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CLINICAL RESEARCH ARTICLE

Near-infrared spectroscopy as a diagnostic tool for necrotizing enterocolitis in preterm infants

Martin van der Heide¹, Jan B. F. Hulscher², Arend F. Bos¹ and Elisabeth M. W. Kooi¹

BACKGROUND: We aimed to investigate whether splanchnic tissue oxygen saturation ($r_s\text{SO}_2$) measured by near-infrared spectroscopy (NIRS) could contribute to the early diagnosis of necrotizing enterocolitis (NEC).

METHODS: We retrospectively included infants with suspected NEC, gestational age <32 weeks and/or birth weight <1200 g in the first 3 weeks after birth. We calculated mean $r_s\text{SO}_2$, cerebral tissue oxygen saturation ($r_c\text{SO}_2$), variability of $r_s\text{SO}_2$ (coefficients of variation [$r_s\text{CoVAR}$] = SD/mean), and splanchnic-cerebral oxygenation ratio ([SCOR] = $r_s\text{SO}_2/r_c\text{SO}_2$) in the period around the abdominal radiograph to confirm or reject NEC.

RESULTS: Of the 75 infants, 21 (28%) had NEC (Bell's stage ≥ 2). Characteristics of infants with and without NEC differed only on mechanical ventilation and nil-per-os status. $r_s\text{SO}_2$ tended to be higher and $r_c\text{SO}_2$ lower in infants with NEC. $r_s\text{CoVAR}$ (median [range]) was lower (0.11 [0.03–0.34]) vs. 0.20 [0.01–0.52], $P = 0.002$) and SCOR higher (0.64 [0.37–1.36]) vs. 0.47 [0.16–1.09], $P = 0.004$) in NEC infants. Adjusted for postnatal age, mechanical ventilation, and nil-per-os status, a 0.1 higher $r_s\text{CoVAR}$ decreased the likelihood of NEC diagnosis with likelihood ratio (LR) 0.38 (95% CI 0.18–0.78) and a 0.1 higher SCOR increased it with LR 1.28 (1.02–1.61).

CONCLUSIONS: Using NIRS, high SCOR may confirm NEC and high variability of $r_s\text{SO}_2$ may rule out NEC, when suspicion arises.

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IMPACT:

- Near-infrared spectroscopy may contribute to the diagnosis of necrotizing enterocolitis.
- When clinical signs are present a high splanchnic-cerebral oxygenation may indicate necrotizing enterocolitis.
- A low splanchnic-cerebral oxygenation ratio and high variability of splanchnic tissue oxygen saturation may rule out necrotizing enterocolitis.
- Whether a bedside real-time availability of the splanchnic-cerebral oxygenation ratio and variability of splanchnic tissue oxygen saturation improves NEC diagnosis needs to be further investigated.

INTRODUCTION

During the past decades, necrotizing enterocolitis (NEC) remained one of the leading causes of morbidity and mortality in preterm infants.^{1,2} In some countries, the incidence of NEC even increased in the past couple of years.^{1,3,4} Despite decades of research, the exact pathogenesis of NEC remains unknown and timely diagnosis remains difficult. Currently, suspicion of NEC is based on clinical signs, and for diagnosis of NEC, abdominal radiographs are used. These clinical signs are not specific for NEC and may be present in various other diseases, such as sepsis.⁵ Infants are usually immediately treated for NEC when only suspicion has arisen (Bell's stage 1),^{6,7} but treatment of NEC with nil by mouth and antibiotics may result in impaired growth and development, liver dysfunction, and need for central lines.^{8,9} Therefore, new diagnostic tools are needed that have the potential to differentiate, at an early stage, between NEC and other diseases in order to prevent unnecessary treatment of infants suspected of but not having NEC. One such a tool may be near-infrared spectroscopy

(NIRS), which has been previously studied in our group as a predictor of the course of NEC.¹⁰ In that study, we showed that, in the first 16 h after diagnosis of NEC, regional splanchnic tissue oxygen saturation ($r_s\text{SO}_2$) could distinguish mild NEC (Bell's stage 2) from more severe NEC (Bell's stage 3), but $r_s\text{SO}_2$ could not distinguish between NEC and other diseases.¹⁰ Variability of $r_s\text{SO}_2$ ($r_s\text{CoVAR}$) is another potential marker as reported in previous studies, showing a decrease in infants with NEC before and after diagnosis.^{10–12} As a side effect of intestinal inflammation, regional cerebral tissue oxygen saturation ($r_c\text{SO}_2$) may also be lower in both mild and severe stage of NEC. As both $r_c\text{SO}_2$ and $r_s\text{SO}_2$ may change during NEC onset, the splanchnic-cerebral oxygenation ratio (SCOR), which is suggested as a marker of ischaemia of the intestines, may be different in NEC infants.^{13,14}

In summary, it is unknown whether several NIRS-derived variables could differentiate between infants with and without NEC at an early stage. Therefore, we aimed to investigate whether $r_s\text{SO}_2$, $r_s\text{CoVAR}$, $r_c\text{SO}_2$, and SCOR could contribute to the diagnosis

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of radiographically confirmed NEC as soon as the first suspicion of NEC arises.

MATERIALS AND METHODS

Patient population

In this retrospective cohort study, we included infants with a gestational age <32 weeks and/or birth weight <1200 g who were admitted to our neonatal intensive care unit (NICU) during the first week after birth between January 2016 and March 2019 and who were clinically suspected of NEC. Our division is a tertiary NICU, meaning that infants doing well after several weeks are transferred to regional hospitals near to their living places for further treatment. As most infants born <32 weeks or <1200 g are still admitted to our NICU during the first 21 days and to avoid bias based on a skewed distribution in postnatal ages (PNA) of cases with and without NEC in our study design, we chose to include only cases suspected of NEC during the first 21 days after birth. Suspicion of NEC was based on clinical signs such as apneas, desaturations and/or bradycardias, lethargy, abdominal distension and feeding intolerance, blood-stained faeces, and abdominal tenderness. For study purposes, we defined the start of (suspected) NEC as the need the neonatologists felt to perform an abdominal radiograph for diagnosis or rejection of NEC. We excluded all infants with chromosomal or congenital anomalies, periventricular/intraventricular haemorrhage (PIVH) grade III/IV,¹⁵ infants with other gastrointestinal diseases such as intestinal atresia and spontaneous intestinal perforation, and infants in whom $r_s\text{SO}_2$ was not routinely measured. Moreover, infants who were clinically suspected of NEC in the first 3 weeks after birth without developing NEC but did develop NEC after this period were also excluded. This study was approved by the ethical review board of the University Medical Center Groningen.

NEC diagnosis

We defined NEC as pneumatosis intestinalis, portal venous gas, or both and classified NEC according to the modified Bell's stage criteria.⁷ Groups were formed according to diagnosis of NEC (i.e. infants with definite NEC [Bell's stage ≥ 2] and infants without NEC [Bell's stage <2]). In case an NEC infant had multiple episodes of clinical suspicion of NEC, we analysed the data of that episode in which NEC was diagnosed within 48 h after the abdominal radiograph. Maximal severity of NEC was classified according to the Bell's criteria by three authors (M.v.d.H., J.B.F.H., and E.M.W.K., in all cases consensus was reached). For subgroup analyses, we further categorized the infants who did not have NEC in infants with sepsis and infants without sepsis. In many of the latter infants, no definitive diagnosis could be identified. The infants with NEC were categorized by final Bell's stage.

Near-infrared spectroscopy

In our hospital, we measure $r_s\text{SO}_2$ and $r_c\text{SO}_2$ in all infants <32 weeks and/or 1200 g as standard clinical care during the first week of life. After this period, both $r_s\text{SO}_2$ and $r_c\text{SO}_2$ were again measured when infants were suspected of NEC, at the discretion of the attending physician. $R_s\text{SO}_2$ and $r_c\text{SO}_2$ were measured using an INVOS™ 5100 C monitor (Medtronic, Dublin, Ireland). The splanchnic neonatal INVOS™ SomaSensor was placed infraumbilically and the cerebral sensor on either frontoparietal side of the head. For skin protection, Mepitel® (Mölnlycke, Sweden) was placed below the sensor to cover the skin. Every 5 s, the INVOS monitor provided a value of $r_s\text{SO}_2$ and $r_c\text{SO}_2$, and from these values we calculated the mean $r_s\text{SO}_2$ and $r_c\text{SO}_2$ during a period of 30 min with at least 80% of valid data. Valid data were defined as data without artefacts. We manually removed artefacts, which were defined as unexplained sudden non-physiological change of the values, documented misplacement of the sensor, or a lack of physiological variability of the values, again indicating sensor

misplacement. The period of the NIRS measurement closest to the abdominal radiograph was chosen for data selection. This measurement was at least within 12 h before or after the first abdominal radiograph, which was obtained for clinical suspicion of NEC. For analysis of variability of $r_s\text{SO}_2$, we calculated coefficients of variation ($r_s\text{CoVAR} = \text{SD}/\text{mean}$) over the full epoch of 30 min, having the advantage that the range of variability is larger than when using smaller epochs.¹⁶ We analysed $\text{SCOR} = r_s\text{SO}_2/r_c\text{SO}_2$ as a marker of intestinal hypoxia.¹³ Finally, we calculated the splanchnic fractional tissue oxygen extraction ($s\text{FTOE} = (\text{SpO}_2 - r_s\text{SO}_2)/\text{SpO}_2$), which reflects the balance between oxygen supply and consumption in tissue.

Clinical variables

From the patient records, we collected various baseline characteristics, including gestational age, birth weight, sex, head circumference at birth, APGAR score at 1 and 5 min, multiple gestations, parity, type of delivery, haemoglobin level, and presence of PIVH.¹⁵ We also collected data on SpO_2 (Nellcor®, Medtronic, Dublin, Ireland) measured during the same 30-min epochs as the NIRS measurements. Of the NEC infants, we recorded Bell's stage and surgical treatment. Of the infants without NEC, we documented the presence of clinical sepsis (sepsis symptoms with a positive blood culture and/or C-reactive protein >20 mg/L) and incidence of positive blood culture within 48 h before or after the abdominal radiograph. Additionally, we documented the PNA at the time of abdominal radiograph, whether <50% of mother's milk was given to the infant of the total amount of enteral feeds in the 24 h before the abdominal radiograph, nil-per-os (npo) status during abdominal radiograph (defined as no feedings for a longer period as the normal feeding interval [2–3 h]), if mechanical ventilation was started 24 h before the abdominal radiograph, mechanical ventilation during the NIRS measurement, administration of inotropes in the 3 h before the NIRS measurement, and data on mortality within the first 21 days after birth.

Statistical analyses

To assess differences between infants with and without NEC, we used independent *t* tests and Mann–Whitney *U* tests for parametric and nonparametric data, expressed as mean \pm SD and median (range), respectively. We used Chi-square tests and Fisher's exact tests for nominal and categorical variables, expressed as number and percentage. For the subgroup analyses, we used the same statistical tests as listed above or, when applicable, one-way analysis of variance or Kruskal–Wallis tests for parametric and nonparametric data, respectively. For the diagnosis or rejection of NEC, receiver operating characteristic (ROC) curve analysis was performed of all NIRS measurements with a *P* value < 0.05 to determine cut-off values with 100% sensitivity and 100% specificity. Next, we performed univariate logistic regression analyses to calculate likelihood ratios (LRs) including 95% confidence interval (CI) for NEC diagnosis regarding each diagnostic NIRS variable. Next we repeated the regression analyses, entering potential confounders in the models. We considered variables as potential confounders if they differed between infants diagnosed with NEC and without NEC at *P* values < 0.1. We decided to enter PNA anyway in the multiple logistic regression models, due to its associations with $r_s\text{SO}_2$ and $r_c\text{SO}_2$.^{17,18} Because the NIRS-related variables $r_s\text{SO}_2$, $r_c\text{SO}_2$, and SCOR are by definition closely related to each other, we did not put them simultaneously into the same regression model to avoid multicollinearity. Regarding $r_s\text{CoVAR}$, we repeated the final multiple regression model including also $r_s\text{SO}_2$, because the variability measured using $r_s\text{CoVAR}$ is influenced by the mean $r_s\text{SO}_2$. In this way, we could differentiate between the diagnostic properties of the variability of $r_s\text{SO}_2$ and the level of $r_s\text{SO}_2$. Two-tailed *P* values of <0.05 were considered statistically significant. IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA) was used for

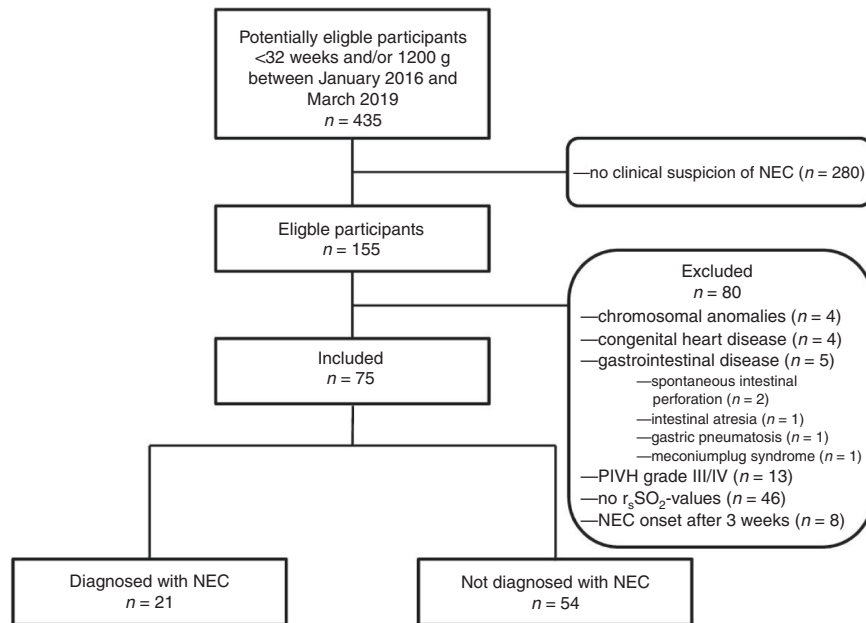


Fig. 1 Flow diagram of the study. NEC necrotizing enterocolitis, PIVH periventricular/intraventricular haemorrhage, r_sSO_2 splanchnic tissue oxygen saturation, NEC onset after 3 weeks NEC development after the inclusion period of 3 weeks after birth.

statistical analyses. We used GraphPad Prism 7.02 (GraphPad Software Inc., La Jolla, CA, USA) for graphical displays.

RESULTS

Of a total of 435 eligible infants, 155 infants (36%) clinically needed an abdominal radiograph for diagnosis or rejection of NEC within the first 3 weeks after birth. We excluded 80 of the 155 infants for various reasons (Fig. 1) of whom 46 had missing r_sSO_2 values. Reasons for missing r_sSO_2 values were: <80% of valid r_sSO_2 values ($n = 1$), no NIRS device available ($n = 3$), measurement of r_sSO_2 started >12 h after abdominal radiograph ($n = 5$), data not stored in our offline database due to technical issues ($n = 6$), an umbilical catheter taped to the infraumbilical skin leaving no space for the NIRS sensor ($n = 12$), and in 19 cases r_sSO_2 was not measured for unknown reasons. Patient characteristics are shown in Table 1. Excluded infants were smaller (lower birth weight and lower head circumference), had lower Apgar score at 1 and 5 min, and had lower SpO_2 than included infants (Supplementary Table S1). Of the 75 infants with suspicion of NEC, 21 (28%) indeed had NEC. Seven infants were diagnosed with Bell's stage 2 and 14 infants with Bell's stage 3. NEC was diagnosed at a median PNA of 10 days (range 4–19) and the median PNA at the day of the first abdominal radiograph for suspected NEC in the group without NEC was 8 days (range 2–21, $P = 0.089$). The median postmenstrual age was 29.0 weeks (range 25.9–35.4) at the time of NEC diagnosis in the NEC group and 29.8 weeks (range 25.7–33.3) in the group without NEC ($P = 0.583$). None of the infants had multiple episodes of NEC. The abdominal radiograph was not conclusive in confirming the diagnosis of NEC in 7 out of 21 infants of the NEC group. It was not conclusive in ruling out the diagnosis of NEC in 11 out of 54 infants in the group without NEC.

NIRS measurements in infants with and without NEC

Around the time of the abdominal radiograph when NEC was suspected, r_sSO_2 tended to be higher in infants with NEC (44.2% [15.6–71.2], $n = 21$) than in infants without NEC (35.5% [15.0–74.8], $n = 54$, $P = 0.05$). Variability (CoVAR) of r_sSO_2 was lower in infants with NEC (0.11 [range 0.03–0.34], $n = 21$) than in infants without NEC (0.20 [range 0.01–0.52], $n = 54$, $P = 0.002$) (Fig. 2). Infants with NEC

demonstrated a lower r_cSO_2 ($63.8\% \pm 11.9$, $n = 21$) than infants without NEC ($72.0\% \pm 11.0$, $n = 53$, $P = 0.006$) (Fig. 2). SCOR was higher in infants with NEC (0.64 [range 0.37–1.36], $n = 21$) than in infants without NEC (0.47 [range 0.16–1.09], $n = 53$, $P = 0.004$) (Fig. 2). The median sFTOE was 0.56 (range 0.27–0.82) in the NEC group and 0.62 (range 0.23–0.83) in the infants without NEC ($P = 0.209$).

In our study, we measured NIRS in 38 (51%) infants in the 12 h before and in 37 (49%) infants in the 12 h after the abdominal radiograph. There was no difference in percentage of NIRS measurements before the abdominal radiograph between infants diagnosed with NEC and without NEC, (38% vs. 56%, $P = 0.176$). Moreover, r_sSO_2 , r_cSO_2 , SCOR, r_sCoVAR , and sFTOE were not different before and after the abdominal radiograph in both the group with NEC and without NEC (data not shown).

More severe NEC (Bell's stage 3) had lower r_sSO_2 (41% vs. 58%), r_cSO_2 (63% vs. 69%), and SCOR (0.64 vs. 0.90) than milder NEC (Bell's stage 2) and higher sFTOE (0.58 vs. 0.36) than milder NEC (Bell's stage 2), but this did not reach statistical significance.

When infants were categorized as infants with NEC, infants with sepsis, and infants without sepsis and NEC, none of the NIRS variables could distinguish NEC from sepsis (Fig. 3). However, r_sCoVAR and r_cSO_2 were lower in infants with NEC and sepsis than in infants without sepsis and NEC. Moreover, SCOR was higher in infants with NEC and sepsis than in infants without sepsis and NEC (Fig. 3).

Diagnostic quality of NIRS

ROC curve analysis showed an area under the curve of 0.73 ($P = 0.002$) for r_sCoVAR , an area under the curve of 0.70 ($P = 0.007$) for r_cSO_2 , and an area under the curve of 0.71 ($P = 0.004$) for SCOR (Table 2). We found that, in our population, NEC could be ruled out with 100% sensitivity if r_sCoVAR was >0.34, if r_cSO_2 was >87.8%, and if SCOR was <0.37 (Table 2). Furthermore, we found that NEC could be diagnosed with 100% specificity if r_sSO_2 was <51.3% or SCOR was >1.10 (Table 2). We could not calculate a cut-off point with 100% specificity for r_sCoVAR because the minimum value in the group of infants without NEC was lower than that in the infants with NEC (Fig. 2).

We calculated LRs for a positive NEC diagnosis regarding r_sSO_2 , r_sCoVAR , r_cSO_2 , and SCOR, both crude and adjusted for potential

Table 1. Patient characteristics of infants with and without NEC.

Variable	NEC		No NEC		P value
		n		n	
Gestational age (weeks)	27.4 (24.3 to 34.4)	21	28.7 (24 to 32.1)	54	0.316
Birth weight (g)	1071 ± 347	21	1140 ± 333	54	0.431
Head circumference (cm)	25.6 ± 2.5	20	26.1 ± 2.4	49	0.509
Male, n (%)	13 (62)	21	33 (61)	54	0.949
Apgar score at 1 min	5.1 ± 2.5	21	5.5 ± 1.8	54	0.508
Apgar score at 5 min	7.1 ± 1.1	21	7.4 ± 1.2	54	0.510
Primipara, n (%)	15 (71)	21	33 (61)	54	0.403
Multiple gestations, n (%)	7 (33)	21	14 (26)	54	0.521
Patent ductus arteriosus, n (%) ^a		21		54	0.122
No PDA	8 (38)		34 (63)		
Non-hsPDA	3 (14)		3 (6)		
hsPDA	10 (48)		17 (31)		
PIVH, n (%)		21		54	0.788
Grade I/II	9 (43)		25 (46)		
Caesarean section, n (%)	7 (33)	21	23 (43)	54	0.462
SpO ₂	91.8 (84.2 to 98.6)	18	94.5 (85.7 to 100)	53	0.101
Mechanical ventilation, n (%) ^b	7 (33)	21	15 (28)	54	0.635
Haemoglobin (mmol/L)	7.9 (6.4 to 10.2)	20	8.0 (5.8 to 12.2)	37	0.514
Mother's milk <50%, n (%) ^c	4 (22)	18	13 (25)	53	0.843
Time of NIRS measurement in relation to the abdominal radiograph (h) ^d	0.65 (−4.00 to 11.68)	21	0 (−8.88 to 11.35)	54	0.109
Mechanical ventilation during NIRS measurement, n (%)	13 (62)	21	17 (32)	54	0.016*
Nil per os during NIRS measurement, n (%)	13 (62)	21	20 (37)	54	0.051
Inotropes before NIRS measurement, n (%)	1 (5)	21	1 (2)	54	0.484
Sepsis in infants without NEC, n (%)	N/A		23 (43)	54	
Positive blood culture in infants without NEC, n (%)	N/A		18 (33)	54	
Deceased, n (%)	9 (43)	21	2 (4)	54	0.001***
Deceased due to NEC, n (%)	9 (43)	21	N/A		
Postnatal age at time of death (days)	13 (7 to 22)	21	N/A		
Cause of death, n (%)					
NEC totalis	4 (44)	9			
Respiratory and/or circulatory failure	4 (44)	9			
Multi-organ failure	1 (12)	9			
Surgical treatment, n (%) ^e	12 (57)	21	N/A		

Data are expressed as median (range), mean ± standard deviation for continuous variables, or as number (percentage) for categorical variables. NEC necrotizing enterocolitis, PDA patent ductus arteriosus, hsPDA haemodynamic significant patent ductus arteriosus, PIVH periventricular/intraventricular haemorrhage, N/A not applicable.

*P < 0.05; ***P < 0.001.

^aScreening of PDA was performed in infants requiring mechanical ventilation or continuous positive airway pressure between day 3 and day 7 after birth.

^bMechanical ventilation before suspicion of necrotizing enterocolitis.

^c<50% of mother's milk 24 h before abdominal radiograph.

^dNegative value indicates measurements before the abdominal radiograph.

^eComfort care was given when there were no further options for treatment.

confounders (Table 3). In the crude analyses, both the r_sCoVAR and r_cSO₂ were associated with a decreased likelihood of NEC, whereas the SCOR was associated with an increased likelihood. For practical reasons, allowing a proper interpretation, we divided r_sSO₂ and r_cSO₂ by 10, and multiplied the r_sCoVAR and SCOR by 10. This means, for example, regarding r_cSO₂, a 10% higher r_cSO₂ value reduces the a priori chance of having the diagnosis NEC by a factor 0.51. Similarly, regarding SCOR, a 0.1 point higher SCOR would increase the chance of a positive diagnosis NEC by approximately 1.33. Of note, r_sSO₂ was not associated with NEC diagnosis (Table 3).

As confounders, we considered PNA, mechanical ventilation, and npo status during the NIRS measurements, being different between infants diagnosed with NEC and without NEC, at P = 0.089, P = 0.016, and P = 0.051, respectively. The LR_s, adjusted for PNA, and next for both PNA and mechanical ventilation, were more or less similar as the crude LR_s, but the significant relation with r_cSO₂ had disappeared (Table 3). Finally, when we included both r_sCoVAR and r_sSO₂ in the multiple regression model, adjusted for PNA and mechanical ventilation, only r_sCoVAR remained significantly in the model with LR 0.38 (95% CI 0.17–0.85, P = 0.019).

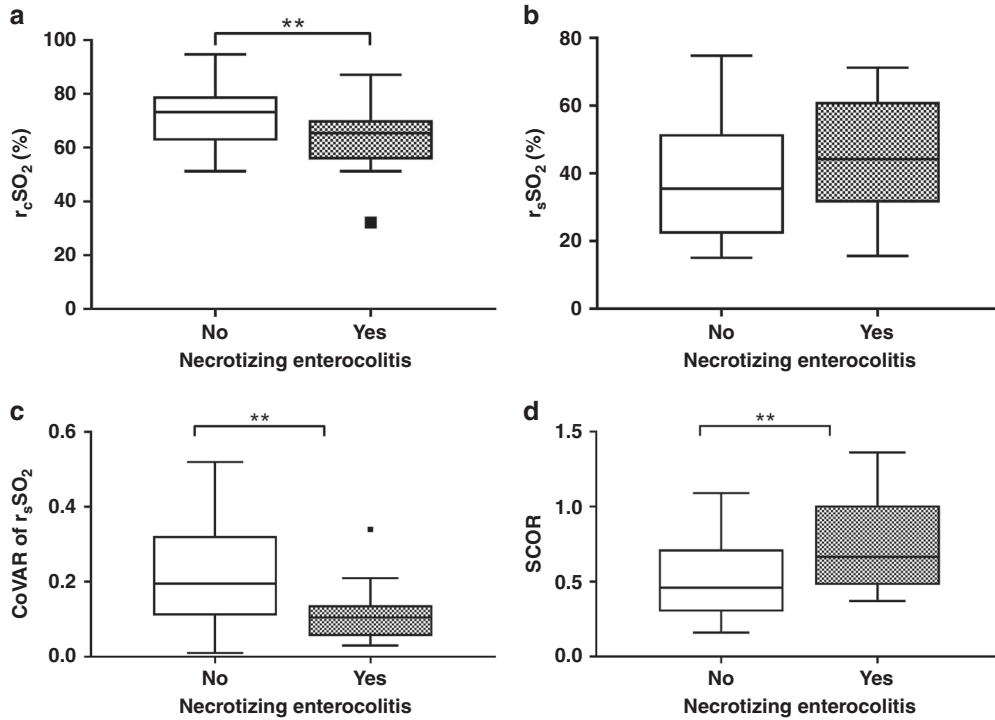


Fig. 2 Levels of r_cSO_2 , r_sSO_2 , CoVAR of r_sSO_2 , and SCOR of infants with and without necrotizing enterocolitis. Box-whisker plots of r_cSO_2 ($n = 21$ vs. 53) (a), r_sSO_2 ($n = 21$ vs. 54) (b), CoVAR of r_sSO_2 ($n = 21$ vs. 54) (c), and SCOR ($n = 21$ vs. 53) (d) of infants with and without necrotizing enterocolitis. The boxes represent the 25th and 75th percentile of the measurements with the median in the center, the whiskers represent the range of the measurements. The black squares represent outliers. r_cSO_2 cerebral tissue oxygen saturation, CoVAR coefficients of variation, r_sSO_2 splanchnic tissue oxygen saturation, SCOR splanchnic-cerebral oxygenation ratio. $**P < 0.01$.

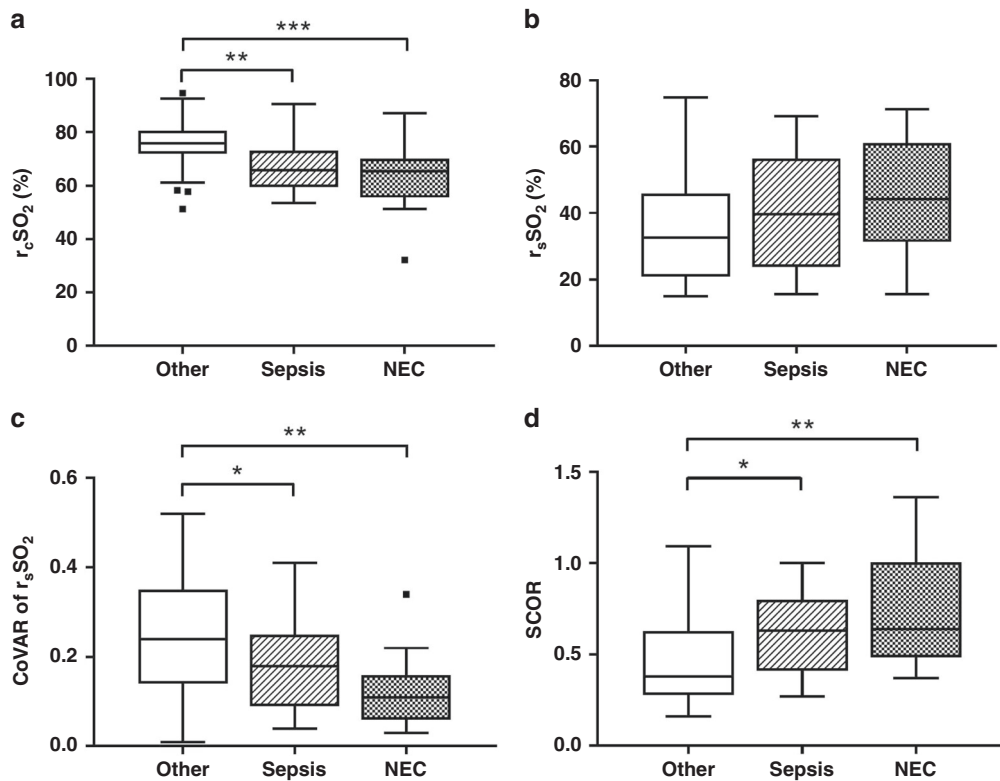


Fig. 3 Levels of r_cSO_2 , r_sSO_2 , CoVAR of r_sSO_2 , and SCOR of infants with necrotizing enterocolitis, sepsis or other causes for abdominal symptoms. Box-whisker plots of r_cSO_2 ($n = 30$ vs. 23 vs. 21) (a), r_sSO_2 ($n = 31$ vs. 23 vs. 21) (b), CoVAR of r_sSO_2 ($n = 31$ vs. 23 vs. 21) (c), and SCOR ($n = 30$ vs. 23 vs. 21) (d) of infants without sepsis or necrotizing enterocolitis, infants with sepsis, and infants with necrotizing enterocolitis. The boxes represent the 25th and 75th percentile of the measurements with the median in the center, the whiskers represent the range of the measurements. The black squares represent outliers. r_cSO_2 cerebral tissue oxygen saturation, CoVAR coefficients of variation, r_sSO_2 splanchnic tissue oxygen saturation, SCOR splanchnic-cerebral oxygenation ratio. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

DISCUSSION

In this study, we demonstrated that the ratio between splanchnic and cerebral tissue oxygen saturation, defined as SCOR, and the variability of r_sSO_2 were promising diagnostic markers for NEC around the time NEC was suspected and an abdominal radiograph was performed. Both differentiated between infants with NEC and infants without NEC. A 0.1 point higher SCOR increased the a priori chance of confirming NEC diagnosis by approximately 28%. This was due to the combination of lower r_cSO_2 values and higher r_sSO_2 values in the infants with NEC. R_sSO_2 and r_cSO_2 alone were not related to NEC diagnosis. Regarding r_sCoVAR , a 0.1 point higher r_sCoVAR decreased the a priori chance of having NEC diagnosed by a factor 0.38, i.e. the a priori chance of ruling out NEC was approximately $1/0.38 = 2.6$ times higher. Using data on r_sCoVAR and SCOR when suspecting NEC may thus be helpful, particularly in ruling out the diagnosis of NEC.

To the best of our knowledge, this is the largest study to date demonstrating the diagnostic properties of a 30-min measurement of r_sSO_2 and r_cSO_2 in a group of preterm infants with clinical signs of NEC. We found that a high SCOR, which represents the ratio between r_sSO_2 and r_cSO_2 , increased the likelihood of a positive early NEC diagnosis, i.e. just before or soon after suspicion has arisen. Moreover, a low SCOR and a high variability of r_sSO_2 , measured as CoVAR, were the best markers to rule out NEC. In a previous smaller study of our group in other infants, a similar lower variability of r_sSO_2 was demonstrated in NEC infants, during the 24 h after suspicion has arisen,¹⁰ whereas this was not the case in infants 48 h before they developed NEC.¹⁹ Therefore, it seems that NIRS, in particular splanchnic CoVAR and SCOR, have the most valid diagnostic qualities in the period immediately before and after NEC onset. In contrast, in a small study describing one infant with NEC and another with a single intestinal perforation by Cortez et al. reported that variability of r_sSO_2 was low several days

before NEC emerged.¹¹ Our findings, in a much larger cohort, indicate, however, that the loss of variability mostly occurs at the time of NEC onset.

We have thought of explanations why r_sCoVAR was lower in infants with NEC. In a healthy intestine, variability might be high due to periods of digestion with a high oxygen demand and periods of rest when less oxygen is required. This was, however, not found in a recent study of ours in relatively healthy preterm infants during the first weeks after birth.²⁰ A better explanation relates to peristaltic movement of the intestines. Variability may increase as different parts of the moving intestines will be measured with potentially different r_sSO_2 values. In NEC infants, peristaltic movements are decreased. Inflammation could also increase the blood flow to the intestines, which might explain the decreased variability with relatively high r_sSO_2 values in NEC infants, although the association between NEC and decreased variability remained after adjusting for r_sSO_2 values in the analyses. Interestingly, r_sCoVAR could not differentiate between Bell's stage 2 and Bell's stage 3 infants. This suggests that, in both Bell's stage 2 and Bell's stage 3 infants, peristaltic movement may be decreased. An alternative explanation could be that the clinical presentation of Bell's stage 2 and Bell's stage 3 infants at that moment are similar, because we used NIRS measurements at an early stage in the course of NEC. We noticed that some infants without NEC also demonstrated rather low r_sCoVAR values. An explanation could be that loss of variability is a general sign of systemic inflammation, which may also be present in infants without NEC. This is supported by the fact that r_sCoVAR could not discriminate between NEC and sepsis. Septic infants may present with feeding difficulties.²¹ We speculate that in those cases poor systemic perfusion decreases intestinal function and peristalsis, potentially associated with decreased splanchnic variability. However, the lack of a difference in r_sSO_2 between sepsis and NEC infants could be very well due to a lack of power. In contrast, because in this study we used a control group with NEC alike symptoms, differences in r_sCoVAR may even be greater than in asymptomatic infants. Further larger studies are required to sort this out.

Apart from r_sCoVAR being discriminative in the early diagnosis of NEC, we also found SCOR to be higher in infants diagnosed with NEC. This was due to a higher r_sSO_2 and a lower r_cSO_2 , albeit non-significant for both. In contrast to our findings, Fortune et al. reported in a small explorative study that SCOR was lower in infants with severe ischaemic gastrointestinal conditions than controls.¹³ The differences between our findings and theirs can be explained by the fact that Fortune et al. only included infants who required surgery. It is known that r_sSO_2 values in infants with severe ischaemia as seen during Bell's stage 3 are lower than in infants having NEC Bell's stage 2.¹⁰ We believe that the change in SCOR that we found in the early stage of the disease is caused by

Table 2. Cut-off points for various NIRS parameters.

	100% sensitivity		100% specificity		P value
	Cut-off value	AUC (95% CI)	Cut-off value	AUC (95% CI)	
R_sCoVAR	0.34	0.73 (0.61–0.85)	N/A	N/A	0.002
r_cSO_2 (%)	87.8	0.70 (0.57–0.83)	51.3	0.70 (0.57–0.83)	0.007
SCOR	0.37	0.71 (0.59–0.84)	1.10	0.71 (0.59–0.84)	0.004

R_sSO_2 cerebral tissue oxygen saturation, *R_sCoVAR* coefficients of variation of splanchnic tissue oxygen saturation, *SCOR* splanchnic-cerebral oxygenation ratio, *N/A* not applicable.

Table 3. Crude and adjusted likelihood ratios (LRs) with 95% confidence intervals (CIs) for NEC diagnosis of various NIRS-related variables adjusted for postnatal age, mechanical ventilation, and nil per os during the NIRS measurement.

Variable	Crude (n = 75)		Adjusted for postnatal age in days (n = 75)		Adjusted for postnatal age in days and mechanical ventilation and nil per os during the NIRS measurement (n = 75)	
	LR (95% CI)	P value	aLR (95% CI)	P value	aLR (95% CI)	P value
R_sSO_2 (per 10%)	1.33 (0.99–1.79)	0.063	1.27 (0.92–1.74)	0.145	1.34 (0.94–1.90)	0.105
R_sCoVAR (*10)	0.41 (0.22–0.76)	0.005	0.42 (0.22–0.80)	0.008	0.38 (0.18–0.78)	0.009
R_cSO_2 (per 10%)	0.51 (0.31–0.86)	0.010	0.55 (0.32–0.95)	0.031	0.70 (0.39–1.23)	0.216
SCOR (*10)	1.33 (1.09–1.63)	0.005	1.30 (1.05–1.61)	0.015	1.28 (1.02–1.61)	0.033

R_sSO_2 cerebral tissue oxygen saturation, *R_sSO_2* splanchnic tissue oxygen saturation, *R_sCoVAR* coefficients of variation of splanchnic tissue oxygen saturation, *SCOR* splanchnic-cerebral oxygenation ratio, *LR* likelihood ratio, *aLR* adjusted likelihood ratio, *CI* confidence interval, *10 multiplied by 10.

haemodynamic instability, potential loss of cerebral autoregulation,²² and increased intestinal blood perfusion due to inflammation resulting in a lower $r_c\text{SO}_2$ and higher $r_s\text{SO}_2$. In line with this, we found that $r_c\text{SO}_2$ was not different between infants with NEC and infants with sepsis.

In our study $r_s\text{SO}_2$ did not differentiate between complicated and uncomplicated NEC. Previously, we found that $r_s\text{SO}_2$ did differentiate if measurements were performed after diagnosis,¹⁰ whereas in the current study, we measured NIRS immediately before or around the time the abdominal radiograph was performed. It might be that at that time NEC is mostly still in an inflammatory stage with increased flow to the intestines, as is also suggested by the slightly higher $r_s\text{SO}_2$ values. Progression into NEC Bell's stage 3 in some infants and not in others may explain why this differentiation is not present already around the onset of NEC.

Interestingly, the splanchnic FTOE was not different between infants with and without NEC. This indicates that the balance between oxygen supply and oxygen consumption of the intestines was similar in both groups. Although the median splanchnic FTOE was not significantly different, NEC infants had a 10% lower splanchnic FTOE compared to infants without NEC. This may suggest that indeed abdominal perfusion is increased or O_2 consumption decreased in NEC infants. Bell's stage 2 infants had even lower splanchnic FTOE values. We speculate that infants with Bell's stage 2 had increased intestinal perfusion without changes in oxygen consumption, whereas infants with Bell's stage 3 had both decreased intestinal perfusion and oxygen consumption, similar as reported by Schat et al.¹⁰ However, as both the differences between infants with and without NEC and between Bell's stage 2 and 3 infants were not significant, this remains speculative.

In this retrospective study, we measured NIRS within the 12 h before or after the abdominal radiograph. Because abdominal radiographs were made already when first suspicion of NEC arose, we believe that the 12 h after the abdominal radiograph still represent the early stage of NEC. Not only a positive diagnosis of NEC is important, ruling out NEC is equally important. Of note, $r_s\text{SO}_2$, $r_c\text{SO}_2$, SCOR, $r_s\text{CoVAR}$, and sFTOE were not different before and after the abdominal radiograph. Perhaps $r_s\text{CoVAR}$ and SCOR may even diagnose or reject NEC before an abdominal radiograph is made.

Clinicians should be aware that a higher SCOR and loss of variability of $r_s\text{SO}_2$ may indicate development of NEC in preterm infants when clinical signs raise suspicion of NEC. We also present cut-off values, although these need to be validated in prospective studies. For example, in our population, none of the infants suspected of NEC indeed had NEC if SCOR was <0.370 or $r_s\text{CoVAR}$ was >0.363 . Ruling out NEC this way would have resulted in refraining from starting NEC treatment in 20–30% of the infants suspected of having NEC at the time of abdominal radiograph in our population. However, this percentage may be different in other hospitals based on the threshold of performing abdominal radiographs in case NEC is suspected. Moreover, whether using the NIRS parameters $r_s\text{CoVAR}$ and SCOR indeed is safe and leads to less unnecessary NEC treatments needs to be confirmed prospectively.

We also recognize several limitations of our study. First, we point at the risk of bias due to the retrospective nature of this study with a high percentage of infants who were excluded. These excluded infants had lower birth weights and head circumference and had lower Apgar scores at 1 and 5 min than the included group. This may be caused by the exclusion of PIVH grade III and IV, which is more common in low birth weight infants.²³ Second, $r_s\text{SO}_2$ could not be measured in some of the smallest infants because the umbilical catheter was taped to the infraumbilical skin. Third, the rates in npo status were lower in infants with NEC than in those without NEC. As feeding may

impact intestinal blood flow and oxygen extraction, this could have influenced our findings. We could not confirm this in this cohort, because adding npo status as covariate to our multiple regression models did not change the LRs. Recently, it was also reported that $r_s\text{SO}_2$ did not change after bolus feeds in preterm infants during the first weeks after birth.²⁰ We believe therefore that the impact of feeding status on our findings is limited. A fourth limitation relates to the CoVAR measurement. Mintzer et al. reported that longer epochs increase variability.¹⁶ $r_s\text{CoVAR}$ will thus be higher if determined in longer epochs, but using too long epochs would be impractical for a fast diagnosis of rejection of NEC. We chose for 30-min epochs for practical reasons, combining enough range in variability concurrently with a fast diagnosis or rejection of NEC. However, it is unknown if 30-min epochs are the best to differentiate between infants with and without NEC. Further research is needed to investigate the optimal epoch with the best diagnostic power. Fifth, a potential disadvantage of COVAR is the mathematical formula. By definition, the CoVAR will be lower when the mean is higher, regardless of a similar SD. In our study, however, we corrected for mean $r_s\text{SO}_2$ and found that the relation between NEC diagnosis and $r_s\text{CoVAR}$ remained unchanged. Finally, SCOR and $r_s\text{CoVAR}$ are not implemented in clinical care and can currently not be shown real time on the monitor.

In conclusion, a high SCOR may contribute to the diagnosis of NEC while a low SCOR and high variability of $r_s\text{SO}_2$ as measured by NIRS may contribute to ruling out NEC when suspicion of NEC arises in infants with clinical signs. In the future, prospective studies on SCOR and variability of $r_s\text{SO}_2$ are required to validate whether these measures are indeed capable of identifying NEC in preterm infants at an early stage.

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AUTHOR CONTRIBUTIONS

M.v.d.H. and E.M.W.K. were involved in the design and execution of the study. All other authors were involved in the final consensus process of the protocol and contributed significantly to the manuscript. M.v.d.H. drafted the manuscript and all other authors read, edited, and approved the final manuscript for publication.

ADDITIONAL INFORMATION

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Consent statement: Due to the retrospective character of the study, no informed consent from the participant's legal guardian was required. Nevertheless, none of the participant's legal guardians objected to participate during admission when they were offered this option.

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