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ORIGINAL RESEARCH

Electromyographic assessment of blink reflex throughout the transition from responsiveness to unresponsiveness during induction with propofol and remifentanil

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Abstract

General anesthesia is a reversible drug-induced state of altered arousal characterized by loss of responsiveness due to brainstem inactivation. Precise identification of the moment in which responsiveness is lost during the induction of general anesthesia is extremely important to provide information regarding an individual's anesthetic requirements and help intraoperative drug titration. To characterize the transition from responsiveness to unresponsiveness more objectively, we studied neurophysiologic-derived parameters of electromyographic records of electrically evoked blink reflex as a means of identifying the precise moment of loss of responsiveness. Twenty-five patients received a slow infusion of propofol until loss of corneal reflex while successive blink reflexes were elicited and recorded every 6 s. The level of anesthesia was assessed using an adapted version of the Richmond Agitation-Sedation Scale. Different variables of the blink reflex components were calculated and compared to the adapted version of the Richmond Agitation-Sedation score and the estimated effect-site propofol concentration. Baselines of the blink reflex responses were similar to those in literature. After propofol infusion started, the most susceptible component of the blink reflex to propofol was R_2 (EC_{50} = 1.358 (95% CI 1.321, 1.396) µg/mL) and the most resistant was R_1 (EC_{50} = 3.025 (95% CI 2.960, 3.090) µg/mL). Most of the patients (24 out of 25) lost the R_1 component when they were still responsive to shaking and shouting and corneal reflex could be elicited clinically (time = 102.48 ± 33.00 s). Habituation was present in R_2 but not in R_1. The R_1 component of the blink reflex was found to have a strong correlation with the adapted version of the Richmond Agitation-Sedation Scale, with amplitude correlating better than areas ($\rho = -0.721$ (0.123) versus $\rho = -0.688$ (0.165)). We found a strong correlation between the R_1 component with the estimated propofol effect-site concentration, with amplitude correlating better than areas ($\rho = -0.838$ (0.113) versus $\rho = -0.823$ (0.153)) and between the clinical scale and the propofol concentration ($\rho = 0.856$ (0.060)). The area and amplitude of the R_1 component showed to be indicators of predicting different levels of anesthesia ($P_k = 0.672$ (0.183) versus $P_k = 0.709$ (0.134)) and these are connected to the propofol concentrations ($P_k = 0.593$ (0.10)). Our results suggest that electrically evoked blink reflex could be used during the induction of anesthesia as a surrogate of the Richmond Agitation-Sedation Scale to provide an objective endpoint as far as a − 4. At this point, at the moment of loss of R_1, the propofol infusion may be stopped, as overshooting increases slightly the effect-site concentration afterward and eventually reaching loss of responsiveness. If the desired target is not achieved, the infusion can then be resumed.

Keywords Personalized anesthesia · Propofol · Blink reflex · Electromyography · Loss of responsiveness · Monitoring

1 Introduction

To induce patient unconsciousness/unresponsiveness, anesthesiologists use a variety of different drug combinations; however, synergisms and interpatient variability may lead to different dose requirements. Precise identification of the moment of loss of responsiveness during general anesthesia induction would allow for the determination of the amount

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of anesthetic that each patient requires in a given situation. This would provide personalized information on anesthetic requirements and could help maintain an adequate level of anesthesia throughout surgery [1], ensuring safe and effective care and balancing the avoidance of intraoperative awareness [2–4] and overdose [5].

Propofol is the most widely used intravenous hypnotic drug for inducing loss of responsiveness and is also used for the maintenance of general anesthesia. Despite its usefulness in several clinical settings, propofol exhibits a dose-dependent relationship with hemodynamic instability, with events such as hypotension and bradycardia increasing as propofol concentration increases during induction [6]. These adverse effects are generally proportional to the dose and rate of administration [7] but slow infusion while titrating for the desired clinical endpoints may reduce them.

Determining which endpoint to use for anesthesia induction with propofol may be chosen based on its pharmacological effects. After propofol is administered intravenously, it reaches multiple sites in the cerebral cortex, brainstem, and thalamus, subsequently altering neurotransmission [8]. This is a continuous process, and as the hypnotic drug concentration is increased in these sites, individuals gradually transit from wakefulness to unresponsiveness [9]. Surrogates of this hypnotic effect can be evaluated by carefully assessing the different stages patients go through until loss of responsiveness. Most of the commonly used clinical endpoints reflect both cortical and brainstem functions. Determining the moment of loss of behavioral responsiveness (LOBR) to stimulation may reflect cortical activity and occurs because hypnosis renders patients unable or unwilling to interact with their external environment [9]. Classifying the adequacy of the hypnotic effect based on the cortical function is possible by using, for example, the Richmond Agitation-Sedation Scale (RASS) [10]. The RASS is a score that ranges from 0 (alert and calm) to − 5 (unarousable) and includes endpoints such as unresponsiveness to name calling, absence of eye-opening to stimulation or loss of response to shaking and shouting.

Another possibility for assessing propofol effect, as it has a profound effect on nerves originating in the brainstem, is the use of brainstem reflexes as clinical endpoints [11], namely the blink, corneal, oculocephalic, gagging and pupillary light reflexes [12].

Blink and corneal reflexes can be elicited by a light touch of the eyelids or cornea, resulting in a response that is usually qualitatively appraised and can be observed clinically. Recording the electromyographic (EMG) activity from the orbicularis oculi, however, provides quantitative information of the reflex circuit. The most commonly used sensory stimulus for eliciting the blink reflex is a brief electrical shock applied to the supraorbital nerve. The reflex response to that stimulus consists of two separate components: R_1 and subsequently R_2. The two responses have different pathways in the brainstem [13]. The R_1 response relates to an oligosynaptic pontine reflex, whereas R_2 is relayed through a more complex route including many interneurons in the pons and lateral medulla [14]. The reflex circuit descends through the spinal trigeminal tract and enters a polysynaptic chain of interneurons in the lateral reticular formation and finally projects to the facial motoneurons bilaterally.

Thus, with the present study, we intended to evaluate whether neurophysiologic-derived parameters of EMG records of electrically evoked blink reflex are useful in identifying the moment of loss of responsiveness determined with a modified version of the RAAS scale, in the continuum to unconsciousness.

2 Methods

2.1 Patients

After Institutional Review Board and Ethics Committee approval (Ref. 068-17 (062-DEFI/062-CES)), twenty-eight consecutive adult patients undergoing routine neurosurgical procedures under general anesthesia at Centro Hospitalar do Porto were considered for this study. Period of enrollment was from June 2018 to July 2018. The anesthesiologist in charge of the room was the same over the 1-month period of the study. All patients gave written informed consent.

Exclusion criteria were: baseline RASS [10] lower than 0 or Bispectral Index < 90 before induction and significant cardiovascular, renal, hepatic or respiratory pathology and obesity (BMI > 35 kg/m^2).

2.2 Anesthesia protocol

In the operating room, after placement of standard monitor and an intravenous line in the dorsum of the hand, an infusion of a balanced electrolytic solution was started at 6 mL/kg/h. The anesthesiologist would then start a target control infusion (TCI) of remifentanil (Minto PKPD model) [15, 16], using a Fresenius Base Primea docking station (Fresenius-Kabi, Bad Homburg, Germany), at an effect-site concentration (Ce) target of 2.5 ng/mL. A bolus of 10 mg of lidocaine was administered to reduce the pain associated with propofol administration. One minute after the remifentanil pseudo equilibration was achieved, baseline blinks were recorded and, then, an infusion of 1% propofol (Schnider PKPD model) [17] was started using a TCI enabled infusion system, in the TCI-View mode, at 3.3 mL/kg/h until loss of responsiveness, determined by the anesthesiologist. This slow velocity of infusion during induction enables a careful titration of the minimum amount of propofol required for loss of responsiveness. Once unresponsiveness was reached,

the propofol infusion was stopped, the estimated propofol Ce concentration was noted and the TCI system was switched to effect-site TCI mode with a Ce of 75% of that at loss of responsiveness. At this point, no additional analgesic/opioid medication was given during induction. Propofol infusion was subsequently titrated to maintain BIS between 40 and 60 (BIS Vista™ monitor—Medtronic, Ireland). The study was terminated just before tracheal intubation.

2.3 Electromyographic stimulations and recordings

The electromyographic stimulations and recordings were conducted with the VikingQuest™ neurophysiological device (VikingQuest, Nicolet, WI, USA) at 200 ms total sweep time with a sample rate of 10 kHz. High pass filter was applied with a cutoff frequency of 20 Hz. Before induction of anesthesia and prior to electrode application, all the patient's head skin surfaces were cleaned with an exfoliating agent. Surface electrodes (1.4 cm^2) coated with conductive paste (electrode impedance < 8 kΩ) were applied to stimulate and record the electromyogram from the right orbicularis oculi muscle. The supraorbital nerve was stimulated transcutaneously with a bipolar electrode with the cathode placed beneath the eyebrow over the supraorbital notch and the anode placed above the eyebrow (interelectrode distance 2 cm). The supraorbital nerve was electrically stimulated with a duration of 0.1 ms at 0.16 Hz. With regard to the recording electrodes, the recording electrode was placed in the middle of the inferior rim of the orbit; the reference halfway on the eye-ear line and the ground electrode was placed on the cheek or on the shoulder of the patient (Fig. 1). The signals were stored in the VikingQuest software provided by the manufacturer. The raw data was exported to a personal computer to be treated and analyzed in MATLAB®.

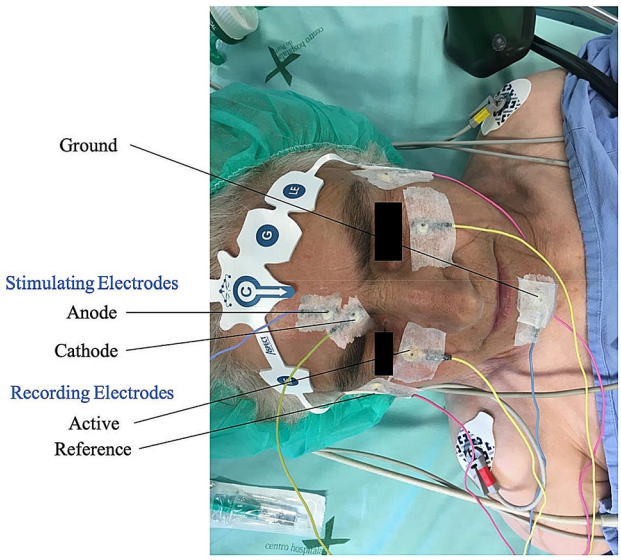

Fig. 1 Patient with the positioned electrodes for stimulation and recording of electrically evoked blink reflexes in the orbicularis oculi muscle

The electromyography records of an electrically evoked blink reflex showed at least two components (R_1 and R_2 components). The first or early response (R_1) is brief and occurs after a latency of approximately 10 ms on the side of stimulation [18]. The second response, (R_2) has a latency of approximately 30 ms, is bilateral and more prolonged in time [18]. The R_2 response causes the actual contraction of the orbicularis oculi muscle [18]. The optimal stimulus intensity was sought by gradually increasing the current until visual observation of the EMG showed that, in the presence of a clearly visible R_1 component, the R_2 component reach its maximum amplitude [19].

2.4 Assessment of levels of responsiveness

The level of responsiveness was assessed every 6 s using an adapted version of the RASS score [10], entitled aRASS and described in Table 1. This scale is a modification of the RASS scale [10], with the addition of the corneal reflex endpoint, yielding an adapted Richmond Agitation-Sedation Scale (aRASS). LOBR, defined as a scale of − 5 in the aRASS scale, was assessed by tapping in the patient's

Table 1 The adapted Richmond Agitation-Sedation Scale (aRASS)

Behavior	Descriptive	Score
Awake	Fully awake and alert	0
Drowsy	Not fully alert, but has sustained awakenings to voice (eye opening and eye contact)	− 1
Light sedation	Briefly awakens to voice (eye opening and eye contact)	− 2
Moderate sedation	Movement, but no eye opening or contact to voice	− 3
Deep sedation	No response to voice, but movement or eye opening to shaking and shouting	− 4
Unarousable	No response to shaking and shouting, but still have corneal reflex	− 5
Unresponsiveness	No corneal reflex	− 6

Definition of seven anesthetic stages based on clinical signs. The main clinical signs of each state are referred, as well as the attributed a numerical scale

forehead. Loss of corneal reflex (LOCR), defined as a score of − 6 in the aRASS scale was evaluated, after the aRASS reached a score of − 5, using a drop of sterile water to the cornea. Hereafter, this evaluation was intercalated with electrical stimulations at 6 s intervals. If the eyes blinked concomitantly, the reflex was intact. If only one eye blinked, the reflex was impaired, and if neither eye blinked, the reflex was absent.

Baseline blink reflexes and evaluation of the aRASS scale were recorded several times before propofol was administered. Patients then received propofol and four successive blink reflexes were elicited and recorded concurrently. After an interval of 6 s another four successive blink reflex stimuli were elicited and recorded continuously until LOCR was achieved.

2.5 Blink reflex parameters

In our study, the 6 s interval between the four stimuli was chosen so habituation was minimal [20] but could still provide the temporal discrimination typically used in a clinical setting to determine LOBR in our center. The mean of the four successive values was used to calculate the response variables (maximum amplitudes, latency, duration and areas under the curves R_1 and R_2). Latency, duration, area under the curve, that is, the mathematical integral of the absolute value of the raw EMG signal, and amplitudes of the R_1 and R_2 components from the electromyographically evoked blink reflexes were measured using the marker tool of the EMG system. An expert neurophysiologist put markers in place after the session took place. Latencies and durations were expressed in milliseconds (ms), area under the curves was expressed as microvolts × millisecond (µVms) and amplitudes in microvolts (µV).

We considered LOR_1 (Loss of R_1 component) and LOR_2 (Loss of R_2 component) as the moments when R_1 and R_2 components were last seen in the EMG signal after an electrically evoked blink reflex. LOBR was defined as the moment at which patients lose behavioral responsiveness, i.e., when patients have no response to shaking and shouting. LOCR was defined as the moment at which patients lose corneal reflex.

2.6 Statistical analysis

Graphical analysis of data preceded formal statistical analysis. We used the prediction probability (P_k) to test the capability of the response variables of the blink reflex to predict the aRASS and the estimated propofol Ce concentrations. P_k is a non-parametric correlation suited to ordinal variables and can accommodate variable scales having any degree of coarseness or fitness, which ranges from 0 to 1. A value of $P_k = 0.5$ means that the indicator correctly predicts the anesthetic depths only 50% of the time, i.e., no better than a 50:50 chance. A value of $P_k = 1$ means that the indicator predicts the anesthetic levels or the concentrations correctly 100% of the time. P_k was calculated for each patient, and an average of these P_k's was then calculated (group P_k). P_k was computed from Somer's measure of association in the SPSS software program (IBM SPSS version 24.0 IL, USA). We also calculated the average of individual Spearman's rank correlation coefficients (group ρ).

A sample size calculation was performed considering the results previously obtained by Mourisse et al. [21] [EC_{50} (LOBR) = 2.44 (2.17–2.79) µg/mL vs EC_{50} (LOR_1) = 2.82 (2.48–3.22) µg/mL vs EC_{50} (LOR_2) = 1.58 (1.39–1.79) µg/mL] [18]. In their study, 20 pairs/patients were used. In the present study, since an opioid was present at induction and considering a 20% exclusion rate, a final sample size of 25 patients was established.

To investigate a relationship between the estimated Ce concentration of propofol and the loss of blink reflex components (LOR_1 and LOR_2), loss of behavioural responsiveness (LOBR) and loss of corneal reflex (LOCR), we used a logistic regression model, since the dependent variable reflects an underlying quantitative variable. The function used in the logistic transformation was the natural logarithm of the odds ratio. Thus, we obtained EC_{50}, representing the concentration of propofol at which 50% of the effect was observed and the Hill's slope, representing the steepness of the curve, and their 95% confidence limits (GraphPad Prism version 7.0a).

Habituation was expressed as the percentage decrease of the area of the reflex component between the first and fourth blinks. Differences between the first and the fourth blinks were evaluated by Student's *t*-test for paired data after Kolmogorov–Smirnov test (IBM SPSS version 24.0 IL, USA).

The Shapiro–Wilk test was used to test whether the data were normally or non-normally distributed. If normally distributed, the data were compared using a paired two-sided *t*-test, whereas if the data were non-normally distributed, a Wilcoxon signed ranks test was used. Comparison of the correlation coefficients and differences between effective values and curve estimates were evaluated by Bonferroni's corrected Student's *t*-test, for paired or unpaired data, when appropriate (IBM SPSS version 24.0 IL, USA).

Data are presented as mean ± standard deviation, unless stated otherwise. A p-value inferior to 0.05 was considered statistically significant (*$p < 0.05$, **$p < 0.001$).

3 Results

Data from twenty-eight patients were analyzed. In 3 patients low quality EMG signals or abnormal baseline measurements (the baseline latencies of R_1 were longer (> 3SD) than the reference values [22, 23]) were present and these

patients were excluded from the study. Twenty-five patients were considered for the analysis, with demographics as follows: 61 ± 13 years old, 71.68 ± 11.59 kg, 162.44 ± 8.89 cm, 1 ASA I, 18 ASA II, 6 ASA III, 9 males and 16 females. No patient had cardiovascular or respiratory problems. All patients reached − 6 in the aRASS.

The supraorbital nerve was stimulated with 26 ± 5 mA, a well-accepted intensity by the patients [24]. Baseline values for all patients were determined. The mean baseline value of the latency of R_1 was 10.69 ± 0.98 ms and R_2 38.16 ± 7.25 ms. The baseline value of the duration of R_1 was 9.16 ± 1.43 ms and R_2 32.41 ± 10.11 ms. The baseline value of the amplitude of R_1 was 405.38 ± 221.71 µV and R_2 416.68 ± 193.61 µV. The baseline value of the area of R_1 was 1173.58 ± 505.78 µV ms and R_2 2440.43 ± 1356.16 µV ms. Figure 2 shows an example of a baseline response from one patient.

After the propofol infusion was started, areas and amplitudes of R_1 and R_2 decreased gradually towards zero. Figure 3 illustrates recordings obtained in one patient during propofol administration. The progression of the events for all patients is presented in figures of the Supplemental Material S1.

Table 2 shows the results of the times at which LOR_2, LOR_1, LOBR and LOCR occurred. The average estimated Ce concentrations of propofol and the aRASS score at those moments are also presented. With the exception of one patient, the corneal reflex (− 6 in aRASS) was still present after LOR_1 disappeared. In 2 patients, LOBR was equal to LOR_1 and in only 1 patient LOCR = LOR_1. All patients were responsive at LOR_2.

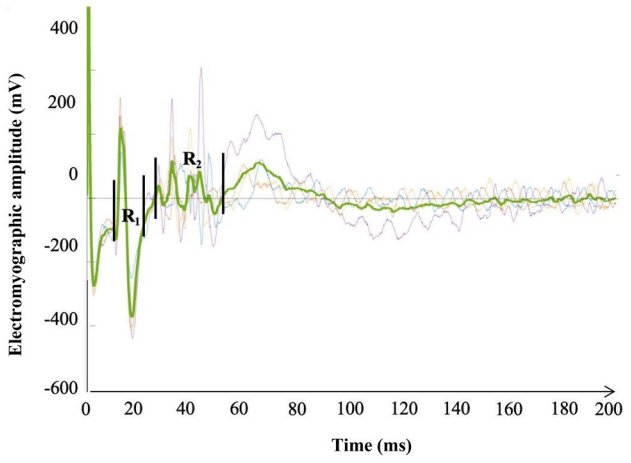

Fig. 2 Example of an electromyographic record of an electrically evoked blink reflex before the infusion of propofol started (baseline). The highlighted line represents the average response from four consecutive blink reflex responses. Vertical lines mark the beginning and end of the individual components (R_1 and R_2), these lines were used to calculate latency, duration, area, and amplitude. These lines were marked by an expert neurophysiologist at the end of the session, using the marker tool of the VikingQuest™ neurophysiological device

The times for endpoints LOR_2, LOR_1, LOBR and LOCR were all statistically different from each other ($p < 0.001$).

The relationship between the estimated propofol Ce concentration and LOR_2, LOR_1, LOBR and LOCR for all 25 patients, was characterized by the EC_{50} and the Hill's slope values (Table 3). Dose–response curves are presented in Fig. 4. We found EC_{50} of LOR_2 to be smallest, followed by the EC_{50} of LOR_1, LOBR and LOCR. The Hill's slopes of LOR_1 and LOR_2 were statistically significant ($p < 0.001$), indicating a different variability between the concentrations of LOR_1 and LOR_2. The following Hill's slopes were not statistically different: LOR_1 versus LOBR ($p = 0.2467$), LOR_1 versus LOCR ($p = 0.2226$), and LOBR versus LOCR ($p = 0.8976$).

Because R_2 responses were quickly abolished and, consequently, there were an insufficient number of data points, only R_1 responses are analyzed. We only analyzed the areas and amplitudes of R_1 because, when a blink reflex component disappears, latency and duration cannot be measured, but areas and amplitudes are zero.

Table 4 shows that areas and amplitudes of R_1 component were correlated with aRASS, with amplitudes of R_1 correlating better than areas. Given this, Spearman's rho correlation coefficients were used to assess the relationship between the change in areas (and amplitudes) from the baseline to aRASS = − 1 (i.e. when the patient is not fully alert, but has sustained awakenings to voice such as eye opening and eye contact) and the required dose of propofol to LOBR (and to LOCR). Results are presented in Table 5. There was a significant correlation between the change in amplitudes of the blink reflex component R_1 from baseline to aRASS = − 1 and the concentration of propofol at LOBR (Rho = − 0.445, $p = 0.026$, $N = 25$). Change in amplitudes from baseline to aRASS = − 1 and the concentration of propofol at LOCR were also correlated (Rho = − 0.424, $p = 0.035$, $N = 25$).

The association between aRASS and the estimated Ce concentration of propofol is given by P_k 0.593 (0.10) and by ρ 0.856 (0.060), showing a correlation, indicating that the clinical scale aRASS increased monotonically and positively with increasing estimated propofol Ce concentrations until LOCR, revealing an increasing deepening of anesthesia.

There was no habituation of the R_1 component (habituation ≈ 2%). Difference between the first and fourth blinks of the R_1 component was not statistically significant ($p = 0.793$). During the baseline recordings (0 mg propofol) and even after induction, R_2 showed a gradual decrease in area (habituation). The area of the fourth blink of R_2 was, on average, 22% lower than the first blink. Difference between the first and fourth blinks of the R_2 component were statistically significant ($p < 0.001$).

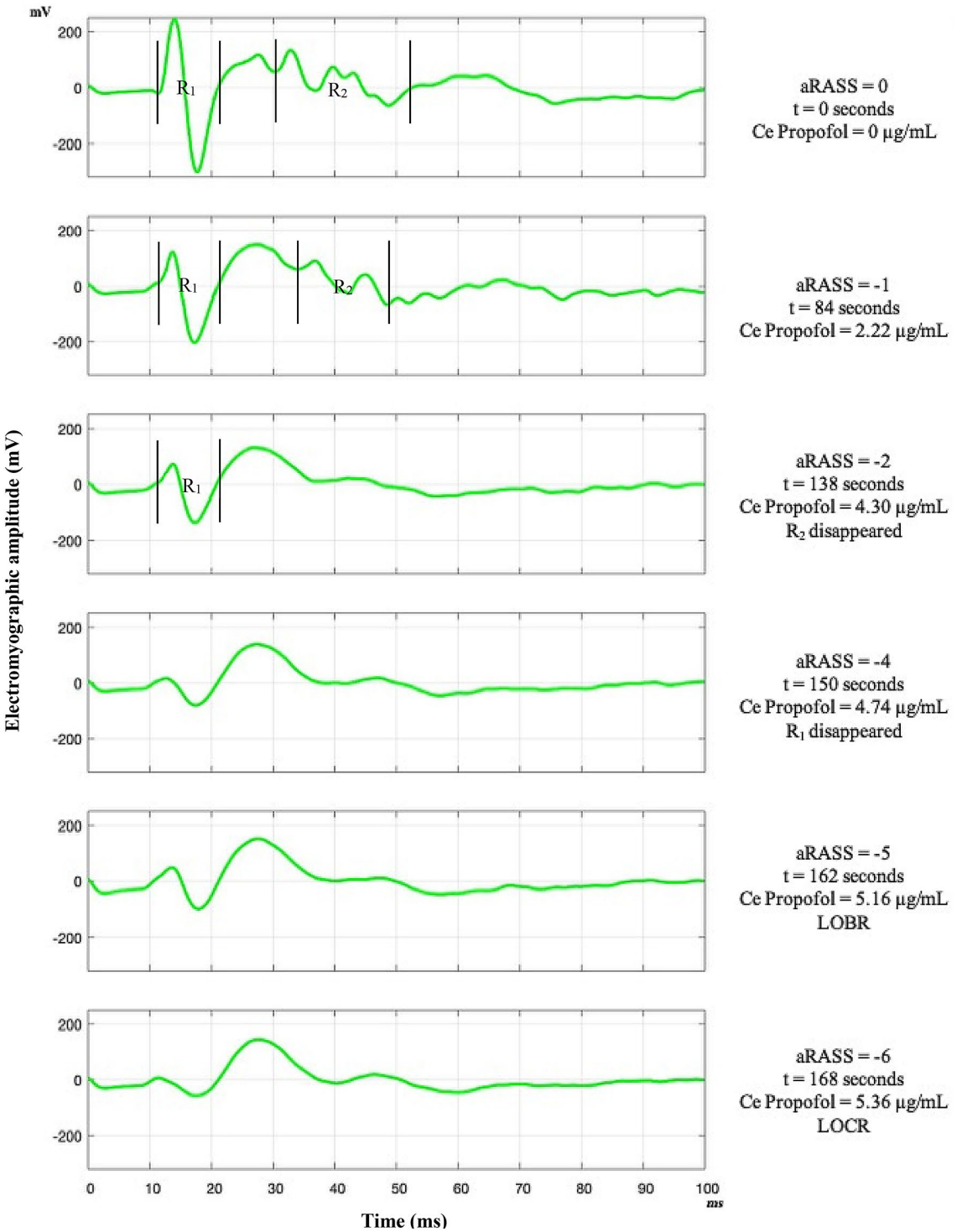

Fig. 3 Example of electromyographic records of the average from four consecutive blink reflex responses of one patient. Each row shows the effects of the increasing administration of propofol. Amplitude and area of R_1 decreased with the depth of anesthesia. After 138 s of propofol infusion (t), the R_2 component disappears. R_1 component was last seen after 150 s. Loss of behavioral responsiveness (aRASS = − 5) occurred after 162 s and loss of corneal reflex (aRASS = − 6) occurred 168 s after propofol infusion, at an effect-site concentration of 5.36 µg/mL. Vertical lines mark the beginning and end of the individual components (R_1 and R_2), marked by an expert neurophysiologist at the end of the session, using the marker tool of the VikingQuest™ neurophysiological device

Table 2 Time since propofol started (seconds), predicted effect-site (Ce) concentrations of propofol (μg/mL), and adapted Richmond Agitation-Sedation Scale (aRASS) score at loss of R_2 (LOR_2), loss of R_1 (LOR_1), loss of behavioural response (LOBR) and loss of corneal reflex (LOCR) in all 25 patients

	Time since propofol started (seconds)	Predicted propofol Ce concentration (μg/mL)	aRASS score
LOR_2	49.68 ± 26.18	1.29 ± 0.82	− 1
LOR_1	102.48 ± 33.00	3.04 ± 1.28	− 2
LOBR	138.24 ± 28.49	4.22 ± 1.28	− 5
LOCR	145.92 ± 28.45	4.50 ± 1.33	− 6

Results are mean ± standard deviation or median

Table 3 Predicted effect-site concentrations of propofol (μg/mL) that produces loss of R_2 (LOR_2), loss of R_1 (LOR_1), loss of behavioural response (LOBR) and loss of corneal reflex (LOCR) in 50% (EC_{50}) of patients

	EC_{50}	(95% CI)	Hill's slope	(95% CI)
LOR_2	1.249	(1.193, 1.304)	− 2.475	(− 2.791, − 2.158)
LOR_1	3.025	(2.960, 3.090)	− 4.368	(− 4.882, − 3.853)
LOBR	4.163	(4.092, 4.234)	− 4.756	(− 5.150, − 4.362)
LOCR	4.470	(4.384, 4.555)	− 4.795	(− 5.234, − 4.355)

According to our clinical protocol for anesthesia, at LOCR, propofol infusion was stopped. However, following LOCR, predicted propofol Ce concentration continued to increase, resulting in a so-called overshoot. Since most individuals still had corneal reflex at LOR_1, we investigated whether the increase of propofol concentration due to the overshoot if one would stop the infusion at LOR_1, would be enough for each individual to reach the required concentration of propofol to lose corneal reflex. Thus, a set of curves was simulated to compute the plasma and effect-site concentrations of propofol if one stops the propofol infusion at the time of LOR_1. Simulated curves ranged from the beginning of propofol infusion until 10 min after propofol was stopped, at LOR_1. The simulations were performed in Simulink/MATLAB, using the parameters of the Schnider pharmacokinetic model, as well as demographics, initial infusion rates and times of LOR_1. Figure 5 shows predicted and simulated plasma and effect-site concentrations (recorded by TCI system) of one patient, a female with 65 years old, 75 kg, 160 cm. Propofol infusion started at 220 mL/h and was kept for 168 s until LOCR, time at which propofol infusion was stopped. At this point, although the plasma concentration begins to decrease, the propofol effect-site concentration continues to increase until a maximum point is reached. Plasma concentrations were used as references to find the stoppage time of the infusion. Estimated overshoot was then calculated, in μg/mL, as the difference between the maximum estimated effect-site concentration achieved after the

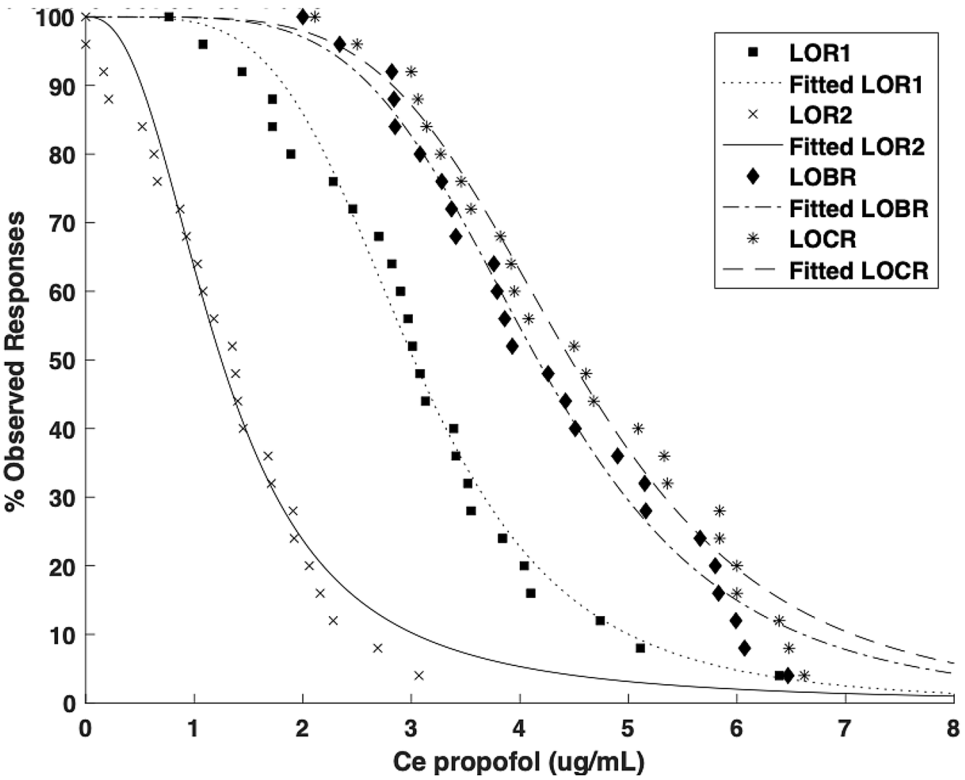

Fig. 4 Propofol dose–response curve for loss of R_2 (LOR_2), loss of R_1 (LOR_1), loss of behavioural response (LOBR) and loss of corneal reflex (LOCR) regarding all patients. Filled squares and their corresponding best-fit dotted line denote LOR_1; crosses and their corresponding best-fit solid line denote LOR_2; filled diamond and their corresponding best-fit dashed-dot line denote LOBR and asterisks and their corresponding best-fit dashed line denote LOCR

Table 4 Correlations [mean prediction probability (group P_k) and mean Spearman's correlations (group ρ)] between the adapted Richmond Agitation-Sedation Scale (aRASS) score and the areas or amplitudes of blink reflex component R_1

	aRASS				Propofol Ce concentration			
	P_k	SD	ρ	SD	P_k	SD	ρ	SD
Area R_1	0.672	0.183	− 0.688	0.165	0.739	0.107	− 0.823	0.153
Amplitude R_1	0.709	0.134	− 0.721	0.123	0.812	0.102	− 0.838	0.113

Correlations were calculated for 25 patients individually and presented as the mean value for all patients and the standard deviation (SD)

Table 5 Spearman's correlations between the change in areas and amplitudes of the blink reflex component R_1 from baseline to adapted Richmond Agitation-Sedation Scale score − 1 (aRASS = − 1) and the effect-site (Ce) concentration of propofol at loss of behavioural response (LOBR) and loss of corneal reflex (LOCR) in all 25 patients

	Propofol Ce concentration at LOBR	Propofol Ce concentration at LOCR
Change in areas from baseline to aRASS = − 1		
Correlation coefficient	− 0.314	− 0.324
Sig. (2-tailed)	0.127	0.114
N	25	25
Change in amplitudes from baseline to aRASS = − 1		
Correlation coefficient	− 0.445*	− 0.424*
Sig. (2-tailed)	0.026	0.035
N	25	25

*Correlation is significant at the 0.05 level (2-tailed)

propofol infusion was stopped and the estimated effect-site concentration at LOCR. Simulated overshoot was calculated, as the difference between the maximum simulated effect-site concentration achieved after the propofol infusion was stopped (if stopped at LOR_1) and the simulated effect-site concentration at LOR_1, in µg/mL. Estimated and simulated overshoots, in the example of Fig. 5 were 0.57 µg/mL and 0.64 µg/mL, respectively.

For all 25 patients, the estimated overshoot was 0.62 ± 0.31 µg/mL and the simulated overshoot was 0.71 ± 0.13 µg/mL. The increase of propofol concentration due to the overshoot if one would stop the infusion at LOR_1 would not be enough to reach the effect-site concentration required to LOBR. The difference between the estimated propofol concentration at LOBR and LOR_1 was on average 4.22–3.04 = 1.18 µg/mL (Table 2).

4 Discussion

With this study, we showed that neurophysiologically-derived parameters of EMG records of electrically evoked blink reflex could be used as surrogates of the Richmond Agitation-Sedation Scale to provide an objective endpoint of the cortical and brainstem effects of propofol in the continuum to unconsciousness.

The baseline blink reflex responses before propofol infusion in this study were similar to those found in the literature. Table in the Supplemental Material S2 compares the values of measurements for the latency, area, duration, and amplitude of R_1 and R_2 in different studies.

After propofol infusion was started, there is a time when the second component of the blink reflex, R_2, was abolished and sometime later, the first component of the blink reflex, R_1, was also abolished. We found that the R_2 component was last seen at an aRASS value of − 1 and R_1 at an aRASS of − 2. The differences in the degree of suppression of the two components of the reflex can be explained neurophysiologically. The R_1 component of the blink reflex is mediated predominantly by an oligosynaptic reflex arc which contains one or two interneurons [25]; however, the R_2 component is relayed through a polysynaptic path, including neurons in the reticular formation, where gamma aminobutyric acid (GABA) receptors are present [26], thereby representing the target for propofol [27]. Besides the GABA effect of propofol, it can also inhibit *N*-methyl-D-aspartate (NMDA) receptors, facilitating the inhibition of these reflex responses [28]. In addition, propofol depresses skeletal muscle excitability [29], thereby influencing the electromyographic response. The presence of remifentanil can also synergistically influence the effects of propofol [30].

The different sensitivity of the blink reflex components to propofol can be seen in Table 3 and Fig. 4. The R_2 component was the most susceptible to the effects of propofol, while R_1 was the most resistant. Each blink reflex component and each endpoint studied (LOBR and LOCR) had a different EC_{50}. The EC_{50} of LOR_2 and LOR_1 are not coincident. The same happened to EC_{50} of LOR_1 and LOBR, with EC_{50} of LOR_1 and LOCR and with EC_{50} of LOBR and LOCR. Almost all patients lost the R_1 component when they still had clinically observed corneal reflex (24 patients). Only one patient lost R_1 at the same time as LOCR and two patients at the same time as LOBR.

Habituation, a gradual quantitative diminution of response to repeated uniform stimuli, is present in R_2 but not in R_1 during baseline recordings and even after induction of

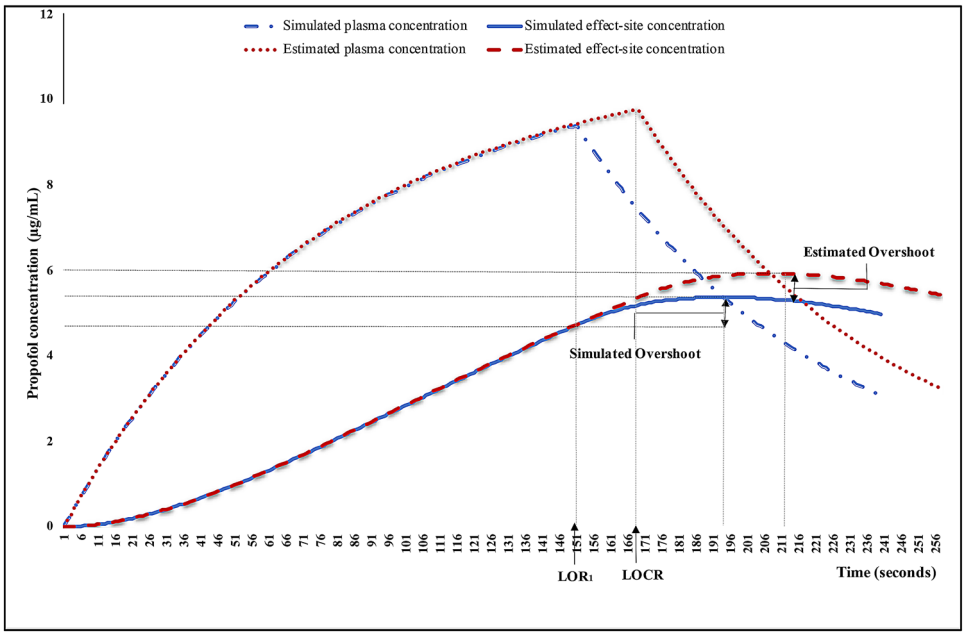

Fig. 5 Effect-site and plasma concentrations of propofol during an intravenous administration with TCI system performed to one patient, female, 65 years old, 75 kg, 160 cm, with an infusion rate of 220 mL/h. Red lines represent the estimated concentrations of propofol, recorded by the TCI system (dashed line representing the effect-site concentrations and dotted line representing the plasma concentration). Blue lines represent the simulated concentrations of propofol, computed if propofol infusion stopped at LOR_1 (solid-line representing effect-site concentration and dash-dot-line representing plasma concentration). LOR_1 occurred 151 s after propofol started and corresponds to the peak maximum of the simulated plasma concentration. LOCR occurred 168 s after propofol started and corresponds to the peak maximum of the estimated effect-site concentration. Overshoot corresponds to the difference between the peak of the effect-site concentration and the effect-site concentrations at which the peak plasma concentrations was maximum

propofol. Habituation of R_2, although small in this study, is statistically significant. It can be prevented by using stimuli at a lower rate, but with the disadvantage of not providing the temporal discrimination usually used clinically in our center to determine LOBR.

Rapid abolition of the R_2 component due to its habituation resulted in an insufficient number of data points for us to analyze. We focused on the analysis of the R_1 component of the blink reflex for this reason. The R_1 component of the blink reflex has a strong correlation with aRASS scale. Amplitudes of R_1 correlated better with aRASS scale than areas. When the R_1 component of the blink reflex disappears, the latency cannot be measured, but area and amplitude are zero. This extra data point for the area and amplitude invariably resulted in a better correlation, because this zero area and amplitude coincided with a lower aRASS scale. The aRASS scale of our study has a minor modification compared with the original RAAS scale, that is the adding of a corneal reflex endpoint because the loss of corneal reflexes is a reliable indicator of impaired brainstem function due to the actions of the hypnotic agent on the nuclei in the pons and midbrain [31].

Another finding was that the R_1 component has a correlation with estimated propofol Ce concentrations and that aRASS scale was strongly correlated with estimated propofol Ce concentrations, indicating that the clinical scale aRASS increased monotonically and positively with increasing estimated propofol Ce concentrations until LOCR. This revealed an increasing deepening of anesthesia. Area and amplitude of the R_1 component showed to be good indicators to predict the different anesthetic levels (aRASS from 0 to − 6) or to predict the estimated propofol Ce. Change in amplitude of the R_1 component from baseline to aRASS = − 1 showed to be correlated with the propofol effect-site concentration required for both LOBR and LOCR. This may be an indication that it would be possible to estimate the individual need of propofol by knowing the amplitude response of the R_1 component right after the transition from the fully awake and alert state to the drowsy state. This could be a very interesting study in the field of modulation that we want to perform in a near future.

To answer the question of whether neurophysiologic-derived parameters of EMG records of electrically evoked blink reflex are useful or not in identifying the moment of loss of responsiveness determined with a modified version of the RAAS scale in the continuum to unconsciousness, the answer is yes. Based on our findings, one can argue that due to overshooting, the proposed moment to stop the propofol

infusion would be after the patient loses the R_1 component of the blink reflex. This would then lead to a subsequent increase in the effect-site concentration from the already circulating plasma propofol and achieve a higher concentration that may be capable of abolishing the behavioral reflex, the endpoint we were targeting. If this subsequent increase was not enough, the infusion could be resumed for an increased dose [32].

The R_1 component of the blink reflex disappears before patients lose consciousness, i.e. $LOCR > LOR_1$. This differs from the results of Mourisse et al. [21], who have studied the relation between the clinical depth of sedation/anesthesia scale with the blink reflex, using a different sedation scale and a different anesthetic protocol. Mourisse's patients did not receive remifentanil, and propofol was administered in a stepwise deepening of anesthesia with different targets (calculated effect-site concentrations of 0 µg/mL, 0.5 µg/mL, 1 µg/mL, 1.5 µg/mL, 2.2 µg/mL, 3.5 µg/mL, 5.0 µg/mL, 6.0 µg/mL and 7.0 µg/mL) and only two minutes after reaching target effect-site concentrations, blink reflexes and depth of anesthesia scores were recorded. In our study, remifentanil infusion starts with an effect-site concentration target of 2.5 ng/mL and then patients received propofol by target-controlled infusion at an infusion rate of 3.3 mL/kg/h, in TCI-View mode, slowly and continuously, until LOBR. Probably due to the use of remifentanil, our R_1 responses were abolished too soon. Although our study did not individualize the effects of propofol alone, which is a limitation of our study, it may be seen as offering the advantage of addressing the real scenario in which clinical anesthesia takes place every day. Another limitation of our approach is the use of estimated Ce concentration values in the absence of pseudo-equilibrium state, which may contribute to less accurate values. In contrast to Mourisse's study, our main goal was to assess whether the blink reflex could be used as a clinical endpoint to identify loss of behavioral response. For this reason, the stimulations of the blink reflex were started before propofol infusion to establish a baseline. A slow infusion of propofol was then used to allow enough time to contemplate the transition from consciousness to unresponsiveness. Our main goal was not to develop a new pharmacokinetic-pharmacodynamic model of propofol and blink reflex, but to investigate it in this transition period.

Nevertheless, our method of electromyographic recording of electrically evoked blink reflex in patients submitted to general anesthesia and in the continuum to unconsciousness, showed to be a possible method to continuously monitor the EMG, in awake, sedated or unconsciousness patients, during the onset to unconsciousness, and in a real scenario in which clinical anesthesia takes place every day. Patients also found the stimuli to be easily tolerable.

Based on our results we conclude that the blink reflex, although being an indicator of immobility, is not useful in precisely determining the absence of responsiveness. However, one can argue that there is an ideal concentration of propofol required to lose corneal reflex, which can be reached after the propofol infusion stops at LOR_1, due to overshooting. Future work would be developing a model capable of identifying this intermediate amount of propofol. We also intend to perform an analysis of the EMG signals obtained from the blink reflex in the frequency domain to evaluate the capacity of several features, such as mean power, median frequency, and band-power, to predict our clinical aRASS scale and to predict the effect-site concentrations of propofol [33].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Ref. 068-17 (062-DEFI/062-CES) and with the Helsinki declaration and its amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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