returns to the
332 normal level or is slightly vasodilated and the heart rate remains low because of the suppression of
333 the sympathetic tone [28, 32]. Furthermore, the vasodilatory effect of propofol [15] and the negative
334 inotropy (decreases cardiac output) caused by isoflurane may have contributed to the lower blood
335 pressure [19, 40]. Invasive blood pressure measurement may be a better way to determine RPP
336 more accurately. However, we still evaluated the trend in blood pressure and RPP in this manner.
337 Third, despite different degrees of postoperative pain, dogs undergoing OHE and orthopedic surgery
338 were still enrolled. More profound analgesia (MLK CRI) was administered after orthopedic surgery
339 to prevent tachycardia induced by nociception. Finally, an SD5 group (dexmedetomidine 5 µg/kg)
340 was not included in this study. However, after the pilot study, we found no increase in the cTnI level
341 in the SD5 group. This supposition combined with the observation that 10 µg/kg dexmedetomidine
342 alone does not increase the cTnI level indicates that the lack of an SD5 group would not affect the
343 interpretation of the results.

344 In conclusion, our findings demonstrate that atropine administration does not increase the cTnI
345 level and prevent bradycardia induced by 5 µg/kg dexmedetomidine. The administration of atropine
346 in combination with 10 µg/kg dexmedetomidine increases the cTnI level, indicating subclinical
347 myocardial damage.

348

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351

POTENTIAL CONFLICTS OF INTEREST

352 The authors have nothing to disclose.

353

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468

469 **Figure legends**

470 Fig. 1. Scatterplot and linear regression of the cTnI level and RPP recorded for 18 dogs. cTnI 6 hr
471 and RPP T10 positively correlated.

472
473 Fig. 2. Scatterplot and linear regression of the cTnI level and RPP recorded for 18 dogs. cTnI 6 hr
474 and RPP T20 positively correlated.

475
476 Fig. 3. Scatterplot and linear regression of the cTnI level and RPP recorded for 18 dogs. cTnI 12 hr
477 and RPP T10 positively correlated.

478

479

Tables

Table 1. Characteristics of the three groups (median, interquartile range).

	AD10 (n = 6)	SD10 (n = 6)	AD5 (n = 6)	<i>P</i> value
Sex (M/F)	0/6	1/5	1/5	
Age (month)	18 (12–24)	30 (24–36)	24 (24–36)	0.4423
Weight (kg)	9.3 (7.2–10.9)	11.3 (9.4–17.5)	9.55 (8–12.4)	0.5195
Propofol dose (mg/kg)	1 (1–2) ^a	0 (0–1)	2 (1.8–2) ^b	0.0079 ^{a-b}
Fentanyl dose for rescue analgesia (μg/kg)	1.5 (0–6)	0 (0–12)	1.5 (0–6)	1.0000
Anesthesia time (min)	80 (60–150)	150 (80–320)	152.5 (80–200)	0.0909

M, male; F, female; AD, atropine-dexmedetomidine; SD, saline-dexmedetomidine; Group AD10, dexmedetomidine 10 μg/kg, intramuscularly (IM) combined with atropine (0.02 mg/kg, IM); group SD10, dexmedetomidine (10 μg/kg, IM); group AD5, dexmedetomidine (5 μg/kg, IM) combined with atropine (0.02 mg/kg, IM); *: Significant differences from baseline (BL) ($P < 0.05$). Different letters indicate statistically significant differences. a-b: $P < 0.05$.

Table 2. Median (interquartile range) of heart rate, systolic blood pressure, mean blood pressure, diastolic blood pressure and rate pressure product at various time points in three groups.

	Group	BL	T0	T10	T20	T30	T45	T60
HR (beats/min)	AD10	117.5 (96-137)	122.5 (89-142)	127 (117-150)	120 (99-138)	100 (84-114)	96.5 (90-105)*	98 (89-98)
	SD10	113 (112-142)	76 (63-97)*	84 (73-94)	88.5 (60-96)	91 (74-95)	91.5 (79-95)*	84.5 (74-106)*
	AD5	116.5 (112-140)	116 (73-135)	109.5 (93-122)	120.5 (96-122)	108 (100-112)	94 (81-111)	91.5 (84-105)
SBP (mmHg)	AD10	149.5 (144-155)	143 (130-155)	129.5 (122-138)*	136.5 (112-150)	128 (119-147)	130 (119-138)*	130 (126-141)*
	SD10	150.5 (136-163)	138 (116-159)	129 (113-138)*	129.5 (117-142)	122 (115-138)*	111.5 (111-113)*	117 (114-120)*
	AD5	152 (144-159)	133.5 (126-156)	114.5 (110-118)*	108 (106-116)*	104.5 (102-110)*	107 (93-118)*	97.5 (84-112)*
MBP (mmHg)	AD10	108 (102-129)	117 (108-135)	100 (94-107)	104.5 (95-109)	102 (89-120)	101 (79-106)	106.5 (80-108)
	SD10	111 (102-123)	113.5 (96-126)	92.5 (75-106)	88.5 (78-103)	84.5 (81-95)	86 (83-89)*	83.5 (81-92)*
	AD5	114.5 (110-125)	105.5 (102-134)	91.5 (81-96)*	83.5 (78-87)*	80.5 (79-88)*	80.5 (72-98)*	75.5 (62-86)*
DBP (mmHg)	AD10	86 (75-113)	103.5 (82-117)	83.5 (76-92)	91 (84-97)	91 (85-103)	86.5 (79-96)	93 (76-94)
	SD10	84.5 (80-99)	98.5 (82-106)	73 (60-84)	68 (64-86)	69.5 (60-81)	72 (61-76) ^b	70 (61-76)
	AD5	93 (85-102)	89.5 (85-119)	76.5 (60-81)	67.5 (57-70)	63.5(58-64)*	64.5 (58-75)	61 (50-72)*
RPP (mmHg×beats/min)	AD10	16,768 (14,880-19,728)	17,321 (12,240-21,390)	16,073 (14,602-18,788) ^a	15,471 (14,541-17,802)	12,723 (12,054-14,098)*	12,682.5 (11,448-13,110)*	12,742.5 (10,486-14,210)*
	SD10	19,015 (15,120-22,720)	11,479 (7,308-11,834)*	10,624 (10,439-11,375)* ^b	10,442.5 (9,204-13,632)*	11,397 (10,465-12,220)*	10,267.5 (8,927-11,009)*	9,926 (8,584-11,310)*
	AD5	17,544 (15,820-20,440)	15,034.5 (9,344-19,311)	11,696 (11,102-12,650)*	12,085 (10,208-13,420)*	10,875 (9,990-12,320)*	9,133.5 (8,505-12,099)*	8,787.5 (8,134-9,072)*

HR, heart rate; SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure; RPP, rate pressure product. *: Significant differences from baseline (BL) ($P < 0.05$). Different letters indicate statistically significant differences. a-b: $P < 0.05$. The value without letter means no significant difference.

Table 3. Median (interquartile range) of cardiac troponin I (cTnI) and lactate levels at various time points in the three groups.

	BL	6hr	12hr	24hr	48hr	72hr
cTnI						
AD10	0.02 (0.01-0.02)	0.07 (0.02-0.12) ^{*,a}	0.06 (0.03-0.10) ^{*,a}	0.04 (0.03-0.07) [*]	0.03 (0.02-0.03)	0.02 (0.01-0.03)
SD10	0.02 (0.00-0.02)	0.01 (0.00-0.01) ^b	0.01 (0.01-0.02) ^b	0.03 (0.01-0.04)	0.01 (0.01-0.02)	0.02 (0.01-0.02)
AD5	0.01 (0.01-0.02)	0.01 (0.00-0.02)	0.02 (0.01-0.02) ^b	0.02 (0.01-0.03)	0.02 (0.01-0.02)	0.02 (0.01-0.03)
Lactate						
AD10	1.0 (1.0–2.2)	1.1 (0.5–1.5)	1.4 (1.2–1.7)	1.5 (1.2–1.6)	1.4 (1.0–1.7)	1.3 (1.2–1.8)
SD10	1.6 (1.5–1.7)	1.5 (1.2–1.7)	1.5 (1.3–2.0)	1.4 (0.7–1.6)	1.0 (1.0–1.9)	1.2 (0.9–1.5)
AD5	1.5 (1.1–1.6)	2.2 (1.7–3.1)	0.9 (0.8–1.5)	1.2 (1.0–1.4)	1.4 (1.2–1.5)	1.1 (1.0–1.1)

BL, baseline; 6hr, 6 hr after premedication; 12hr, 12 hr after premedication; 24hr, 24 hr after premedication; 48hr, 48 hr after premedication; 72hr, 72 hr after premedication; *: Significant differences from baseline (BL) ($P < 0.05$). Different letters indicate statistically significant differences. a-b: $P < 0.05$. The value without letter means no significant difference.

Fig. 1.

Fig. 2.

Fig. 3.