Journal of Perinatology (2013) 33, 282–287

www.nature.com/jp

ORIGINAL ARTICLE

Changes in regional tissue oxygenation saturation and desaturations after red blood cell transfusion in preterm infants

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OBJECTIVE: The study investigated the ability of near-infrared spectroscopy (NIRS) to detect subgroups of preterm infants who benefit most from red blood cell (RBC) transfusion in regard to cerebral/renal tissue oxygenation (i) and the number of general oxygen desaturation below 80% (SaO_2 <80%) (ii).

STUDY DESIGN: Cerebral regional ($crSO_2$) and peripheral regional ($prSO_2$) NIRS parameters were recorded before, during, immediately after and 24 h after transfusion in 76 infants. Simultaneously, SaO_2 <80% were recorded by pulse oximetry. To answer the basic question of the study, all preterm infants were divided into two subgroups according to their pretransfusion $crSO_2$ values (<55% and ⩾55%). This cutoff was determined by a k-means clustering analysis.

RESULT: $crSO_2$ and $prSO_2$ increased significantly in the whole study population. A stronger increase ($P<0.0005$) of both was found in the subgroup with pretransfusion $crSO_2$ values <55%. Regarding the whole population, a significant decrease ($P<0.05$) of episodes with SaO_2 <80% was observed. The subgroup with $crSO_2$ baselines <55% had significant ($P<0.05$) more episodes with SaO_2 <80% before transfusion. During and after transfusion, the frequency of episodes with SaO_2 <80% decreased more in this group compared with the group with $crSO_2$ baselines ⩾55%.

CONCLUSION: NIRS measurement is a simple, non-invasive method to monitor regional tissue oxygenation and the efficacy of RBC transfusion. Infants with low initial NIRS values benefited most from blood transfusions regarding SaO_2 <80%, which may be important for their general outcome.

Journal of Perinatology (2013) **33,** 282–287; doi:10.1038/jp.2012.108; published online 30 August 2012

Keywords: cerebral oxygen saturation; near-infrared spectroscopy; oxygen arterial saturation; peripheral oxygen saturation

INTRODUCTION

The administration of red blood cell (RBC) transfusions is still one of the most controversial topics in neonatology. Nevertheless, 80% of preterm infants will receive at least one blood transfusion until the end of hospitalization due to frequent blood draws or anaemia of prematurity.[1] Although studies on liberal and restrictive transfusion guidelines exist,[2,3] the results with regard to long-term effects, especially the neurological outcome,[4–6] and short-term effects on apnoea, tachycardia and bradycardia[7,8] are contradictory.

In recent years, the measurement of regional tissue oxygenation (rSO_2) by near-infrared spectroscopy (NIRS) has become more and more established in neonatal care units.[9,10] NIRS has been used in several ways to examine the cerebral oxygenation of preterm infants. So cerebral NIRS has been used during reanimation of preterm infants,[11] for analysing hyperoxygenation after desaturation[12] and for monitoring term infants during periodic breathing.[13] Demirel *et al.*[14] have used NIRS to investigate the appropriate positioning in stable preterm infants. Heldt *et al.*[15] recently tried to reflect titrated cerebral oxygen supply by using NIRS during conventional ventilation. The utility of NIRS in neonates has been reviewed in several papers.[16–18] Furthermore, the role of NIRS with regard to neonatal surgery was analysed by Giliberti *et al.*[19] All these authors concluded that the measurement of rSO_2 might be a significant tool in neonatology with increasing importance in clinical practise. Ten years ago, Nicklin *et al.*[20] published their opinion on NIRS in neonatology and summarized that it was 'still very much a developmental technique'. Greisen *et al.*[21] recently reviewed the usefulness of NIRS as a routine clinical tool in neonatology and suggested the NIRS-probe should be miniaturized for a better handling, especially to enable further randomized trials.

Some studies investigated the rSO_2 with NIRS during RBC transfusions in infants.[2,22] It has been shown that both cerebral and peripheral regional oxygen saturation ($crSO_2$, $prSO_2$) decrease when the oxygen transport capacity becomes compromised,[23] and increase during blood transfusion.[2,9] Zaramella *et al.*[24] showed that a blood transfusion by late cord clamping immediately after birth did not change cerebral NIRS values. However, the Hct values were 0.54 before and 0.62 after transfusion. May be such high Hct levels are the reason for that $crSO_2$ values did not change after transfusion. In contrast, Baenziger *et al.*[25] observed an increase of $crSO_2$ after delayed cord clamping. Bailey *et al.*[26] detected the 'splanchnic-cerebral oxygenation ratio as a marker of preterm infant blood transfusion needs'. However, the role of NIRS with regard to transfusion management is not finally resolved, yet.

The management and prevention of apnoe-bradycardia episodes in preterm infants is still an unsolved problem in neonatology. It is known that methylxanthine treatment is effective in reducing the number of apnoe episodes,[27] but long-term results are contradictory. Although caffeine is the preferred methylxanthine, Cochrane Collaboration and its Neonatal Review Group did not find long-term benefits after prophylactic use of

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Received 28 December 2011; revised 6 July 2012; accepted 16 July 2012; published online 30 August 2012

caffeine.[28] The widely known CAP study found a benefit for preterm infants after 18 to 21 months,[29] but the effect was abolished 5 years later.[30] Martin *et al.*[31] and Zhao *et al.*[32] reviewed the literature and concluded that a lot of questions, especially about long-term effects of desaturations are still unclear. However, other studies found that a higher apnoe rate is associated with neurodevelopmental impairment.[6,33]

Thus, it might be interesting to investigate whether the brain indeed has hypoxic episodes during apnoea. Therefore, NIRS has been used for analysing apnoe episodes in different ways. Urlesberger *et al.*[34] showed that $crSO_2$ decreases during apnoea. However, Baerts *et al.*[12] observed that an increase in FiO_2 after apnoe episodes may cause hyperoxygenation of the brain, which may be harmful in very preterm infants.[12] Some authors investigated the relation between oxygen desaturations and the number of RBC, following the assumption that if more oxygen carriers are available the oxygen transport is more effective and episodes with desaturations thereby can be prevented. Therefore, a RBC transfusion might be able to reduce oxygen desaturations. However, the currently available data investigating this question are sparse and the results are contradictory.

The aim of this study was to analyse whether preterm infants benefit from RBC transfusions with regard to the increase of tissue oxygenation, and especially, with regard to the number of desaturations. An association of short episodes of desaturations down to 70 to 80% of arterial oxygen saturation (SaO_2) with decreased $crSO_2$ and somatic rSO_2 was already demonstrated.[23] Therefore, we expected a decline of desaturations after transfusing neonates.

In this study, we investigated if NIRS measurements before and after RBC transfusions are able to answer the question who really benefited from these transfusions and who did not. To answer this question in a short observation period, we chose a simple but very important tool: the number of oxygen desaturations before and after transfusion.

We hypothesized that with the help of NIRS measurements, it is possible to determine a subgroup of infants that will have the most clinical benefit in view of oxygen desaturations associated with an RBC transfusion.

METHODS

Patients

Infants who qualified for a RBC transfusion due to anaemia of prematurity according to our unit guidelines (Table 1) were studied over the period from July 2009 to July 2010 at the Division of Neonatology of the University of Leipzig, Germany, a tertiary referral centre. Exclusion criteria were changes in respiratory support or oxygen demand, further transfusions within the following 24 h after the transfusion took place and acute haemorrhage. As oxygenation and NIRS values depend on oxygen supply, special attention has been paid to exclude that respiratory support and oxygen demand changed during the study period (4 h before until 24(±2) h after transfusion). Changes in oxygen demand of not more than $FiO_2 \leqslant 0.05$ were deemed acceptable.

Methods/Measurements

In addition to our standard monitoring, the transfused patients were studied continuously by multi-probe NIRS (INVOS 5100C, Neonatal OxyAlert NIRSensor, Somanetics Corporation, Troy, MI, USA) from 4 h before the beginning of transfusion to 4 h after ending it and 24 ± 2) hours after the end of the transfusion for another 4 h. The sampling interval was 6 s. One sensor for measuring $crSO_2$ was fixed to the patient's forehead and another one for measuring $prSO_2$ to the posterior flank (thoracolumbar projection of the kidney). The instrument enables non-invasive, real-time measurement of rSO_2 by emitting near-infrared light (730 and 810 nm) from a sensor that contains a LED, placed on the skin. After penetrating the subjacent tissue, these particular wavelengths are absorbed by oxygenated (oxy) and deoxygenated (deoxy) haemoglobin (Hb). At the sensor, two detectors are placed at different distances from the LED allowing the measurement of two penetration depths. The device detects the light reflected to the sensor and subtracts the shallow from the deep measurement so that the influence of superficial tissue on the measured values is subtracted. By using two different wavelengths of near-infrared light, the device calculates the rSO_2, that is, the percentage of oxy-Hb in relation to the total amount of Hb (oxy-Hb/deoxy-Hb + total Hb). It is a mixed measurement of arterial, capillary and venous blood supply and this value is generally referred to as rSO_2.[35] The INVOS device was also used by the group of Dani *et al.*[2], where a more detailed description of the algorithm can be found. The infants were transfused with cytomegalovirus-negative, gamma-irradiated red cell concentrate according to our unit guidelines (Table 1). The transfusion volume was calculated by using the formula: 80 × weight (kg) × (desired Hct–current Hct)/Hct of donor unit (Hct = haematocrit) and administered using a peripheral intravenous cannula within 4 h. The NIRS data were recorded 4 h before beginning the RBC transfusion (T0); at 1 (T1), 2 (T2), 3 (T3) hours after the beginning of RBC transfusion; at the end (T4) of transfusion; 4 h after the end of transfusion (T5); and during another 4-h period 24 h after the end of the transfusion (T6). The resulting data were analysed after download and transferred to a standard computer. Heart rate, blood pressure, SaO_2 and SaO_2 <80% were continuously recorded during the whole investigation period (IntelliView Mp50, Koninklijke Philips Electronics NV, Amsterdam, the Netherlands). The following variables were recorded for each patient: gestational age, sex, age at the time of transfusion, weight at the time of transfusion, as well as FiO_2, and the need of respiratory support (continuous positive airway pressure, mechanical ventilation) during the whole investigation period. Hct values were measured before and after RBC transfusion.

Table 1. Transfusion guidelines for infants at the University of Leipzig

Haematocrit (%)	*Artificial respiration*	*With respiratory support*	*Without respiratory support/FiO_2 0.21*
⩽40	FiO_2 >0.4 MAWP >8 cm H_2O		
⩽30	FiO2 ⩽0.4 MAWP ⩽8 cm H_2O	CPAP >6 cm H_2O	
⩽25		FiO_2 >0.21 + Tachycardia/ -apnoea (>24 h) + CPAP-increase (>48 h) + Exacerbation of apnoea-bradycardia-syndrome despite respiratory stimulant + operation	
⩽20			Absolute reticulocyte count <100 000 μl^{-1}

Abbreviation: CPAP, continuous positive airway pressure; MAWP, mean airway pressure.

Subgroups

For dividing all patients into two groups with high or low basic NIRS values, a k-means clustering analysis was performed. Using this procedure, we were able to perform an objective statistical subgroup analysis. Owing to the outstanding role of cerebral saturation, the $crSO_2$ values were used for this analysis.

Data analysis

SSPS 11.5.1 for Windows was used for data analysis. The NIRS variables, $crSO_2$ and $prSO_2$, were normally distributed (analysed by Kolmogorov-Smirnov test) and the means (±s.d.) were calculated by averaging over each measurement period. A repeated measures ANOVA was used to analyse the differences in NIRS variables at the different points in time. The median number of SaO_2 <80% ($1\,h^{-1}$) and the differences at the various periods were analysed by using the Wilcoxon test. After analysing the $crSO_2$ baseline data and the number of SaO_2 <80% before RBC transfusion

Table 2. Main characteristics of studied patients

Characteristics	*All patients (n = 76)*	*Patients $crSO_2$ <55% (n = 25)*	*Patients $crSO_2$ >55% (n = 51)*
Gestational age (weeks)	27 (± 3)	26 (± 3)	27 (± 3)
Male	38 (50%)	11 (44%)	27 (53%)
Postnatal age at time of transfusion (days)	38 (± 22)	42 (± 21)	36 (± 23)
Weight at time of transfusion (g)	1568 (± 673)	1506 (± 639)<	1597 (± 679)
Ventilated at time of transfusion	8 (10%)	2 (8%)	6 (12%)
Non-invasive ventilation at time of transfusion	41 (54%)	17 (68%)	23 (45%)
Room air	27 (36%)	6 (24%)	11(22%)
Receiving antibiotics at time of transfusion	30 (40%)	5 (20%)	15 (29%)

Data is reported as mean ± s.d. or rate (%).

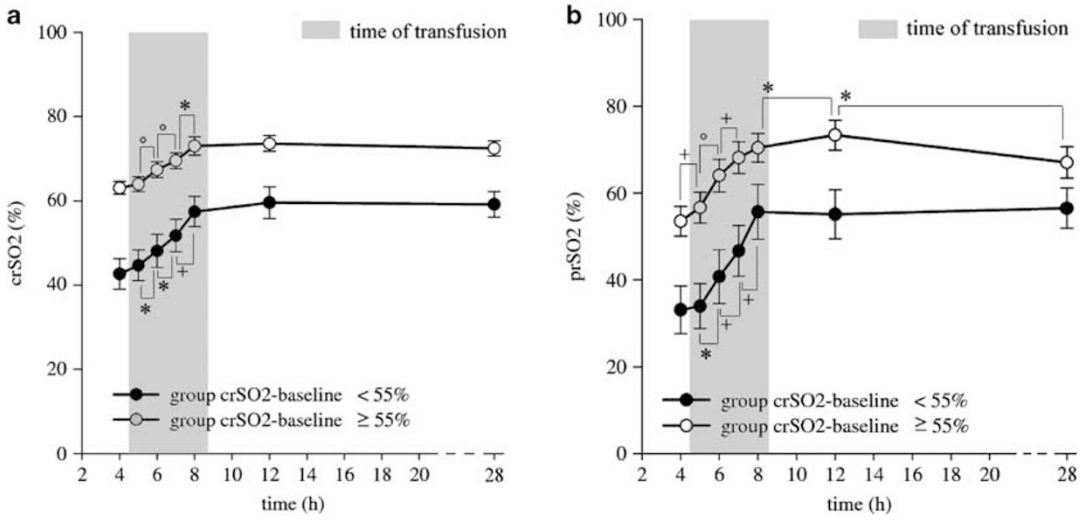

Figure 1. (**a**) Increase of crSO2 during transfusion. CrSO2 4 h before transfusion (T0); at first (T1), second (T2), third (T3) hour after the beginning of RBC transfusion; at the end (T4) of transfusion; 4 h (T5) and 24 h (T6) after the end of transfusion. Mean + s.d. $^{*}P<0.005$;. $^{+}P<0.05$; $^{\circ}P<0.0001$. (**b**) Increase of prSO2 during transfusion. PrSO2 4 h before transfusion (T0); at first (T1), second (T2), third (T3) hour after the beginning of RBC transfusion; at the end (T4) of transfusion; 4 h (T5) and 24 h (T6) after the end of transfusion. Mean + s.d. $^{+}P<0.005$; $^{*}P<0.05$; $^{\circ}P<0.0001$.

by a k-means clustering, the patients were divided into two groups. Unpaired *t*-tests were used to compare $crSO_2$ and $prSO_2$ between the subgroups. The Mann–Whitney *U* test was used to compare the numbers of desaturations between the groups. A *P* value of less than 0.05 was considered significant.

RESULTS

Basic data

A total of 93 patients were included in the study, of which 76 (gestational age 27 ± 3 weeks) were included in the final data analysis. Twelve patients were excluded due to incomplete $crSO_2$ or $prSO_2$ data, five patients due to changes in respiratory support or oxygen supply. The patients' baseline characteristics are given in Table 2. They were transfused at the mean age of 38 ± 22 days of life and a mean weight of 1555 ± 663 g at the time of transfusion. Twenty-seven of the seventy-six patients received no respiratory support, whereas forty-one received continuous positive airway pressure and eight were on a conventional ventilator.

Whole study group—increase of NIRS parameters and decrease in SaO_2 <80%

The average $crSO_2$ and $prSO_2$ of the whole study population increased significantly ($P<0.05$) during transfusion and the effect lasted from 4 h until 24 h after the end of the transfusion. In the whole study population, the pretransfusion Hct level did not correlate with either the $crSO_2$ baseline ($r=-0.09$; $P=0.45$) or the $prSO_2$ baseline ($r=-1.14$; $P=0.22$).

The median number of SaO_2 <80% decreased very slightly from 0.5 (0–2) h^{-1} to 0.4 (0–1.2) h^{-1} during the transfusion ($P<0.005$) and remained low 4 h after transfusion (0.2 (0–1) h^{-1}) until 24 h after the end of the transfusion (0.4 (0–0.8) h^{-1}) in the whole study population. There was no correlation (r = 0.15; $P=0.2$) between pretransfusion Hct level and the number of SaO_2 <80% before transfusion. However, the frequency of desaturations correlated weakly negative ($r=-0.25$; $P<0.05$) with the $crSO_2$ baselines. So we found a relation between $crSO_2$ and the frequency of desaturations: the lower the $crSO_2$ values the more the patients suffering from desaturations.

Subgroups with low or high pretransfusion oxygenation values

Based on the baseline $crSO_2$, all patients were divided into two groups (<55% $crSO_2$ baseline; ⩾55% $crSO_2$ baseline) by a k-means clustering. Clinical data of these two groups are given in Table 2. These two groups did not differ with regard to blood pressure, heart rate, SaO_2 and FiO_2 (data not shown). Especially, the pretransfusion Hct level were not different between the two subgroups (27.6 ± 2.5 vs 27.3 ± 4.2; $P=0.23$), as well as the posttransfusion Hct level (48.3 ± 3.6 vs 47.7 ± 4.7; $P=0.59$).

Significant increases in $crSO_2$ and $prSO_2$ during transfusion were observed in both the groups (Figure 1a and b).

(a) *Increase of $crSO_2$*: As expected, the increase in $crSO_2$ was significantly ($P<0.005$) higher in the group with low $crSO_2$ baselines. Compared with the data immediately after transfusion, no decrease in $crSO_2$ could be observed in either of the two groups 24 h after the transfusion (T6).
(b) *Increase of $prSO_2$*: There was no significant difference concerning the magnitude of the increase in $prSO_2$ between the subgroups immediately after RBC transfusion (T5). However, 24 h after the end of the transfusion, the subgroup with high $crSO_2$ baselines showed a significant decrease (T6, $P<0.05$) in $prSO_2$, compared with the measurements immediately after the transfusion.
(c) *Decrease in SaO_2 <80%:* The patients with low $crSO_2$ baselines <55% had significantly ($P<0.05$) more desaturations before the RBC transfusion (Figure 2) than the patients with initially higher $crSO_2$. Analysing the data immediately after transfusion, we found a much stronger decrease in the number of desaturations in the subgroup with low $crSO_2$ baselines ($-0.79\,h^{-1}$, $P<0.05$) compared with the subgroup with high baselines ($-0.27\,h^{-1}$, $P<0.05$). Similar results were found 24 h after the end of the transfusion (-0.5 vs $-0.16\,h^{-1}$; $P<0.05$).

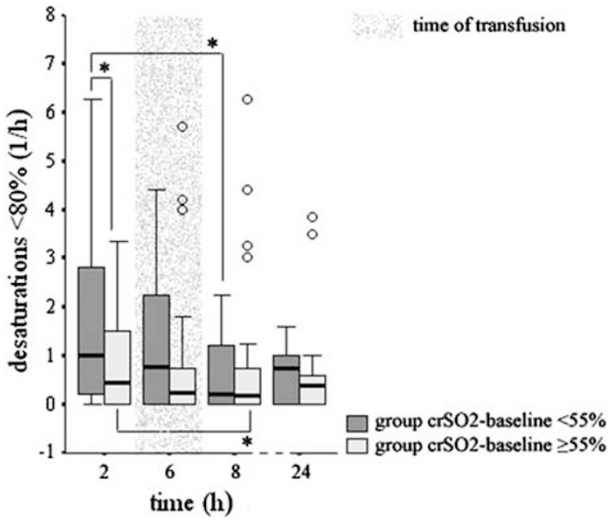

Figure 2. Episodes with SaO2 <80% decrease during transfusion. Episodes of SaO2 <80% 4 h before transfusion (T1), during RBC transfusion (T2), 4 h (T3) and 24 h (T4) after the end of transfusion. Median (interquartile range). *$P<0.05$; °outliers.

DISCUSSION

The study confirmed data of others that RBC transfusions perceptibly increase $crSO_2$ values. Furthermore, we found an increased $prSO_2$ measured at the thoracolumbar site (in projection of the kidney). The most important result of this study was that infants with very low NIRS values before RBC transfusion also had the highest number of oxygen desaturations, and benefited most from the transfusion because they had the most reduction in the number of desaturations. These observations were independent of the Hct values that are generally used to guide transfusion therapy. In view of the common insecurity in finding of clear indications for transfusion of RBC, a simple NIRS measurement may be a very useful tool to decide when a transfusion is actually going to benefit a preterm infant.

The data of different studies investigating normative $crSO_2$ values using NIRS were collected by van Bel *et al.*[16] The $crSO_2$ values of these studies were gathered in term and preterm neonates during the first week of life and ranged from 57 to 75%. Similar values were found in brain-injured children by Grant *et al.*[36] McNeill *et al.*[37] investigated 14 stable preterm infants during the first weeks of life and found normal $crSO_2$ values between 66 and 83% and $prSO_2$ values between 64 and 87%. Roche-Labarbe *et al.*[38] measured $crSO_2$ in 11 premature infants without known brain injury during the first 6 weeks of life and showed a decrease of $crSO_2$ from the first to the sixth week of life from 73 to 57%. Summarizing these data, $crSO_2$ values between 57 and 75% could be assumed as cerebral reference range in preterm infants during the first weeks of life. In view of these results, our limit of 55% determined by a k-means clustering seems to be adequate, as this value is slightly below the lower normal range.

Despite the risks of blood transfusions, almost every infant in a neonatal care unit will receive at least one RBC transfusion during hospitalization.[39] Therefore, it is important to investigate the indications and benefits of transfusions. This study clearly shows that $crSO_2$ and $prSO_2$ increased significantly throughout the RBC transfusion and remained high after transfusion, as described previously.[2,22] The effect was expected because an increase of the Hb concentration after RBC transfusion leads to a higher oxygen transport capacity, which in turn results in an increased tissue oxygen delivery. The reverse proof of decreasing $crSO_2$ and $prSO_2$ with declining oxygen carrying capacity was already given by Petrova and Mehta.[23] Therefore, NIRS measurement is an excellent tool for monitoring the efficacy of blood transfusions in preterm infants. However, NIRS measurement by itself will not improve the care of preterm infants. In contrast to our observations regarding the $crSO_2$ after transfusion, it has been found that in adults RBC transfusion did not change muscle tissue oxygenation in critically ill patients.[40] A possible explanation therefore might be the different organs that were measured (muscle and brain) and the different patients that were observed (healthy preterm infants and septic adults).

To improve patient-oriented transfusion therapy, we sought to determine whether NIRS measurement can detect patients who benefit more from RBC transfusion than others. Only a few studies investigated the utility of NIRS in detecting the need of RBC transfusion. Wardle *et al.*[41] found that peripheral fractional oxygen extraction failed to predict the usefulness of blood transfusion in VLBW infants. Recently, it has been shown that the splanchnic-cerebral oxygenation ratio might be a good marker for preterm infant blood transfusion needs.[26] So the authors observed that symptomatic premature infants who had low splanchnic-cerebral oxygenation ratio values ($\leqslant 0.73$) before RBC transfusion clinically improved after transfusion. In our study, RBC transfusions perceptibly increased $crSO_2$ in general, but the response was stronger in infants with very low $crSO_2$ baselines. These infants may be a particularly vulnerable subgroup, and the detection of this subgroup as well as the subsequent offer of an appropriate treatment can lead to better clinical outcomes. As the SaO_2 was maintained in a similar range for all infants, a low $crSO_2$ means a higher degree of oxygen extraction. An increased fractional oxygen extraction is linked to low $crSO_2$, as has been shown by Van Hoften *et al.*[22] Thus, an already high oxygen extraction under baseline conditions leaves little reserve to meet the demands of brain tissue during oxygen desaturations. The oxygen supply, which is required for the energy metabolism of the brain, may become compromised more easily in these infants, and may threaten the integrity of brain tissue. Therefore, it may be clinically important to identify infants with low $crSO_2$ values, to administer transfusions in a timely manner.

As has been shown normal values for $crSO_2$ ranged between 60 and 75%. In our study, patients with low $crSO_2$ baselines (<55%)

had a mean $crSO_2$ of only $43 \pm 9\%$ before RBC transfusion. This value increased to almost $60 \pm 9\%$ after transfusion, which means that these patients increased from very low, possibly insufficient $crSO_2$, to almost normal $crSO_2$.

Standard values for $prSO_2$ can be found in studies investigating preterm infants[37] and infants.[42] Whereas the published data of normal $prSO_2$ differ widely between 40 and 95%, we focused on the study of McNeill *et al.*[37] who observed $prSO_2$ values between 55 and 75% in stable preterm infants in the third week of life and assumed this as $prSO_2$ reference range. Analysing our study groups again, we found very low $prSO_2$ values in the subgroup with initial low $crSO_2$ baselines ($<55\%$), which increased from $33 \pm 13\%$ before to $55 \pm 14\%$ after RBC transfusion, achieving approximately the $prSO_2$ baselines of the patients with high $crSO_2$ baselines ($>55\%$) before transfusion. However, also the $prSO_2$ values of the infants with initial high $crSO_2$ seem to be at the lower normal range of the $prSO_2$ data given by McNeill *et al.*[37] On the one hand, this may mean that the patients with initial low $crSO_2$ ($<55\%$) definitively had very low $prSO_2$ values before and reached a minimum of sufficient $prSO_2$ after RBC transfusion. On the other hand, the data show that also in the group with initial high $crSO_2$ baselines ($\geqslant 55\%$) the $prSO_2$ values were at the lower normal range. So, we assumed that regardless of a normal $crSO_2$ they may have an inadequate $prSO_2$. This may be a manifestation of cerebral autoregulation.

Bernal *et al.*[42] observed that in healthy term infants $prSO_2$ values were higher than $crSO_2$ values. Maturational differences may be responsible for the lower values observed in the present study.

It has been shown that SaO_2 $<80\%$ are associated with a decreased $crSO_2$ and $prSO_2$ in ventilated infants.[23] We observed that the subgroups differ in the median number of desaturations before the RBC transfusion. The infants with initial low $crSO_2$ values ($<55\%$) had significantly more frequent desaturations than the patients with higher baselines (Figure 2). We also observed that the number of episodes with SaO_2 $<80\%$ decreased during RBC transfusion in the whole study population. However, this decrease was significantly higher in the group with initial low $crSO_2$ baselines ($<55\%$). So we concluded that the administration of RBC transfusion is especially useful for patients with $crSO_2$ below 55%, regardless of their actual Hct. Yamamoto *et al.*[43] observed that SaO_2 values below 85% are associated with decreased cerebral circulation, suggesting that the subgroup with significantly more desaturations before RBC transfusion probably also had a lower cerebral circulation. Even if the significant but small reduction in the number of desaturations does not seem to be clinically important, frequent desaturations together with a generally precarious oxygen delivery may put brain cells at risk. Therefore, the benefit of RBC transfusion is double: a better overall oxygenation and less desaturations. Although the number of desaturations in preterm infants decreases usually over time, we assumed that this phenomenon does not change our results significantly, because of the short observation period of our study.

Research using NIRS measurements has several limitations that may influence the interpretation of the results. To clearly demonstrate changes in rSO_2 and desaturations resulting from RBC transfusion, we have to consider a couple of confounding factors. We excluded patients who changed in respiratory status or showed significant changes in inspired fraction of oxygen after transfusion, as beside the Hb level, the administration of oxygen and the respiratory support greatly influence the oxygen carrying capacity, thus invalidating the measurements. Furthermore, tissue oxygen saturation depends on the blood pressure, heart rate and SaO_2.[44] We investigated carefully if there were any significant changes in these parameters between the various points in time in the two subgroups and found none. Tissue oxygen saturation may also depend on the cardiac output (CO). This was not investigated. However, Leipälä *et al.*[45] demonstrated that CO did not change during RBC transfusion, whereas Hudson *et al.*[46] observed a decrease in CO after transfusion only in infants with a RBC volume of less than $25\ ml\ kg^{-1}$ before transfusion. Thus, a confounding effect on our data is unlikely. Physiological stress and infections may also influence tissue oxygen consumption,[47,48] but we minimized stress situations during NIRS measurements and no patient developed any new infection during the study period.

Apart from the upper mentioned, there are some more limitations regarding our investigation. First of all it is doubtful if the frontal cortex is the most interesting region of the premature brain. Possibly other cortical areas or even subcortical structures are more decisive. Second, this was only a pilot study with only a small study population. Furthermore, cerebral blood flow depends on several factors. Probably pCO_2 is the most important regulator of cerebral blood flow. Booth *et al.*[49] found that cerebral blood flow and $crSO_2$ correlated with pCO_2 in ventilated piglets. It has also been shown that end-tidal CO_2 influences cerebral blood flow.[50] So it should be noted that cerebral blood flow correlates strongly with CO_2 levels. This fact might be especially important in ventilated infants. Mostly the infants we investigated were spontaneously breathing, so we assumed that most of the investigated infants had consistent CO_2 levels within the normal range. Therefore, we presume that CO_2 changes are not responsible for the observed increase of $crSO_2$. However, in further studies the measurement of pH and CO_2 level should be added to the study design to prove that these values are stable during the whole investigation period.

Current transfusion guidelines in neonatal care units differ as to the extent of medical support requirements in addition to ranges of the Hb level to determine when a transfusion is indicated.[4] Nevertheless, clinicians are often uncertain whether to transfuse or not. Like Bailey *et al.*,[9] we observed that the pretransfusion Hct level does not correlate with the initial $crSO_2$ and $prSO_2$, which confirms that the Hct level alone is a poor predictor of tissue oxygenation. Therefore, we considered the incorporation of NIRS measurements in conjunction to the Hb level and clinical parameters to determine when a transfusion is indicated. The data of our pilot study demonstrates that children with low $crSO_2$ values ($<55\%$), especially if they suffer from frequent desaturations, most benefits from transfusion. According to our data, the clinical condition in regard to the frequency of desaturations improved after RBC transfusion, which may represent an actual improvement of the clinical course of preterm infants. NIRS is a simple, non-invasive method may be with the potential of modifying current transfusion management. However, these data were obtained by a non-randomized pilot study, though the results are limited. Larger prospective controlled studies are needed to confirm our results and determine whether $crSO_2$-guided transfusions result in better clinical and neurological outcomes. We hope that this study leads to more interest investigating the role of NIRS in neonatology.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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