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Effects of general anaesthesia in dorsal recumbency with and without vatinoxan on bronchoalveolar lavage cytology of healthy horses

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ABSTRACT

Pneumonia is one of the potential complications of general anaesthesia in horses. Anaesthesia is known to increase neutrophils in bronchoalveolar lavage fluid (BALF) of horses after lateral recumbency, but studies after dorsal recumbency are lacking. Our primary aim was to determine when lung inflammation reaches its maximum and how rapidly BALF cytology returns to baseline after anaesthesia in dorsal recumbency. A secondary aim was to investigate the possible effect of vatinoxan, a novel drug, on the BALF cytology results.

Six healthy experimental horses were enrolled in this observational crossover study. The horses were subject to repeated BALF and blood sampling for 7 days after general anaesthesia with two treatment protocols, and without anaesthesia (control). During the two treatments, the horses received either medetomidine-vatinoxan or medetomidine-placebo as premedication, and anaesthesia was induced with ketamine-midazolam and maintained with isoflurane for 1 h in dorsal recumbency. The differences in BALF and blood variables between the two anaesthesia protocols and control were analysed with repeated measures analysis of variance models.

In this study, anaesthesia in dorsal recumbency resulted in no clinically relevant changes in airway cytology that could be differentiated from the effect of repeated BALF sampling. No differences in BALF matrix metalloproteinase gelatinolytic activity could be detected between the two treatments or the control series. Marked increase in serum amyloid A was detected in some animals. Vatinoxan as premedication did not consistently affect lung cytology or blood inflammatory markers after anaesthesia.
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Introduction

The complication rate in equine general anaesthesia is considered high compared with that in several other species (Johnston et al., 2002; Brodbelt, 2009). Postanaesthetic pneumonia has been described as one of the potential complications of general anaesthesia in horses (Grubb and Muir, 2005; Monticelli and Adami, 2019). In humans, pre-existing pulmonary disease, advanced age of the patient, atelectasis during anaesthesia, and aspiration of gastric contents have been considered as predisposing factors for postanaesthetic pneumonia (Ephgrave et al., 1993; Fujita and Sakurai, 1995; Kozlow et al., 2003). In horses, general anaesthesia causes atelectasis and gas-exchange impairment in the lungs (Nyman et al., 1990). Furthermore, horses are often subjected to mechanical ventilation during general anaesthesia, which can cause trauma and inflammation, as reported in other species (Dos Santos and Slutsky, 2000). However, the effects of general anaesthesia on inflammatory responses in the lungs and on systemic inflammatory markers, such as serum amyloid A (SAA) are poorly understood in horses.

In healthy horses anaesthetised in lateral recumbency, neutrophils increase in the epithelial lining fluid in the dependent lung (Ito et al., 2003), but it is not known what level and duration of lower-airway neutrophilia can be expected after anaesthesia in dorsal recumbency. This information is warranted in clinical practice, when early differentiation of bacterial pneumonia from other causes of post-anaesthetic fever is crucial.

In horses, α_2-adrenoceptor agonists such as medetomidine are used as pre-anaesthetic agents and in constant-rate infusions in general anaesthesia. Vatinoxan (previously MK-467 and L-659,066) is an α_2-adrenoceptor antagonist that poorly penetrates the blood-brain barrier and therefore selectively targets peripherally located α_2-adrenoceptors (Clineschmidt et al., 1988). Combining vatinoxan with an α_2-adrenoceptor agonist improved

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arterial oxygen tension (PaO_2) in anaesthetised sheep (Adam et al., 2018) and horses (Pakkanen et al., 2015), in addition to its cardiovascular effects. In sheep, vatinoxan reduced dexmedetomidine-induced bronchoconstriction and pulmonary oedema (Adam et al., 2018). However, in horses, the mechanism leading to improvement in PaO_2 is currently unknown. Vatinoxan also alleviates the reduction in gastrointestinal motility induced by α_2-adrenoceptor agonists in horses (Vainionpää et al., 2013; de Vries et al., 2016; Tapio et al., 2018), which may improve oesophageal tonus and reduce the risk of aspiration of gastric fluids during general anaesthesia and the recovery period.

The primary aim of this study was to investigate the effect of general anaesthesia in dorsal recumbency on equine lower airway cytology and selected inflammatory biomarkers in clinically healthy horses, and to evaluate how rapidly the cytology recovers after anaesthesia. A secondary aim was to assess the possible effect of vatinoxan on airway cytology. We hypothesised that anaesthesia would induce a transient increase in the percentage of neutrophils in bronchoalveolar lavage fluid (BALF) and that vatinoxan would reduce atelectasis or potential micro-aspiration during general anaesthesia resulting in reduced lung inflammation after anaesthesia.

Materials and methods

Study design

In this observational crossover study, three series of BALF sampling were performed in each horse. Two of the series were performed after two different anaesthetic protocols (treatments) and one as a control series without anaesthesia. Each horse was anaesthetised twice for 1 h in dorsal recumbency using medetomidine-vatinoxan (MED + V) or medetomidine-placebo (MED) as premedication. The order of the anaesthetic protocols was randomised with a two-treatment paired crossover Latin-square design. A minimum of 2-week washout period was used between both treatments and control BALF sampling series.

Animals

Six experimental horses (age range 5–19 years, median 10 years), three mares and three geldings, four Standardbreds and two Warmbloods, median body mass of 521 kg (range 476–571 kg) were used. The horses were considered clinically healthy based on clinical examination, blood sample results (haematology and serum biochemistry) and the lack of clinical respiratory signs. During the study period, physical examination of the horses was performed daily, and possible signs of cough, nasal discharge or other abnormal signs were recorded. The study was approved by the Committee for Experimental Animals of Southern Finland (Approval number ESAVI/4789/04.10.07/2014; Approval date 19 June 2014).

Anaesthetic protocol

The horses were not fed for 12 h prior to each anaesthesia. A venous catheter was placed into the left jugular vein (Intraflon, Kruuse). Ten min before induction, all horses received 7 μg/kg medetomidine (Dorbene, Vetcare) intravenously (IV) either with 140 μg/kg vatinoxan (Vetcare; MED + V) or with an equal volume of saline placebo (MED). Premedications were mixed in the same syringe. Anaesthesia was induced with 2.2 mg/kg ketamine (Ketaminol, Intervet) and 0.06 mg/kg midazolam (Midazolam Hameln, Hameln Pharmaceuticals) mixed in the same syringe and administered IV. After intubation, the horses were positioned in dorsal recumbency and connected to the breathing system (Tafonius, Hallowell). Anaesthesia was maintained with $3.5\ \mu g\ kg^{-1}\ h^{-1}$ medetomidine infusion and isoflurane (Isoba, Schering-Plough) in 100% oxygen. The lungs were mechanically ventilated (7 times per minute, 1.2 L/100 kg), resulting in positive inspiratory pressures of 16–26 mmHg). Dobutamine was infused when needed to maintain the mean arterial blood pressure above 70 mmHg. The horses recovered in left lateral recumbency.

Bronchoscopy and airway sampling

Bronchoscopy was performed and BALF samples collected 1 h, 20 h, 2 days, 3 days and 7 days after anaesthesia as described previously (Couëtil and Hawkins, 2013) with a 220 cm-long, 11 mm-diameter video endoscope (Pentax Medical). For bronchoalveolar lavage (BAL), 240 mL of sterile 0.9% saline at room temperature per lung was used. To decrease coughing, 60 mL of 1% lidocaine (Lidocain, Orion Pharma) was injected onto the carina and mainstem bronchi. Syringes with the retrieved BALF samples were immediately placed on ice and submitted for laboratory analysis within 30 min of collection. The samples were considered adequate when they contained a foamy surfactant layer. No samples had to be discarded due to inadequate quality. Observational bronchoscopy findings before sampling were recorded. Similar series of BALF samples (control) were obtained from the same horses without anaesthesia a minimum of 2 weeks before the first anaesthesia (3/6 horses) or 2 weeks after the second anaesthesia (3/6 horses). Cytological analysis of the BALF samples was performed, as described previously (Rossi et al., 2017). Reference values for the BALF cell types were obtained from the American College of Veterinary Internal Medicine revised consensus statement (Couëtil et al., 2016) and literature (Couëtil and Hawkins, 2013), with a neutrophil count of >5% considered abnormal. Matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9) combined gelatinolytic activity was measured in BALF from both lungs using the method described by Rossi et al. (2017).

Blood samples

An arterial blood sample was anaerobically collected for blood-gas analysis at the end of anaesthesia (Pico 50, Radiometer Medical). The sample was placed in ice water and analysed (ABL800 Flex; Radiometer Medical) within 10 min, and the values were temperature-corrected to concurrent measurements of rectal temperature. Ethylenediaminetetraacetic acid (EDTA) plasma and serum samples were collected 24 h before each anaesthesia (Pre-ane) and 20 h, 2 days, 3 days and 7 days after anaesthesia from the jugular vein, and analysed within 1 h after collection. The SAA concentration was measured with an immunoturbidometric assay (Eiken SAA LZ, Eiken Chemical). Serum activity of aspartate aminotransferase (AST) and creatine kinase (CK) were measured with an automatic clinical chemistry analyser (Konelab 30i, Thermo Fischer Scientific). The total leukocyte count was determined (ADVIA 2120i Haematology System, Siemens Healthcare Diagnostics) and the fibrinogen concentration was measured with the heat precipitation method.

Statistical analysis

Considering BALF neutrophil percentage between treatments as the primary outcome and with an expected mean of the paired differences of 16 and an expected standard deviation of 8, a sample size of five horses was required to achieve a power of 80% and a level of significance of 5% (two sided).

The differences between the three treatments (control, MED and MED + V) in the change in the BALF variables from the baseline were analysed with repeated measures analysis of variance models. The models included treatment, time point, period and the interaction between the treatment and time point as fixed terms and the horse as the random factor. The baseline was defined as the first sample of the control series within each horse. The sequence effect was excluded from the models, because the randomisation was conducted only for the MED and MED + V treatments. The sides of the lung (right/left) were analysed separately.

The blood samples were drawn during the MED and MED + V treatments only; therefore, the corresponding variables were analysed assuming a 2 × 2–crossover design. The changes from baseline (within the period) were analysed with repeated measures analysis of covariance models. The models included baseline covariate, treatment, time point, sequence, period and treatment*time point as fixed terms. The horse effect (within the sequence) together with the interaction terms of treatment*horse (sequence) and time point*horse (sequence) were used as random terms in the models.

Spearman's rank correlation coefficients were calculated for SAA and neutrophil percentage or gelatinolytic activity in each lung. Due to the exploratory nature and small number of horses, no multiplicity adjustment methods were applied. Statistical analyses were performed using SAS System for Windows, version 9.3 (SAS Institute) and IBM SPSS statistical package version 22 (IBM). The differences were considered statistically significant at $P < 0.05$.

Results

The horses remained in good health during the study period, and none developed clinical signs of pneumonia (e.g. fever). All recoveries from anaesthesia were uneventful. However, one horse presented with cough on a single day during the washout period after the first anaesthesia (MED), and two horses presented with cough during the BALF sampling period after the MED + V anaesthesia. In all horses, bronchoscopy revealed serous fluid accumulation (from mild to marked) in the trachea at the level of the thoracic inlet, 1 h after both anaesthetic episodes. Fluid accumulation was no longer detected at later time points. The results of the cytological analysis of BALF during the three treatments are presented in Table 1 for the right lung and in Table 2 for the left lung. There were no consistent differences regarding cytology or the total gelatinolytic activity of MMP-2

Table 1
Mean ± standard deviation (SD) and range (min–max) bronchoalveolar lavage fluid (BALF) cytology and gelatin zymography results of the right lung at 1 h, 20 h, 2 days, 3 days and 7 days after general anaesthesia in dorsal recumbency with medetomidine-placebo (MED) and medetomidine-vatinoxan (MED + V) premedication and control.

		1 h	20 h	2 days	3 days	7 days
Neutrophils (%)	Control	3.2 ± 1.2 (1.4–4.7)	10.9 ± 8.5 (3.0–22.7)	10.4 ± 10.7 (2.7–31.7)	8.0 ± 4.8 (1.4–13.0)	3.9 ± 2.4 (1.4–7.4)
	MED	4.4 ± 3.6 (0.4–9.4)	9.3 ± 7.2 (1.4–18.0)	14.1 ± 13.8 (1.0–41.0)	13.4 ± 6.0 (5.7–20.0)	2.9 ± 1.7 (1.0–5.0)
	MED + V	4.1 ± 3.2 (1.0–9.7)	4.9 ± 2.7 (1.7–8.0)	5.2 ± 4.7[c] (0.4–12.4)	8.0 ± 3.8 (2.4–12.7)	3.8 ± 3.6 (0.0–10.4)
Eosinophils (%)	Control	0.2 ± 0.3 (0.0–0.7)	1.5 ± 2.9 (0.0–7.4)	0.3 ± 0.3 (0.0–0.7)	0.4 ± 0.3 (0.0–0.7)	0.3 ± 0.3 (0.0–0.7)
	MED	0.1 ± 0.3 (0.0–0.7)	0.5 ± 0.8[a] (0.0–2.0)	0.1 ± 0.3 (0.0–0.7)	0.4 ± 0.4 (0.0–1.0)	0.3 ± 0.3 (0.0–0.7)
	MED + V	0.1 ± 0.2 (0.0–0.4)	0.2 ± 0.3 (0.0–0.7)	0.3 ± 0.4 (0.0–1.0)	0.3 ± 0.3 (0.0–0.7)	0.0 ± 0.0 (0.0–0.4)
Mast cells (%)	Control	1.7 ± 0.6 (0.7–2.4)	1.3 ± 0.3 (1.0–1.7)	0.9 ± 0.8 (0.0–2.0)	1.5 ± 1.1 (0.4–3.4)	1.6 ± 1.2 (0.0–3.4)
	MED	2.3 ± 1.7 (0.7–5.4)	1.8 ± 0.5 (1.4–2.7)	2.0 ± 2.0 (0.0–5.0)	1.5 ± 1.1 (0.4–3.7)	2.3 ± 1.5 (0.7–4.0)
	MED + V	2.6 ± 2.1 (0.4–5.7)	2.1 ± 1.3 (0.7–4.0)	2.7 ± 3.4[b] (0.0–9.0)	1.7 ± 1.1 (0.7–3.7)	1.2 ± 0.6 (0.4–2.0)
Lymphocytes (%)	Control	56.6 ± 5.4 (49.0–62.7)	58.7 ± 15.5 (33.4–73.0)	58.3 ± 16.3 (40.0–76.4)	62.1 ± 11.0 (48.4–79.0)	52.3 ± 7.0 (44.7–63.0)
	MED	59.1 ± 6.6 (49.7–68.0)	66.1 ± 8.7 (55.7–78.0)	55.6 ± 9.3 (44.7–68.4)	60.7 ± 9.8 (42.0–68.7)	55.3 ± 10.2 (41.7–70.7)
	MED + V	56.8 ± 10.9 (39.7–72.4)	64.0 ± 7.0 (55.7–72.4)	65.5 ± 12.3 (48.7–79.4)	68.7 ± 9.9 (52.4–80.4)	58.2 ± 10.1 (43.0–69.0)
Macrophages (%)	Control	38.6 ± 6.0 (32.0–47.4)	28.0 ± 5.8 (21.4–37.7)	30.3 ± 14.4 (16.4–54.4)	28.2 ± 8.6 (16.4–41.0)	42.0 ± 9.1 (27.4–51.0)
	MED	33.3 ± 8.9 (23.4–46.4)	24.0 ± 6.1 (16.0–31.0)	28.0 ± 12.2 (13.7–43.7)	24.1 ± 9.7 (13.0–38.7)	39.4 ± 8.7 (27.7–53.0)
	MED + V	36.6 ± 14.1 (18.4–58.7)	28.8 ± 7.9 (20.4–40.7)	26.2 ± 12.3 (12.0–46.7)	21.6 ± 10.2 (14.4–39.4)	36.7 ± 10.9 (20.4–52.7)
Total gelatinolytic activity (arbitrary units)	Control	5.6 ± 4.0 (2.0–12.2)	26.1 ± 31.3 (3.7–88.0)	10.9 ± 10.7 (1.7–29.5)	13.8 ± 15.4 (3.4–44.6)	6.8 ± 9.2 (1.8–25.5)
	MED	6.8 ± 4.8 (2.0–12.9)	8.0 ± 7.2 (2.7–21.7)	19.3 ± 32.2 (1.7–84.8)	14.8 ± 12.0 (4.0–33.2)	3.7 ± 2.1 (1.9–7.7)
	MED + V	10.9 ± 10.4 (1.9–27.0)	7.1 ± 4.0 (1.2–10.5)	4.6 ± 3.1 (2.5–10.7)	6.9 ± 3.9 (3.1–12.9)	6.0 ± 8.6 (1.2–23.3)

[a] $P < 0.05$ between control and MED for change from common baseline.
[b] $P < 0.05$ between control and MED + V for change from common baseline.
[c] $P < 0.05$ between MED and MED + V for change from common baseline.

and -9 among the right or left lung sides during either anaesthesia or control.

After MED anaesthesia, the BALF neutrophil percentage increased in both lungs above the reference range in all horses and peaked in the right lung at 20 h in 2/6 horses, at 2 days in 1/6 horses and at 3 days in 3/6 horses. In the left lung, the neutrophil percentage peaked at 20 h in 1/6 horses, at 2 days in 3/6 horses and at 3 days in 2/6 horses. At 7 days after anaesthesia, all horses showed BALF neutrophil percentages within the reference range in the right lung and all horses except one (no. 2) showed normal BALF neutrophil percentages in the left lung. The overall increase in neutrophils from the baseline was significant in the right lung ($P = 0.014$).

Following the MED + V anaesthesia, the BALF neutrophil percentage increased in both lungs above the reference range in all horses except one and peaked in the right lung at 2 days in 1/5 horses and at 3 days in 4/5 horses. In the left lung, the neutrophil percentage peaked at 20 h in 2/5 horses, at 2 days in 1/5 horses, at 3 days in 1/5 horses and at 7 days in 1/5 horses. While one horse (no. 2) showed peak values at 7 days, all other horses showed normal cytology in both lungs 7 days after anaesthesia.

In the control series, the BALF neutrophil percentage increased above the reference range in the right lung in all horses and peaked at 20 h in 3/6 horses, at 2 days in 2/6 horses and at 3 days in 1/6 horses. In the left lung, the neutrophil percentage increased above the reference range in 3/6 horses, and peaked at 20 h in 1/3 horses, at 2 days in 1/3 horses and at 3 days in 1/3 horses. At day 7 in the control series, 2/6 horses showed BALF neutrophil percentage slightly above reference range in the right lung (horses no. 2, 4). All horses had normal neutrophil percentage in the left lung 7 days after anaesthesia.

The SAA concentration showed marked interindividual variation (Table 3), peaked in most horses at 2 days and returned to the reference range at 7 days in all horses except one (no. 2), which also showed the highest peak (923.9 mg/L). Two horses showed no increase in SAA above the reference limit of 20 mg/L (Jacobsen et al., 2006) after the MED anaesthesia. Overall, no statistically significant changes from baseline values or within any of the individual time points with either treatment were detected. The SAA concentration correlated slightly with the BALF neutrophil percentage in the right lung ($\rho = 0.324$, $P = 0.011$) and left lung ($\rho = 0.400$, $P = 0.001$) and also with the BALF total gelatinolytic activity of MMP-2 and -9 in the right lung ($\rho = 0.338$, $P = 0.008$) and left lung ($\rho = 0.487$, $P < 0.001$). At the end of anaesthesia, the PaO_2 was 250 ± 112 mmHg (range 133–386; MED) and 260 ± 118 mmHg (range 109–420; MED + V), and there was no significant difference between the two anaesthetic protocols. Serum muscle enzyme activities (AST and CK) were within the reference range at all time points in all horses.

Discussion

In this study, we primarily investigated the effect of general anaesthesia in dorsal recumbency on equine lower airway cytology

Table 2
Mean ± standard deviation (SD) and range (min–max) bronchoalveolar lavage fluid (BALF) cytology and gelatin zymography results of the left lung at 1 h, 20 h, 2 days, 3 days and 7 days after general anaesthesia in dorsal recumbency with medetomidine-placebo (MED) and medetomidine-vatinoxan (MED + V) premedication and control.

		1 h	20 h	2 days	3 days	7 days
Neutrophils (%)	Control	4.4 ± 3.1 (1.0–9.0)	4.5 ± 4.0 (1.0–9.4)	5.4 ± 5.4 (1.4–16.0)	3.1 ± 3.8 (0.7–10.7)	2.0 ± 1.8 (0.0–4.0)
	MED	5.5 ± 3.9 (1.0–11.7)	10.5 ± 11.0 (2.4–29.4)	10.2 ± 9.1 (1.4–24.4)	11.8 ± 12.2[a] (2.4–35.4)	4.2 ± 3.9 (1.7–12.0)
	MED + V	4.0 ± 3.0 (2.0–10.0)	7.4 ± 6.2 (1.7–15.4)	10.0 ± 15.1 (2.0–40.7)	4.0 ± 1.9[c] (1.0–6.0)	3.4 ± 3.8 (0.7–10.7)
Eosinophils (%)	Control	0.3 ± 0.2 (0.0–0.4)	0.5 ± 0.7 (0.0–1.7)	0.4 ± 0.4 (0.0–1.0)	0.4 ± 0.3 (0.0–0.7)	0.0 ± 0.0 (0.0–0.0)
	MED	0.4 ± 0.4 (0.0–0.7)	0.2 ± 0.3[a] (0.0–0.7)	0.1 ± 0.3[a] (0.0–0.7)	0.1 ± 0.2[a] (0.0–0.4)	0.2 ± 0.2 (0.0–0.4)
	MED + V	0.4 ± 0.4 (0.0–1.0)	0.5 ± 0.6[c] (0.0–1.4)	0.2 ± 0.3 (0.0–0.7)	0.1 ± 0.2[b] (0.0–0.4)	0.3 ± 0.2 (0.0–0.4)
Mast cells (%)	Control	2.0 ± 0.8 (1.0–3.0)	2.2 ± 1.1 (1.4–4.4)	1.8 ± 0.8 (0.4–2.7)	3.5 ± 2.3 (1.4–7.0)	1.7 ± 0.9 (0.7–2.7)
	MED	3.0 ± 1.9 (1.0–5.7)	3.0 ± 1.1 (1.4–4.4)	1.7 ± 1.0 (1.0–3.4)	2.4 ± 1.7 (1.0–5.4)	1.8 ± 1.0 (0.7–3.0)
	MED + V	1.6 ± 0.8[c] (1.0–2.7)	1.6 ± 1.5[c] (0.0–4.4)	1.7 ± 0.3 (1.4–2.0)	2.8 ± 1.6 (1.0–5.4)	2.1 ± 0.9 (1.4–3.7)
Lymphocytes (%)	Control	54.7 ± 7.3 (44.0–65.0)	54.7 ± 8.9 (45.4–68.0)	63.7 ± 7.3 (51.4–71.4)	55.1 ± 11.8 (42.7–71.7)	56.8 ± 8.5 (45.4–68.4)
	MED	62.3 ± 5.5 (58.0–71.7)	55.3 ± 10.4 (36.0–63.4)	58.0 ± 2.7 (54.7–61.4)	58.8 ± 9.5 (46.7–73.0)	57.9 ± 12.1 (36.0–69.7)
	MED + V	63.4 ± 9.1[b] (45.7–70.7)	64.3 ± 10.4[b,c] (49.7–75.7)	55.0 ± 15.4 (32.0–68.0)	63.3 ± 4.1[b] (59.7–70.4)	52.3 ± 12.0[d] (38.7–68.7)
Macrophages (%)	Control	38.8 ± 5.3 (32.4–43.7)	39.4 ± 9.4 (28.4–50.7)	28.9 ± 10.4 (15.4–44.7)	38.1 ± 12.4 (21.4–52.7)	39.7 ± 7.3 (29.7–49.4)
	MED	28.7 ± 6.3[a] (22.4–37.7)	31.2 ± 9.1[a] (17.0–43.4)	30.1 ± 9.8 (13.4–40.0)	27.1 ± 10.1[a] (12.5–38.4)	36.1 ± 12.8 (23.7–58.7)
	MED + V	30.7 ± 10.1[b] (19.7–49.5)	26.0 ± 4.9[b] (20.4–33.4)	33.0 ± 15.4 (17.0–62.0)	29.8 ± 3.7[b] (22.7–33.4)	42.0 ± 10.9[d] (29.4–56.0)
Total gelatinolytic activity (arbitrary units)	Control	3.0 ± 1.4 (1.5–5.6)	8.3 ± 7.4 (3.7–23.3)	7.8 ± 6.9 (2.6–21.1)	4.2 ± 1.9 (1.3–6.1)	4.0 ± 3.5 (1.3–11.0)
	MED	7.2 ± 5.0 (0.9–12.8)	8.3 ± 8.2 (0.0–22.5)	10.3 ± 11.2 (0.9–28.3)	10.8 ± 10.5 (0.0–25.7)	5.1 ± 5.7 (1.5–16.4)
	MED + V	5.6 ± 4.1 (2.2–13.3)	10.1 ± 9.7 (1.7–24.1)	6.9 ± 5.6 (1.5–17.2)	7.8 ± 8.3 (2.2–24.0)	5.0 ± 5.6 (1.5–16.2)

[a] $P < 0.05$ between control and MED for change from common baseline.
[b] $P < 0.05$ between control and MED + V for change from common baseline.
[c] $P < 0.05$ between MED and MED + V for change from common baseline.
[d] $P < 0.05$ between 1 h and 7 days within treatment.

Table 3
Mean ± standard deviation (SD) and range (min–max) total blood leukocyte count, fibrinogen and serum amyloid A (SAA) concentration 24 h before the general anaesthesia (Pre-ane) and 20 h, 2 days, 3 days and 7 days after general anaesthesia in dorsal recumbency with medetomidine-placebo (MED) and medetomidine-vatinoxan (MED + V) premedication.

	Pre-ane		20 h	2 days	3 days	7 days
Leukocyte count ($\times 10^9$/L)	7.0 ± 1.7 (5.7–9.3)	MED	9.4 ± 2.0[a] (6.8–11.9)	8.3 ± 1.3 (6.0–9.7)	8.3 ± 1.8 (6.3–11.3)	7.1 ± 1.3 (5.0–9.4)
	6.7 ± 1.2 (4.9–8.0)	MED + V	10.4 ± 2.0 [a,b] (8.3–13.3)	8.9 ± 1.4[a] (7.2–10.6)	7.6 ± 1.2 (6.5–9.7)	6.7 ± 1.5 (5.3–8.8)
Fibrinogen (g/L)	3.4 ± 0.5 (2.8–4.1)	MED	3.9 ± 0.4 (3.4–4.4)	4.1 ± 0.5[a] (3.3–4.6)	4.2 ± 0.6[a] (3.5–4.8)	3.8 ± 0.7 (3.0–4.7)
	3.2 ± 0.4 (2.6–3.6)	MED + V	3.8 ± 0.4[a] (3.1–4.2)	3.8 ± 0.5[a] (3.4–4.7)	4.0 ± 0.4[a] (3.7–4.7)	3.8 ± 0.4 (3.5–4.5)
SAA (mg/L)	0.3 ± 0.7 (0.0–1.7)	MED	83.4 ± 117.8 (0.5–246.8)	212.1 ± 369.5 (0.7–923.9)	191.1 ± 332.9 (0.5–817.8)	27.4 ± 63.0 (0.3–155.8)
	0.5 ± 0.7 (0.0–1.7)	MED + V	131.9 ± 82.8 (24.3–221.7)	207.1 ± 175.0[a] (20.3–476.0)	134.3 ± 121.3 (1.4–259.7)	4.0 ± 8.0 (0.0–20.2)

[a] $P < 0.05$ for change from Pre-ane within treatment.
[b] $P < 0.05$ between MED and MED + V.

in clinically healthy horses. General anaesthesia did not cause neutrophilia in the lungs since no consistent, clinically relevant differences were detected in BALF cytology between the control series and general anaesthesia. However, neutrophils increased in the BALF after both anaesthetic episodes, as well as in the control series, and returned to baseline within 7 days in most horses. These findings suggest that the increase in neutrophils was likely due to the effect of repeated BALF sampling and not anaesthesia. This is in contrast to previous reports in which repeated sampling did not increase the neutrophil counts (Clark et al., 1995; Tee et al., 2012). On the other hand, Sweeney et al. (1994) demonstrated that BALF sampling caused a local infiltration of neutrophils to the sampling site that persisted at least 48 h, although it did not cause a diffuse inflammation when the airway adjacent to the first BAL site was sampled. Therefore, the increase in the relative neutrophil counts in this study observed in the control, MED and MED + V sampling

series may have been due to repeated sampling of the same part of the distal airway, which could have possibly been avoided by sampling the adjacent bronchus of the previously sampled airway. However, in humans, neutrophil recruitment has also been observed in adjacent bronchi after BALF sampling (Von Essen et al., 1991). In the present study, lidocaine was used to reduce coughing during BALF sampling in both treatments and the control group. The use of lidocaine is unlikely to affect the quantity or quality of BALF cytology results in horses (Westermann et al., 2005), and therefore should not have affected the conclusions in this study.

Ito et al. (2003) demonstrated that during an anesthetic episode of 2 h in lateral recumbency in healthy horses, neutrophils increase in the BALF of the dependent lung and return to normal within 7 days. The increase was interpreted to be mostly due to compression atelectasis of the dependent lung, since no changes were detected in the other lung (Ito et al., 2003). In our study, the horses were placed in dorsal recumbency, which causes atelectasis to both dorsal lung fields (Nyman et al., 1990). This could explain why no consistent or clinically relevant differences could be found between right and left lung cytology. On the other hand, general anaesthesia itself did not cause a significant increase in neutrophils in comparison to the control series. This discrepancy between our results and the study by Ito et al. (2003) might be caused by a shorter anaesthetic time (1 h) in our experiment, and consequently, a shorter duration of atelectasis. Moreover, Ito et al. (2003) did not use controls to study the possible effect of repeated BALF sampling which might have contributed to the BALF neutrophilia detected in that study.

Our results showed large individual variation in BALF cytology in healthy horses, which did not develop signs of pneumonia after general anaesthesia. However, some horses presented with occasional coughing during the BALF sampling series. In the absence of other clinical signs or significant increases in fibrinogen or body temperature, this was considered to be associated with the local irritation of airways as a consequence of repeated sampling and not pneumonia. However, neither tracheal fluid microbiology nor lung ultrasonography or radiography were performed to confirm the absence of pneumonia. Pneumonia or pleuropneumonia in horses often causes changes in the airway cytology, although Rossier et al. (1991) reported that only 10/22 horses with pneumonia or pleuropneumonia showed abnormal BALF cytology, including an increase in absolute or relative number of neutrophils. This highlights the fact that it is not possible to diagnose pneumonia based solely on BALF cytology.

In all horses, a mild to marked serous fluid accumulation was detected in the trachea but not in the mainstem bronchi shortly after anaesthesia, and this disappeared within 1 day. The origin of the fluid remains unclear; however, it may have originated from the upper airways and trachea due to local mucous membrane irritation and congestion, because oedema fluid originating from the alveoli would probably have been frothy (Kollias-Baker et al., 1993; Ball and Trim, 1996; Kaartinen et al., 2010).

As a secondary aim, we investigated the possible effect of vatinoxan on airway inflammation and cytology, but we did not find significant differences between the two anaesthesia protocols. We hypothesised that vatinoxan would reduce atelectasis or potential micro-aspiration during general anaesthesia resulting in reduced lung inflammation after anaesthesia. Although macro-aspiration of gastric contents has been reported as a peri-anaesthetic complication in horses with colic (Monticelli and Adami, 2019), there is currently no evidence whether micro-aspiration of gastric contents can occur during general anaesthesia in horses similarly to people (Ephgrave et al., 1993). In our study, the horses did not develop marked lung inflammation related to anaesthesia, and therefore macro- or micro-aspiration seems unlikely in these horses, which might explain why no effect of vatinoxan on the airway cytology was observed. Moreover, in this study, vatinoxan did not affect the PaO_2 at the end of either anaesthetic protocol, when atelectasis was expected to peak. Pakkanen et al. (2015) found that PaO_2 was higher at 40 min of anaesthesia in horses anaesthetised with vatinoxan as part of the premedication protocol, but the difference was no longer found at 60 min, which is in agreement with our results. Therefore, it is possible that vatinoxan affects lung function earlier in the anaesthetic episode, although in our study, no difference was detected in lung inflammation after either anaesthetic protocol.

SAA is a major acute-phase protein and may increase in several conditions, including various inflammatory and infectious diseases and surgical trauma in horses (Pepys et al., 1989). In our study, some of the horses showed marked elevations in SAA after both anaesthetic episodes, in contrast to others presenting almost no or only mild elevation. Furthermore, a significant but clinically irrelevant increase was also detected in plasma fibrinogen concentration in some horses. SAA was weakly correlated with both neutrophil percentage and total gelatinolytic activity of MMP-2 and -9 in BALF. SAA was also measured during the control series in four horses where it remained in the reference range. Unfortunately, due to technical problems, SAA was not measured during the control series in two horses, which would have allowed statistical comparison with the concentration after anaesthesia. Nevertheless, marked interindividual variation in the SAA response to general anaesthesia was observed. Further studies are warranted to explain the wide variation detected and to establish reference ranges for SAA in horses after anaesthesia, which could aid in the early detection of post-anaesthetic pneumonia or other infections.

Several limitations of the study need to be pointed out. The number of horses was calculated to be sufficient to detect clinically significant changes in cytology. However, less variation was expected in the results, and therefore the number of animals may have been too small to identify clinically relevant changes. One of the six horses (no. 2) showed marked neutrophilia both in the control series and after both anaesthetic episodes. This horse also showed the most substantial SAA increase and failure of normalising SAA at 7 days. The marked neutrophilia and increase in SAA detected in this horse may suggest the presence of an underlying subclinical problem, such as mild asthma or other inflammatory or even infectious disease, although the physical examination and haematology screening before anaesthesia were normal. Moreover, other extrapulmonary causes for an increase in SAA after anaesthesia are possible, including myopathy due to hypotension or rough recovery or any other inflammation or infection. However, in this study, myopathy due to hypotension seems an unlikely reason for the increase in SAA since the muscle enzyme activities remained within the reference ranges, and rough recoveries were not detected.

The control series BALF collection was performed before both anaesthetic episodes in half of the horses and after a 2-week washout period following the second anaesthesia in the other half. Theoretically, the anaesthetic episodes could have caused a subclinical inflammation in the lungs of these three horses that may have affected the control series results. However, the neutrophils were within the reference range in all of these horses in the first sample of the control series. The first sample of the control series was used as a baseline for statistical comparison in all treatments. The horses were also used for another study in which respiratory and cardiovascular parameters were measured during anaesthesia (Tapio et al., 2019), and therefore, we were unable to obtain baseline BALF samples prior to general anaesthesia for the purpose of not affecting lung function during anaesthesia. The baseline may have changed over time; however, the total study period including both anaesthetic episodes and all three BALF sampling series was short. In our patient population, typical duration of anaesthesia for common procedures (e.g. castration) is approximately 1 h,

and therefore this duration was set as the length of the anaesthesia. However, longer duration of anaesthesia might have led to different results regarding BALF and blood variables.

Conclusions

In this study, anaesthesia in dorsal recumbency caused no clinically significant changes in airway cytology that could be differentiated from the effect of repeated BALF sampling. Inclusion of vatinoxan in the premedication did not result in clinically relevant effects on lung cytology after anaesthesia.

Conflict of interest statement

Vatinoxan was provided for this experiment by Vetcare Oy, Finland, which also provided financial support for the study expenses, materials and maintenance of the horses. Vetcare played no role in the study design, collection or analysis of the data or the decision to submit the manuscript for publication. None of the authors has any financial or personal relationships that could have inappropriately influenced or biased the content of the article.

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