Red Blood Cell Transfusions Affect Intestinal and Cerebral Oxygenation Differently in Preterm Infants with and without Subsequent Necrotizing Enterocolitis

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Abstract

Objective To assess intestinal and cerebral oxygenation during and after red blood cell (RBC) transfusions in preterms with or without subsequent transfusion-associated necrotizing enterocolitis (TANEC).

Study Design In preterms of < 32 weeks' gestational age, we measured intestinal and cerebral regional tissue oxygen saturation ($r_{int}SO_2$, r_cSO_2) and their variabilities using near-infrared spectroscopy during and after transfusions. We compared eight infants who developed TANEC 6 to 48 hours after RBC transfusions with 16 controls.

Results In TANEC infants, r_cSO_2 was lower during and after RBC transfusions than in controls, median (interquartile range) 55% (50–62) versus 72% (65–75), $p < 0.01$. There were no differences regarding $r_{int}SO_2$. Individual $r_{int}SO_2$ and r_cSO_2 ranges were smaller after transfusions in TANEC infants, 28% (9–36) versus 49% (40–65), $p < 0.01$, and 17% (14–33) versus 36% (26–57), $p = 0.01$, as was short-term $r_{int}SO_2$ variability. For each 10% higher r_cSO_2, the risk of developing TANEC decreased (odds ratio 0.09; 95% confidence interval 0.01–0.63). The smaller the $r_{int}SO_2$ range after transfusion, the higher the risk of developing TANEC.

Conclusion In preterm infants lower r_cSO_2, but not $r_{int}SO_2$, values during and after RBC transfusions are associated with TANEC. Lower $r_{int}SO_2$ and r_cSO_2 variabilities after RBC transfusions may represent a diminished capacity for vascular adaptation, possibly leading to TANEC.

Keywords
- red blood cell transfusion
- intestinal regional tissue oxygen saturation
- cerebral regional tissue oxygen saturation
- necrotizing enterocolitis
- preterm infants
- variability
- hemoglobin
- near-infrared spectroscopy

Most preterm infants receive red blood cell (RBC) transfusions.[1] Such transfusions are effective in treating acute anemia and may reduce anemic hypoxia-associated morbidity.[2] Previously, RBC transfusions were associated with an increased risk of necrotizing enterocolitis (NEC).[3,4] The clinical presentation of transfusion-associated NEC (TANEC) was first described by Mally et al.[5] Other authors reported TANEC in 39 to 56% of cases.[6,7] A possible explanation for the development of TANEC is ischemia–reperfusion damage of the intestine.[8] An alternative explanation for developing TANEC is that RBC transfusions serve as incidental markers for clinically significant anemia in infants with incipient NEC.[9,10]

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Intestinal oxygenation can be assessed by using near-infrared spectroscopy (NIRS).[11] This method, which is non-invasive, can be used to monitor regional organ tissue oxygen saturation (rSO_2) continuously and to simultaneously determine its variability at several sites.[12] Several studies reported oxygenation values in preterm infants during and after RBC transfusions.[1,13,14] The only study that offered detailed information on site-specific oxygenation variability reported that baseline rSO_2 variability differs between monitoring sites and that briefer monitoring resulted in less rSO_2 variability.[12] Previously, NIRS proved helpful in predicting complicated NEC.[15] It has also proved a useful diagnostic tool in other disease processes that affect tissue perfusion and oxygenation.[16,17]

Even though intestinal rSO_2 ($r_{int}SO_2$) in TANEC has been investigated previously, most studies lacked controls and no data were provided on cerebral oxygenation as a global measure of a compromised systemic circulation.[18] Moreover, results were inconclusive. Lower $r_{int}SO_2$,[19] and increased[20] or decreased[11] variability of $r_{int}SO_2$, were described during and after RBC transfusions in infants with TANEC. Such patterns may be the result of immature intestinal vasculature.[21]

Our aim was to provide insight into the possible mechanisms underlying these inconsistencies by presenting multisite NIRS data from three prospective studies in preterm infants.[15,18,22] Furthermore, we aimed to determine whether the course of intestinal and cerebral oxygenation, and its variability during and after RBC transfusions, was different in preterm infants who subsequently developed NEC in comparison to preterm infants who did not. We hypothesized that we would find a different intestinal and cerebral vascular response, as manifested in lower median oxygen saturation values in the NEC group, and a difference in the oxygenation variability between these two groups.

Methods

Study Design and Study Population

Retrospectively, we included preterm infants from three prospective cohort studies that investigated the value of multisite NIRS monitoring to assess organ perfusion or to predict NEC: the NEMO,[18] NoNEC,[15] and California trials.[22] The studies were approved by the ethical review board of University Medical Center Groningen.

All subsequent infants were included in the current substudy if they met the following criteria: gestational age (GA) $<$ 32 weeks, $r_{int}SO_2$, and cerebral rSO_2 (r_cSO_2) measurements within 12 hours after a completed RBC transfusion and, in case the infants developed NEC, a completed transfusion at least 6 hours before the onset of NEC. Depending on the original prospective study design, in half the cases data were also available during RBC transfusions. Because data on baseline rSO_2 were limited, we chose not to analyze these data, but to provide a complete overview of the data instead. During RBC transfusions, all infants received one or two bolus feedings consisting of mother's milk or formula.

According to the local guideline, a RBC transfusion consists of a leukocyte-reduced "pedipack" of erythrocytes of 15 mL/kg administered intravenously over a 3-hour period. Depending on the infant's clinical condition, the amount could be adjusted and the duration extended. In accordance with our local guidelines, all infants needed RBC transfusions (►**Table 1**). NEC, defined as modified Bell's Stage $\geq$ 2, was confirmed by a pediatric radiologist from an abdominal X-ray.[16] The time of onset of NEC was defined retrospectively as the moment when nil per os was initiated. We defined TANEC as NEC that developed within 6 to 48 hours after a completed RBC transfusion. This delay was necessary to prevent our including infants who had received a RBC transfusion because of NEC-induced anemia.

We divided the infants into two groups: the index group comprising preterm infants who developed TANEC and the control group comprising preterm infants who had received a RBC transfusion without subsequently developing NEC.

Near-Infrared Spectroscopy

The $r_{int}SO_2$ and r_cSO_2 were measured using an INVOS 5100c oximeter and neonatal sensors (Medtronic, Boulder, CO). Mepitel®, used as a skin barrier below each sensor, does not adversely affect INVOS signal integrity or validity.[23] The sensor was placed infraumbilically on the central abdomen to measure $r_{int}SO_2$ and on the frontoparietal side of the infant's head to measure r_cSO_2. In accordance with the original study protocol, NIRS measurements were performed for either 2 hours a day or for 48 to 72 hours continuously.

Mean rSO_2 values were calculated 2 hours before the RBC transfusion, during the entire transfusion period, and for 2 hours of available NIRS data closest to the RBC transfusion within 12 hours after transfusion. On account of the rather high intra-individual baseline variability of $r_{int}SO_2$, we also calculated mean $r_{int}SO_2$ during 15-minute periods of the recordings before, during, and after the RBC transfusion of each individual infant. Furthermore, we assessed the variability of rSO_2 during the same three periods before, during, and after the RBC transfusion, in two ways. First, we used the individual range between the highest and lowest rSO_2 value

Table 1 Indications for red blood cell transfusion in relation to clinical variables

Threshold level	Clinical variables
Hemoglobin $<$ 8 mmol/L (12.9 mg/dL)	First 24 hours postpartum
	Ventilator dependency
	Cardiorespiratory instability
Hemoglobin $<$ 7 mmol/L (11.3 mg/dL)	Cardiorespiratory problems with stable clinical condition
Hemoglobin $<$ 6 mmol/L (9.7 mg/dL)	Clinical symptoms of anemia, such as tachycardia, tachypnea, apneas/bradycardias, poor weight gain in an otherwise stable neonate
Hemoglobin $<$ 5 mmol/L (8.1 mg/dL)	Stable neonate, $>$ 4 weeks post-term

for each individual patient to focus on extreme values within each patient.[11] Second, for individual short-term variability, we used the root mean square of successive differences (RMSSD) to assess small changes in time.[24] Finally, we calculated the cerebrosplanchnic oxygenation ratio (CSOR), that is r_cSO_2 divided by $r_{int}SO_2$.[25]

Clinical Characteristics

The clinical data we collected consisted of GA, birth weight (BW), closest hemoglobin (Hb) value before and after the RBC transfusions, duration and volume of the RBC transfusions, illness severity using the score for neonatal acute physiology—perinatal extension II,[26] presence of a patent ductus arteriosus, ventilator mode, inotrope administration, and volume expansion during measurements.

Statistical Analysis

Statistical analyses were performed with IBM SPSS Statistics for Windows 23.0 (IBM Corp., Armonk, NY). Patient characteristics were described as median and interquartile range. The Mann–Whitney and chi-square tests were used to analyze the differences between groups. Because of the small dataset, we used exact *p*-values, tested two-sided. Missing data were not replaced.

Logistic regression analyses were used to determine the relationship between intestinal and cerebral oxygenation variables and TANEC development. Multivariate logistic regression models were constructed entering oxygenation variables into the model that were univariately different between the two groups with $p < 0.1$ after RBC transfusion. We also included Hb levels before and after transfusion in the multivariate model to adjust for pre-existing anemia and transfusion success. Statistical significance was defined as $p < 0.05$.

Results

Patient Characteristics

From the three prospective studies, we identified 139 infants with GA < 32 weeks (►**Fig. 1**). A total of 24 infants with a median GA of 27.3 weeks (range, 26.0–28.3) and a BW of 900 g (743–1190) were included. Of these 24 infants, eight (33%) were diagnosed with TANEC. ►**Table 2** provides the patient characteristics.

Intestinal and Cerebral rSO_2 and rSO_2 Variability during and After RBC Transfusions

Intestinal rSO_2 was comparable (►**Fig. 2A** and **2B**, ►**Supplementary Tables 1** and **2** [available in the online version]), while r_cSO_2 was consistently lower during and after RBC transfusions in index patients in comparison to controls (►**Fig. 2A**, ►**Supplementary Table 3** [available in the online version]).

During RBC transfusions, the variability parameters between the TANEC and control groups were comparable (►**Supplementary Tables 1** and **3** [available in the online version]). After RBC transfusions, individual ranges of both $r_{int}SO_2$ and r_cSO_2 (►**Fig. 2C**), and the RMSSD of $r_{int}SO_2$, were smaller in TANEC patients than in controls, whereas the RMSSD of r_cSO_2 did not differ between the two groups (►**Supplementary Tables 1** and **3** [available in the online version], ►**Fig. 2D**).

During and after transfusions, the CSOR values between the two groups were also comparable (►**Supplementary Table 1** [available in the online version]).

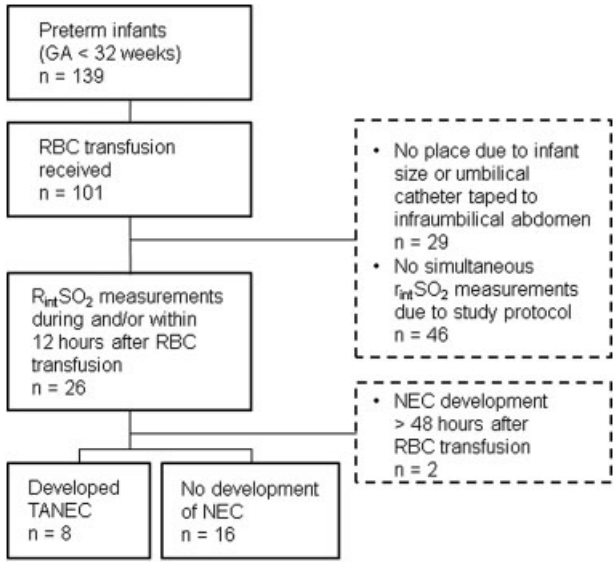

Fig. 1 Flowchart of the study. GA, gestational age; NEC, necrotizing enterocolitis; RBC, red blood cell; $r_{int}SO_2$, intestinal regional tissue oxygen saturation; TANEC, transfusion-associated NEC, defined as NEC between six and 48 hours after a RBC transfusion.

NIRS Parameters, Hb, and TANEC

The two groups had similar Hb levels before the RBC transfusions. In the index infants, we found smaller increases in Hb values after RBC transfusion and lower Hb values after the RBC transfusions than in the controls (►**Table 2**).

In ►**Table 3**, we present odds ratios (ORs) for rSO_2 values and variability parameters after RBC transfusions and the risk of developing TANEC, unadjusted and adjusted for Hb levels before and after RBC transfusions. The risk of developing TANEC decreased with OR 0.09 (95% confidence interval, 0.01–0.63) per 10% increase of r_cSO_2. This association remained significant following adjustment for Hb values before and after RBC transfusions. The univariate analysis revealed that the smaller the individual range of $r_{int}SO_2$ after a RBC transfusion, the higher the risk of developing TANEC. This association remained significant after adjusting for Hb values before, but not after, RBC transfusion (►**Table 3**).

Discussion

This study demonstrated that in preterm infants who developed TANEC r_cSO_2 was lower during and after RBC transfusions than in controls, whereas $r_{int}SO_2$ did not differ between the two groups. Furthermore, in comparison to the control group, the variability of both r_cSO_2 and $r_{int}SO_2$ after RBC transfusions, expressed by smaller individual ranges and a

Table 2 Patient characteristics

	NEC+ $n = 8$	NEC− $n = 16$	*p*-value
Gestational age (wk)	26.4 (25.7–27.7)	27.8 (26.0–28.5)	0.24
Birth weight (g)	930 (825–1190)	835 (710–1208)	0.53
Sex (boy/girl)	5/3	9/7	1.00
Hb before RBC transfusion (mmol/L)	7.1 (6.5–7.3)	6.7 (6.2–7.0)	0.21
Hb after RBC transfusion (mmol/L)	7.5 (6.7–8.1)	8.7 (7.7–9.3)	0.03[a]
Hb increase during RBC transfusion (mmol/L)	0.35 (0.03–1.1)	1.7 (0.8–2.8)	<0.01[a]
Postnatal day of RBC transfusion	14 (10–23)	20 (11–29)	0.35
Corrected gestational age (wk)	28.9 (27.8–30.4)	30.6 (28.4–31.5)	0.11
RBC volume (mL/kg)	13 (11–24)	15 (14–18)	0.21
Duration of RBC transfusion (h)	3 (3–4)	4 (2–4)	0.40
Mother's milk during RBC transfusion	6 (75)	11 (69)	0.75
Feeding volume during RBC transfusion (mL/kg)	10 (7–22)	16 (8–21)	0.85
Time between the RBC transfusion and NEC onset (hours)	17 (8–43)		
SNAPPE-II score	46 (12–58)	40 (18–61)	0.79
hsPDA	2 (25)	8 (50)	0.23
Mechanical ventilation	3 (38)	7 (44)	0.56
$paCO_2$ during study period (kPa)	6.7 (5.7–7.0)	7.3 (6.1–7.6)	0.32
Indomethacin during study period	1 (13)	4 (25)	0.45
Inotropes during study period	3 (38)	1 (6)	0.09
Volume expansion during study period	6 (75)	5 (31)	0.06

Abbreviations: Hb, hemoglobin; hsPDA, hemodynamically significant patent ductus arteriosus; NEC, necrotizing enterocolitis; $paCO_2$, arterial partial pressure of carbon dioxide; RBC, red blood cell; SNAPPE-II, Score for Neonatal Acute Physiology - Perinatal Extension II.
Note: Data are displayed as median (interquartile range) or as *n* (%). Mechanical ventilation indicates synchronized intermittent positive pressure ventilation, synchronized intermittent mechanical ventilation, or high frequency oscillation.
[a]*p*-value < 0.05.

smaller RMSSD of $r_{int}SO_2$, was lower in index infants, while variability measurements during RBC transfusion did not differ between the two groups.

Recently, the association between RBC transfusions and the development of NEC has received much attention.[8,19,20] Some researchers suggested that the oxygen load resulting from a RBC transfusion in combination with an immature intestinal vasculature may induce reoxygenation injury. Additionally, immune mechanisms similar to those seen in transfusion-related lung injury were reported to contribute to the development of TANEC.[20,21,27] This response of the immature intestinal vasculature to changes in oxygen supply may be reflected by the variability of $r_{int}SO_2$ after RBC transfusions.

We found a lower variability of $r_{int}SO_2$ after RBC transfusions in infants who developed TANEC in comparison to controls. These results are in line with those reported by Cortez et al.[11] They also found a decrease in variability of $r_{int}SO_2$ after RBC transfusions in infants who developed TANEC. Bailey et al[20] and Marin[19] et al, however, found the opposite and suggested that the higher $r_{int}SO_2$ variability after RBC transfusions they found points to a potential mechanism through which TANEC develops. They suggested that the extremes of tissue perfusion may make the intestine vulnerable to reperfusion injury, a potential factor in the pathogenesis of NEC. Nevertheless, in one of these studies,[20] no patients actually developed NEC after transfusion, and the infants in the other study[19] who did develop NEC were born after lower GAs with lower BWs than the controls. The higher variability they found after RBC transfusions may actually reflect adequate vascular regulation capabilities. Our data and those of Cortez et al[11] suggest that it might be the loss of normal variability in intestinal oxygenation that increases the risk of developing TANEC, possibly due to a reduced capacity of the immature intestinal vasculature to cope with the increased oxygen delivery after a RBC transfusion, or as a result of anemic hypoxia.

Other authors suggested that reduced $r_{int}SO_2$ variability may not only be a reflection of immaturity of the intestinal vasculature, but that it may also be a reflection of intestinal underperfusion.[28] The findings of Cortez et al[11] and Marin[19] et al did indeed suggest intestinal underperfusion, as they reported a lower median $r_{int}SO_2$ after RBC transfusions in infants who subsequently developed TANEC, in comparison to infants who did not. We could not confirm intestinal underperfusion or intestinal anemic hypoxia on the basis

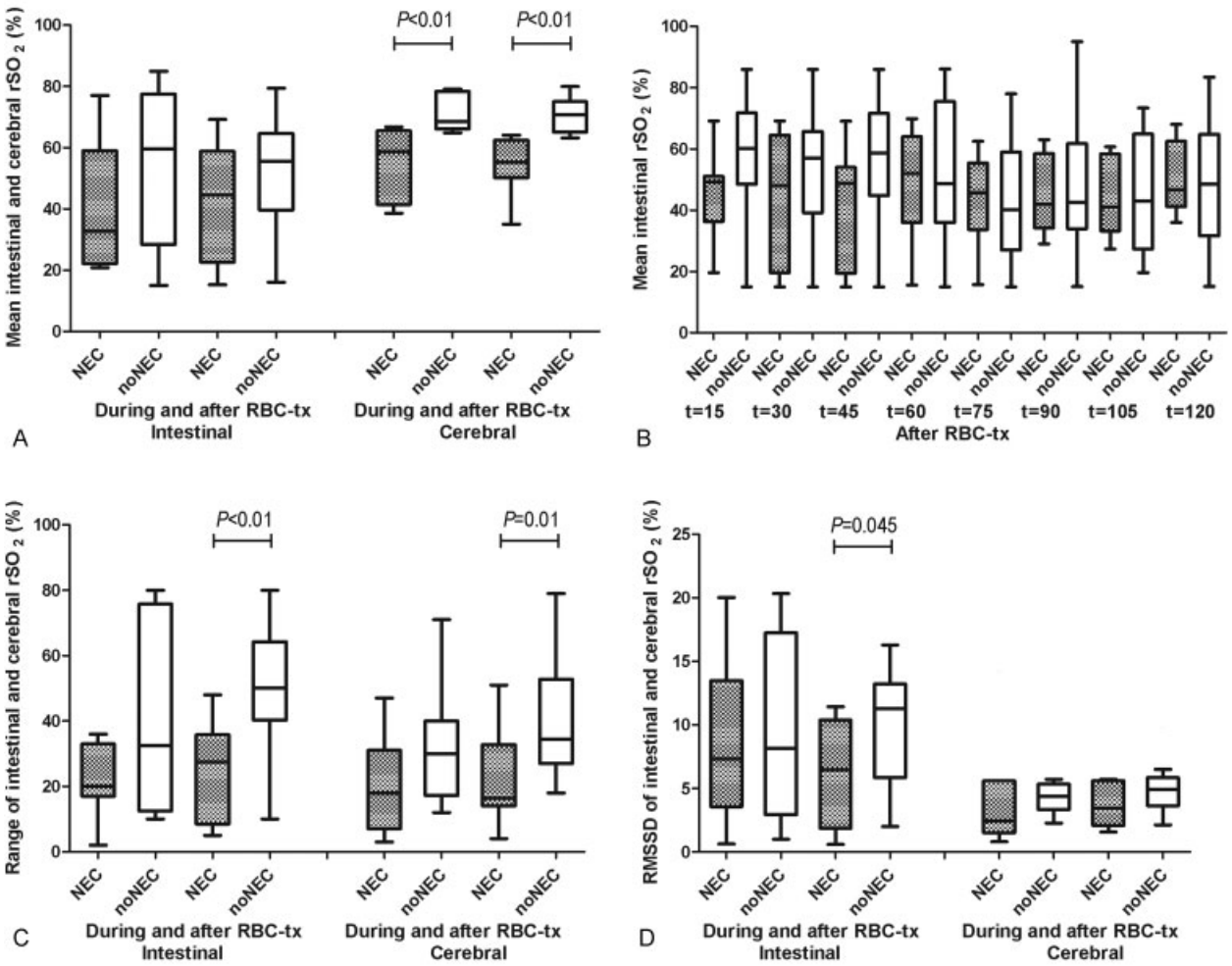

Fig. 2 Intestinal and cerebral oxygenation values during and after RBC transfusions. (**A**) Mean $r_{int}SO_2$ and r_cSO_2 during and after RBC transfusions. (**B**) Mean $r_{int}SO_2$ in 15-minute periods after RBC transfusions. (**C**) Range of $r_{int}SO_2$ and r_cSO_2 during and after RBC transfusions. (**D**) RMSSD of $r_{int}SO_2$ and r_cSO_2 during and after RBC transfusions. RBC, red blood cell; RBC-tx, red blood cell transfusion; r_cSO_2, cerebral regional tissue oxygen saturation; $r_{int}SO_2$, intestinal regional tissue oxygen saturation; RMSSD, root mean square of successive differences; rSO_2, regional tissue oxygen saturation. Index group—preterm infants who developed transfusion-associated necrotizing enterocolitis. Control group—preterm infants without development of necrotizing enterocolitis. 't' indicates the duration in minutes from the start of the measurement.

of the $r_{int}SO_2$ values. The intestinal rSO_2 values, however, are extremely variable, especially compared with the measurements obtained by r_cSO_2 monitoring.[12,23] When we analyzed mean $r_{int}SO_2$ values using 15-minute periods, we still did not find statistically significant differences between the two groups. We cannot exclude the possibility that the large individual variability of $r_{int}SO_2$ in this small sample still prevented us from finding differences, or that an immature vascular response plays a more important role than intestinal oxygenation itself. Future research investigating the association between superior mesenteric artery Doppler and intestinal NIRS measurements may shed light on this potential mechanism.

In this study, we did find lower r_cSO_2 levels during and after RBC transfusions in infants with TANEC in comparison to controls. This could be the result of anything that either lowers total cerebral perfusion or that increases cerebral oxygen utilization. The association between low r_cSO_2 and TANEC development remained significant after correcting for Hb levels. This might suggest that the low cerebral oxygen status represents low cerebral perfusion. Low cerebral perfusion might be a reflection of systemic underperfusion as a possible mechanism leading to TANEC. This is not, however, reflected by differences in other clinical characteristics. The $r_{int}SO_2$ and CSOR were also not different between the two groups. A possible alternative explanation for the difference in r_cSO_2 between the two groups is the fact that the TANEC group is slightly less mature. Further research is needed to elucidate what factors are associated with the decreased r_cSO_2 and why r_cSO_2 is lower among infants who developed TANEC than among those who did not.

We did not withhold enteral feeds during RBC transfusion which might have influenced the NIRS measurements. Withholding feeds during RBC transfusion is becoming common practice, because some studies suggested that enteral feeding during RBC transfusion may play a role in the development of TANEC.[6,7] One study demonstrated diminished superior mesenteric artery blood flow subsequent to the

Table 3 ORs for selected intestinal and cerebral oxygenation values after RBC transfusion in relation to the development of TANEC, unadjusted and adjusted for Hb levels before or after RBC transfusion using logistic regression analysis

	Univariate analysis	Multivariate, adjusted for Hb levels before RBC transfusion	Multivariate, adjusted for Hb levels after RBC transfusion
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Range $r_{int}SO_2$ per 10%	0.50 (0.27–0.91)[a]	0.49 (0.27–0.91)[a]	0.56 (0.30–1.05)
RMSSD $r_{int}SO_2$ per 1%	0.81 (0.64–1.02)	0.82 (0.65–1.04)	0.79 (0.60–1.04)
R_cSO_2 per 10%	0.09 (0.01–0.63)[a]	0.05 (0.003–0.66)[a]	0.08 (0.01–0.83)[a]
Range r_cSO_2 per 10%	0.41 (0.16–1.02)	0.44 (0.18–1.09)	0.49 (0.20–1.24)

Abbreviations: CI, confidence interval; Hb, hemoglobin; NIRS, near-infrared spectroscopy; OR, odds ratio; RBC, red blood cell; r_cSO_2, cerebral regional tissue oxygen saturation; $r_{int}SO_2$, intestinal regional tissue oxygen saturation; RMSSD, root mean square of successive differences; TANEC, transfusion-associated necrotizing enterocolitis.
Note: The values were obtained using NIRS.
[a]p-value < 0.05.

RBC transfusion in the postprandial state, including the suggestion of changes in $r_{int}SO_2$ values.[29] Marin et al suggested a possible physiological mechanism for the association between withholding feeds and the decreased incidence of TANEC. They reported that mesenteric oxygenation during RBC transfusion was not influenced by feeding status, but feeding during the transfusion was associated with negative trends in postprandial mesenteric tissue oxygenation.[30] Cortez et al found a lower mean $r_{int}SO_2$ in infants with than in infants without feeding intolerance.[11] They did not, however, investigate the variability of the NIRS measurements during continuous and bolus enteral feedings. Possibly our findings might have been different if we had withheld enteral feeds. Further research is needed to elucidate the influence of withholding enteral feeds on baseline rSO_2 variability during the transfusion.

Some researchers suggested that the severity of anemia is associated with the risk of intestinal injury leading to TANEC due to impaired tissue oxygen delivery.[9,10] The severity of anemia before RBC transfusions was, however, not different between infants who did and infants who did not develop TANEC. These results are in line with the study by Wallenstein et al.[31] Furthermore, less $r_{int}SO_2$ variability after RBC transfusions was still related to the development of TANEC following adjustment for Hb levels before the transfusions. Nevertheless, the Hb gain during RBC transfusions, that is the success of transfusions, was lower in comparison to the control infants. More information is needed to determine why infants who went on to develop TANEC did not respond better to the transfusions, which is what one would expect.

We recognize several limitations of this study. First, we selected a small number of infants who were retrospectively included from three existing, prospectively collected datasets. Nevertheless, we applied similar time windows for all infants and clinical practice, for example, the volumes and durations of the RBC transfusions did not differ between cohorts. Thus, we believe these data allow for a methodologically sound comparison. Second, the retrospective study design has some limitations. The NIRS measurements were mainly performed according to a study protocol instead of routinely, and this resulted in limited rSO_2 data before RBC transfusions. Because baseline rSO_2 data were limited, we chose not to submit these data to analysis. Instead, we provided a complete overview of these data. We cannot exclude the possibility that rSO_2 values might already have been different between the groups beforehand, even though this was not represented by the small sample of available data nor by clinical parameters. Third, the nature of the NoNEC trial may have led to bias because we included infants suspected of NEC. To prevent contamination with infants who had received a RBC transfusion because of NEC-induced anemia, we only included infants with a confirmed NEC diagnosis at least 6 hours after completed RBC transfusions, or who did not develop NEC. Fourth, NIRS has limitations concerning validity and precision. There are several concerns about assessing intestinal perfusion with NIRS. One such concern is bowel wall movements produced by peristalsis.[28] Nevertheless, NIRS monitoring has increasingly proved its clinical relevance despite concerns about precision. Even though the high degree of real-time $r_{int}SO_2$ variability makes direct intestinal monitoring difficult for clinicians on account of the need to process the data before it can be used in clinical decision making.

Conclusion

Less intestinal variability after RBC transfusion is associated with the development of TANEC, irrespective of the severity of pre-existing anemia. This is in line with the hypothesis that reduced adaptability of the intestinal vasculature to oxygen supply and demand may be associated with the development of TANEC. Furthermore, there is an increased risk of developing TANEC in infants with lower cerebral tissue oxygenation during and after RBC transfusions regardless of their Hb levels before and after the transfusions. This may reflect the overall impaired circulatory condition and vulnerability of these infants and suggest that decreased Hb levels are not solely associated with the development of TANEC.

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Conflict of Interest
None.

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