Reliability of pulse oximetry at four different attachment sites in immobilized white rhinoceros (*Ceratotherium simum*)

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Abstract

Objectives: To determine the reliability of peripheral oxygen haemoglobin saturation (SpO_2), measured by a Nonin PalmSAT 2500A pulse oximeter with 2000T transflectance probes at four attachment sites (third eyelid, cheek, rectum and tail), by comparing these measurements to arterial oxygen haemoglobin saturation (SaO_2), measured by an AVOXimeter 4000 co-oximeter reference method in immobilized white rhinoceros (*Ceratotherium simum*).

Study design: Randomized crossover study.

Animals: A convenience sample of eight wild-caught male white rhinoceros.

Methods: White rhinoceros were immobilized with etorphine (0.0026 ± 0.0002 mg kg^{-1}, mean ± standard deviation) intramuscularly, after which the pinna was aseptically prepared for arterial blood sample collection, and four pulse oximeters with transflectance probes were fixed securely to their attachment sites (third eyelid, cheek, rectum and tail). At 30 minutes following recumbency resulting from etorphine administration, the animals were given either butorphanol (0.026 ± 0.0001 mg kg^{-1}) or an equivalent volume of saline intravenously. At 60 minutes following recumbency, insufflated oxygen (15 L $minute^{-1}$ flow rate) was provided intranasally. In total, the SpO_2 paired measurements from the third eyelid (n = 80), cheek (n = 67), rectum (n = 59) and tail (n = 76) were compared with near-simultaneous SaO_2 measurements using Bland-Altman to assess bias (accuracy), precision, and the area root mean squares (ARMS) method.

Results: Compared with SaO_2, SpO_2 measurements from the third eyelid were reliable (i.e., accurate and precise) above an SaO_2 range of 70% (bias = 1, precision = 3, ARMS = 3).

However, SpO_2 measurements from the cheek, rectum and tail were unreliable (i.e., inaccurate or imprecise).

Conclusions and clinical relevance: A Nonin PalmSAT pulse oximeter with a transflectance probe inserted into the space between the third eyelid and the sclera provided reliable SpO_2 measurements when SaO_2 was > 70%, in immobilized white rhinoceros.

Keywords: chemical immobilization; co-oximetry; oxygen haemoglobin saturation; pulse oximetry

Introduction

Hypoxaemia is a life-threatening complication that often accompanies chemical immobilization of white rhinoceros (*Ceratotherium simum*) (Reiners et al. 2019). If untreated, hypoxaemia can cause organ failure, leading to morbidity and mortality. Hence, reliable and field-friendly techniques are needed to monitor hypoxaemia, for early detection and treatment in rhinoceros.

Arterial oxygen haemoglobin saturation (SaO_2) is a useful index of blood oxygenation that can be indirectly estimated by pulse oximetry, or directly measured by co-oximetry. Co-oximetry is a spectrophotometric method that directly measures SaO_2 and is regarded as a 'reference method' for humans. Although untested in the rhinoceros, the spectrophotometric characteristics of haemoglobin (i.e., infrared absorbance of oxy- and deoxyhaemoglobin) are almost identical among humans, horse (Grosenbaugh et al. 1997) and rhinoceros (Reiners et al. 2019), suggesting that co-oximetry algorithms are likely transferable to the rhinoceros.

Unlike co-oximetry, pulse oximetry measures peripheral arterial oxygen haemoglobin saturation (SpO_2), noninvasively and continuously, making it a more convenient method to indirectly monitor blood oxygenation in mammals (Reiners et al. 2018). Apart from the theoretical-based work of Haymerle et al. (2016), no study has investigated the reliability of pulse oximetry and probe attachment sites in immobilized white rhinoceros.

In this study, a co-oximeter was used to directly measure SaO_2 and thereby assess the reliability of pulse oximetry at different attachment sites, specifically the third eyelid, cheek, rectum and tail. We hypothesized that pulse oximetry, when used with appropriate probe placement, can reliably measure SaO_2 in immobilized white rhinoceros.

Materials and methods

Animals

This study was approved by the University of Pretoria Animal Ethics Committee (V035-17) and the South African Nation Parks Animal Use and Care Committee (001/16). All procedures were conducted at the Veterinary Wildlife Services, Kruger National Park, South Africa (23° 49' 60 S, 31° 30' 0 E; altitude 320 m). A convenience sample of eight wild-caught male white rhinoceros (*Ceratotherium simum*) (4–5 years old) were captured and relocated to a holding facility (bomas) where they were used for the study. The animals were fed lucerne and teff, and water was provided *ad libitum*.

Immobilization

Data were collected during a larger investigation into the effects of etorphine, with and without butorphanol. Animals were immobilized twice with etorphine—once to test the effects of butorphanol on etorphine immobilization and once for control (saline) purposes—in a random order (www.randomiser.org), with a 2 week washout period between treatments. Etorphine [0.0026 ± 0.0002 mg kg^{-1}, mean ± standard deviation (SD); M99; Voluplex, Mnandi, South Africa; see drug doses in Table S1], was administered intramuscularly in the nuchal hump using a 3 mL dart with 60 mm uncollared needle propelled by a compressed air rifle (DAN-INJECT International SA, Skukuza, South Africa). Butorphanol (0.0260 ± 0.0001 mg kg^{-1}, Butonil; Wildlife Pharmaceuticals Pty Ltd, South Africa), or an equivalent volume of saline, was administered intravenously after 30 minutes following recumbency from the etorphine administration. Insufflated oxygen was provided intranasally (15 L $minute^{-1}$ flow rate) after 60 minutes through a nasogastric tube, inserted into one nostril to the level of the medial canthus of the eye, coupled to an oxygen tank with a pressure regulator and a flowmeter. Immobilization was reversed with 0.11 mg kg^{-1} (median) naltrexone (Kyron Laboratories, Benrose, South Africa) administered intramuscularly at the end of data collection.

Experimental procedure

A 25 cm, 22 gauge intravenous catheter (Nipro Medical Corporation, NJ, USA) was inserted into the medial auricular artery for arterial blood collection. Four Nonin PalmSAT 2500A pulse oximeters (Kyron Laboratories (Pty) Ltd., South Africa) with Nonin 2000T transflectance pulse oximeter probes (Kyron Laboratories (Pty) Ltd) (Fig. 1a) were placed at the following attachment sites:

1) Third eyelid (nictitating membrane mucosa), where the probe was covered in lubricant gel and gently inserted (after applying gentle pressure onto the eye orbit through the upper eyelid) into the space between the third eyelid and sclera of the eye, away from the cornea and towards the medial canthus, such that it apposed the third eyelid (Fig. 1b). Once the probe was in place, the eyes were covered with a blind fold.
2) Cheek (buccal mucosa), where the probe was attached to a modified pair of tongs (Reiners et al. 2018) and inserted inside the mouth so that it lay against the buccal mucosa, approximately 90 mm from the commissure of the lip (level with the maxillary premolars). The tongs were gently clamped to secure the probe (Fig. 1c).
3) Rectum (rectal mucosa), where the probe was inserted approximately 50 mm into the rectal cavity (Fig. 1d,i) and secured in position against the rectal wall alongside a rectal temperature probe (Fig. 1d,ii).
4) Tail (ventral tail skin), where the probe was secured gently with adhesive tape, with the probe apposing, a non-pigmented area of the skin, on the ventral midline of the tail base (Fig. 1d,iii).

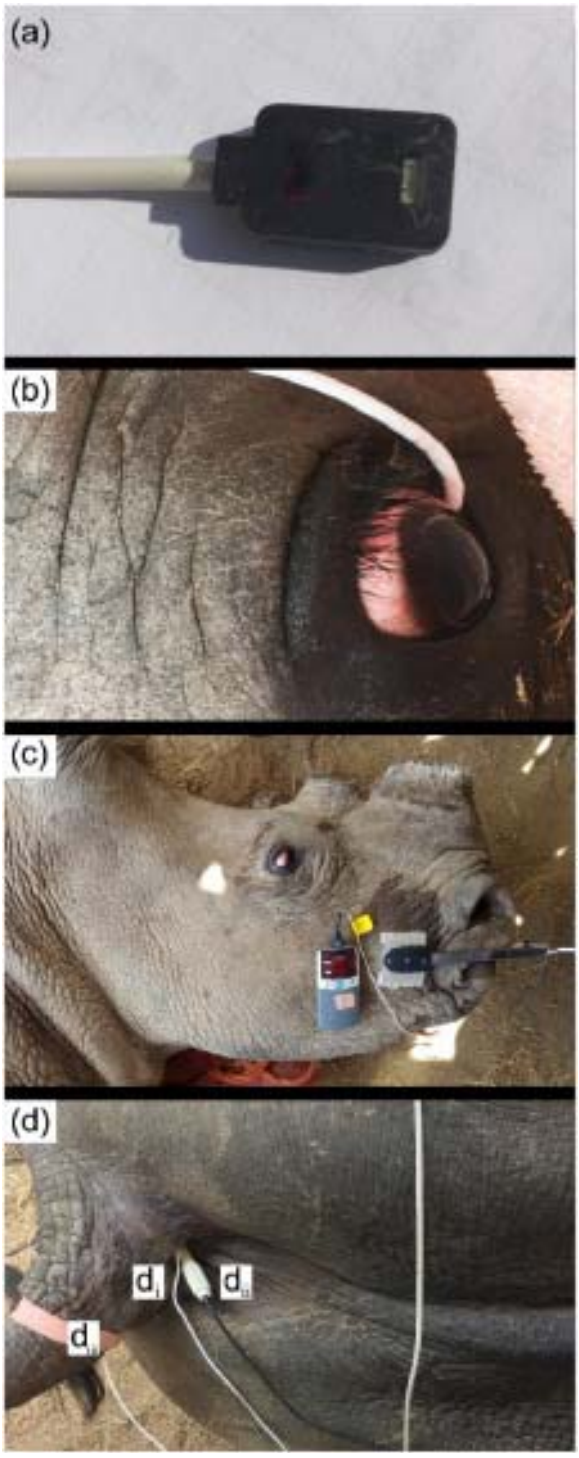

Figure 1. Four Nonin PalmSAT pulse oximeters and 2000T transflectance probes used at four different attachment sites (third eyelid, cheek, rectum and tail) in eight immobilized white rhinoceroses (*Ceratotherium simum*) (a) The transflectance probes (Nonin 2000T) were placed against (b) the third eyelid towards the medial canthus of the eye, (c) the mucosa of the cheek, approximately 90 mm from the commissure of the lip (using a technique described by Reiners et al. 2018), (d) (i) the rectal mucosa, approximately 50 mm into the rectal cavity (ii, temperature probe) and (iii) the ventral midline of the tail where it was secured gently with adhesive tape on a non-pigmented area of the skin.

We had convenient access to these attachment sites as our study was conducted concurrently with another study.

Animals were instrumented immediately following recumbency. The first arterial blood sample (3 mL) was collected after instrumentation and the remaining six samples were collected approximately every 10 minutes thereafter until reversal. Blood samples were collected anaerobically into heparinized syringes (BD Present A-Line; BD Medical, New Jersey, USA) and immediately stored on ice for co-oximeter measurements. At the same time, pulse oximetry measurements were recorded from the four attachment sites.

Pulse oximetry data collection

The SpO_2 values, pulse rate and pulse quality were recorded in sets of three (i.e., triplicate), from each of the four pulse oximeters. Pulse quality (signal strength) was indicated by a flashing, coloured, light-emitting diode, where green indicated good, amber intermediate and red poor quality. Pulse oximetry measurements were recorded in this order: third eyelid, cheek, rectum, tail. A 10 second averaging time was applied for each pulse oximeter reading.

Co-oximetry data collection

Arterial blood samples for measurement of SaO_2 were collected within 30 seconds of recording SpO_2 measurements. Blood samples were analysed within 15 minutes using a co-oximeter (AVOXimeter 4000; Surgical Innovations, South Africa), calibrated daily with test cuvettes (Surgical Innovations), and used as a reference method for comparisons with the SpO_2 measurements (Batchelder & Raley 2007).

Statistical analyses

Mean and SD were calculated for the triplicate SpO_2 measurements. 'Pass data' comprised triplicate SpO_2 measurements, where the pulse quality was indicated by a green (good) or amber light (intermediate), and the SD between the triplicate SpO_2 measurements was $< 3\%$, indicating stable and consistent measurements. 'Excluded data' comprised triplicate SpO_2 measurements, where poor pulse quality was indicated by a red light or the SD between the triplicate SpO_2 measurements was $\geq 3\%$, indicating unstable and variable measurements.

The Bland-Altman method for multiple observations was performed on 'pass data' to compare the mean SpO_2 measurements at the four attachment sites with SaO_2 (Bland & Altman 2007). The Bland-Altman method determined the bias (accuracy), precision between the different devices (SD), and the limits of agreement (LOA) between the paired measurements (bias $\pm$ 1.96 SD). For Bland-Altman method analysis to meet 80% power at an α of 0.05 and β of 0.90, a minimum of 17 paired data sets (SpO_2-SaO_2) were required. Area root mean squares (ARMS), a combined measure of accuracy and precision, were also calculated between the paired measurements to determine the overall reliability of pulse oximeter measurements (Batchelder & Raley 2007).

Results

Immobilizations were effective, and animals recovered successfully upon reversal (Boesch 2019).

Quality of pulse oximetry data

The pulse quality of the pulse oximetry data was mostly acceptable at each probe attachment site (Table S2). Despite data from the cheek and rectal probes showing good and intermediate pulse quality, compared with the other attachment sites, there was greater variability in the SpO_2 measurements (triplicate SpO_2 measurements with SD ≥ 3%). Whereas the tail had some SpO_2 measurements that indicated poor pulse quality (Table S2). Thus, most of the SpO_2 data from the rectum (29%) and cheek (15%), were excluded with the least data excluded from the third eyelid (8%) and tail (4%) (Table S2).

Table 1. Performance of pulse oximetry (SpO_2) compared with the co-oximetry (SaO_2) reference method at four probe attachment sites (eyelid, cheek, rectum and tail) in eight white rhinoceros immobilized with etorphine [0.0026 ± 0.0002 mg kg^{-1}, mean ± standard deviation (SD)] after the exclusion criteria were applied (i.e., only 'Pass data' presented). Excluded data were triplicate SpO_2 readings where the pulse quality was poor (red light), or SD was ≥ 3%. Pass data are triplicate SpO_2 readings where the pulse quality was good or intermediate (green or amber light) and SD was < 3%. ARMS, area root mean squares; LOA, limits of agreement (maximum and minimum); *n,* the sample size of the pass data.

Probe site	Ranges	*n*	Bias (%)	Precision (%)	ARMS (%)	LOA
Eyelid	0–100	80	–2∗	10	10	18–21
	70–100	41	1∗	3∗	3∗	8–5
	< 70	39	–5	13	17	20–30
	70–79	13	1∗	2∗	2∗	4–3
	80–89	13	0∗	4	4∗	8–8
	90–100	15	3∗	3∗	4∗	5–3
Cheek	0–100	67	–10	16	19	21–42
	70–100	33	0∗	10	10	19–20
	< 70	34	–20	15	25	9–49
	70–79	13	–5	5	7	5–15
	80–89	10	–3∗	10	10	–1–22
	90–100	10	8	11	12	28–13
Rectum	0–100	59	–23	20	30	16–62
	70–100	33	–9	11	14	11–30
	< 70	26	–40	14	43	–12–68
	70–79	11	–20	5	21	–11–29
	80–89	10	–9	7	11	5–23
	90–100	12	0∗	5	5	10–9
Tail	0–100	76	–34	21	40	8–76
	70–100	35	–14	8	16	1–30
	< 70	41	–51	12	52	–27–74
	70–79	12	–22	5	23	–12–33
	80–89	11	–15	3∗	15	–10–21
	90–100	12	–6	3∗	6	1–12

∗Results that are considered acceptable according to manufacturer guidelines, United States Food and Drug Administration (FDA) and International Organization for Standardization (ISO) (bias ≤ ± 3%, precision ≤ 3% and ARMS ≤ 4%).

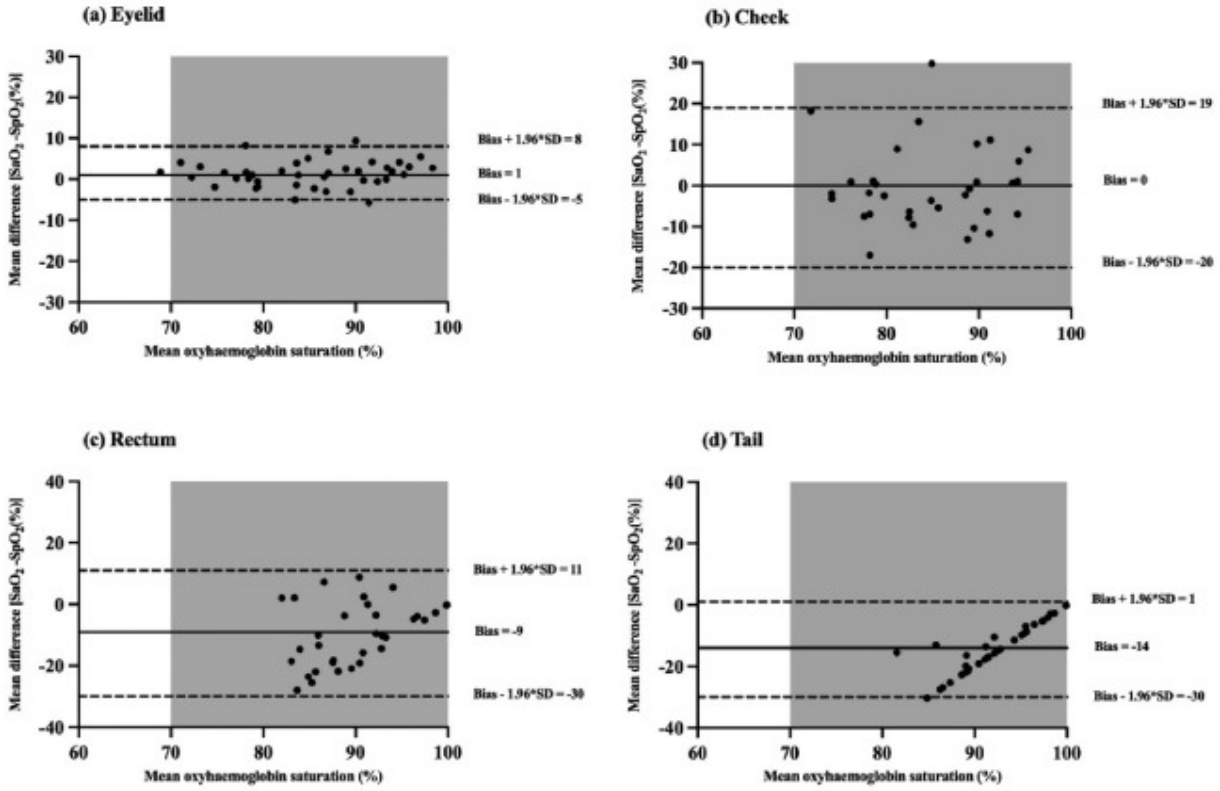

Figure 2. Bland-Altman plots showing the level of agreement between arterial oxygen haemoglobin saturation (SaO_2) measured by the co-oximeter reference method and the peripheral arterial oxygen haemoglobin saturation (SpO_2) measured by the Nonin PalmSAT pulse oximeters and 2000T transflectance probes attached at four different attachment sites in eight immobilized white rhinoceroses (*Ceratotherium simum*). The probes were placed against (a) the third eyelid, (b) the mucosa of the cheek, (c) the rectal mucosa and (d) the ventral midline of the tail on a non-pigmented area of the skin. The percentage mean difference between SaO_2 and SpO_2 is plotted against the percentage mean arterial oxygen haemoglobin saturation measurements obtained from the co-oximeter and the pulse oximeters (SaO_2 and SpO_2) at the saturation range of 70-100%. The grey shaded regions represent the manufacturer's claimed performance range of 70-100%. The estimated bias is represented by the solid line and limits of agreement [bias ± 1.96 ∗ standard deviation (SD)] is represented by the dashed lines. Each datum point represents the paired measurements taken from the rhinoceroses. The number of paired measurements from each attachment site are as follows: third eyelid (n = 41), cheek (n = 33), rectum (n = 33) and tail (n = 35).

Reliability of pulse oximetry data

The performance of pulse oximetry is reported in Table 1 and Fig. 2. Pulse oximetry measurements from the third eyelid were accurate, but imprecise over the entire SaO_2 range (0-100%). However, at the manufacturer's claimed performance range of 70-100%, the measurements were accurate and precise (Fig. 2a), whereas at values < 70%, the measurements were inaccurate and imprecise. Between 80% and 89%, the measurements were accurate but imprecise; however, the ARMS indicated overall reliability. Between 70% and 79%, and between 90% and 100%, the measurements were accurate and precise. In contrast, SpO_2 measurements from the cheek, rectum and tail were found to be unreliable

(i.e., inaccurate or imprecise) across all SaO_2 ranges (Figs 2b–d). The SpO_2 measurement from the tail systematically overestimated SaO_2 measured by the co-oximeter, providing SpO_2 values > 90% even when the animal was hypoxaemic (SaO_2 < 90%). This is shown by the systematic pattern seen in Fig. 2d. The poor reliability observed from the probe attached to the tail may result from the potentially unsuitable characteristics of the skin at that location.

Discussion

In immobilized white rhinoceros, the Nonin PalmSAT 2500A pulse oximeter with a 2000T transflectance probe placed between the third eyelid and the sclera reliably measured SaO_2 at the manufacturer's claimed performance range of 70-100%. Although mostly good pulse quality measurements were obtained across all sites, the reliability of pulse oximetry was poor when the probes were placed on the cheek area, rectal mucosa and the skin of the tail. There were no triplicate SpO_2 with SD > 3% (no variability) from the tail, and the highest variability was from the rectum (29%). Differences in the reliability and variability of the pulse oximeter measurements between sites highlight the importance of probe placement when assessing blood oxygenation in immobilized white rhinoceros.

Pulse oximetry requires the detection of a pulse to measure SpO_2, so some studies compare pulse oximetry pulse rate with heart rate to exclude potentially erroneous SpO_2 data, as a weak pulse signal may also indicate an error in SpO_2 measurements (Reiners et al. 2018). In this study, we did not measure heart rates simultaneously with pulse oximetry measurements, but instead used the internal pulse quality indicator and variability of the SpO_2 measurements (within the reading time) as exclusion criteria.

In humans, the United States Food and Drug Administration and International Organization for Standardization (ISO) have certified pulse oximetry as reliable when the comparisons with co-oximetry are $\leq \pm 3\%$ for bias, $\leq 3\%$ for precision and $\leq 4\%$ for ARMS across the SaO_2 range of 70-100% (Batchelder & Raley 2007). In immobilized white rhinoceros, we showed that at SaO_2 values < 70%, the SpO_2 measurements were unreliable, which was unsurprising given that ISO has set a limit that pulse oximetry devices can only be calibrated at high saturation levels (Weininger 2007). The reliability of pulse oximetry also depends on probe attachment sites in different species. In llamas and alpacas, pulse oximetry is reliable when the probe is placed on the tongue (Grubb & Anderson 2017). In thick-tongued cattle (Coghe et al. 1999) and impala (Mtetwa et al. 2020), it is reliable at the base of the tail. In horses (Reiners et al. 2018) and cynomolgus monkeys (Young et al. 2002), the most reliable site is the cheek. It is possible that site-specific differences between attachment sites (e.g., skin and coat thickness, pigmentation and vascular perfusion), and possibly the effect of ambient light and movement, explain the variation in reliability of pulse oximetry measurements (Reiners et al. 2018).

To our knowledge, this is the first study to validate pulse oximetry at different attachment sites in white rhinoceros and we show that the third eyelid is the most reliable attachment site. The mucous membrane of the third eyelid has a well-perfused vascular bed with little pigmentation, which probably facilitates reliable measurement. The probe was also secured in position by the surrounding tissues and shielded from ambient light. The animals tolerated the probe well during immobilization, and no adverse effects to the eye were observed over the study period, or for 2 weeks thereafter. The transflectance probe used was an appropriate size with smooth edges, for ease of placement without causing abrasion to the surface of the eye.

Conclusions

In conclusion, the Nonin PalmSAT 2500A device with a 2000T transflectance probe placed under and on the mucous membrane of the third eyelid can be used reliably in white rhinoceros at saturation levels between 70% and 100%. The cheek, rectum and tail attachment sites were unreliable. Furthermore, if saturation levels < 70% are expected, then alternative methods such as co-oximetry should be used.

Authors' contributions

TKM: preparation of equipment, data collection, statistical analysis, preparation of manuscript. EPS: data interpretation, preparation of manuscript and editing of the manuscript. GEZ and PB: data collection and editing of the manuscript. LCRM: conceived the study, data collection, data interpretation, preparation of manuscript.

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Conflict of interest statement

The authors declare no conflict of interest.

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