

# Performance of the Parasympathetic Tone activity (PTA) index to predict mean arterial pressure variations in anaesthetized horses with different health conditions

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## Research article

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# Abstract

## Background

The parasympathetic tone activity (PTA) is an index based on heart rate variability recently developed in animals to assess their relative parasympathetic tone and their analgesia nociception balance. The present study aimed to evaluate the variation of PTA in anaesthetized horses according to haemodynamic status and health conditions and to determine the performance of dynamic variations of PTA ( $\Delta$ PTA) to predict mean arterial pressure (MAP) variations.

## Results

Thirty-nine client-horses admitted to the Veterinary Campus of Lyon were anaesthetized for elective or emergency surgery and divided into "Colic" and "Elective" groups. During anaesthesia, dobutamine was administered as treatment of hypotension (MAP < 60 mmHg). No significant variation of PTA and MAP were detected at steady-state and following cutaneous incision. PTA increased before each hypotension (+ 15% in Elective and + 11.4% in Colic group), conversely, the administration of dobutamine was accompanied by a significant decrease in PTA (-12.7% in Elective and - 9% in Colic group). Horses of the Colic group had lower PTA values than those in Elective group, whereas MAP didn't differ between groups. Globally, to predict a 10% increase in MAP,  $\Delta$ PTA performance was associated with: AUC ROC [95% CI] = 0.77 [0.70 to 0.83] ( $p < 0.0001$ ), with a sensitivity of 88.2% and a specificity of 57.7% for a threshold value of - 1%. Besides, to predict a 10% decrease in MAP,  $\Delta$ PTA performance was associated with: AUC ROC [95% CI] = 0.80 [0.73 to 0.85] ( $p < 0.0001$ ), with a sensitivity of 62.5% and a specificity of 94.6% for a threshold value of 25%.

## Conclusions

The PTA index in anaesthetized horses appears to be influenced by the haemodynamic status and the health condition. The shift toward lower PTA values in colic horses may potentially reflect a sympathetic tone predominance. Of clinical significance, a PTA increase of > 25% in 1 min showed fair performance to predict a MAP decrease of > 10% within 5 min but a decrease in PTA was poorly specific to predict an increase in MAP. Even though these results require further evaluation, this index may thus help to predict potential autonomic dysfunctions in sick animals.

## Background

The assessment of intra operative nociception remains a challenge for the veterinary anaesthetist. An appropriate analgesia nociception balance can provide haemodynamic stability for the anaesthetized patient, pain relief and prevent the effects of analgesic overdose [1]. Recently, similarly to human

medicine, the use of devices based on heart rate variability (HRV) has been proposed for veterinary purpose, including equine species [2].

Spectral analysis of HRV is a non-invasive objective technique that examines the beat-to-beat variations in heart rate (HR) and can characterize the autonomic nervous system activity [3]. Stress from various origin (including nociception, anxiety, aggression, etc...) can shift the autonomic balance towards a sympathetic nervous prevalence with a decrease in HRV [4]. HRV has been used as a diagnostic and prognostic tool in a variety of conditions including anaesthesia [5], aiming to optimize intraoperative haemodynamics [6]. It is commonly used to characterize the relative activity of the sympathetic and parasympathetic nervous systems in animals and humans [7].

Recently, a monitor has been launched to assess the sympathovagal balance in animals (PTA Monitor®; MDoloris Medical Systems, Lille, France). It displays an index called PTA (Parasympathetic Tone Activity), which is similar to the Analgesia Nociception Index (ANI), an index validated to detect nociception during anaesthesia and critical care in human patients. It is based on the analysis of HRV and reflects the relative parasympathetic tone and the sympathovagal balance of the patient [8].

The autonomic nervous system (ANS) is a major regulator of the cardiovascular system: it maintains internal physiologic homeostasis, but can be altered by a variety of perioperative factors, including anaesthetic drugs and clinical condition, with resulting haemodynamic changes [9] [10]. As horses are prone to develop pronounced cardiovascular alterations during general anaesthesia, it is probable that their sympathovagal balance can be modified during anaesthesia, which may interfere with HRV analysis. Alternately, apart from the evaluation of analgesia nociception balance, monitoring the sympathovagal balance during anaesthesia may provide useful information to early detect ANS dysfunction and optimize the cardiovascular support of the anaesthetized horse [11].

To our knowledge, no study has been carried out to evaluate the performance of the PTA index in anaesthetized horses. The present study aimed to evaluate the variation of PTA in anaesthetized horses according to different intraoperative and health conditions and to determine the performance of dynamic variations of PTA ( $\Delta$ PTA) to predict an increase or a decrease of MAP. We hypothesized that the PTA index would vary in conjunction with cardiovascular changes and could help to predict mean arterial pressure (MAP) changes in anaesthetized horses.

## Statistical analysis

Statistical analysis was performed using MedCalc® 12.1.4.0 (MedCalc Software®, Ostend, Belgium). The number of horses in each group was chosen arbitrarily. Normality of distribution was assessed using the Shapiro-Wilk test. Normal data were expressed as mean  $\pm$  standard deviation (SD) whereas skewed data were expressed as median and interquartile range [IQR]. Demographic data was compared between groups using Student's *t* test. An analysis of variance (ANOVA) for repeated measures was used to detect any significant variations of PTA and MAP within 1 min and 5 min at each time-point for both Colic and Elective groups. In case of significant variation, *post-hoc* Tukey multiple paired comparisons were

performed. Variations of PTA and MAP within time were compared between groups using two-way ANOVA with Bonferroni correction for post hoc analysis. The performance of  $\Delta$ PTA to predict an increase or decrease of MAP within 5 minutes after predefined time-points was assessed by calculation of the area under curve (AUC) of a receiver operating characteristic (ROC) curve using pooled data from the defined times. The threshold value showing the best sensitivity and specificity was determined using Youden index. A p-value < 0.05 was considered statistically significant.

## Results

### Animals

No significant difference in age, sex and weight was found between groups whereas the total surgical time was significantly longer in Colic group ( $p = 0.003$ ) (Table 1).

Table 1  
Demographic data

	n (horses)	Gender	Age (years)	Weight (kg)	Total length of surgery (min)
Elective	24	8 G, 3 S, 4 M	7 ± 8	448 ± 107	121 ± 42
Colic	15	3 G, 15 S, 6 M	10 ± 6	505 ± 105	176 ± 74*
G, gelding; S, stallion; M, mare.					
* indicates a significant difference ( $p < 0.05$ ) between Elective and Colic group.					

Colic group (24 horses) included horses admitted for colic surgery, whereas Elective group (15 horses) comprised healthy horses admitted for elective surgery. Elective surgeries in the latter group consisted of castration ( $n = 9$ ), cutaneous surgery (sarcoïdosis  $n = 3$ , epidermoid carcinoma  $n = 1$ ), abdominal hernia ( $n = 2$ ). Among horses of Colic group, nineteen horses recovered from anaesthesia and five were euthanized prior to the end of surgery. All horses, except of 1 horse in Colic group, developed episodes of hypotension (MAP  $\leq 60$  mmHg) that required the administration of dobutamine.

## Pta And Map Evolution At The Predefined Time-points

### Variation of parameters for horses of the Elective group

For each predefined time-point of interest in the “Elective group”, the initial PTA and MAP as well as their evolution at 1 and 5 min thereafter are shown in Fig. 2.

During  $T_{SS}$  and  $T_{Cut}$ , no significant variation occurred for PTA or MAP.

During  $T_{\text{Pre-Hypo}}$ , no significant difference was observed between  $PTA_{1\text{min}}$  and  $PTA_0$ . However, at 5 min, a significant increase in PTA of 15% ( $p = 0.03$ ) was observed compared to  $PTA_0$ . In addition, the results showed a significant decrease in  $MAP_{1\text{min}}$  (-4%,  $p = 0.03$ ) and  $MAP_{5\text{min}}$  (-20%,  $p < 0.0001$ ) compared to  $MAP_0$ .

At  $T_{\text{Dobut}}$ , a decrease in PTA was observed 1 min after initiation of dobutamine (-12.7%,  $p = 0.08$ ), whereas  $PTA_{5\text{min}}$  did not vary compared to  $PTA_0$ . After dobutamine initiation, MAP significantly increased at 1 min (+20%,  $p = 0.009$ ) as well as at 5 min (+27%,  $p < 0.0001$ ).

After dobutamine discontinuation ( $T_{\text{Post-dobut}}$ ), no significant change occurred in PTA, whereas a decrease of 8% of MAP was noticed after 5 min ( $p = 0.002$ ).

### Variation of parameters for horses of the Colic group

For each surgical time-point of interest in the “Colic group”, the initial PTA and MAP as well as their evolution at 1 and 5 min thereafter are shown Fig. 3.

During  $T_{\text{SS}}$  and  $T_{\text{Cut}}$ , no significant difference was found neither in PTA or MAP, whereas a decrease of 8% compared to  $MAP_0$  was noticed for  $MAP_{5\text{min}}$  at  $T_{\text{SS}}$  ( $p = 0.059$ ).

At  $T_{\text{Pre-Hypo}}$ , an increase of PTA was noticed within 5 min (11.4%,  $p = 0.057$ ), whereas  $MAP_{5\text{min}}$  decreased significantly (-13%,  $p < 0.0001$ ).

At  $T_{\text{Dobut}}$ , a decrease in PTA was observed at 1 min (-9%,  $p = 0.07$ ) as well as at 5 min (-12.9%,  $p = 0.03$ ). MAP increased significantly at 1 min (8%,  $p < 0.0001$ ) as well as at 5 min (21%,  $p < 0.0001$ ) compared to  $MAP_0$ .

At  $T_{\text{Post-dobut}}$ , no difference in PTA was found whereas a significant decrease in MAP (-8%,  $p < 0.0001$ ) was found 5 minutes after dobutamine discontinuation.

## Elective vs Colic group

Figure 4 (a and b) compares PTA and MAP values at each predefined time-point between Elective group and Colic group. The PTA values were significantly lower in the Colic group compared to the Elective group (group effect,  $p = 0.001$ ) (Fig. 4, a). However, there was no significant PTA variations within time for both groups (time effect,  $p = 0.260$ ) and no interaction between time and groups (time by group effect,  $p = 0.598$ ) (Fig. 4, a).

There was no significant difference in MAP values at each time-point between groups (group effect,  $p = 0.719$ ) and no interaction between time and groups (time by group effect,  $p = 0.187$ ) (Fig. 4, b). Yet, in

both groups, MAP was shown to be significantly the lowest at the time of dobutamine administration ( $T_{\text{Dobut}}$ ) (time effect,  $p < 0.001$ ).

## Relationship between $\Delta$ PTA and $\Delta$ MAP at the predefined time-points of each group

The ROC analysis of the pooled data of  $\Delta$ PTA at each predefined time-points assumed to anticipate an increase or decrease in MAP was performed with the totality of horses. The  $\Delta$ PTA was associated with an AUC ROC [95% CI] of 0.77 [0.70 to 0.83] ( $p < 0.0001$ ), showing a fair performance to predict an increase of 10% in MAP with 88.2% sensitivity and 57.7% specificity for a threshold value of -1% (Fig. 5). On the other hand,  $\Delta$ PTA was associated with an AUC ROC [95% CI] of 0.80 [0.73 to 0.85] ( $p < 0.0001$ ), showing a fair performance to predict a decrease of 10% in MAP with 62.5% sensitivity 94.6% specificity for a threshold value of + 25% (Fig. 5).

## Discussion

This study describes the variations of the PTA index in anaesthetized horses according to haemodynamic variations and to their physical status. The main findings revealed significant variations of the PTA index during hypotension and administration of dobutamine, but no significant variation of the PTA following cutaneous incision. Horses of the Colic group demonstrated lower PTA values for several predefined time-points in comparison with those of the elective group, whereas MAP did not differ between groups. Finally, the PTA index showed a fair performance to predict MAP changes, in particular with high specificity of a 25% increase in PTA in 1 min to predict a 10% decrease in MAP with 5 min. However, although statistically significant, the threshold of 1% decrease in  $\Delta$ PTA to predict a 10% increase in MAP is not clinically relevant.

The analysis of heart rate variability (HRV) is a non-invasive method, which can detect the fluctuations in the autonomic input to the sinoatrial node and the activity of the individual components of the ANS [12]. To evaluate the ANS, HRV uses a frequency domain-based analysis [5]. Classically, three spectral domains are considered: the very low frequency (VLF) reflecting the peripheral vasomotor tone, thermoregulation and the rennin–angiotensin-system; the low frequency (LF) related to sympathetic and parasympathetic tones modulations; and the high frequency (HF) mainly associated with the parasympathetic tone and influenced by efferent vagal activity and respiratory sinus arrhythmia [13]. Compared to other methods such as stress hormone dosage, HRV is simpler, non-invasive and can provide continuous recording [14]. During anaesthesia, HRV has been used in humans for various purposes, in particular for the prediction of blood pressure variation [15], quality of recovery and the evaluation of analgesia nociception balance [16] [17]. However, data remain conflicting with some studies reporting a failure to detect sudden shifts in ANS activity before hypotension and during recovery [18]. The PTA index is similar to the ANI, validated in human medicine to detect perioperative nociception [17]. The ANI index has also been used to predict intraoperative haemodynamic reactions [19] and

hypotension caused by spinal anaesthesia [20], as well as a tool to investigate the process of emotional regulation in humans [23].

Hitherto, the use of HRV analysis in anaesthetized animal has been sparsely reported. Recently, the dynamic variation of the PTA index ( $\Delta$ PTA) has been evaluated in anaesthetized dogs, with a correct performance to predict haemodynamic reactivity associated with intraoperative nociceptive stimuli [2] [21], but to our knowledge, this index has not been evaluated in anaesthetized horse. In horses, HRV power spectrum has been reported to the power spectrum of humans, rats and pigs [5] with two main frequency ranges: a HF range set at 0.07–0.6 Hz and a LF range set at 0.01–0.07 Hz [14]. The normal resting horse is considered as having a prevailing parasympathetic tone, which was confirmed by HRV analysis [22]. A recent study in horses has reported that HRV is a sensitive and non-invasive method to detect sympathovagal stimulation during ocular surgery [14]. It has also been used as a prognostic information for postoperative horses with severe gastrointestinal disease, with a significantly better performance for predicting poor outcome than HR at admission [7]. However, HRV failed to demonstrate a correlation between preanaesthetic ECG variables and the recovery scores after general anaesthesia in horses [7].

Maintaining a stable cardiovascular function in the anaesthetized animal is often challenging particularly in large animals. In addition to the physical status and the recumbency, many drugs used in anaesthesia interfere with the cardiovascular system either directly or through their action on the ANS [23]. Inhaled anaesthetics can induce hypotension through peripheral vasodilation and depression of baroreceptor responsiveness,  $\alpha$ 2-agonists may promote hypotension through a decrease of cardiac output and heart rate following inhibition of the sympathetic pathway [24]. Conversely, intraoperative noxious stimulation may cause a shift toward sympathetic dominance [25].

In the present study, predefined time-points were chosen to allow a comparison between animals of different physical status undergoing different surgical procedures. The time-point  $T_{SS}$  was designed to evaluate the stability of the signal without any surgical stimulation, whereas  $T_{Cut}$  was designed to evaluate the potential influence of a nociceptive stimulation on PTA. The time-points  $T_{Pre-Hypo}$ ,  $T_{Dobut}$  and  $T_{Post-dobut}$  were designed to assess the influence of hypotension and administration of inotropes on the index, as these events may influence the sympathovagal balance [26].

At steady-state ( $T_{SS}$ ), the absence of significant difference within and between groups was expected, as no surgical or pharmacological stimulus was carried out during this time-point.

In comparison with previous results in dogs, no significant variation was registered at  $T_{Cut}$ , [2]. However, as no concomitant haemodynamic reaction was registered at this time-point, which is likely due to an adequate level of analgesia. This can be explained by the use of xylazine and morphine as premedicants. Xylazine mediates a sympatholytic action with a reported duration of action of 20 to 30 minutes [27], morphine has a reported plasma half-life of elimination of 1.6 hours [28]. This association most probably provided an appropriate analgesia at the time of cutaneous incision. No other nociceptive time-points

were selected as the main objective of the study was to compare the influence of the haemodynamic and health status on the index.

The variations of PTA observed during blood pressure variations appeared to be inversely related to those of arterial pressure, and thus, seem to follow modifications of the sympathovagal balance. During hypotension, the increase in PTA reflects a shift toward a parasympathetic predominance or a decrease of the sympathetic tone. Similar results have been reported in human medicine with the analgesia nociception index [29] [30]. This shift was blunted by dobutamine initiation ( $T_{\text{Dobut}}$ ) with a decrease in the PTA and a concomitant increase in blood pressure values noticed during this time-point. We assume that dobutamine administration, because of its sympathomimetic activity, caused a shift toward sympathetic predominance, as described after cardiac  $\beta_1$ -adrenergic receptors stimulation [31].

In general, lower PTA values were found in the horses of the Colic group, in comparison with those of the Elective group. This is in accordance with a presumed predominance of the sympathetic tone in Colic horses, associated with the stress response due to the critical condition. Moreover, autonomic dysfunctions are common during septic shock or associated disease. Similar findings have been reported in an experimental model of sepsis [32] and in human patients presented with endotoxaemia; the patients presented an uncoupling of autonomic nervous system and cardiovascular function leading to an impaired sympathetic modulation and maintenance of blood pressure [33]. Indeed, patients with autonomic dysfunction are described as presenting an ineffective baroreflex and impaired regulation of blood pressure, failing to compensate the anaesthetics-induced hypotension [6]. This dysfunction may potentially be explained in the septic patients by the endotoxins which contribute to a vasodilatory effect, a downregulation of the sympathetic myocardial responsiveness and an alteration of autonomic reflexes [11] [34]. Our findings confirm thereby a previous report where horses with gastrointestinal disease, especially with ischemic gastrointestinal lesions, had an increased sympathetic tone and a reduced HRV [7].

The variations of MAP at the different time-points did not differ horses of the colic group and those from the Elective group. These results are probably related to the blood-pressure directed therapy that was guided to optimize MAP and maintain a systemic haemodynamic pressure above 60 mmHg. An additional potential explanation could be attributed to the sympathetic activation associated with the early stages of sepsis [35].

The ROC analysis revealed a fair performance of the dynamic variation of PTA to predict a MAP variation, in particular a decrease in MAP following an increase in PTA. This result is, to some extent, in agreement with several human studies that reported a good performance of ANI to predict intraoperative haemodynamic reactivity and hypotension in human patients [8] [19]. However, other studies failed to show such a similar performance for the ANI [36].

We acknowledge several limitations for this study. Because of the small number of horses, this study was probably underpowered to show significant differences in PTA variations in Colic and Elective groups.

There was a lack of homogeneity in the inclusion criteria, leading to different surgical stimulations, and different intestinal lesions in the Colic group, which could have biased the homogeneity of PTA measurements. However, one main objective of the study was to assess the influence of health status on the PTA index, which had to be performed in a clinical setting. Thus, it was particularly challenging to obtain a homogeneity of the cases. In addition, several other factors may have influence the HRV analysis, including posture, medication, preoperative stress, noxious stimulation and different ages and breeds [5] [7] [37]. Even though surgical procedures with the same recumbency were chosen to limit the influence of the posture, the anaesthetic protocols were slightly different between group, with intravenous lidocaine that was added in the Colic group and acepromazine that was used for horses of the Elective group. As this study was performed in a clinical setting, it was first difficult to change the anaesthetic protocol routinely used by the clinicians, and we were unable to provide a reference technique in order to analyse HRV and validate our results. Further studies should be considered in a more standardized condition to evaluate the performance of the PTA index to anticipate nociception in horses.

## Conclusions

In the present study, the values of the PTA index were influenced by the health status of the animal, with emergency conditions associated with lower values, corresponding to higher sympathetic tone. Moreover, independently from the health status, intraoperative blood pressure variations were also associated with PTA variations. Dynamic variations of PTA showed fair performance to predict a decrease in MAP but the performance of  $\Delta$ PTA to predict an increase in MAP was not clinically relevant. These results are in accordance with the influence of the sympathovagal balance on HRV. Consequently, the PTA index may provide interesting information regarding the sympathovagal balance of the anaesthetized animal. This should be also considered for the interpretation of the PTA index when used for assessment of the analgesia nociception balance. Further studies are needed in particular to evaluate the effects of different intraoperative drugs on the PTA performance in horses, but also to assess if this index could serve as a prognosis factor with regard to critically ill animals.

## Methods

### Animals

After obtaining the institutional approval of the ethical committee of VetAgro Sup (n°1514) as well as the informed consent of the owners, this study prospectively enrolled thirty-nine client-owned horses (10 stallions, 17 geldings and 12 mares; mean age  $10 \pm 7$  years; mean body weight  $484 \pm 96$  kg). These horses were admitted to the Equine Hospital of VetAgro Sup (Veterinary Campus of Lyon, France) for elective surgery (castration and cutaneous surgery) or emergency colic surgery. Horses anaesthetized for elective surgery were determined to be healthy, based on preanaesthetic physical examination, and graded 1 and 2 on the American Society of Anaesthesiologists (ASA) physical status classification, whereas horses admitted for colic surgery were classified ASA 3 to 5 E.

Foals were not included in the study and horses requiring an intraoperative  $\alpha_2$ -agonists or ketamine continuous infusion or cardiopulmonary resuscitation were excluded.

The animals were assigned to two groups: horses requiring an emergency colic surgery were included in the “Colic group”, whereas those admitted for elective surgery were defined as “Elective group”. After the surgery, horses were hospitalized in the Equine Hospital of VetAgro Sup until full recovery.

## Anaesthetic protocol

Horses of the Elective group had free access to water and food was withheld for at least six hours before anaesthesia, whereas horses of Colic group had no food or water restriction before anaesthesia due to their emergency condition. After placement of a catheter into a jugular vein, horses from the Elective group were premedicated with acepromazine (Calmivet, Vetoquinol, Paris, France)  $0.03 \text{ mg kg}^{-1}$  intramuscularly (IM), followed 30 minutes later by a combination of  $0.6 \text{ mg kg}^{-1}$  of xylazine hydrochloride (Rompun™, Bayer, Lille, France) and  $0.1 \text{ mg kg}^{-1}$  of morphine (Morphine chlorhydrate, Aguettant, Lyon, France) intravenously (IV). Horses of the Colic group received  $0.4 \text{ mg kg}^{-1}$  of xylazine hydrochloride combined to  $0.1 \text{ mg kg}^{-1}$  of morphine IV as a premedication. Once sedation was achieved, anaesthesia was induced with  $2.2 \text{ mg kg}^{-1}$  of ketamine hydrochloride (Imalgene 1000, Merial, Lyon, France) and  $0.05 \text{ mg kg}^{-1}$  of diazepam (Diazepam TVM, TVM, Clermont-Ferrand, France) intravenously (IV).

After orotracheal intubation, horses were positioned in dorsal recumbency on the surgical table and anaesthesia was maintained in both groups with sevoflurane (SevoFlo, Zoetis, Malakoff, France) delivered in 60%  $\text{O}_2$  using a large animal rebreathing circuit (Tafonius; Vetronic Services Ltd, Abbotskerswell, UK). The horses were mechanically ventilated (Tafonius; Vetronic Services Ltd, Abbotskerswell, UK) with an initial respiratory rate of  $8 \text{ breaths minute}^{-1}$ , a tidal volume ( $\dot{V}_T$ ) of  $10 \text{ mL kg}^{-1}$ , adjusted to maintain a  $P_{E'}\text{CO}_2$  of 4.6 to 6.0 kPa (35–45 mmHg). Ringer lactate solution was administered IV during anaesthesia at a rate of  $10 \text{ mL kg}^{-1} \text{ h}^{-1}$ . Horses of the Colic group received a lidocaine (Lurocaine, Vetoquinol, Paris, France) infusion of  $0.05 \text{ mg kg}^{-1} \text{ min}^{-1}$  preceded by a loading dose of  $1.5 \text{ mg kg}^{-1}$  over 20 minutes. In case of a prolonged surgery, a supplementary bolus of morphine  $0.1 \text{ mg kg}^{-1}$  IM was given once intra-operatively, 2 hours after the initial dose. At the end of the anaesthesia, xylazine ( $0.1\text{--}0.2 \text{ mg kg}^{-1}$ ) IV was administered in every horse of the “Elective” group, and in horses of the Colic group according to the presence of early signs of excitation and / or consciousness. Horses were then transferred to a padded recovery box. After removal of the endotracheal tube, oxygen ( $15 \text{ L min}^{-1}$ ) was administered flow-by through a nasal tube during recovery. Horses of both groups received flunixin meglumine (Finadyne, MSD Santé animale, Beaucouzé, France) ( $1.1 \text{ mg kg}^{-1}$  IV) and antimicrobial agents adapted to the surgical condition.

## Monitoring

Heart rate (HR), invasive blood pressure, respiratory rate, end-tidal carbon dioxide tension ( $P_{E'}CO_2$ ), end-tidal oxygen tension ( $P_{E'}O_2$ ), end-tidal sevoflurane concentration ( $P_{E'}Sevo$ ), inspired oxygen fraction ( $FiO_2$ ) and oxygen saturation of haemoglobin ( $SpO_2$ ) were measured continuously using a multi-parameter monitor (Tafonius; Vetronic Services Ltd, Abbotskerswell, UK) and recorded manually every 5 minutes. Arterial blood samples were taken from the facial artery at 1-hour intervals to determine blood gas values (VetStat analyzer, Idexx, Hoofddorp, The Netherlands). The urinary bladder was catheterized for passive urine collection until the end of anaesthesia. The PTA index was monitored continuously during anaesthesia using a dedicated monitor (Physiodoloris®, MDoloris Medical System, Lille, France).

Signs of anaesthetic depth were monitored every 5 minute, and presence or absence of spontaneous palpebral reflex and nystagmus was recorded, as well as skeletal muscle relaxation. Ketamine  $0.5 \text{ mg kg}^{-1}$  IV was injected in case of signs of insufficient depth anaesthesia.

In case of hypotension, defined as  $MAP < 60 \text{ mmHg}$ , anaesthesia depth was lightened if possible and a dobutamine continuous infusion (Dobutamine Aguettant, Laboratoires Aguettant, Lyon, France) was administered at a dose-rate of  $2 \text{ to } 10 \mu\text{g kg}^{-1} \text{ min}^{-1}$  IV, with step-incremental doses until a MAP above  $60 \text{ mmHg}$  was reached.

## Pta Measurement

The PTA monitor uses the ECG signal to evaluate HRV. It records a base-apex surface ECG (lead II), using a 3-lead system with flattened crocodile clips attached to the skin. In our setting, the clips were moistened with electrode gel to maintain electrical contact; the red and yellow electrodes were positioned at the level of the right and left jugular groove respectively, the black electrode was placed over the right olecranon.

From the ECG signal, the PTA monitor detects R waves and calculates RR intervals. The RR series are filtered in real time using a non-linear artefact removal algorithm preventing from artefacts-induced inaccurate measurement of these series. After mean centring, RR series are resampled at 8 Hz and normalized using the vectorial norm of the RR series over 64 seconds for inter subject comparability. The mean centred and normalized RR series are then band pass filtered from 0.15 Hz to 0.5 Hz using a 4 coefficient Daubeuchies wavelet based filter. This provides RRhf in order to keep only HF variations and display the influence of respiratory sinus arrhythmia in the RR series, which corresponds to the parasympathetic tone of the patient. The amplitude of the normalized and filtered RR series is comprised between 0 and 0.2 (normalized unit).

The parasympathetic tone is assessed by computing the area under the RRhf series curve values as described elsewhere [8]. Local minima and maxima are detected, and the areas A1, A2, A3 and A4 are measured as the area between the lower and upper envelopes in each 16 sec subwindows. The minimal Area Under the Curve (AUCmin) is the smallest among the 4 surfaces, such as  $AUCmin = \min$

(A1,A2,A3,A4). The PTA index is then calculated in order to express a fraction of the total window surface, based on the following formula:

$PTA = (100 * [\alpha * AUC_{min} + \beta] / 12.8) * 100 / 163$ , where  $\alpha = 5.1$  and  $\beta = 1.2$  have been determined in order to keep the coherence between the visual effect of respiratory influence on RR series and the quantitative measurement of ANI; 100/163 is a coefficient determined for the horse in order to obtain PTA values between 0 and 100.

The PTA monitor continuously displays an instantaneous index (PTAi) calculated over the last 56 seconds and an average measurement (PTAm) over the previous 176 s. PTA values are scored between 0 and 100: a value of 100 corresponds to a maximum parasympathetic tone; conversely, a value of 0 corresponds to a decreased parasympathetic tone with maximum sympathetic tone.

## Study design

For each anaesthetized animal, different predefined time-points of 5 minute-duration were considered (Fig. 1):  $T_{SS}$  (steady-state time after induction of anaesthesia and before any surgical stimulus),  $T_{Cut}$  (after surgical noxious stimulation defined as cutaneous incision),  $T_{Pre-Hypo}$  (retrospectively assessed 5 minutes before each hypotension),  $T_{Dobut}$  (after each dobutamine initiation) and  $T_{Post-dobut}$  (after each dobutamine discontinuation). These different time-points were designed to allow a comparison between groups despite different clinical conditions and surgical procedures.

In order to assess the performance of the PTA index to predict a decrease or an increase in MAP, PTA and MAP were recorded initially, 1 minute and 5 minutes thereafter for each predefined time-point. Based on a recent report showing a better performance of the dynamic variations of ANI over static values to detect haemodynamic reactions in human patients [19], dynamic variations of PTA ( $\Delta PTA$ ) and MAP ( $\Delta MAP$ ) were calculated at each time-point as follow:

Over 1 minute period:  $\Delta X_{1min} = [(X_{1min} - X_0) / (X_{1min} + X_0) / 2] * 100$ .

Over 5 minutes period:  $\Delta X_{5min} = [(X_{5min} - X_0) / (X_{5min} + X_0) / 2] * 100$ .

Where  $X_0$ ,  $X_{1min}$  and  $X_{5min}$  are respectively the values of PTA and MAP at the predefined time, 1 min and 5 min thereafter.

This calculation was used *posteriori* to evaluate the performance of  $\Delta PTA_{1min}$  to anticipate a variation of MAP over the following 5 minutes.

## Abbreviations

PTA  
Parasympathetic Tone Activity

MAP  
Mean Arterial Pressure

$T_{SS}$   
steady-state, before surgical stimulus

$T_{Cut}$   
after surgical stimulation

$T_{Dobut}$   
retrospectively assessed 5 minutes before hypotension

$T_{Pre-Hypo}$   
after dobutamine initiation

$T_{Post-Dobut}$   
after dobutamine discontinuation

ANS  
Autonomic Nervous System

HRV  
Heart Rate Variability

ANI  
Analgesia Nociception Index

AUC  
area under curve

ROC curve  
receiver operating characteristic curve

VLF  
Very Low Frequency

LF  
Low Frequency

HF  
High Frequency

$\Delta PTA$   
Dynamic variation of the Parasympathetic Tone Activity Index

$\Delta MAP$   
Dynamic variation of the Mean Arterial Pressure

$\Delta HR$   
Dynamic variation of the Heart Rate

ASA  
American Society of Anesthesiologists

IM  
Intramuscular

IV

Intravenous

HR

Heart Rate

Vt

Tidal Volume

$P_E'Sevo$

End-tidal sevoflurane concentration

$P_E'CO_2$

End-tidal carbon dioxide tension

$FiO_2$

inspired fraction of oxygen

## Declarations

## Ethics approval and consent to participate

This prospective clinical study was approved by the ethical committee of VetAgro Sup (n°1514).

A written informed consent was obtained from the owners before using the animals in the study.

## Consent for publication

“Not applicable”.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Competing interests

Christelle Mansour has received a travel grants from MDoloris Medical Systems for short communications related to PTA.

Emmanuel Boselli has received honoraria and travel grants from MDoloris Medical Systems for lectures related to ANI.

The other authors have no conflict of interest to disclose.

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## Authors' contributions

All authors of the manuscript have read and agreed to its content.

CM: collected, analyzed and interpreted the horses' data and wrote the manuscript.

RM: helped in data collection.

BS: helped in data collection.

RC: assisted in data interpretation.

BA: contributed in data analysis.

JMBG: supported in writing the manuscript.

EB: advised in data interpretation.

SJ: major contributor in writing the article and data collection, analysis, and interpretation.

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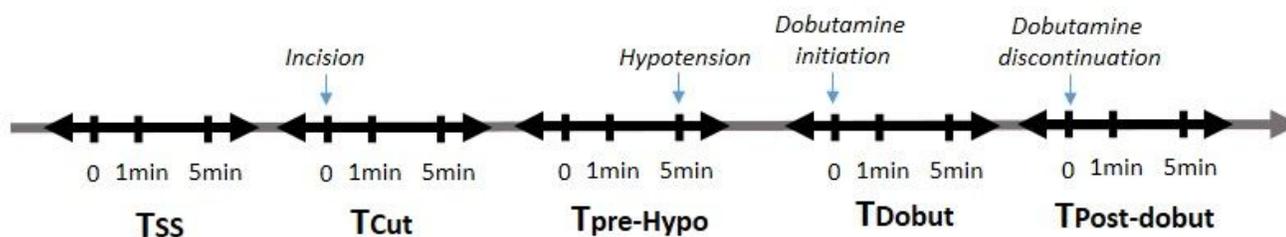
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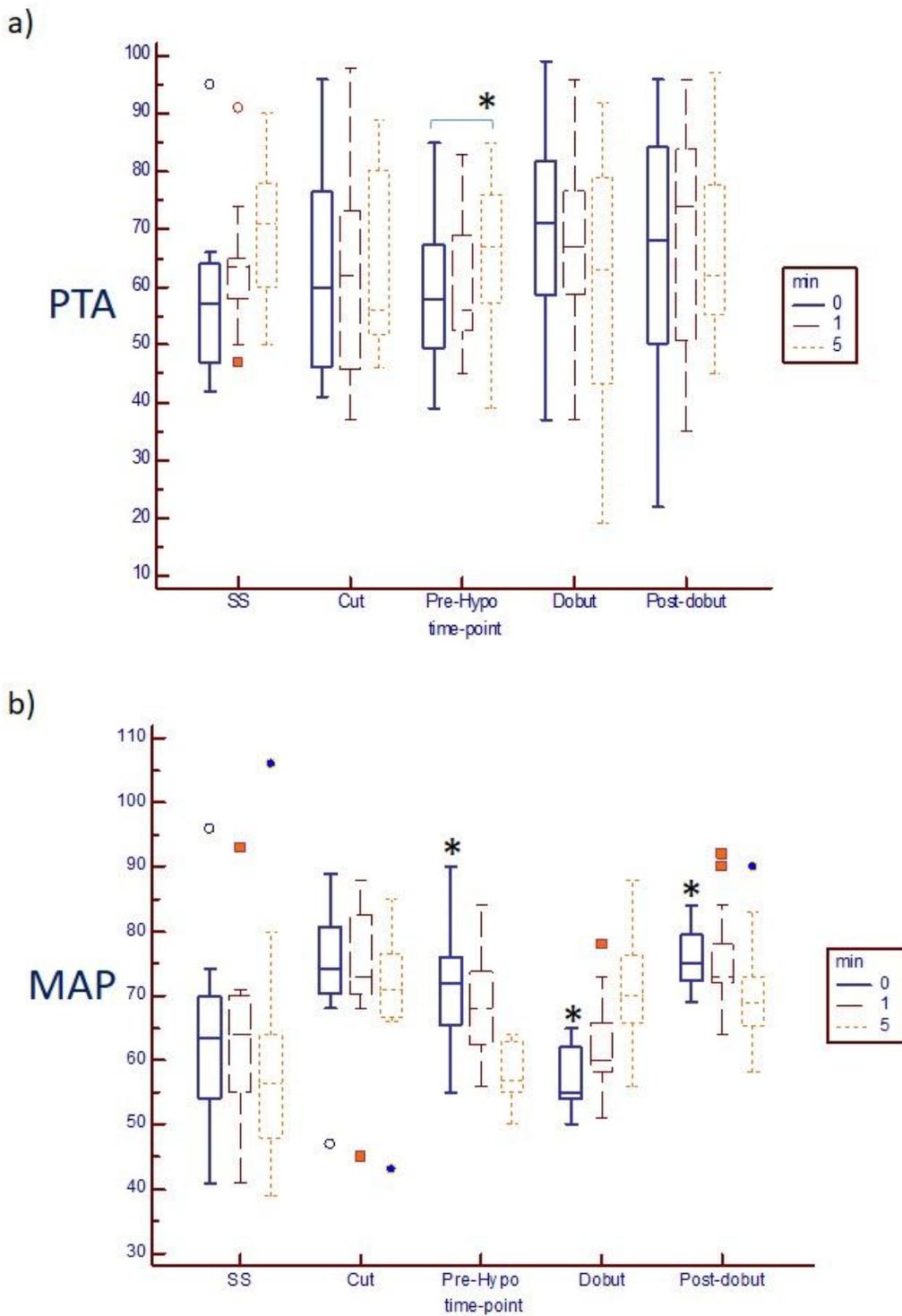
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## Figures



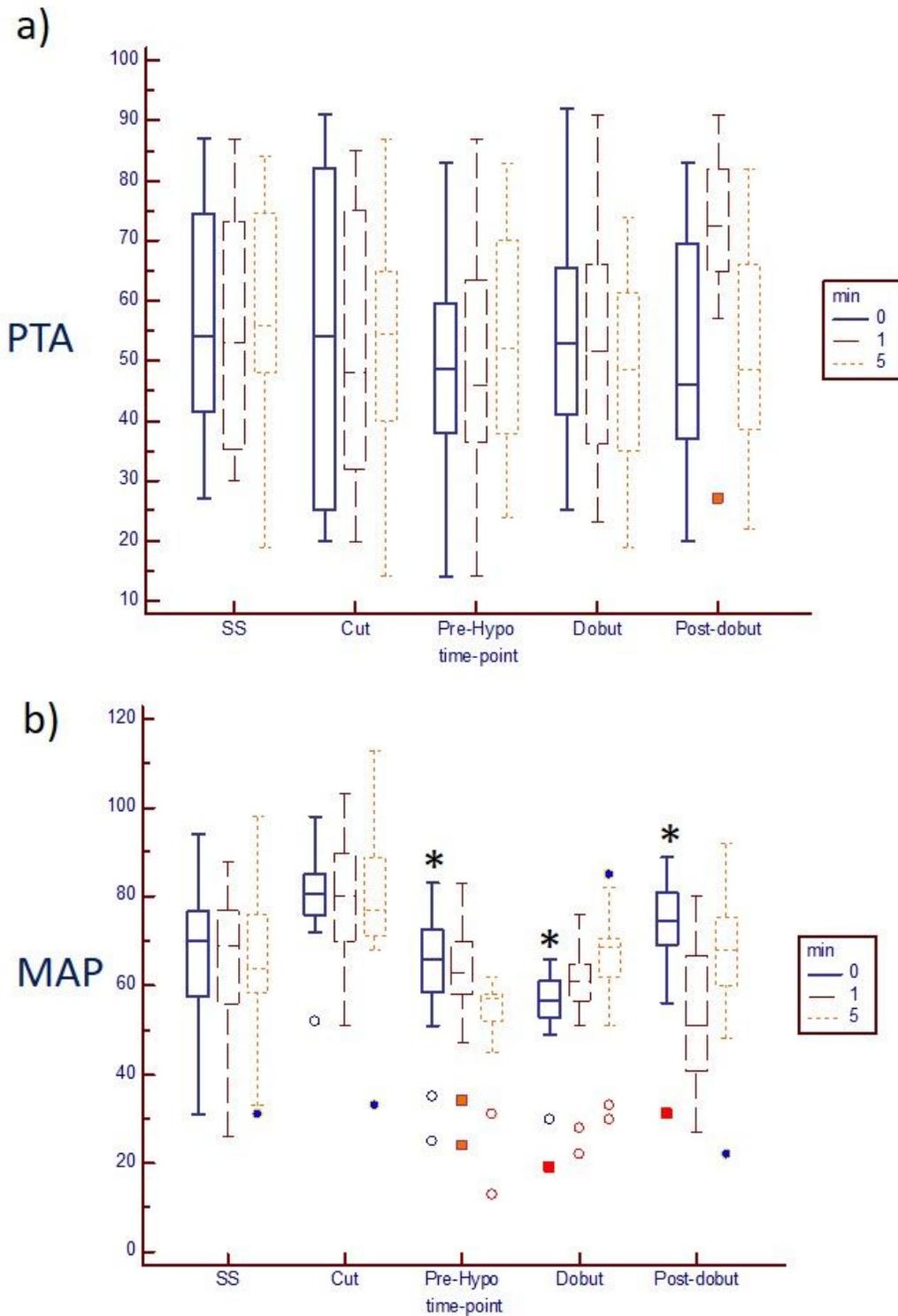
**Figure 1**

Schematic presentation of the predefined time-points. T<sub>ss</sub>, steady-state period; T<sub>cut</sub>, period after noxious stimulation; T<sub>pre-Hypo</sub>, retrospective period before each hypotension; T<sub>dobut</sub>, period after each dobutamine initiation; T<sub>post-dobut</sub>, period after each dobutamine discontinuation.



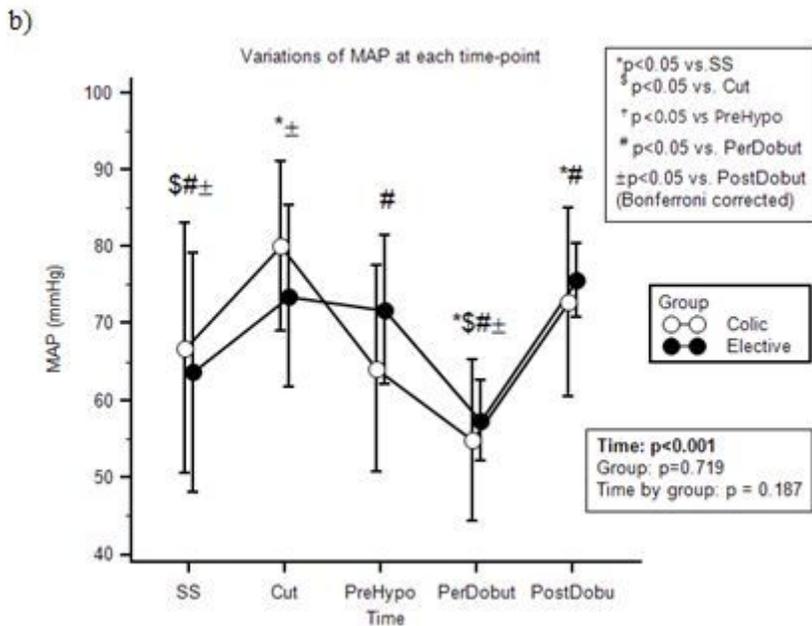
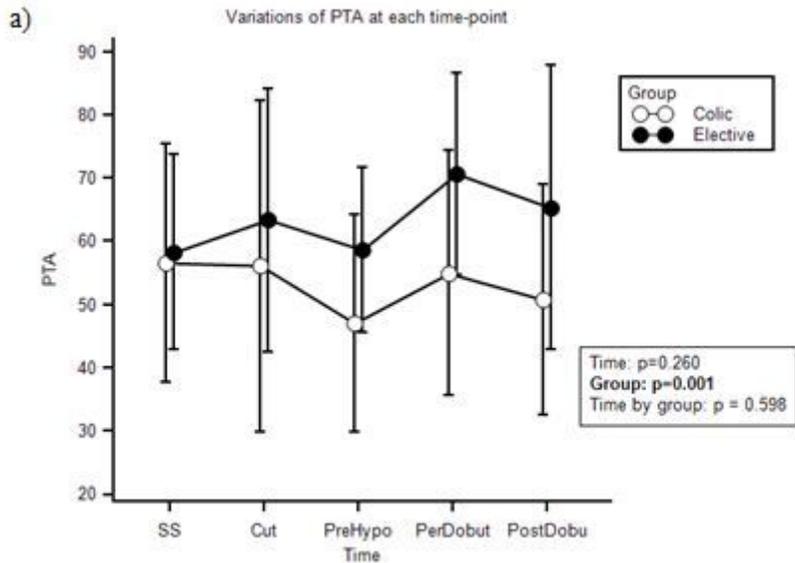
**Figure 2**

(a) PTA (Parasympathetic Tone Activity) and (b) MAP (Mean arterial pressure) evolution in Elective group at the predefined time-points. \* indicates a significant difference ( $p < 0.05$ ) of PTA and MAP between the predefined-time, 1 and 5 minutes thereafter. Values are expressed as median [IQR]. SS, steady-state; Cut, after noxious stimulation; Pre-Hypo, retrospectively before each hypotension; Dobut, after each dobutamine initiation; Post-dobut, after each dobutamine discontinuation.



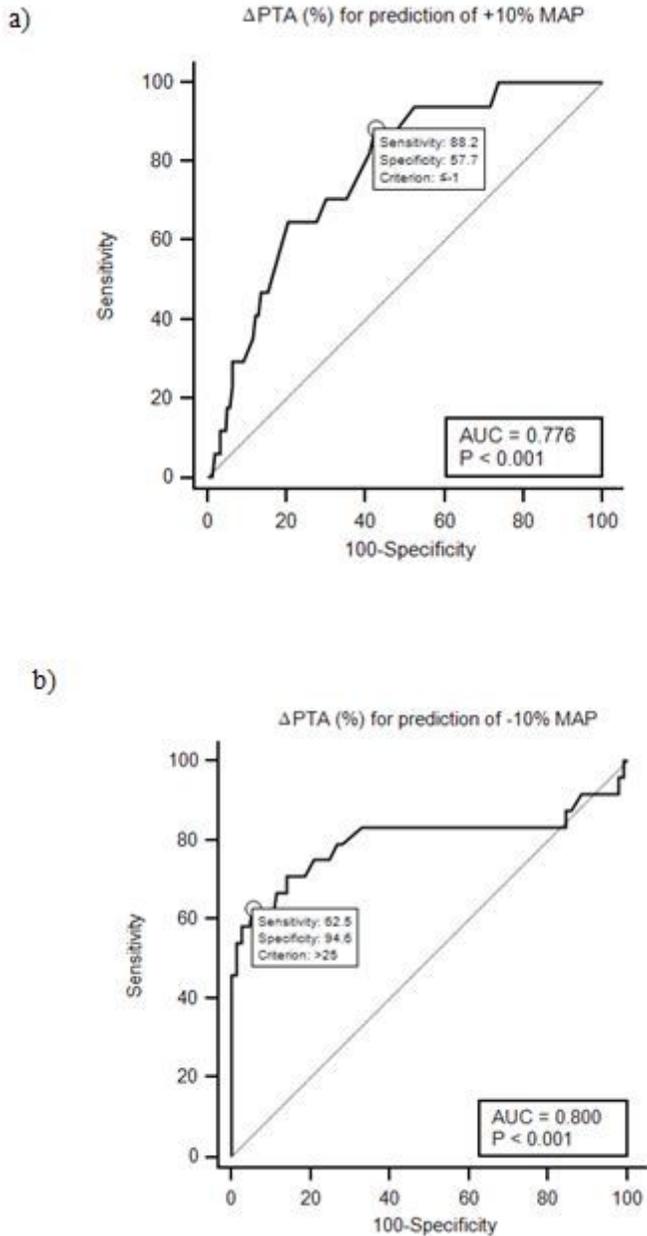
**Figure 3**

(a) PTA (Parasympathetic Tone Activity) and (b) MAP (Mean arterial pressure) evolution in Colic group at the predefined time-points. \* indicates a significant difference ( $p < 0.05$ ) of PTA and MAP between the predefined-time, 1 and 5 minutes thereafter. Values are expressed as median [IQR]. SS, steady-state; Cut, after noxious stimulation; Pre-Hypo, retrospectively before each hypotension; Dobut, after each dobutamine initiation; Post-dobut, after each dobutamine discontinuation.



**Figure 4**

Time by group effect at the baseline (T0) of the predefined time-points of Elective group (closed circles ●) versus Colic group (open circles ○) for (a) Parasympathetic Tone Activity index values (PTA) and (b) mean arterial pressure (MAP). Values are expressed as median [IQR]. SS, steady-state; Cut, after noxious stimulation; Pre-Hypo, retrospectively before each hypotension; Dobut, after each dobutamine initiation; Post-dobut, after each dobutamine discontinuation.



**Figure 5**

Performance of PTA to predict 10% increase and decrease in MAP in both groups. (a) 10% increase in MAP. AUC ROC = 0.77 [0.70 to 0.83] ( $p < 0.0001$ ), sensitivity = 88.2 %, specificity = 57.7 % and a threshold value of -1% for  $\Delta$ PTA. (b) 10% decrease in MAP. AUC ROC = 0.80 [0.73 to 0.85] ( $p < 0.0001$ ), sensitivity = 62.5 %, specificity = 94.6 % and a threshold value of 25% for  $\Delta$ PTA

## Supplementary Files

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