Comparison of haemoglobin measurement methods in the operating theatre

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Editor’s key points

- This study compared the accuracy of four bedside methods for haemoglobin (Hb) assessment (non-invasive and continuous haemoglobin measurement with Pulse CO-Oximetry (SpHb), arterial blood measurement by satellite CO-Oximetry (HbSat), and HemoCue arterial (HcueArt) and capillary (HcueCap) blood) with a laboratory haematology analyser (LHA).
- HcueArt is closest to LHA even when Hb concentrations change rapidly.
- HbSat provided values in close agreement LHA but requires sample preparation and handling.
- SpHb is less invasive, less accurate, but measures continuously.
- When absolute accuracy is essential then invasive measurements are needed to confirm SpHb or HcueCap values before transfusion.

Background. Various methods of haemoglobin (Hb) measurement are available to guide transfusion including several methods that allow for measurement at the bedside. This study directly compared their absolute and trend accuracy compared with values from the central lab (reference method).

Methods. Adult patients undergoing surgery with expected blood loss wore a rainbow ReSposable sensor connected to a Radical-7 Pulse CO-Oximeter (SpHb). Arterial samples were analysed with a haematology analyser (HbLab), a satellite CO-Oximeter (HbSat), and a point-of-care haemoglobinometer (HemoCue; HcueArt). Concomitantly, ear capillary blood was tested using the same haemoglobinometer (HcueCap). Absolute accuracy and the clinical significance of error were assessed with Bland–Altman plots and three-zone error grids. Trend analysis was performed using a modified polar plot, testing both directionality and magnitude of Hb changes compared with the reference.

Results. Two hundred and nineteen measurements from 53 patients with HbLab ranging between 6.8 and 16.3 g dl⁻¹ (4.2 and 10.1 mmol litre⁻¹) were recorded. Compared with the reference method, bias (precision) was 0.2 (0.2) g dl⁻¹ [0.1 (0.1) mmol litre⁻¹] for HcueArt, 0.8 (0.3) g dl⁻¹ [0.5 (0.2) mmol litre⁻¹] for HbSat, 0.5 (0.5) g dl⁻¹ [0.3 (0.3) mmol litre⁻¹] for HcueCap and 1.0 (1.2) g dl⁻¹ [0.6 (0.7) mmol litre⁻¹] for SpHb. None of the devices tested would have led to unnecessary or delayed transfusion according to 2006 ASA transfusion criteria. Trend accuracy was better for HcueArt and HbSat than for HcueCap and SpHb.

Conclusion. Bedside Hb measurement methods differ in their agreement to a laboratory haematology analyser but none would have led to transfusion errors.

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Anaesthetists need tools to monitor haemoglobin (Hb) quickly and accurately in order to avoid delayed transfusions, which can result in patient death, and unnecessary transfusions which increase patient morbidity. For this purpose, several invasive methods of estimating Hb at the bedside such as satellite CO-Oximeters and point-of-care haemoglobinometers have been developed. Although they provide rapid measurement of Hb, they still require a blood sample, and therefore allow only intermittent monitoring. Pulse CO-Oximetry provides continuous and non-invasive monitoring of Hb with a multiple wavelength optical sensor. Several studies have compared the point-to-point accuracy of Pulse CO-Oximetry in emergency departments, intensive care units (ICUs), and operating theatres but few have evaluated trend accuracy in comparison with other invasive bedside devices.

This study aims to determine the absolute and trend accuracy of four bedside methods for Hb assessment (non-invasive and continuous Hb measurement with Pulse CO-Oximetry (SpHb), arterial blood measurement by satellite CO-Oximetry (HbSat), and HemoCue measurement with arterial (HcueArt) and capillary blood (HcueCap)) during surgery at high risk of bleeding, compared with a laboratory haematology analyser as the reference method.
Methods

This prospective observational study was conducted at the University Hospital of Poitiers, France. After obtaining ethics committee approval (Comité de Protection des Personnes Ouest III; Eudract ID RCB: 2009-A01144-53) and informed consent, adult patients undergoing major surgery with expected significant blood loss were recruited.

Patients with suspected methaemoglobinemia, carbon oxide poisoning or hyperbilirubinaemia, and those undergoing emergency surgery were excluded.

Anaesthesia was induced with propofol 2.5 mg kg\(^{-1}\) and sufentanil 0.3 mg kg\(^{-1}\). Tracheal intubation was facilitated with rocuronium 0.6 mg kg\(^{-1}\) and additional rocuronium administration was guided by neuromuscular monitoring during the procedure. Anaesthesia was maintained with desflurane and sufentanil. If necessary, norepinephrine infusion was titrated to obtain a mean arterial pressure > 65 mm Hg. Subjects were ventilated using volume-controlled mechanical ventilation (tidal volume: 6–8 ml kg\(^{-1}\)) with a mixture of oxygen and air (inspired oxygen fraction, \(F_{O_2}\) 0.50). Respiratory rate was adjusted to maintain normocapnia. No patient received additional epidural anaesthesia or regional anaesthesia.

Patients wore rainbow adult ReSposable sensors (R2–25, Revision E) connected to a Radical-7 Pulse CO-Oximeter, software version 7.6.0.1 (Masimo, Irvine, CA, USA), for continuous and non-invasive measurement of total Hb (SpHb), \(S_pO_2\), heart rate, and perfusion index, an indicator of localized perfusion. Sensors were applied to the patient according to the directions for use provided by the manufacturer. This included the application of the adhesive portion of the sensor so that the emitter and detector were precisely aligned on the finger. Sensors were covered with opaque shields to prevent optical interference. The sensor position was checked before every reading and readjusted if the adhesive portion became misaligned. If perfusion index was <1%, the sensor was repositioned and recalibrated by switching the monitor off and on. The first SpHb measurement was recorded after the device had been reporting SpHb data for at least 15 min.

For invasive measures of Hb, arterial blood was drawn through a radial arterial catheter placed in the wrist contralateral to the SpHb sensor, for continuous arterial pressure monitoring and intermittent blood analysis. Blood was collected into standard blood collection tubes appropriate for the method of analysis. Reference Hb values (HbLab) were obtained by analysing arterial blood samples at the central laboratory using a Sysmex™ XT-2100i automated haematology analyser (Roche™ Diagnostics, Paris, France). Central laboratory analysers vary by institution but the Sysmex automated haematology analyser is of a type which is typical for many hospital laboratories and has been shown to have good concordance with the cyanmethaemoglobin assay, the international standard for Hb measurement.\(^{19}\) The confidence limits provided by the manufacturer for the Sysmex analyser are 0.2 g dl\(^{-1}\) (0.12 mmol litre\(^{-1}\)). The same samples were also analysed with a satellite CO-Oximeter (Siemens™ RapidPoint 405, Siemens™, Munich, Germany; HbSat) and a point-of-care haemoglobinometer (HemoCue™, Hb201, Ängelholm, Sweden; HcueArt). Concomitantly, the fourth drop of blood after skin puncture on the ear was obtained for testing of capillary blood with the same HemoCue™ point-of-care device (HcueCap). The anaesthetist was blinded to all Hb values except those of the arterial HemoCue which was used for clinical care.

The Pulse CO-Oximeter is self-calibrating. The Sysmex™ measures Hb by colorimetry using the cyanide-free, sodium lauryl sulphate method, and is calibrated daily according to manufacturer’s instructions and good laboratory practice. The Siemens™ RapidPoint CO-Oximeter is calibrated daily under the control of the central laboratory. The HemoCue™ point-of-care device is factory calibrated against the cyanmethaemoglobin method and does not require recalibration.

Simultaneous recording of SpHb, HbLab, HbSat, HcueArt, and HcueCap values were manually collected before surgical incision and then approximately hourly or more often if clinically indicated. Measures ended after completion of the surgical procedure.

Statistical analyses

Categorical data are expressed as number and percentage. Quantitative data are reported as mean values and standard deviation (SD) if normally distributed and as median values and 25th–75th percentiles (25p–75p) if the distribution is non-normal.

Agreement between HbLab (reference method) and Hb values provided by the test devices was performed as described by Bland and Altman.\(^{20}\) In this study, multiple Hb measurements per patient provided unequal numbers of replicated data in pairs. With such clustered observations, adjustment is necessary, so bias, precision (1 SD) and limits of agreement (bias (1.96 SD)) were adjusted by a component of variance technique (estimating inter-individual and intra-individual variance with non-linear mixed effect model).\(^{21}\)

Paired Hb values provided by test devices and the reference method were also plotted using the three zones Hb error grid analysis proposed by Morey and colleagues,\(^{22}\) which takes into account the clinical significance of the difference. The ability of the test devices to follow Hb changes reported by the reference method was analysed by a modified version of the polar plot proposed by Critchley and colleagues\(^{23}\) for cardiac output monitoring. Variations of Hb between two successive measurements were expressed as relative differences in percentage. Only data points with relative variations of HbLab of >10% between two consecutive measurements were used for analysis (see Supplementary material for description of these two statistical methods).

The association of tested methods absolute accuracy with HbLab levels, and SpHb absolute accuracy with perfusion index and use of vasopressors, were assessed in univariate analysis with Pearson’s correlation with variance adjustment for multiple measurements and linear regression, and Student’s t-test when required. For two-tailed tests, a P-value of <0.05 was considered statistically significant. All data
management and statistical analysis were made with R software version 2.11.0 (R Development Core Team, Vienna, Austria), except for the conversion of Cartesian data to polar coordinates for trending analysis which was performed using Excel 2007 (Microsoft® Office Excel® 2007; Microsoft® Corp., Redmond, WA, USA) and SigmaPlot® 12.0 (Systat® Software, San Jose, CA, USA).

**Sample size determination**

Using the method described, the standard error of the 95% limit of agreement (95% CI) is approximately root (3 s² n⁻¹), where s is the SD of the differences between measurements by the two methods and n the sample size. Expecting a SD of the differences between measurements by the two methods of 1 g dl⁻¹, a sample size of 210 measurements gives a 95% CI of about (0.22 s). Considering that four measurements will be performed per patient on average, at least 52 patients should be included.

**Results**

Fifty-six patients were enrolled for the study but three were excluded because of the inability to obtain SpHb signal. Characteristics of the remaining 53 patients are summarized in Table 1. A median (25p–75p) of 4 (3–5) Hb assessments were performed per patient, leading to a total of 219 measurements. The interval between two measurements was 62 (19) min on average (sd). Among the 219 measurements, 25% were performed while the patient was receiving vasopressors (norepinephrine at a concentration up to 0.8 µg kg⁻¹ min⁻¹). The perfusion index [median (25p–75p)] was 3.4% (2.1–4.9). Twenty-four patients received a blood transfusion (45%). Among them, 15 patients received only cell saver salvaged blood [median (25p–75p), 483 ml (245–665)], 7 patients received only red blood cell package [median (25p–75p), 3 packages (2–5)] and two patients received both.

HbLab was between 6.8 and 16.3 g dl⁻¹ (4.2 and 10.1 mmol litre⁻¹) [median (25p–75p), 11 (9.6–12.1) g dl⁻¹ (6.0–7.5) mmol litre⁻¹]; 32.4% of HbLab values were <10 g dl⁻¹ (6.2 mmol litre⁻¹) and 2.3% were <8 g dl⁻¹ (5.0 mmol litre⁻¹). Relative HbLab variations between two consecutive measurements were comprised between 0 and 37%, corresponding to absolute Hb variations comprised between 0 and 3.6 g dl⁻¹ (0 and 2.2 mmol litre⁻¹). Absolute and relative HbLab variations were >1 g dl⁻¹ (0.6 mmol litre⁻¹) in 34.3% assessments and >10 in 31.9% of assessments.

The Bland and Altman plots for test devices are displayed in Figure 1. Compared with the reference method, bias (precision) was 0.2 (0.2) g dl⁻¹ [0.1 (0.1) mmol litre⁻¹] for HcueArt, 0.8 (0.3) g dl⁻¹ [0.5 (0.2) mmol litre⁻¹] for HbSat, 0.5 (0.5) g dl⁻¹ [0.3 (0.3) mmol litre⁻¹] for HcueCap, and 1.0 (1.2) g dl⁻¹ [0.6 (0.7) mmol litre⁻¹] for SpHb.

The three zones Hb error grids for test devices are displayed in Figure 2. Zone A encompassed 100% of data pairs for HcueArt, 89% of data pairs for HbSat, 85% of data pairs for HcueCap, and 74% of data pairs for SpHb. When only HbLab values between 6 and 10 g dl⁻¹ (3.7 and 6.2 mmol litre⁻¹) were considered, (n=71 data pairs) these proportions were 54% for HbSat, 65% for HcueCap, and 18% for SpHb. No data pairs fell into Zone C for any of the test devices.

The modified Critchley’s polar plots for each test device are depicted in Figure 3. A total of 53 HbLab changes exceeding 10% between two assessments were included for analysis. The angular bias (precision) was −1.3 (6.9)° for HcueArt, −2.5 (8.1)° for HbSat, −4.1 (17.4)° for HcueCap, and −17.3 (19.2)° for SpHb. Hb changes with test devices and the reference method were in opposite directions only with HcueCap (n=2, 4%) and SpHb (n=6, 11%).

SpHb absolute accuracy neither correlated with perfusion index value (r=0.39, P=ns) nor with HbLab values (r=0.32, P=ns) or with the use of vasopressors (P=0.08). Absolute accuracy of HcueArt, HcueCap and HbSat were not proportional to HbLab levels. Medium correlation was observed only between SpHb bias and HbLab (r=0.45, P<0.0001). SpHb overestimates HbLab in the lower values, while it underestimates HbLab in the higher values.

**Discussion**

This study compared the absolute and trend accuracy of four bedside devices for Hb assessment in the operating theatre, using a haematology analyser from the central laboratory as the reference method.

Although none of the devices tested would have led to unnecessary or delayed transfusions (according to the 2006 recommendations of ASA), the four methods did differ in their agreement with the reference method. During different surgeries with high risk of bleeding, where haemodynamics...
could be unstable, SpHb and HcueCap had moderate accuracy which suggest that caution should be exercised using these methods when absolute accuracy is essential. HcueArt was the method with the smallest bias and precision and the most reliable method in trend analysis, indicating that this technique can be recommended to guide transfusion.

HcueArt in our study performed similarly in paediatric surgery and adults aortic surgery where bias were 0.1 (0.3) g dl\(^{-1}\) [0.6 (0.2) mmol litre\(^{-1}\)] and 0.0 (0.2) g dl\(^{-1}\) [0.0 (0.1) mmol litre\(^{-1}\)]. Two other studies conducted in ICU patients also reported similar bias, but wider precision of 0.5 and 1.0 g dl\(^{-1}\) (0.3 and 0.6 mmol litre\(^{-1}\)). Errors in blood sample handling may explain these findings as the same blood sample was used for measuring HcueArt and HbLab. Excess blood outside of micro-cuvette, samples containing air bubbles or not immediately placed in the device may lead to inaccurate readings.

Studies performed in the emergency departments, in the operating theatre and in the ICU reported better precision for HcueCap measurements [as low as 0.2 g dl\(^{-1}\) (0.1 mmol litre\(^{-1}\))] while other studies reported greater imprecision, as high as 2.4 g dl\(^{-1}\) [15 mmol litre\(^{-1}\)]. In our study, capillary blood was collected at the ear; this site is more accessible during surgery and gives similar results to blood samples collected from the finger. Errors in blood sample handling may explain the variability of the findings between studies, as previously discussed. Additionally, large volume fluid administration and the presence of an inflammatory state, which causes the passage of water into the interstitial space, may increase the imprecision of HcueCap. Volumes of fluids infused were not precisely quantified in our study. Their influence on the accuracy of HcueCap could not be evaluated.

Bias (precision) of the satellite lab CO-Oximeter in our study is similar to those observed in our previous study (0.9 [0.3] g dl\(^{-1}\) [0.6 (0.2) mmol litre\(^{-1}\)]). Since the two studies used the same device, the bias may have been affected by a problem with internal calibration. Although the overall performance of this device seems satisfactory, several measures had an imprecision of >1.5 g dl\(^{-1}\) (0.9 mmol litre\(^{-1}\)) compared with the reference method, which may seem unusual for this type of device. However, Gehring and colleagues documented variations as large as 1.2 g dl\(^{-1}\) (0.7 mmol litre\(^{-1}\)) from the same blood sample analysed on two identical CO-Oximeters, so inter- and intra-device differences can be a significant source of measurement variation.

The bias (precision) of Pulse CO-Oximetry found in our study were in accordance with the precision (0.9 g dl\(^{-1}\) [0.6 mmol litre\(^{-1}\)]) reported in healthy volunteers after isovolemic haemodilution. Studies performed during spine surgery, gastrointestinal surgery, cardiac surgery, hepatic surgery, Caesarean section, paediatric neurosurgery, or acute gastrointestinal bleeding, reported precision comprised between 1.0 and 2.7 g dl\(^{-1}\) (0.6 and 1.7 mmol litre\(^{-1}\)). Three studies used the same software and sensors versions as in our
study. The first two ones conducted during spinal or hepatic surgery did not find any bias [0.1 mmol litre\(^{-1}\)], but precision was comparable with ours [1.0 mmol litre\(^{-1}\)] for both studies. The third study performed in ICU patients shows different results with no bias and better precision of 0.5 mmol litre\(^{-1}\). In this study, 54% of patients had non-surgical diseases and changes in Hb were slow and of small magnitude. Less than one-third of patients had changes of more than 1.0 mmol litre\(^{-1}\) between two successive measurements while the collection interval was several hours.

SpHb values displayed by the monitor are an average of measurements calculated over several minutes (2–8 min depending on the setting). If the Hb change is rapid and of large magnitude, several minutes are required before the changed value is displayed by the monitor. During spinal surgery, the imprecision of SpHb was higher in patients with the largest blood losses. In contrast, the good precision of SpHb in the ICU study of Frasca and colleagues may be linked to small Hb variations and/or to extended time intervals between two measurements.

Another hypothesis has been advanced to explain the imprecision of SpHb during rapid Hb changes. Pulse CO-Oximetry measures the light absorption of blood in both the microvascular and macrovascular network of the fingertip. During acute haemorrhage, microvascular Hb remains high to maintain tissue oxygenation, while macrovascular Hb measured in a blood sample decreases. The microvascular Hb therefore contributes more to the SpHb estimation during conditions of acute blood loss, increasing the discrepancy between SpHb and an invasive arterial Hb derived value. This hypothesis, while interesting, has not been validated with clinical evidence. The accuracy of SpHb may be influenced by several factors. The infusion of colloids instead of crystalloids appears to alter SpHb accuracy. In our study, crystalloids and colloids were frequently infused simultaneously, and the influence of fluids infused on SpHb accuracy could not be evaluated. Like others, we have observed a medium correlation between SpHb bias and HbLab values. SpHb overestimates Hb in the lower values, while it underestimates Hb in the higher values. Conflicting results have been reported by others. During paediatric neurosurgery, the SpHb accuracy was similar before and after rapid volume resuscitation.

Fig 2  Paired Hb values provided by test devices and the reference method (HbLab) plotted using the three zones Hb error grid taking into account the clinical significance of the difference. (a) Arterial haemoglobinometer (HcueArt); (b) satellite lab CO-Oximeter (HbSat); (c) capillary haemoglobinometer (HcueCap); and (d) Pulse CO-Oximeter (SpHb). Zone A (blue) represents the area where difference between HbLab and test device is clinically acceptable. Zone C (green) represents the area of major therapeutic error leading to unnecessary or delayed transfusion according to the ASA recommendation transfusion thresholds. (1 g dl\(^{-1}\)=0.63 mmol litre\(^{-1}\))
Similarly, SpHb accuracy was comparable during steady state (fluid administration restricted) and dynamic phases (administration of large volumes of fluids) of major hepatic surgery.\(^{18}\)

The accuracy of SpHb seems to decrease in patients with impaired tissue perfusion, hypotension or receiving vasopressors.\(^{6,8,10,14,18}\) Using regional anaesthesia to increase the perfusion to the finger wearing the SpHb sensor has been shown to increase the accuracy of SpHb.\(^{45}\) As other authors, we did not observe any link between the accuracy of SpHb and the value of perfusion index\(^{5,7,11,42}\) or the use of vasopressors.\(^{7,11}\) It is unclear why some investigators observed this limitation and others did not. It may be related to the severity of the vasoconstriction or to the version of the SpHb sensor used. However, it has been shown that the use of norepinephrine increases the likelihood of not being able to obtain a SpHb signal.\(^{7}\) We were unable to obtain a SpHb signal in 3 (5%) patients despite many attempts to reposition the sensor. The inability of the device to display a SpHb value has been reported by others. In one study, SpHb values were not displayed for 1% of the monitoring time and the poor signal quality indicator (‘low SiQ’) was displayed 7% of the time.\(^{12}\) The poor signal quality indicator was displayed for 2.4% of measurements in another study.\(^{42}\) In our study, the position of the sensor was changed if perfusion index was < 1% or the message ‘low SiQ’ was displayed, but we did not quantify the incidence of these events. It is important to note, however, that even though SpHb monitoring may not provide continuous values

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**Fig 3** Trending analysis by polar plot method for arterial haemoglobinometer (HcueArt, a), satellite lab CO-Oximeter (HbSat, b), capillary haemoglobinometer (HcueCap, c), and Pulse CO-Oximeter (SpHb, d) vs haematology analyser (HbLab). The mean angular bias is represented by continuous line and limits of agreement by dashed lines. Only absolute variations HbLab of > 10% between two consecutive measurements were selected for the analysis. The angle is negative if the test method underestimates the variations of HbLab and positive if it overestimates them. If the angle is lower than -45°, the variation of Hb displayed by the test device is in opposite direction to the reference Hb variation.
for 100% of the monitoring time, this method of Hb measurement provides far more data than any intermittent, invasive method of measurement.

Finally, some of the imprecision of SpHb measurements may be attributable to the choice of reference device. In vivo adjustment of Pulse CO-Oximeter values is a feature available on the Radical-7 in some countries and is pending Food and Drug Administration clearance for the US market. In vivo adjustment allows the clinician to manually adjust the initial SpHb measurement to match a value of their preferred reference device. Thereafter, the SpHb value is automatically adjusted by the user-entered bias. A recent study conducted in surgical patients showed retrospective in vivo adjustment of SpHb values reduced both the bias and so by 0.5 g dl⁻¹. retrospective in vivo adjustment of the data collected from our patients reduced the bias and precision from 1.0 (1.2) g dl⁻¹ to 0.4 (1.0) g dl⁻¹ [0.6 (0.7) mmol litre⁻¹ to 0.2 (0.6) mmol litre⁻¹] calculated from 42 matched pairs. This suggests that some, but not all of the bias was constant.

The error grid proposed by Morey and colleagues allows assessment of inaccuracy of devices according to their impact on patient care. In our study, none of the test devices had a negative angular bias, indicating an underestimation of Hb changes, and higher precision reflecting poor tracking accuracy of Hb changes. Moreover, SpHb varied in the opposite direction of reference Hb in 11% of assessments in our study. Similar findings in the operating theatre were reported by other authors, with inverse Hb variations displayed in 6, 7, and 16% measurements.

This study has several limitations. Despite inclusion of patients undergoing surgery at high risk of bleeding, only 32% of measurements were conducted while Hb concentration was <10 g dl⁻¹ (6.2 mmol litre⁻¹) and only 2.3% when it was <8 g dl⁻¹ (5.0 mmol litre⁻¹). Similarly, only 32% of Hb variation between two measurements exceed 10 and 2 exceed 30%. Further studies are necessary to confirm the performance of these devices for low concentrations and larger variations of Hb.

In conclusion, bedside methods of Hb measurement during surgery have different advantages and limitations. During haemorrhagic surgery, a point of care haemoglobinometer measuring arterial blood provided values closest to central laboratory measurements even when Hb concentrations were rapidly changing. CO-Oximetry provided values in close agreement with laboratory measurements but this method requires more sample preparation and handling. The point of care haemoglobinometer measuring capillary blood is less invasive but also less accurate. SpHb by Pulse CO-Oximetry underestimated rapid changes in Hb but is the only method that provides continuous data. Non-invasive and continuous Hb measurement can be valuable as a trend monitor, in patients where absolute accuracy is not essential—or if blood collection is a concern (Jehovah Witness patients for example). When absolute accuracy is essential then invasive measurements are needed to confirm SpHb or HcueCap values before treating (transfusion).

Supplementary material

Supplementary material is available at British Journal of Anaesthesia online.

Authors’ contributions

B.G. chose the patients to be included and helped to draft the manuscript. O.M. and B.D. designed the study and helped to draft the manuscript. D.F. contributed to the design of the study, helped out with the statistical analysis, and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of interest

D.F. and O.M. received lecture fees and travel expenses from Masimo Corp.

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