Combination of different noninvasive measuring techniques: a new approach to increase accuracy of peripheral near infrared spectroscopy

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Abstract. One of the problems of near-infrared-spectroscopy (NIRS) measurements is low reproducibility. The aim of the present study was to introduce quality criteria to increase reproducibility of peripheral NIRS measurements. In a prospective cohort study in 40 neonates, repeated NIRS measurements were performed on the calf. During five "reapplication" periods (of NIRS optodes), five "measurements" (venous occlusions) were performed. Tissue oxygenation index (TOI), mixed venous oxygenation (SvO2), fractional oxygen extraction (FOE), hemoglobin flow (Hbflow), oxygen delivery (DO2), and oxygen consumption (VO2) were assessed. Measurements with linear changes during venous occlusions were included for further analysis (first quality criterion: $R^2 > 0.95$). The second quality criterion was the equation $0 \leqslant \mathrm{TOI} - \mathrm{SvO2} \leqslant (\mathrm{SaO2} - \mathrm{SvO2}) \times 0.2$. Variance components and mean standard deviations were analyzed after introduction of the quality criteria. Variance components of reapplication and measurement decreased after introduction of the second quality criterion (TOI: 46.6–35.0%, SvO2: 76.8–38.2%, FOE: 73.1–37.5%, Hbflow: 70.3–51.9%, DO2: 71.5–52.7%, and VO2: 70.9–63.8%). Mean standard deviations of TOI (6.6 ± 3.0 to $4.7 \pm 3.2\%$), SvO2 (11.1 ± 4.8 to $5.7 \pm 3.9\%$), FOE (11.3 ± 4.8 to $5.9 \pm 4.0\%$), Hbflow (4.3 ± 2.0 to 2.9 ± 1.6 μmol/100 mL/min), and DO2 (17.8 ± 7.6 to 11.4 ± 6.2 μmol/100 mL/min) decreased significantly, too. Only 12% of measurements fulfilled both quality criteria. With the introduction of two quality criteria, test–retest variability of peripheral NIRS measurements decreased significantly and reproducibility increased significantly. [DOI: 10.1117/1.3076193]

Keywords: near-infrared spectroscopy; peripheral muscle; reproducibility; quality criteria.

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1 Introduction

Near-infrared spectroscopy (NIRS) enables noninvasive measurement of oxygenation in regions of interest, e.g., cerebral, renal, intestinal and "peripheral muscle" tissue. Since the first description of NIRS by Jöbsis[1] in 1977 more than 30 years ago, an increasing number of studies with NIRS have been published.[2–5] Some of these studies of "peripheral muscle" NIRS measurements using venous/arterial occlusion were performed in adults[6–10] and some in newborn infants.[11–23]

Until now, doubts concerning the accuracy of measurements in clinical settings have still remained. Furthermore, validation of measurement accuracy was difficult due to missing gold standard methods for comparison. Concerning measurements with arterial occlusion in neonates, Hassan et al. showed that for oxygen consumption (VO2), accepting only measurements with $R^2 > 0.96$ (assessed with regression analysis) led to more consistent results.[14] Therefore, the present study group used $R^2 > 0.95$ in regression analysis as quality criterion in peripheral NIRS measurements in newborn infants.[15–17]

Nevertheless, accuracy and reproducibility of single NIRS measurements are still a problem.[19] The purpose of the present study was to increase reproducibility, and thus accuracy, of "peripheral muscle" NIRS measurements by the introduction of a new quality criterion.

2 Methods

2.1 *Patients*

Term and preterm neonates who were admitted to the Neonatal Ward of the Department of Pediatrics, Medical University of Graz, from January 2007 to December 2007 were consid-

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ered for inclusion in the study. Because peripheral application of NIRS probes in neonates below 2000 g is difficult to perform, study entry criterion was a body weight above 2000 g at the time of measurement. No other specific entry criteria were defined. The neonates were prospectively enrolled. The study was approved by the local ethical committee. Informed consent was obtained for each subject from the parents before starting measurements.

2.2 *NIRS*

NIRS measurements were carried out with the NIRO 300 (Hamamatsu Photonics, Japan). The optodes were placed over the left lateral calf; the interoptode distance was 3.0 cm, and the sampling rate was 2/s. Circular fixation of optodes was avoided to produce no pressure.[24] A differential path length factor of 5.51 was used.[13] The spatially resolved method (NIRO 300) enables the noninvasive continuous measurement of tissue oxygenation index (TOI) and of changes in the concentration of oxygenated hemoglobin (HbO2) and deoxygenated hemoglobin (Hb). Changes in the concentration of total hemoglobin (Hbtot) were calculated from the sum of changes in HbO2 and Hb. HbO2, Hb, and Hbtot were measured in micromole units.

2.3 *Venous Occlusion*

Venous occlusion was performed using a pneumatic cuff placed around the thigh. Venous occlusion causes an increase of calf blood volume by undisturbed calf arterial (in)flow and interrupted venous (out)flow. Thus, changes in HbO2, Hb, and Hbtot during venous occlusion are caused only by arterial inflow and oxygen consumption of tissue.

2.4 *Protocol*

Measurements were performed under standardized conditions[24] during undisturbed daytime sleep after feeding. The infants lay in a supine position tilted up (10 deg), and the calf was positioned just above the level of mid-sternum. Heart rate and SaO2 were measured by pulse oximetry using the ipsilateral foot. A rectal sensor and a skin sensor placed on the ipsilateral calf continuously measured central and peripheral temperatures. After positioning of the NIRS optodes, pneumatic cuff, temperature, and pulse-oximetry sensors, a calm-down period was introduced until there was at least a 3-min rest period without any body movements. Afterward, arterial blood pressure was measured oscillometrically with the pneumatic cuff on the left thigh. After a rest period of 1 min again, the pneumatic cuff was inflated within 0.5–1 s to a pressure below the diastolic arterial pressure and above the venous pressure (i.e., 20–30 mm Hg). The cuff was maintained in the inflated state for 20 s, and NIRS data were recorded. This procedure was repeated with a rest period of at least 40 s between inflations until five measurements were achieved. After the five venous occlusions, the optodes were reapplied at the very same area, and after a further 3-min rest period without body movements, another five venous occlusions were performed. Five reapplications were performed in each infant. Subcutaneous adipose tissue thickness and diameter of the calf were measured by ultrasound.

2.5 *Calculations*

Oxygen delivery (DO2) was calculated from hemoglobin flow (Hbflow), $\text{Hbflow} \times 4 \times \text{SaO2}$, whereby SaO2 was arterial oxygen saturation measured by pulse oximetry. Hb (in)flow corresponds to the increase of Hbtot. Mixed venous oxygenation (SvO2) was calculated from HbO2/Hbtot.[21] VO2 was calculated from $\text{Hbflow} \times 4 \times [\text{SaO2} - \text{SvO2}]$. Oxygen extraction (OE) was calculated from SaO2 – SvO2 and fractional oxygen extraction (FOE) from (SaO2 – SvO2)/SaO2. SaO2, TOI, SvO2, OE, and FOE are given as percentages.

Two quality criteria were introduced consecutively.

2.6 *First Quality Criterion*

Measurements had to have, during venous occlusions, linear changes of Hbtot with $R^2 > 0.95$ assessed with linear-regression analysis. The remaining data underwent the following further criterion:

2.7 *Second Quality Criterion*

A combination of two equations was introduced.

NIRS measures hemoglobin oxygenation in venules, capillaries, and arterioles. TOI represents mean hemoglobin oxygenation/saturation of the venous, capillary, and arteriolar compartments of a regional tissue. SvO2 is calculated out of changes only in the venous compartment, if NIRS is used in combination with venous occlusion. Therefore, TOI has to be higher than SvO2:

$$\text{TOI} \geqslant \text{SvO2} \tag{1}$$

In peripheral muscle tissue, the volume proportions of the venous, capillary, and arteriolar compartments were described as 70:20:10%.[25,26] Assuming that "regional tissue" oxygen extraction corresponds in venules to OE (SaO2–SvO2) and that it is between zero and OE (half of OE) in capillaries and not significant in arterioles,[27] the "regional tissue" oxygen extraction can be calculated from: $(\text{OE} \times 0.7) \times 1 + (\text{OE} \times 0.2) \times 0.5 + (\text{OE} \times 0.1) \times 0 = \text{OE} \times 0.8$. Thus, the regional-tissue oxygen extraction should be 20% lower than OE. However, recent studies using *in vivo* microelectrode, phosphorescence, or hemoglobin saturation methods have shown that, especially at rest, OE starts in arterioles and continues in capillaries.[28] Therefore, "regional tissue" oxygen extraction can be assumed to be even less than 20% lower than OE. TOI, which corresponds to regional-tissue hemoglobin oxygenation/saturation including the venous, capillary, and arteriolar compartments, should therefore be up to 20% of OE higher than SvO2:

$$\text{TOI} - \text{SvO2} \leqslant \text{OE} \times 0.2 \tag{2}$$

Taking into account Eqs. (1) and (2), measurements have to fulfill the following second quality criterion: $0 \leqslant \text{TOI} - \text{SvO2} \leqslant \text{OE} \times 0.2$. With the replacement of OE by SaO2 – SvO2, the equation for the second quality criterion can be transformed to: $0 \leqslant \text{TOI} - \text{SvO2} \leqslant (\text{SaO2} - \text{SvO2}) \times 0.2$.

2.8 *Analysis*

Only neonates with data fulfilling the first quality criterion were included for further analysis. Descriptive analysis of de-

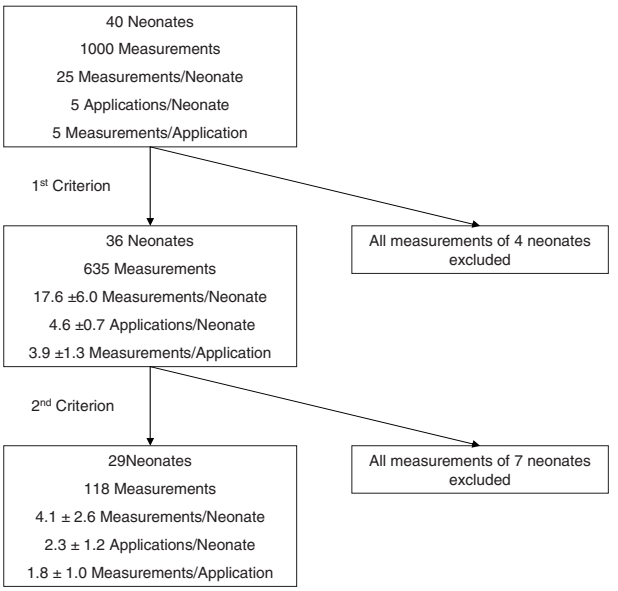

Fig. 1 Reapeated NIRS measurements in 40 neonates.

mographic, clinical, and NIRS data are given as the mean with standard deviation.

In each neonate, the means of remaining NIRS data have been calculated after introduction of first and second quality criterion, respectively. In neonates with NIRS data fulfilling the second quality criterion, data obtained after introduction of the first quality criterion were compared to data obtained after introduction of the second quality criterion by a paired t-Test. To estimate the size of the components contributing to the variation in the NIRS parameters TOI, HbFlow, DO2, VO2, SvO2, and FOE, we set up a variance components model. We used a mixed-effects model with "patient" as a random effect and, as a nested random effect, "optodes reapplication within patient" with five factor levels. For the method we chose the minimum norm quadratic unbiased estimation (MINQUE) model with unit *a priori* values for the ratios of the variance components to the residual variance and for the residual itself. The estimated variance components of the patients ("patient"), optodes reapplications within the patient ("reapplication"), and the residual variance caused by the repetition of measurements ("measurement") for NIRS data on which the first quality criterion was applied were compared with the variance components of NIRS data on which the first and second quality criteria were applied. Variance components are reported as percentages.

To analyze reproducibilty and test–retest variability in patients with at least two remaining measurements, standard deviations of TOI, HbFlow, DO2, VO2, SvO2, and FOE after introduction of the first and second quality criterion were compared by a paired t-Test. All computations were done using the statistical package SPSS for Windows version 14.

3 Results

Forty infants were enrolled in the study, representing a number of 1000 measurements (Fig. 1). Four infants had to be excluded, three infants because of unsettlement during measurements, which forced discontinuation, and one infant due to the fact that no measurement passed the first criterion. Thus, 635 measurements in 36 neonates passed the first criterion and were further analyzed.

Table 1 Demographic and clinical characteristics of the neonates after introduction of the first and second quality criterion.

Patients—no.	First criterion 36	Second criterion 29
Gestational age (weeks)	35.0±3.2	35.0±3.1
Birth weight (g)	2295±683	2330±708
ph-umbilical cord artery	7.23±0.13	7.26±0.12
Apgar 1 min	7.2±2.1	7.4±1.9
Postnatal age (days)	13.3±15.7	13.0±15.6
Actual weight (g)	2420±554	2405±566
Diameter calf (cm)	3.1±0.2	3.1±0.25
Diameter subcutaneous adipose tissue (cm)	0.27±0.06	0.27±0.06
Mean arterial blood pressure (mm Hg)	44.6±7.0	42.9±5.7
Temperature rectal (°C)	36.7±0.4	36.7±0.5
Temperature peripheral (°C)	34.8±0.7	34.9±0.8
Recapillarisation time central (s)	2.6±0.7	2.5±0.7
Recapillarisation time peripheral (s)	2.7±0.7	2.6±0.6

In these infants, the male/female ratio was 23/13. At the time of measurements, nine neonates received respiratory support because of respiratory distress syndrome (RDS) ($n=6$), infection ($n=1$), and asphyxia ($n=2$). Two infants received catecholamines [asphyxia ($n=1$) and infection ($n=1$)]. The remaining 27 neonates were without cardiocirculatory or respiratory support.

There were 118 measurements in 29 neonates that passed the second quality criterion. The exclusion of the seven patients due to the second criterion caused no relevant changes in demographic and clinical characteristics of the neonates (Table 1).

The 11 patients with no measurement passing both criterias had a gestational age of 35.2 ± 3.1 weeks, a birth weight of 2276 ± 494 g, and a postnatal age of 13 ± 12 days. In these infants, the male/female ratio was 6/5. At the time of measurements, five neonates received respiratory support because of RDS ($n=3$), infection ($n=1$), and asphyxia ($n=1$). One infant received catecholamines (asphyxia). The remaining six neonates were without cardio-circulatory or respiratory support.

NIRS parameters of the 29 neonates passing both criteria are presented in Table 2. After introduction of the second criterion, mean HbFlow, DO2, and SvO2 decreased, whereas

Table 2 Mean NIRS parameters of the 29 neonates with NIRS measurements fulfilling the first and second quality criterion.

	First criterion	Second criterion	p-value
TOI (%)	62.7±7.3	64.1±8.2	0.073
SvO2 (%)	64.6±7.7	60.7±9.4	0.001
FOE (%)	33.6±7.0	36.6±9.1	0.001
Hbflow (μmol/100 mL/min)	10.0±3.4	8.8±3.9	0.036
DO2 (μmol/100 mL/min)	39.0±13.3	34.3±15.3	0.037
VO2 (μmol/100 mL/min)	11.7±3.7	11.9±4.1	0.836

Table 4 Mean standard deviations of NIRS parameters of 23 neonates with ≥2 NIRS measurements/neonate fulfilling the first and second quality criterion.

	First criterion	Second criterion	p-value
TOI (%)	6.6±3.0	4.7±3.2	0.012
SvO2 (%)	11.1±4.8	5.7±3.9	<0.001
FOE (%)	11.3±4.8	5.9±4.0	<0.001
Hbflow (μmol/100 mL/min)	4.3±2.0	2.9±1.6	0.003
DO2 (μmol/100 mL/min)	17.8±7.6	11.4±6.2	0.004
VO2 (μmol/100 mL/min)	4.5±2.1	3.6±2.3	0.126

FOE increased. TOI and VO2 did not change.

The NIRS parameters' patient, reapplication, and measurement variance components after introduction of the first and second criterion are presented in Table 3. The variance components of reapplication and measurement decreased with the introduction of quality criteria in all parameters: TOI from 46.6 to 35.0%, SvO2 from 76.8 to 38.2%, FOE from 73.1 to 37.5%, Hbflow from 70.3 to 51.9%, DO2 from 71.5 to 52.7%, and VO2 from 70.9 to 63.8%.

Mean standard deviations of TOI, SvO2, FOE, Hbflow, and DO2 decreased significantly after introduction of the second quality criterion. There was also a decrease of the standard deviation in VO2, but the difference did not reach significance (Table 4). The mean standard deviation of TOI–SvO2 decreased significantly from 10.74 ± 5.10 to 2.34 ± 1.14% after introduction of the second quality criterion. The mean difference of TOI and SvO2 (TOI–SvO2) shifted from -1.9 ± 4.9 to 3.4 ± 1.9%.

4 Discussion

With the introduction of two quality criteria, we were able to increase the reproducibility and accuracy of peripheral-muscle NIRS measurements and decrease the test–retest variability of TOI, SvO2, FOE, Hbflow, DO2, and VO2 measurements. Variance components of application and measurement of SvO2 had the highest drop. Before introduction of the second quality criterion, 76.8% of the differences of SvO2 values were due to the application and measurement procedures. The contribution of application and measurement procedures to differences dropped to 38.2% after introduction of the second quality criterion.

Before the introduction of the second quality criterion, the mean TOI was lower than the mean SvO2. Because TOI is a combination of the venous, capillary, and arteriolar compartments, TOI has to be higher than SvO2. With the second quality criterion, mainly data of measurements were excluded, where TOI was too low in relation to SvO2 and/or SvO2 was too high in relation to TOI and SaO2. As a consequence, TOI tended to increase, SvO2 decreased, and FOE increased.

In the present study, as in recent studies of our group, measurements were performed according to the recommendation at rest, which constitutes the basis for measurement reproducibility in neonates.[15–17,24] Measurements during body movements, which raise blood flow and O2 consumption,[10,29]

Table 3 Variance components (%) of patients, reapplications, and repeated measurements after introduction of the first and second quality criterion.

	Variance components after first criterion			Variance components after second criterion		
	Patient	Application	Measurement	Patient	Application	Measurement
TOI (%)	53.4	30.2	16.4	65.0	18.7	16.3
SvO2 (%)	23.2	16.2	60.6	61.8	23.7	14.5
FOE (%)	26.9	15.0	58.1	62.5	21.4	16.1
Hbflow (%)	29.7	31.6	38.7	48.1	34.3	17.6
DO2 (%)	28.5	32.0	39.5	47.3	35.1	17.6
VO2 (%)	29.1	30.2	40.7	36.2	49.2	14.6

are not comparable to measurements at rest. Body movements can also cause artifacts due to pressure changes on the sensor and displacement of sensors. Contrary to adults, a standardization of body movements and exercise is nearly impossible in neonates.

Venous occlusion in combination with NIRS is a technique that allows analyzing changes only in the venous compartment. This technique has been shown to agree with plethysmography and the Fick method and was therefore used in the present study.[30,31]

There are different approaches in the analysis of changes during venous occlusion. In several studies, the first seconds after occlusion were used for analysis.[18,20–23] Nevertheless, this period might have some artifacts from inflating the cuff. Analyzing the first 15 s after the beginning of venous occlusion, as done in the present study, showed lower test–retest variability than analysis of the first 5 s.[11]

In addition, in the present study the quality of linearity of parameter changes by coefficient of determination (R^2) was defined. This was the first quality criterion and represents an approach leading to more consistent results.[14–17] This approach easily allows the exclusion of tracings with artifacts (e.g., caused by movements), even if they might have been small or invisible.

Apart from the problem of excluding movement artifacts, reproducibility of peripheral NIRS measurements might be influenced by other confounding factors in neonates. Some of these confounding factors are difficult to recognize immediately during measurements. One major technical problem is the application of the light source and detector in such a way that good optical contact is secured. A loss of fixation to the skin may cause loss of light shielding, thus allowing direct passage of light from source to detector. Quite a number of oximeters and imagers are commercially available,[4] though the fixation systems differ between these devices. Nevertheless, tight fixation for optical contact and light shielding still remain a problem and may cause falsified results. Furthermore, dust, hair, or skin pigmentation just below the light source or detector might disturb optical contact and falsify results. These are well-known limitations for NIRS measurements and are probably the main reason for the low reproducibility of NIRS measurements. It was for this reason that the second quality criterion was introduced in the present study to recognize values, which are not possible due to physiological concerns. It represents a new approach, strengthened by the fact that parameters of three different techniques were included: TOI, which is measured continuously by the spatially resolved method, SvO2, which is calculated from changes during venous occlusion, and SaO2, which is measured by pulse oximetry. Because only relative values are used within calculations, this quality criterion is independent of path length and path-length factors. Furthermore, the quality criterion is applicable with any device and is not restricted to one device which is produced by a certain company.

Underwood et al.[19] observed a high-ranging inter- and intraobserver variability using NIRS as a screening tool for "patent ductus arteriosus." In a recent study in eight neonates in need of intensive care, a low test–retest variation defined by standard deviations of SvO2 measurement series after reapplication of the NIRS optodes was found.[11] Furthermore, this increased reproducibility was obtained by visual exclusion of artifacts. With the present quality criterion, an increase of reproducibility can be achieved automatically without the need for visual controlling, which is substantial for clinical routine.

With introduction of the quality criteria, standard deviations of TOI, SvO2, and FOE decreased and were close to those accepted for SaO2 measured with pulse oximetry. Pulse oximetry has become a vital clinical device in the care of neonates despite continuing problems with reliability, accuracy, and clinical utility. Manufacturers claim confidence limits for tracing values above 70% to be between $\pm 2\%$ and $\pm 4\%$, although newer technologies have improved, especially in the reduction of false alarms.[32,33] The limitations of pulse oximetry are well known and very similar to those of NIRS (e.g., movement artifacts, ambient light, and skin pigmentation).[33]

Measurement of cerebral oxygenation in term and preterm infants is also still of questionable reproducibility.[12,34] For this reason, Sorensen et al. increased the precision of measurements of cerebral TOI by calculating the mean value from five reapplications of the probes.[35] However, such a procedure is difficult to perform in clinical settings. The application of quality criteria by introducing equations that immediately give feedback of accuracy of measurement results should be of interest in this field in the future.

In the present study, only 118 of 1000 initial measurements (12%) passed both quality criteria. This seems to be low, but such strict quality criteria are necessary to obtain measurements with high accuracy. Furthermore, the present quality criteria enable, by immediate analysis, accuracy to be assessed and any possible disturbing factor to be eliminated immediately.

In adults, the adipose tissue thickness ranges from 1 to 9 mm and thus strongly affects peripheral NIRS measurements.[9] In neonates >2000 g, the adipose tissue thickness is about 3 mm. Therefore, the influence can be assumed to be negligible in this group. But adipose tissue thickness should be measured, especially if study populations have a wide range in body weight, to rule out any influence. In adults, peripheral muscle tissue shows a spatial distribution pattern within different regions of a muscle.[8] The calf and forearm in neonates are rather small. Therefore, the light source and detector can be fixed on a well-defined position in each subject, eliminating influence of spatial heterogeneity.

The contribution of myoglobin to the NIRS signal is discussed controversially and has been assumed to be 10%. In hemoglobin-free perfused animal hearts, myoglobin accounted for nearly 50% of the NIRS signal. Myoglobin should be fully saturated under most conditions, because P_{50} for myoglobin is very low (5 mm Hg).[36] Therefore, changes occurring during venous occlusions can be assumed to be due to changes of Hb and HbO2, because myglobin content of the tissue will not change in such short periods.

One possible domain of peripheral-muscle NIRS measurements in the future might be detection of (occult) shock and monitoring of therapy.[6,7,37] Whether NIRS will become the basis for clinical decisions will depend on measurement reproducibility and accuracy. Technical improvement of devices will play an important role in improving reproducibility, but there will still be artifact problems due to application and

settings. With the present quality criteria, we were able to increase reproducibility substantially. The present combination of different parameters obtained by different techniques and devices to establish a quality criterion represents a new approach to increase accuracy of *in vivo* measurements. In addition, the application of these quality criteria might be independent of NIRS device, study population, and setting.

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