Electronic supplementary material (ESM)

Manuscript entitled "Analgesia Nociception Index for the assessment of pain in critically ill patients: a diagnostic accuracy study" by Chanques et al.

METHODS

Ethics approval

The protocol was approved by the Ethics committee: Comité de Protection des Personnes (CPP) Sud-Méditerranée IV (N°ID - RCB : 2014-A00337-40; Protocol Version: March 19, 2014; Consent Version: April 17, 2014). Qualified as a "usual care protocol" according to French law, oral consent was required from the legally authorized representative or a proxy/surrogate decision maker (patient's next of kin) who gave consent on the patient's behalf, followed by patient's consent as soon as they were able to communicate.

Patient population

The study took place in the 16-bed medical-surgical ICU of the University of Montpellier Saint Eloi Hospital, an academic tertiary care hospital, from April 2014 to June 2015 (14 months). All consecutive patients ≥ 18 yrs old, mechanically ventilated and/or receiving vasopressors were eligible for enrolment if they had a Richmond Agitation Sedation Scale (RASS)[1-3] above -4 and were unable to self-rate their pain intensity with the Visually Enlarged 0-10 Numeric Rating Scale (0-10 V-NRS). This scale is adapted to ICU patients and demonstrated to be the most feasible self-report pain scale in the ICU setting.[4] Exclusion criteria were decision to withdraw life-support or unstable condition preventing planned routine procedures of care, as well as conditions precluding the use of ANI: absence of sinus cardiac rhythm, respiratory rate < 10 b/min.

Conduct of the study

Investigators screened patients daily for eligibility including RASS assessment, self-pain-report ability and possibilities to plan routine procedures of care with the bed-side nurse. After having obtained consent from the surrogate decision maker and having enrolled the patient into the study, investigators planned different procedures of care with the bed-side

nurse including: 1) a central venous catheter or arterial catheter dressing change, 2) a complete turning of the patient on both sides in order to wash the back and change the bed sheets, and 3) a tracheal suctioning if relevant (intubated patients).

Data handling

1) Pain

Pain was measured in two different ways: 1) clinically, using the BPS performed by a clinical investigator, 2) electrophysiologically, using ANI. The clinical investigator who assessed the BPS was unaware to the ANI which was continuously recorded by the PhysioDoloris® monitor (MDoloris Medical Systems, Lille, France) at an acquisition frequency of 60 Hz. ANI data were subsequently extracted and analyzed by an independent research investigator who was unaware to the BPS value. While the clinical investigator assessed the BPS, another investigator indicated the timing of each procedure on the ANI monitor for subsequent ANI analysis. Because the ANI analyst needed to know the timing of the procedure, he could not be blinded to the procedure. ANI is a non-invasive device that takes an ECG analogue output from the patient ICU monitor (Carescape™ B850, GE-Healthcare, Helsinki, Finland) and displays an average measurement of ANI.[5] Two ANI values provided by the monitor were analyzed after data extraction: 1) Mean-ANI (ANIm), an average calculated over the previous 4 min, and 2) Instant-ANI (ANIi), an average calculated over a shorter period of time (64 seconds). ANI is calculated from analysis of heart rate variability, which is based on small beat-to-beat oscillations of the heart rate. High-frequency fluctuations in heart rate (0.15–0.5 Hz) are mediated predominantly by changes in parasympathetic outflow during respiratory cycles, corresponding to the "respiratory sinus arrhythmia".[6] Low-frequency fluctuations in heart rate are mediated by both parasympathetic and sympathetic activity. The ANI monitor uses mathematical analysis which permits differentiation of sympathetic and parasympathetic effects.[5] The calculated values of ANI range from 100 to 0, based on the degree of parasympathetic activation. 100 means a high

parasympathetic modulation (low stress level = low risk of pain) and 0 means extremely low parasympathetic modulation (high stress level = high risk of pain).

Pain assessments were made under three conditions for each patient: 1) at rest, before any procedure; 2) during the procedure of care; and 3) after the procedure. Study design was shown in figure 1.

2) Demographic and medical data

Age, gender, height and weight, co-morbidities, and reason for admission to the ICU were recorded. Simplified Acute Physiological Score II (SAPS II) score[7] and Sequential Organ Failure Assessment (SOFA) score[8] were calculated within 24-hrs after ICU admission. Body Mass Index (BMI) was calculated as the ratio (kg/m^2) between weight (kg) and height squared (m^2). Type and doses of sedative and analgesic drugs were collected before all procedures, as well as physiological parameters (heart and respiratory rates, systolic, diastolic and mean arterial blood pressure, pulse oximetry) that were continuously measured. As well as mechanical ventilator settings, Tidal volume was recorded as the ratio (ml/kg) between Tidal volume (ml) and Ideal Body Weight (kg) determined by usual formula.[9] Vigilance level was measured by investigators using the Richmond Agitation Sedation Scale (RASS).[1-3]

Statistical analysis

1) Primary endpoint: ANI performance to detect pain defined as a BPS ≥ 5

Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value were calculated according to standardized definitions.[10, 11] To determine relevant thresholds of Mean and Instant ANI values, Receiving Operator Characteristics (ROC) curves were constructed based on the definition of pain as a BPS ≥ 5. This threshold was chosen because it is the lower limit of the interquartile range in ICU patients during a painful procedure,[12, 13] and because this threshold is now used in routine protocols that have been shown to be feasible and safe in the ICU setting, such as analgesia based sedation

protocol[14] and protocol for procedural analgesia[15]. These protocols are provided online in French and in English as additional files in Critical Care Forum (http://ccforum.biomedcentral.com/articles/10.1186/cc12683).[15] BPS was used as the Gold standard to measure pain in the present population of ICU patients unable to communicate according to guidelines.[16-18] With the Critical Care Pain Observation Tool (CPOT),[19] BPS has demonstrated the best psychometric properties among different behavioural pain tools,[13, 20] and high responsiveness well adapted for research.[13] The Youden index was used to determined the ANI threshold.[21] The graphic correlation with BPS was also shown and measured using Spearman's test.

2) Secondary endpoint: variables associated with ANI

To explore patients' baseline variable and variables associated with critical illness that could impact on ANI, a mixed linear regression model was used to determine which would be associated with a greater or a lower ANI value by univariate and multivariate analysis. Variables whose p value were under 0.15 (univariate analysis) were considered for a multivariate analysis. Forward selection (according to Akaike Information Criterion) was used to determine the final regression model (see Table E.1). To better highlight the relevance of the previous model and significance of each variable retained by the forward selection, we also performed a stepwise selection ($p<0.15$ to enter the model, $p<0.05$ to remain in the final model) (see Table E.2).

3) Post-Hoc analysis

A post-hoc analysis was performed to explain the performance of ANI compared to the BPS. While recommended for clinical practice, BPS remains an "imperfect" gold-standard due to its subjective nature and the impossibility of monitoring it continuously (punctual measurements). Thus, we used the procedure itself rather than the BPS to define the gold-standard. This was done to show the performance of each tool (ANI and BPS) according to

the three different procedures. ROC curves were constructed using the procedure as the Gold standard and Hawley and McNeil's method was used to compare ROC curves.[22]

4) Number of patients and procedures necessary to include for analysis

Expecting ANI to have a sensitivity of at least 80% based on previous data in patients studied in a postanaesthesia care unit,[23] and expecting that 75% of patients would have a BPS ≥ 5 during a procedure of care,[12] with an estimation of ± 7% (half-distance of the 95% Confidence Interval), 102 paired measurements of ANI and BPS were necessary for each procedure of care*,* that meant including at least 102 patients undergoing each procedure.

5) Presentation of data

Quantitative data are shown as medians and 25^{th}-75^{th} percentiles. A p-value of ≤ 0.05 was considered statistically significant. Data were analysed using the SAS Enterprise Guide version 7.12 (2016) (SAS Institute, Cary, NC) and the R software version 3. 3.1 (21 June 2016).

FIGURE AND TABLE LEGENDS

Figure E1. Design of the study

Pain was measured by two different ways: 1) clinically, using the Behavioral Pain Scale (BPS) performed by a clinical investigator, 2) electrophysiologically, using the Analgesia Nociception Index (ANI). BPS was used independently to the ANI that was continuously recorded at an acquisition frequency of 60 Hz. ANI data were subsequently extracted and analyzed by an independent research investigator based on the timing indicated by the clinical investigator on the ANI monitor. The two ANI values provided by the monitor were analyzed after data extraction: 1) Mean ANI (ANIm), an average of ANI values made over the previous 4 min, and 2) Instant ANI (ANIi), an average of ANI values made over a shorter period of time (64 s). Pain assessments were made in three conditions for each patient: 1) at rest, before any procedure; 2) during the procedure of care; and 3) at rest, after the procedure. To take into consideration that ANI provides a measure during a period of time (4 min and 64 s for ANIm and ANIi, respectively), minimal ANI values were determined within 5 min after the end of the procedure to take into account all the procedure; in the same way, after procedure's ANI values were determined 10 min after the end of the procedure to avoid any procedure's overlapping.

Figure E2. Study flow chart

Figure E3. Receiver operating characteristics (ROC) curves for BPS and ANIi associated with procedures

All paired measurements of BPS and ANIi obtained before during and after each of the three procedures of care (catheter's dressing change: n=330 paired measurements; turning: n=330 paired measurements; endotracheal suctioning: n=309 paired measurements) were included for analysis.

All 969 paired measurements of ANIi and BPS were taken into account for analysis.

ANIi was more predictive of dressing change than BPS (right panel, $p<0.001$ for comparison between areas under the curves). No significant difference was shown between ANIi and BPS for prediction of turning (middle panel) and suctioning (right panel).

Thresholds determined by ROC analysis for dressing change, turning and suctioning were $\leq$ 45.5, 42.5 and 42.5 for ANIi, and $\geq$ 4, 4 and 5 for BPS, respectively.

ANI: Analgesia Nociceptive Index; ANIm: mean ANI; ANIi: instant ANI; BPS: Behavioral Pain Scale

Table E1. Univariate and multivariate analysis of variables associated with ANIi values

This table shows the variables associated with ANIi values by univariate and multivariate analysis. Only variables for which a p value was < 0.10 were taken into account for multivariate analysis. If a qualitative variable has a positive (negative) estimate number this means that ANIi value increases (decreases) when the variable is present. If a continuous variable has a positive (negative) estimate number this means that ANIi value increases (decreases) for every unity of the continuous variable.

ANI: Analgesia Nociceptive Index; ANIm: mean ANI; ANIi: instant ANI; BMI: Body Mass Index; ICU: Intensive Care Unit; SAPS II: Simplified Acute Physiological Score II; SOFA: Sequential Organ Failure Assessment score; RASS: Richmond Agitation Sedation Scale

Table E2. Comparison between final models obtained using Forward and Stepwise procedures (multivariate analysis of variables associated with ANIi)

Forward and stepwise procedures for selection of variables provided similar models, but the stepwise procedure provided a more restrictive set of variables (7 variables compared to 9 variables for the forward selection). We chose to retain the larger set of variables (forward procedure), and to discuss all 9 clinically relevant variables in the manuscript.

ANIi: instant Analgesia Nociception Index; BMI: Body Mass Index; SAPS II: Simplified Acute Physiological Score II; RASS: Richmond Agitation Sedation Scale

Figure E1

Design of the study

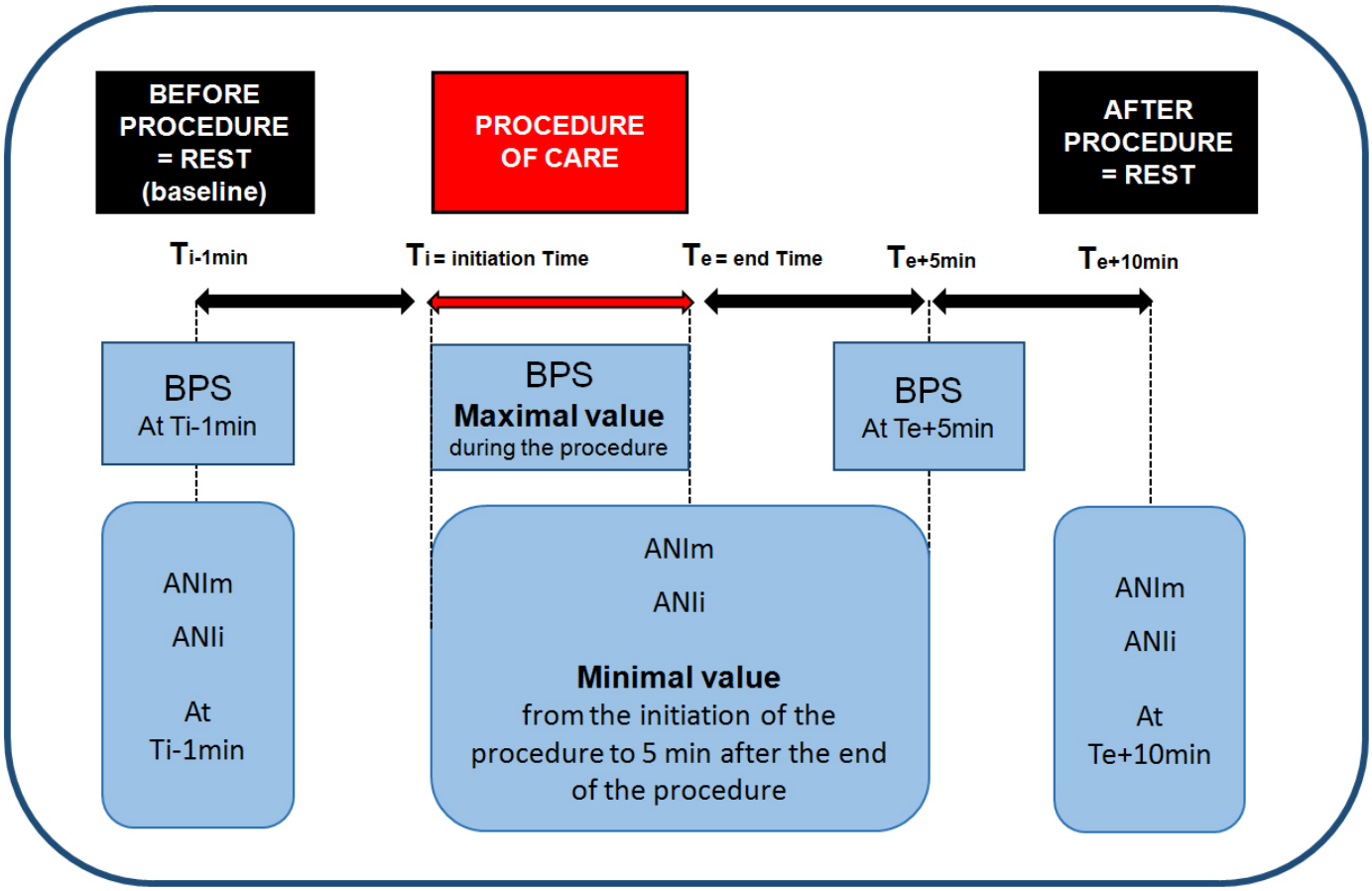

Figure E2

Study flow-chart

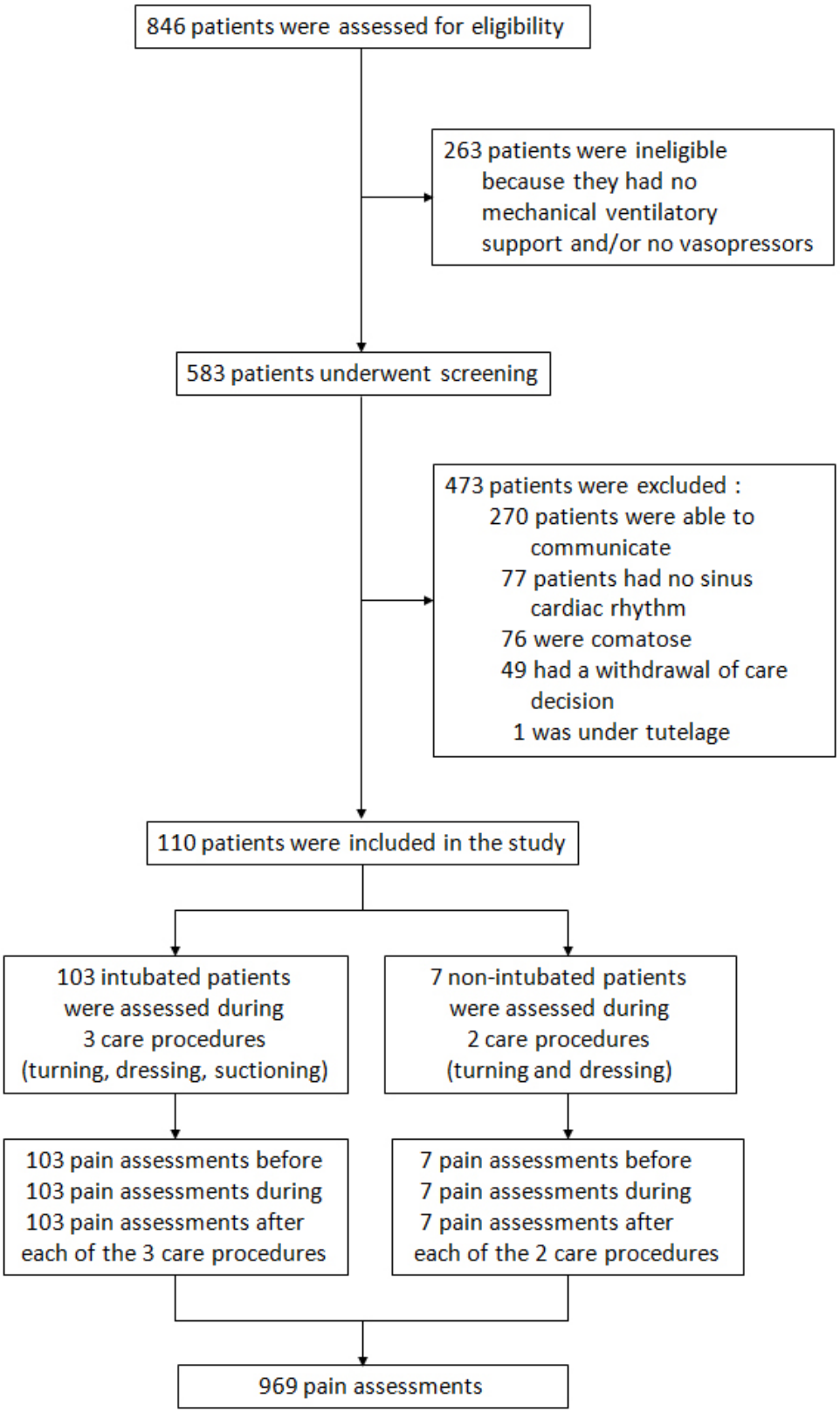

Figure E3

Receiver operating characteristics (ROC) curves for BPS and ANIi associated with procedures

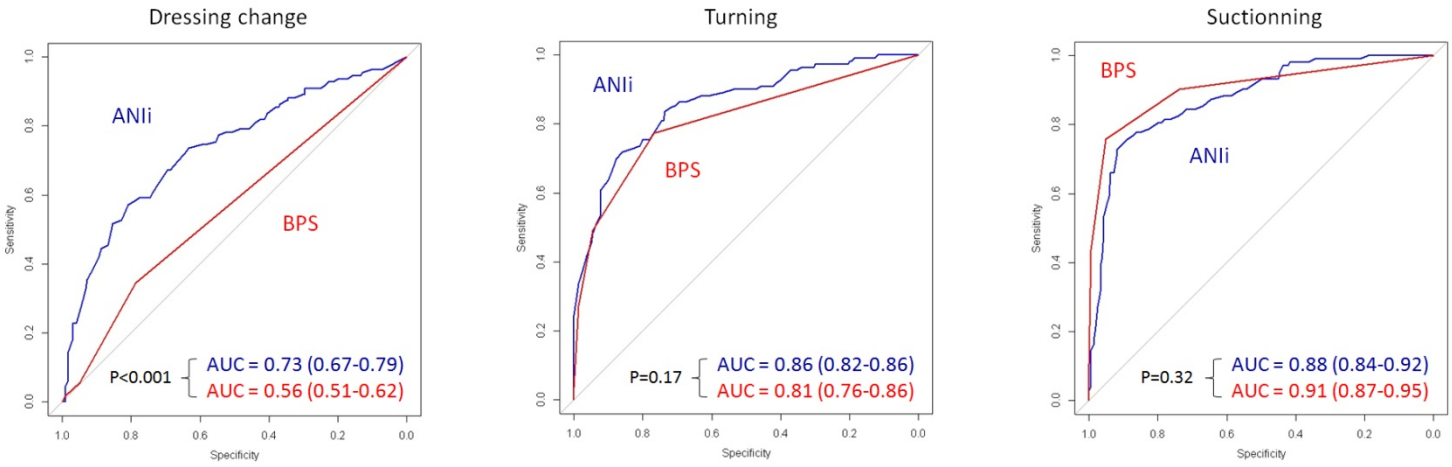

Table E1

Univariate and multivariate analysis (Forward procedure) of variables associated with ANIi

	Univariate analysis					Multivariate analysis				
Variable	Estimate	Standard Error	95% CI		Pr > |t|	Estimate	Standard Error	95% CI		Pr > |t|
Age (1 year)	0.21	0.05	0.12	0.31	**<0.001**	0.22	0.04	0.14	0.30	**<0.001**
Sex M	Ref									
Sex F	-1.04	1.59	-4.16	2.08	0.51					
BMI < 30 kg.m^{-2}	Ref					Ref				
BMI ≥ 30 kg.m^{-2}	8.23	1.69	4.91	11.55	**<0.001**	8.78	1.42	6.00	11.57	**<0.001**
Hypertension : No	Ref									
Hypertension : Yes	5.03	1.50	2.08	7.98	**<0.001**					
Diabetes : No	Ref									
Diabetes : Yes	3.18	1.79	0.32	6.69	**0.07**					
Chronic pain : No	Ref									
Chronic pain: Yes	2.97	2.20	-1.35	7.29	0.18					
Type of ICU admission *Surgical from ward*	Ref									
Surgical from OR	-0.63	2.79	-6.11	4.85	0.82					
Medical	-2.28	2.73	-7.65	3.08	0.40					
SAPS II score (1 point)	0.15	0.05	0.06	0.24	**<0.001**	0.17	0.04	0.08	0.25	**<0.001**
SOFA score (1 point)	0.13	0.18	-0.22	0.49	0.47					
RASS (1 point)	-2.34	0.67	-3.64	-1.03	**<0.001**	-1.25	0.63	-2.48	-0.01	**0.04**
Heart Rate (1 b/min)	-0.09	0.04	-0.16	-0.02	**0.01**					
Mean Arterial Blood Pressure (1 mmHg)	-0.27	0.06	-0.39	-0.15	**<0.001**					
Respiratory Rate (1 b/min)	-0.43	0.10	-0.63	-0.22	**<0.001**	-0.29	0.09	-0.47	-0.11	**0.002**
Oxygen saturation (1%)	-0.08	0.34	-0.74	0.58	0.81					
Ventilatory support										
Not intubated	Ref					Ref				
Pressure support	7.47	3.63	0.35	14.58	**0.04**	4.24	3.22	-2.08	10.56	0.19
Assist control ventilation	11.29	3.72	0.3.99	18.58	**0.003**	8.71	3.43	1.97	15.45	**0.02**
Tidal volume (1 ml/kg of Ideal body weight)	0.27	0.36	-0.43	0.98	0.45					
Plateau airway pressure (1 cmH2O)	0.16	0.18	-0.20	0.53	0.38					
Positive End Expiratory Pressure (1 cmH2O)	1.44	0.39	0.68	2.21	**<0.001**					
Vasopressors : No	Ref					Ref				
Vasopressors : Yes	6.91	1.45	4.06	9.76	**<0.001**	2.74	1.34	0.11	5.38	**0.04**
Norepinephrine dose (1µg.kg^{-1}.min^{-1})	-1.59	3.38	-8.22	5.05	0.64					

To be continued

Variable	Estimate	Standard Error	95% CI		Pr > |t|	Estimate	Standard Error	95% CI		Pr > |t|
Sedation : No	Ref									
Sedation : Yes	1.31	1.47	-1.57	4.20	0.37					
propofol : No	Ref									
propofol : Yes	3.06	1.46	0.19	5.94	**0.04**					
midazolam : No	Ref									
midazolam : Yes	-4.46	3.88	-12.07	3.15	0.25					
propofol dose (1µg.kg^{-1}.min^{-1})	-0.03	0.06	-0.14	0.09	0.68					
midazolam dose (1µg.kg^{-1}.min^{-1})	-1.27	0.99	-3.28	0.74	0.21					
Analgesia : No	Ref									
Analgesia : Yes	3.72	1.54	0.70	6.74	**0.02**					
acetaminophen : No	Ref					Ref				
acetaminophen : Yes	3.77	2.18	-0.50	8.05	**0.08**	5.66	1.82	2.09	9.24	**0.002**
nefopam : No	Ref									
nefopam : Yes	0.85	1.75	-2.58	4.27	0.63					
tramadol : No	Ref									
tramadol : Yes	-1.07	1.71	-4.43	2.29	0.53					
major opioids : No	Ref									
major opioids : Yes	-0.11	1.62	-3.29	3.07	0.95					
acetaminophen dose (1mg.kg^{-1}.d^{-1})	-0.02	0.14	-0.30	0.26	0.88					
nefopam dose (1mg.kg^{-1}.d^{-1})	-6.18	3.49	-13.05	0.69	**0.08**					
tramadol dose (1mg.kg^{-1}.d^{-1})	-2.32	0.89	-4.08	-0.56	**0.01**					
sufentanil equivalent dose (1µg.kg^{-1}.h^{-1})	-15.29	5.86	-26.82	-3.76	**0.01**					
Type of procedure										
Turning	Ref									
Suctioning	0.92	1.81	-2.63	4.46	0.61					
Dressing change	2.98	1.78	-0.50	6.47	**0.09**					
Timing										
During the procedure	Ref									
Before the procedure	23.76	1.55	20.71	26.80	**<0.001**	23.05	1.48	20.13	25.96	**<0.001**
After the procedure	24.89	1.55	21.84	27.94	**<0.001**	24.23	1.48	21.32	27.14	**<0.001**

ANIi: instant Analgesia Nociception Index; BMI: Body Mass Index; ICU: Intensive Care Unit; SAPS II: Simplified Acute Physiological Score II; SOFA: Sequential Organ Failure Assessment score; RASS: Richmond Agitation Sedation Scale

Table E2

Comparison between final models obtained using Forward and Stepwise procedures

(**multivariate analysis of variables associated with ANIi)**

	Forward procedure					Stepwise procedure				
Variable	Estimate	Standard Error	95% CI		Pr > |t|	Estimate	Standard Error	95% CI		Pr > |t|
Age (1 year)	0.22	0.04	0.14	0.30	**<0.001**	0.19	0.04	0.11	0.27	**<0.001**
BMI < 30 kg.m^{-2}	Ref					Ref				
BMI ≥ 30 kg.m^{-2}	8.78	1.42	6.00	11.57	**<0.001**	9.76	1.52	6.78	12.74	**<0.001**
SAPS II score (1 point)	0.17	0.04	0.08	0.25	**<0.001**	0.15	0.05	0.07	0.24	**<0.001**
RASS (1 point)	-1.25	0.63	-2.48	-0.01	**0.04**	-2.54	0.64	-3.79	-1.29	**<0.001**
Respiratory Rate (1 b/min)	-0.29	0.09	-0.47	-0.11	**0.002**	-0.33	0.10	-0.53	-0.14	**<0.001**
Ventilatory support										
Not intubated	Ref									
Pressure support	4.24	3.22	-2.08	10.56	0.19					
Assist control ventilation	8.71	3.43	1.97	15.45	**0.02**	-	-			-
Vasopressors : No	Ref									
Vasopressors : Yes	2.74	1.34	0.11	5.38	**0.04**	-	-			-
Acetaminophen : No	Ref					Ref				
Acetaminophen : Yes	5.66	1.82	2.09	9.24	**0.002**	6.09	1.87	2.43	9.76	**<0.01**
Timing										
During the procedure	Ref					Ref				
Before the procedure	23.05	1.48	20.13	25.96	**<0.001**	22.98	1.58	19.89	26.08	**<0.001**
After the procedure	24.23	1.48	21.32	27.14	**<0.001**	23.79	1.58	20.70	26.88	**<0.001**

Forward and stepwise procedures for selection of variables provided similar models, but the stepwise procedure provided a more restrictive set of variables (7 variables compared to 9 variables for the forward selection). We chose to retain the larger set of variables (forward procedure), and to discuss all 9 clinically relevant variables in the manuscript.

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