Cardiovascular and cerebrovascular responses to cardio-respiratory events in preterm infants during the transitional period

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Abstract

This observational prospective study aimed to investigate cerebral and cardiovascular haemodynamic responses to different types of cardio-respiratory events (CRE) in preterm infants during postnatal transition, and to evaluate the impact of relevant clinical characteristics.

Infants with gestational age (GA) <32weeks and/or birth weight <1500g were enrolled after birth. Cerebral oxygenation index (cTOI), fractional oxygen extraction (cFTOE), cardiac output (CO), cardiac contractility (iCON) and systemic vascular resistances (sVR) were simultaneously monitored over the first 72h by near-infrared spectroscopy and electrical velocimetry. CRE were clustered into isolated bradycardia (IB), isolated desaturation (ID), combined desaturation/bradycardia (DB). For each parameter, percentage changes from baseline (%Δ) were calculated. The impact of different CRE types and clinical variables on %Δ was evaluated with generalized estimating equations.

A total of 1426 events were analysed. %ΔcTOI significantly differed among ID, IB and DB ($p<0.001$), the latter showing the greatest drop. %ΔcFTOE decreased significantly during DB ($p<0.001$) and ID ($p<0.001$) compared to IB. DB and IB were associated with more negative %ΔCO ($p<0.001$) and more positive %ΔsVR ($p<0.001$) compared to ID. A slight iCON reduction was observed during DB compared to ID ($p=0.043$). Antenatal umbilical Doppler impairment, GA and the presence of a haemodynamically significant patent ductus arteriosus had a significant independent impact on %ΔcTOI, %ΔcFTOE and %ΔCO.

During the transitional period, the haemodynamic responses to CRE are influenced by the event type and by specific neonatal characteristics, suggesting the importance for targeted individualized approaches to minimize the risk of cerebral injury in the preterm population.

Key points summary

- Non-invasive simultaneous multiparametric monitoring allows the *in vivo* evaluation of cerebral and cardiovascular haemodynamic responses to different types of recurrent episodes of

intermittent hypoxia and/or bradycardia, also defined as cardio-respiratory events (CRE), in preterm neonates during postnatal transition.

- By decreasing left cardiac output, bradycardia further contributes to cerebral hypoxia during CRE.
- The presence of a haemodynamically significant patent ductus arteriosus results in a deeper impairment of cerebral oxygen status in response to CRE, whereas the brain-sparing remodelling of the fetal circulation resulting from placental insufficiency is associated with more favourable haemodynamic responses to intermittent hypoxia.
- During transition, the haemodynamic impact of CRE is influenced not only by the event type, but also by specific clinical features; this highlights the importance of developing individualized approaches to reduce the hypoxic burden in this delicate phase.

Introduction

Due to the immaturity of the physiological mechanisms involved in respiratory control and oxygenation, intermittent episodes of hypoxia and/or bradycardia are very frequent among premature infants (Di Fiore *et al.*, 2016). These episodes, also defined as cardio-respiratory events (CRE), have been associated with several adverse neonatal outcomes, including higher rates of retinopathy of prematurity, bronchopulmonary dysplasia, sleep disordered breathing and poor neurodevelopment (Di Fiore *et al.*, 2019).

The unfavourable effects of CRE on cerebral haemodynamics in the preterm population have also been established (Schmid *et al.*, 2015*a*; Garvey *et al.*, 2018). A transient decrease in cerebral oxygenation has been documented during both bradycardic and hypoxic episodes, which can be deeper if the two events occur simultaneously (Schmid *et al.*, 2015*b*; Walter *et al.*, 2018). Fluctuations between cerebral hypoxia and hyperoxia activate a pro-oxidant cascade that triggers an inflammatory response and alters the balance of neurotransmitters (Di Fiore *et al.*, 2019). These mechanisms have been associated not only with the development of white matter injury in animal studies (Juliano *et al.*, 2015), but also with different patterns of neurocognitive impairment in preterm neonates (Pillekamp *et al.*, 2007; Greene *et al.*, 2014; Poets *et al.*, 2015).

The first 72 hours after premature birth represent a critical phase of transition from a fetal to a neonatal circulation. This phase, also referred to as the transitional period, consists of a complex interplay between cardiac function, blood pressure and systemic blood flow. Due to the immature cardiovascular physiology of preterm neonates, the transitional period is often characterized by

enhanced haemodynamic instability (Wu *et al.*, 2016). This, in turn, may worsen the impact of CRE on cerebral haemodynamics throughout this period, with possible impairment of cerebrovascular autoregulation. This is suggested by recent evidence of the association between early hypoxic burden and higher rates of intraventricular haemorrhage (IVH) (Vesoulis *et al.*, 2019; Ng *et al.*, 2019).

The current literature on CRE and their impact on cerebral perfusion and oxygenation is mostly based on infants aged from few weeks to few months. During the transitional period, specific conditions, such as a patent ductus arteriosus (PDA), may influence the haemodynamic status of premature neonates (Deshpande *et al.*, 2018). To the best of our knowledge, the haemodynamic impact of CRE in these situations still has to be elucidated. Moreover, evidence on functional cardiac changes during CRE in the preterm population is very scarce, and only related to bradycardic episodes without desaturation (de Waal *et al.*, 2016).

The aim of this study was to investigate combined cardiovascular and cerebrovascular changes in response to different types of CRE in preterm infants during the transitional period, and to evaluate whether these haemodynamic changes were associated with specific antenatal, perinatal or post-natal characteristics.

Methods

Ethical Approval

This study was conducted in conformity with principles and regulations of the Helsinki Declaration. The study protocol was approved by the Ethics Committee of St. Orsola-Malpighi Hospital, Bologna (Italy) and is registered in the Protocol Registration System ClinicalTrials.gov (NCT04184245). Written informed consent was obtained from the parents/legal guardians of each infant. The study protocol is compliant with the ethics policies of *The Journal of Physiology*.

Study population

Infants with a gestational age (GA) <32 weeks and/or a birth weight <1500 g, admitted to the Neonatal Intensive Care Unit (NICU) of S. Orsola-Malpighi Hospital between March 2018 and December 2019, were consecutively enrolled in this observational prospective study in the first 12 hours of life. For the sake of data homogeneity, major congenital malformations, including congenital heart disease, and ongoing mechanical ventilation were exclusion criteria. Moreover, in order to rule

out possible confounding factors on haemodynamic parameters, infants requiring inotropes during the study period were also excluded from data analysis.

For each enrolled infant, the following data were collected: GA; antenatal steroids (complete course vs. incomplete course or not given); evidence of reversed end-diastolic flow on antenatal umbilical Doppler (uREDF); ventilatory status over the first 72 hours of life (continuous positive airway pressure [CPAP] vs. nasal cannulas or self-ventilating in air [SVIA]); daily weight and body surface area (BSA).

A screening echocardiogram aimed at evaluating the ductal status was routinely performed at the time of enrolment using an ultrasound scanner CX50 (Philips Healthcare) with a 12-MHz probe, and repeated 6-12 hourly in the presence of a PDA, or 12-24 hourly if there was no evidence of PDA. The ductal diameter was measured from the high parasternal view at the point of maximum constriction, caring to avoid colour-Doppler interference outside the vessel wall. The ductal flow pattern was evaluated from the parasternal short axis using continuous-wave Doppler and, based on the ratio of end-diastolic to peak-systolic velocity, was defined as pulsatile (≥0.5) or restrictive (<0.5) (Jain & Shah, 2015). Flow velocity in the descending aorta (DAo) and in the anterior cerebral artery (ACA) was measured using pulsed-wave Doppler from low subcostal sagittal view and from sagittal view through the anterior fontanel, respectively. The left atrium to aortic root (LA:Ao) ratio was measured on M-Mode scans from the parasternal long axis view, using the leading-edge-to-leading-edge technique. Based on these echocardiographic features, the ductal status was classified as follows: haemodynamically significant PDA (hsPDA; pulsatile shunt pattern and LA:Ao ratio ≥1.5 or evidence of reversed end-diastolic flow in the DAo or ACA) (van Laere *et al.*, 2018); restrictive PDA (rPDA; restrictive shunt pattern and LA:Ao ratio <1.5); no PDA evidence (noPDA).

Multi-parametric monitoring

Non-invasive, multi-parametric haemodynamic monitoring was routinely performed during the transitional period in infants <32 weeks' gestation and/or <1500 g. Peripheral oxygen saturation (SpO_2) and heart rate (HR) were detected using a pulse oximeter Masimo Radical 7 (Masimo Corporation, Irvine, CA, USA) with an averaging time set at 2sec. HR, weight-indexed stroke volume (SV), weight-indexed cardiac output (CO), an index of heart contractility (iCON) and BSA-indexed systemic vascular resistance (sVR) and were collected beat-to-beat by means of electrical velocimetry (ICON®, Osypka Medical, Berlin, Germany), a non-invasive haemodynamic monitoring technique based on the measurement of thoracic electrical bioimpedance (Noori *et al.*, 2012). Blood pressure

(BP) was measured at regular intervals by the oscillometric technique and the obtained values were entered in the ICON ® device for sVR calculation.

Cerebral tissue oxygenation index (cTOI) was monitored with a NIRO-200nx oximeter (Hamamatsu Phototonics, Japan), set on a 1Hz sampling rate; disposable neonatal sensors were placed on the central forehead.

In the enrolled infants, each monitoring device was connected via a RS232 cable to a laptop running ICM+® (*https://icmplus.neurosurg.cam.ac.uk*, Cambridge Enterprise, UK), a software tool that allows a real-time synchronized multiparametric data collection. Using this software, the above-mentioned parameters were recorded continuously from the time of the enrolment up to 72 hours of life. Cerebral fraction of tissue oxygen extraction (cFTOE), which reflects the balance between oxygen delivery and consumption (Naulaers *et al.*, 2007), was also calculated from SpO_2 and cTOI values according to the following formula: $(SpO_2\text{-cTOI})/SpO_2$. The ICM+ traces were visually inspected; time periods with signal interruptions or showing a HR discrepancy >20% between pulse oximeter and electrical velocimetry were considered as likely artefactual and, as such, were excluded from data analysis.

Based on SpO_2/HR values, CRE were defined as follows: isolated desaturations (ID), defined as SpO_2 <85% (Finer *et al.*, 2006; Corvaglia *et al.*, 2014); isolated bradycardias (IB), defined as any HR fall <100 bpm or >30% below the baseline before the event (Henderson-Smart *et al.*, 1986; Corvaglia *et al.*, 2014; Eichenwald, 2016); combined desaturation and bradycardia (DB), if the two events occurred within a 30-sec time window (Poets *et al.*, 1993). Event duration was calculated as the period spent below the SpO_2/HR thresholds used for CRE definition; due to their doubtful clinical relevance, events <10 sec were ruled out from the analysis (Finer *et al.*, 2006). Moreover, CRE with baseline SpO_2 <90% were ruled out from the analysis.

For each parameter, the last stable values before the event onset (baseline) and their zenit/ nadir, concomitant to the lowest SpO_2 and/or HR (event), were tracked down (Figure 1); percentage changes (%Δ) between baseline and event values were then calculated and used for statistical analysis.

Statistical analysis

The analytical strategy used in this paper is based on generalized estimating equations (GEEs), introduced by Zeger and Liang (Zeger & Liang, 1986). This method is appropriate for the analysis of correlated data that arise from longitudinal studies, in which measurements/data from different

subjects are collected at different time points; the interpretation of regression coefficients is the same as in regression models.

In particular, GEEs with a normal distribution and autoregressive correlation structure, which is appropriate when time-varying outcomes or covariates are investigated (Ballinger, 2004), were used to analyse the effects of different CRE types on the %Δ of cerebrovascular (cTOI and cFTOE) and cardiovascular parameters (HR, SV, CO, iCON, sVR), to compare %Δ between bradycardic events with HR nadir < or ≥ 80bpm and to compare SpO_2 during and after desaturations in relation to oxygen adjustments. GEEs with normal distribution and autoregressive correlation structure were also used to assess the impact of different antenatal and neonatal variables on %ΔcTOI, %ΔcFTOE and %ΔCO. In these models, the PDA status and the mode of ventilatory support were used as time-dependent covariates, and all the events were nested within individuals. Standard errors were adjusted for the number of non-redundant parameters. A pool of >1000 events nested within 40 infants was adequately powered to test the relationship between cerebrovascular and cardiovascular responses and the independent variables included in the GEE models.

Baseline and event values for each monitored parameter were compared using Wilcoxon signed-rank test, whereas repeated measures analysis of variance (RM-ANOVA) for continuous variables and χ^2 square or Fisher's exact test for categorical variables were used to analyse changes of clinical, anthropometric and haemodynamic characteristics of the study population over the study period.

IBM SPSS, version 25.0, was used for the statistical analyses. Significance level was set at $p<0.05$.

Results

Forty neonates were included in this study, as illustrated in the study flow chart (Figure 2). The characteristics of the study population are shown in Table 1. Daily anthropometric, clinical and haemodynamic features during the study period are detailed in Table 2. All the infants received caffeine prophylaxis for apnoea of prematurity.

A total of 1426 events were recorded from the study cohort over the first 72 hours of life; of these, 903 were ID (63.3%), 224 IB (15.7%) and 299 DB (21%). Baseline and event values of each study parameter for each event type are provided in Table 3, together with the results of their comparison.

Of the 1202 events characterized by a SpO_2 drop, 795 events (66.1%), of which 638 ID and 157 DB, resolved spontaneously or with physical stimulation, whereas the remaining 407 (33.9%), of which 265 ID and 142 DB, required an increase in the fraction of inspired oxygen (FiO_2). This latter group

was characterized by significantly deeper nadir values of SpO_2 (70 [interquartile range, IQR, 63.3-74.2]%) and HR (127 [IQR 103-143] bpm) compared to those events which did not require FiO_2 adjustments (SpO_2 81 [IQR 78.8-82.7]%, p<0.001; HR 142 [IQR 121-156] bpm, p<0.001). The zenit SpO_2 in the recovery phase was significantly higher after those desaturation events requiring a FiO_2 adjustment compared to those who resolved without FiO_2 changes (SpO_2 97 [IQR 95.7-98.2] vs. 95.5 [IQR 93-97.6], p=0.002).

Percentage changes of cerebrovascular parameters in response to different CRE types are shown in Figure 3; %ΔcTOI significantly differed among CRE types, with the deepest reductions observed during DB compared to ID (p<0.001) and IB (p<0.001), whereas IB had the mildest impact. Both ID (p<0.001) and DB (p<0.001) led to a significant reduction of %ΔcFTOE compared to IB, which showed minimal effects on this parameter. A significant difference in %ΔcFTOE was also observed between ID and DB (p=0.019).

Figure 4 illustrates percentage variations of cardiovascular parameters in response to different CRE types. Consistent with the definition of DB and IB, a significant HR drop was observed during these events compared to ID (p<0.001 for both comparisons); however, the HR decrease was significantly deeper if bradycardia was accompanied by a concomitant desaturation (p=0.020). Despite the %ΔSV increase, there was significant overall reduction of %ΔCO during bradycardic episodes, either when alone or associated with desaturation, compared to ID (p<0.001 for both comparisons). Conversely, when compared to ID, %ΔsVR significantly increased during DB (p<0.001) and IB (p<0.001). The combination of desaturation and bradycardia also resulted in significant reduction of %ΔiCON compared to desaturations alone (p=0.043).

For bradycardic events (DB and IB), % Δof cardiovascular and cerebrovascular parameters were compared between CRE with a nadir HR ≥ (n=419) or <80 bpm (n=104). As shown in Figure 5, a nadir HR <80 bpm was associated with significantly deeper %ΔcTOI (p=0.015), %ΔCO (p<0.001) and more positive %ΔsVR (p<0.001) compared to nadir HR values ≥ 80 bpm, while no significant effect was observed for %ΔcFTOE, %ΔSV and %ΔiCON.

The results of GEE models, detailed in Table 4, confirmed the impact of different CRE types on %ΔcTOI, %ΔcFTOE and %ΔCO and showed that different neonatal characteristics exert a significant influence on these parameters. In particular, the presence of an hsPDA was independently associated with greater cTOI reductions (p=0.033) and higher cFTOE values (p=0.039) in response to CRE, whereas antenatal evidence of uREDF resulted in more negative variations of % ΔcFTOE (p=0.043). A significant positive correlation was observed between %ΔCO and GA (p=0.008), and significantly higher %ΔCO were also found in uREDF neonates (p=0.003).

Discussion

To the best of our knowledge, this is the first study providing a combined non-invasive investigation of cardiac and cerebral haemodynamics in response to CRE in preterm infants during the transitional period. Our findings indicate that different types of CRE elicit variable cardiovascular and cerebrovascular fluctuations. These in turn, are significantly influenced not only by the event type, but also by specific neonatal characteristics, such as abnormal umbilical Doppler or the presence of a hsPDA.

CRE characterized by a combined drop in SpO_2 and HR had the highest impact on cerebral oxygenation; this is consistent with previous findings describing a significant reduction of this parameter during DB when compared to isolated events (Schmid *et al.*, 2015*b*; Walter *et al.*, 2018).

In 1985, Perlman and Volpe (Perlman & Volpe, 1985) carried out a Doppler evaluation of blood flow velocity fluctuations in the anterior cerebral arteries during apnoeic episodes accompanied by bradycardia in preterm infants aged 2 to 45 days. During a bradycardia, especially if the HR fell below 80 bpm, they observed a significant decrease in cerebral blood flow velocity and associated reduction in BP. A causal relationship between the two findings was postulated, raising important questions on the efficacy of the physiological mechanisms of cerebral autoregulation during these events, characterized by a sudden onset and a relatively short duration.

Later on, Pichler et al. (Pichler *et al.*, 2003) performed a combined evaluation of cerebral oxygenation and cerebral blood volume during a small number of apnoeic episodes, characterized by desaturation with or without bradycardia (HR <80 bpm). In the presence of bradycardia, a greater reduction of cerebral oxygenation and of cerebral blood volume occurred compared to desaturation alone. The authors hypothesized that a decrease in cerebral blood flow occurs during bradycardic episodes, highlighting the importance of associated cardio-vascular changes on cerebral haemodynamics.

Our study provides simultaneous evaluation of cerebral and cardiovascular haemodynamic changes in response to CRE in preterm infants during the transitional period. We observed a significant reduction in CO during bradycardic episodes, with lower values if the bradycardia was accompanied by desaturation. CO is the product of HR and SV. With a fall in HR, there was a compensatory increase in SV. The inverse correlation between HR and the left-ventricular ejection time, which is a main determinant for SV (Miyamoto *et al.*, 1983), can explain this finding. While HR decreased, SV significantly increased during IB and DB, compared to ID. However, according to our results, the effect of HR drop predominated over the increase in SV resulting in a net decrease in CO during bradycardic events. Moreover, the occurrence of bradycardia and desaturation together was associated

with a significant reduction of cardiac contractility, which could play a role in the deeper reduction of CO observed during DB.

The effect of bradycardia on myocardial function has been previously evaluated by de Waal et al. on a small number of episodes by means of functional echocardiography (de Waal *et al.*, 2016). In line with the present findings, the authors reported an increased SV but a lower total CO during bradycardic events; they also observed a deficit in left atrial contractility.

Bradycardic episodes, with or without desaturation, were also associated also with a significant increase in sVR. This might represent a physiological attempt to compensate the reduction in CO occurring with the bradycardia by increasing the systemic vascular tone. However, as there was no continuous invasive BP monitoring when collecting this data, this finding should be interpreted with caution.

In the present cohort, the presence of a hsPDA led to a greater fall in cTOI and increase in cFTOE during CRE, independently of their type. A significantly lower cerebral oxygenation has been previously reported by several NIRS studies in preterm infants with a hsPDA compared to those with a non-significant PDA or with a closed duct (Lemmers *et al.*, 2008; Dix *et al.*, 2016; Chock *et al.*, 2016; Poon & Tagamolila, 2019), reflecting reduced cerebral blood flow due to the significant left-to-right transductal shunting (Kluckow & Lemmers, 2018). These findings suggest that, in infants with a hsPDA, CRE are more likely to result in a greater degree of cerebral hypoxia, which has been recently proposed as a possible risk factor for IVH development over the first days of life (Vesoulis *et al.*, 2019; Ng *et al.*, 2019). Moreover, both hsPDA (Shortland *et al.*, 1990) and intermittent hypoxia (Juliano *et al.*, 2015; Darnall *et al.*, 2017) have been associated with a higher risk of periventricular leukomalacia. We speculate that particular attention should be made in infants with a hsPDA experiencing repeated CRE. However, the evaluation of a possible association with IVH/PVL was not feasible in the present study, as only few infants had low-grade IVH during the study period and none went on to develop PVL.

We observed that antenatal uREDF evidence was independently associated with a lower increase in cFTOE and with a smaller CO reduction during CRE. Identifying fetuses with placental-related intrauterine growth restriction (IUGR), characterized by the antenatal Doppler evidence of REDF in the umbilical artery and/or in the ductus venosus, is very important. This condition is associated with chronic fetal hypoxia, which ultimately leads to a circulatory redistribution aimed at favouring brain perfusion (Baschat & Harman, 2001). Our findings suggest that the physiological adaptations occurring antenatally in response to the persistently reduced blood and oxygen delivery through the placenta, and to the subsequent compensatory cardiovascular remodelling (Leipälä *et al.*, 2003), aimed at guaranteeing an adequate blood perfusion and oxygenation of the brain, may persist over the

3 days after birth (Cohen *et al.*, 2016). The high prevalence of uREDF in the study cohort reflects the characteristics of the local preterm population as our hospital is a tertiary referral centre for placental-related pregnancy complications, including placental-related IUGR. We believe that this represents a point of strength of the present study, because it allowed an adequately powered evaluation of the effects of this condition in the study population.

According to our results, lower GAs resulted in a greater drop in CO during CRE associated with bradycardia. It has been shown that preterm infants have an immature myocardial tissue, with fewer contractile elements, higher water content, greater surface-to-volume ratio, and a reliance on L-type calcium channels that utilize extracellular calcium as a source of the second messenger driving cardiomyocyte contraction (Wu *et al.*, 2016). A GA-dependent impairment of myocardial function has been demonstrated by functional echocardiographic studies (Saleemi *et al.*, 2014; Hirose *et al.*, 2015), and a positive correlation between GA and baseline CO has also been documented using electrical cardiometry in healthy and stable preterm neonates (Hsu *et al.*, 2016; Boet *et al.*, 2016). Hence, the immature cardiac function associated with lower GAs may explain the greater impact of CO that we observed in more premature infants during CRE.

To date, increasing evidence correlates vital parameters during the first golden minutes after birth with postnatal adaptation (Lara-Cantón *et al.*, 2019). As illustrated by the physiological transitional modifications of the clinical and hemodynamic parameters reported in Table 2 (e.g., progressive weaning of respiratory support, decreasing rates of hemodynamically significant ductal patency, increasing BP etc.), most of the study infants showed an adequate early postnatal adaptation, and 1 in 3 did not require positive pressure ventilation in the delivery room. Consistently with the mentioned evidence, these infants maintained overall stable condition during the transitional period and a low number of complications possibly triggered by poor neonatal adaption were observed.

An important limitation of the present study is the lack of invasive continuous BP monitoring (i.e., through an indwelling arterial catheter). However, this was an observational study based on relatively stable preterm infants, and in our routine practice arterial lines are not placed in infants without a significant haemodynamic instability (e.g., hypotension requiring inotropes).

Furthermore, the small number of infants with extremely low gestational ages (e.g., <28 weeks' gestation) or with adverse postnatal adaptation also needs to be acknowledged among the study limitations. This is mainly due to the exclusion of invasively ventilated neonates, in order to rule out several factors related to mechanical ventilation (e.g., ventilation settings, respiratory rate, endotracheal suctioning, tube obstruction or dislodgement) which may have influenced not only CRE features but also cardiovascular and cerebrovascular parameters.

As a consequence, however, the infants included were generally well-adapted and developed very few complications during transition. While this cohort may constitute a reference range for cerebral and cardiovascular hemodynamic parameters, larger studies including ventilated and haemodynamically unstable neonates, may elucidate the role of BP and the integrity of the physiological mechanisms of cerebral autoregulation during intermittent hypoxia and bradycardia in sicker preterm infants.

Conclusions

This study provides a combined evaluation of cardiovascular and cerebrovascular responses to different CRE types in preterm infants during the transition period. We report significant correlations between cerebral haemodynamics and cardiac output in response to CRE and such clinical characteristics as GA, antenatal uREDF and hsPDA. The present data sheds light on the haemodynamic effects of CRE and provides important information to understand not only their impact on cerebral perfusion and oxygen delivery, but also the modulative effects of different neonatal characteristics on the observed hemodynamic responses. The ability to assess the cardio- and cerebrovascular physiology continuously and non-invasively at the cot side in preterm infants is important to develop targeted and individualized approaches designed to minimize cerebral injury in this vulnerable population. Further studies including ventilated preterm infants or with lower gestational ages are warranted to implement the generalizability of the present results.

Additional information

Competing interests: PS and MC have a financial interest in a fraction of the licensing fees for the software ICM+, used in this research project. SM, GFr, PR, SG, AGC, GFa, LC and TA have no conflict of interest to declare.

Authors contribution: SM, TA and LC designed the study. GFa contributed to the study design. SM and GFr enrolled the patients and acquired the data; SG and AG contributed to data acquisition. Data analysis was performed by SM and PR. MC and PS contributed to data acquisition and interpretation. SM wrote the first draft of the manuscript. GFr, PR, MC, PS, SG, AGC, GFa, LC and TA critically revised the manuscript for important intellectual content. All the authors have approved the approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring

that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Table 1. Clinical characteristics of the study population.

Clinical characteristics (n=40)	
Gestational age (weeks), mean ± standard deviation (SD)	30.5 ± 2.2
Birth weight (g), mean ± SD	1279 ± 326
Sex (males), n (%)	20 (50)
Twinhood, n (%)	11 (27.5)
Delivery mode (C-section), n (%)	37 (92.5)
Antenatal steroids, n (%)	
Complete course	30 (75)
Incomplete course or not given	10 (25)
Apgar score, mean ± SD	
1 min	7 ± 1
5 min	9 ± 1
Delivery room respiratory management, n (%)	
Need for positive pressure ventilation	27 (67.5)
Fraction of inspired oxygen (FiO_2) ≤ 0.30	17 (42.5)
FiO_2 0.30-0.49	8 (20)
FiO_2 ≥ 50%	2 (5)
No oxygen supplementation	13 (32.5)
Umbilical absent or reversed end-diastolic flow	17 (42.5)

Small for gestational age, n (%)	13 (32.5)

Table 2. Relevant clinical, anthropometric and haemodynamic characteristics of the study population (n=40) during the first 72 hours of life.

Monitoring period (hours of life)	*0-24h*	*24-48h*	*48-72h*	*P-value °*
Weight (g), mean ± SD	1266 ± 330	1219 ± 328	1161 ± 316	<0.001
Blood pressure, mean ± SD				
Systolic	49 ± 4	53 ± 5	55 ± 4	<0.001
Diastolic	29 ± 3	33 ± 4	32 ± 5	<0.001
Status of ductus arteriosus, n (%)				
Haemodynamically significant	13 (32.5)	8 (20)	3 (7.5)	
Restrictive	18 (45)	14 (35)	7 (17.5)	<0.001
Closed	9 (22.5)	18 (45)	30 (75)	
Ventilatory support, n (%)				
Continuous Positive Airway Pressure	34 (85)	31 (77.5)	27 (57.5)	0.016
Nasal cannulas or self-ventilating in air	6 (15)	9 (22.5)	17 (42.5)	
Baseline fraction of inspired oxygen, n (%)				
21%	32 (80)	30 (75)	31 (77.5)	
22-25%	5 (12.5)	9 (22.5)	7 (17.5)	0.698
25-30%	3 (7.5)	1 (2.5)	2 (5)	

Intraventricular haemorrhage, n (%)				
Grade I	4 (10)	4 (10)	6 (15)	0.724
Grade II to IV	0 (0)	0 (0)	0 (0)	
Baseline values, mean ± SD				
Peripheral arterial oxygen saturation (%)	96.5 ± 2.8	96 ± 3.2	95.8 ± 2.7	0.253
Cerebral tissue oxygenation index (%)	74.3 ± 8.2	75.9 ± 8.0	75.2 ± 7.7	0.143
Cerebral fraction of oxygen extraction	0.23 ± 0.09	0.21 ± 0.08	0.22 ± 0.08	0.294
Heart rate (bpm)	150.5 ± 16.7	147.1 ± 17.8	152.3 ± 18.2	0.406
Stroke volume (ml/kg)	2.23 ± 0.65	2.49 ± 0.7	2.38 ± 0.61	0.150
Cardiac output (ml/kg/min)	331.3 ± 94.7	356.6 ± 91.5	357.3 ± 99.1	0.583
Index of cardiac contractility	104.6 ± 45.3	120.2 ± 44.7	115.7 ± 40.9	0.661
Systemic vascular resistances (dyn/sec/cm^5)	9704 ± 3936	8729 ± 3421	9904 ± 3373	0.219

° RM-ANOVA for continuous variables; χ^2 square or Fisher's exact test for categorical variables.

Table 3. Comparison between baseline and event values of the study parameters for isolated desaturations, combined desaturations and bradycardias, isolated bradycardias.

	Baseline (mean ± SD)	*Event (mean ± SD)*	*P-value°*
Isolated desaturations (n=903)			
Peripheral arterial oxygen saturation (%)	95.6 ± 3.1	76.9 ± 8.3	<0.001
Cerebral tissue oxygenation index (%)	75.2 ± 7.9	67.3 ± 8.5	<0.001
Cerebral fraction of oxygen extraction	0.21 ± 0.08	0.14 ± 0.11	<0.001
Heart rate (bpm)	152.1 ± 15.6	144.7 ± 18	<0.001

Stroke volume (ml/kg)	2.44 ± 0.65	2.52 ± 0.68	<0.001
Cardiac output (ml/kg/min)	362.3 ± 95.5	360.1 ± 103.8	0.086
Index of cardiac contractility	117.6 ± 46.3	115.9 ± 48.7	0.058
Systemic vascular resistances (dyn/sec/cm^5)	9211 ± 4623	9206 ± 4690	0.526
Combined desaturations and bradycardias (n=299)			
Peripheral arterial oxygen saturation (%)	96.3 ± 2.7	73.6 ± 9.3	<0.001
Cerebral tissue oxygenation index (%)	76.4 ± 7.7	63.6 ± 8.9	<0.001
Cerebral fraction of oxygen extraction	0.20 ± 0.08	0.16 ± 0.11	<0.001
Heart rate (bpm)	156.8 ± 17.6	86.5 ± 13.4	<0.001
Stroke volume (ml/kg)	2.22 ± 0.6	2.60 ± 0.67	<0.001
Cardiac output (ml/kg/min)	340.1 ± 93.8	239.8 ± 70.9	<0.001
Index of cardiac contractility	107.7 ± 36.1	97.2 ± 34.3	<0.001
Systemic vascular resistances (dyn/sec/cm^5)	11558 ± 5190	16220 ± 10439	<0.001
Isolated bradycardias (n=224)			
Peripheral arterial oxygen saturation (%)	96.4 ± 2.2	94.5 ± 4.6	<0.001
Cerebral tissue oxygenation index (%)	75.1 ± 7.9	72.6 ± 7.1	<0.001
Cerebral fraction of oxygen extraction	0.22 ± 0.08	0.23 ± 0.07	0.004
Heart rate (bpm)	137.7 ± 19.1	89.8 ± 9.8	<0.001
Stroke volume (ml/kg)	2.54 ± 0.67	2.77 ± 0.57	<0.001
Cardiac output (ml/kg/min)	335.8 ± 87.1	263.4 ± 58.6	<0.001
Index of cardiac contractility	109.3 ± 34.8	107.4 ± 28.2	0.829
Systemic vascular resistances (dyn/sec/cm^5)	11378 ± 5027	13805 ± 6710	<0.001

° Wilcoxon signed-rank test

Table 4. Results of three generalized estimating equation models predicting percentage changes of cerebral tissue oxygenation index (%ΔcTOI), cerebral fraction of oxygen extraction (%ΔcFTOE) and cardiac output (%ΔCO), respectively. *b* are regression coefficients that can be interpreted as in a linear regression model. The confidence interval reflects the error of the estimate of the regression coefficient; when the confidence interval includes zero, the association between the dependent and the independent variable is not significant. When the independent variable is categorical, one group is used as the reference category ([§]).

Abbreviations: CI, confidence interval; GA, gestational age; uREDF, umbilical reversed end-diastolic flow; CPAP, continuous positive airway pressure; SVIA, self-ventilating in air; ID, isolated desaturation; DB, desaturation with bradycardia; IB, isolated bradycardia.

	%ΔcTOI (n=1426)		**%ΔcFTOE** (n=1426)		**%ΔCO** (n=1426)	
Variable	*b (95%CI)*	*P-value*	*b(95%CI)*	*P-value*	*b (95%CI)*	*P-value*
GA, weeks	-0.290 (-0.834, 0.254)	0.296	0.564 (-0.148, 1.277)	0.120	1.076 (0.279, 1.873)	**0.008**
Antenatal Doppler						
uREDF	1.547 (-0.904, 3.998)	0.216	-3.420 (-6.726, -0.114)	**0.043**	4.507 (1.510, 7.504)	**0.003**
normal	0[§]	.	0[§]	.	0[§]	.
Antenatal steroids,						
complete course	-1.500 (-3.714, 0.714)	0.184	-0.284 (-2.643, 2.075)	0.813	-0.864 (-4.532, 2.804)	0.644
incomplete/not given	0[§]	.	0[§]	.	0[§]	.
Ductal status,						
haemodynamically sign.	-2.835 (-5.435, -0.235)	**0.033**	5.337 (0.263, 10.411)	**0.039**	3.948 (-1.336, 9.232)	0.143
restrictive	-0.978 (-3.421, 1.465)	0.433	-0.587 (-3.907, 2.734)	0.729	-1.582 (-5.015, 1.852)	0.367
closed	0[§]	.	0[§]	.	0[§]	.
Respiratory support,						
CPAP	0.801 (-1.507, 3.108)	0.497	-0.700 (-3.686, 2.286)	0.646	-2.225 (-6.616, 2.165)	0.320
nasal cannulas/SVIA	0[§]	.	0[§]	.	0[§]	.

Event type,						
IB	6.325 (4.117, 8.533)	**<0.001**	0[§]	.	-20.084 (-25.628, -14.540)	**<0.001**
DB	-6.648 (-8.842, -4.454)	**<0.001**	-6.8 (-8.923, -4.793)	**<0.001**	-21.303 (-26.047, -16.558)	**<0.001**
ID	0[§]	.	-4.458 (-6.367, -2.548)	**<0.001**	0[§]	.

[§]*Reference category*

Figure legends

Figure 1. Example of an isolated desaturation on ICM+ software. The arrows indicate the time points for baseline and event values. Abbreviations: cTOI, cerebral tissue oxygenation index; nTHI, normalized total haemoglobin index; O2Hb, oxygenated haemoglobin index; HHb, deoxygenated haemoglobin index; SpO2, peripheral arterial oxygen saturation; cFTOE, cerebral fraction of oxygen extraction; HR, heart rate measured by electrical velocimetry; BPM, heart rate measured by pulse oximeter; SV, stroke volume; CO, cardiac output; iCON, index of cardiac contractility; SVR, systemic vascular resistances.

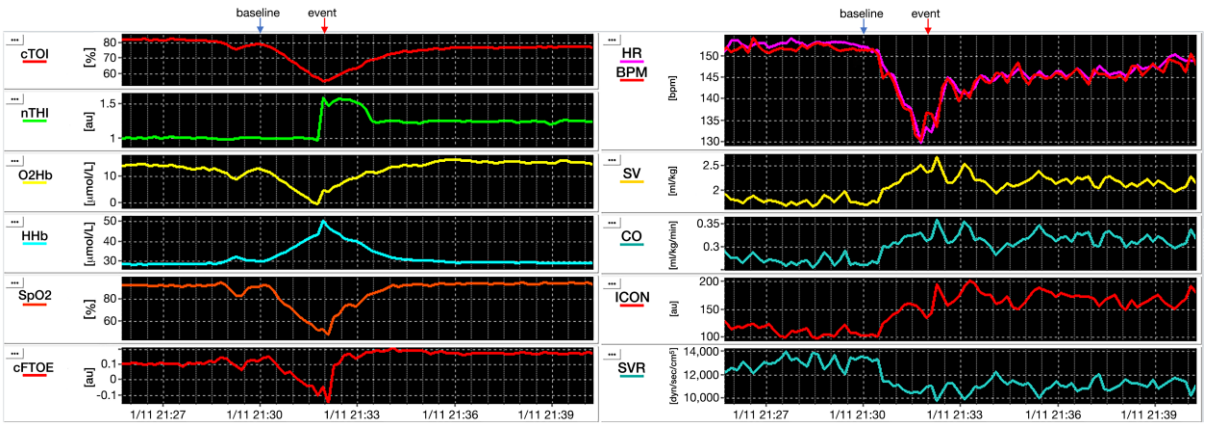


Figure 2. Flow chart of the study phases.

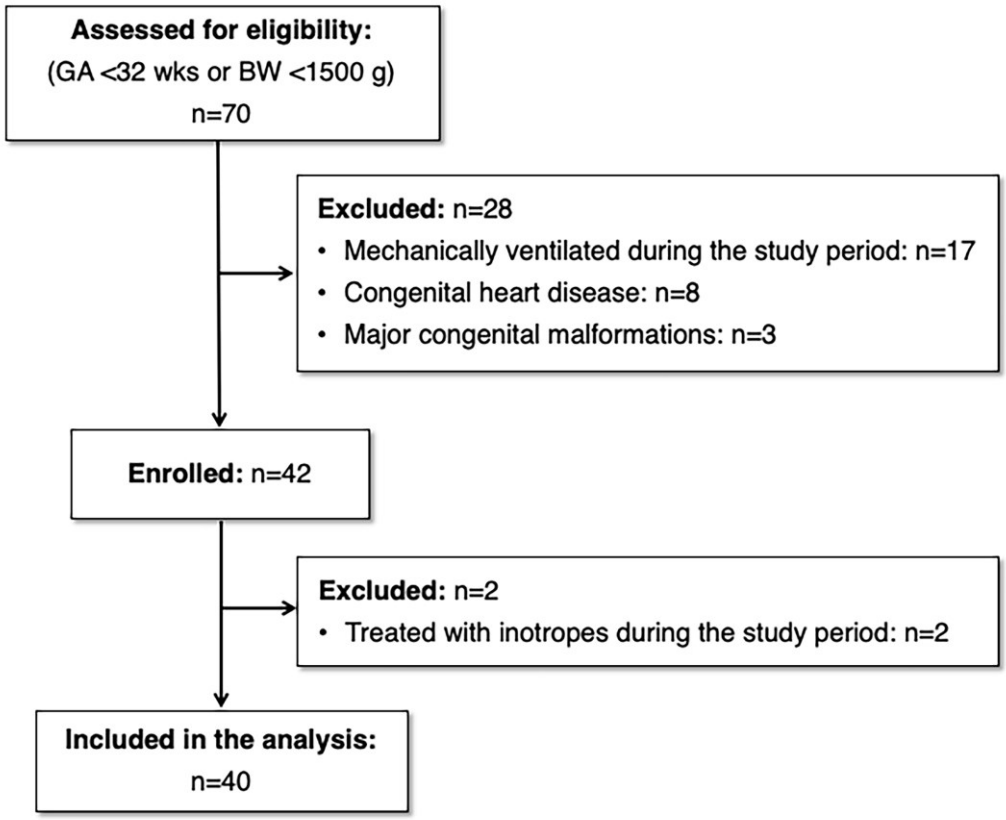


Figure 3. Percentage change (%Δ) of cerebral tissue oxygenation index (cTOI) and cerebral fraction of tissue oxygen extraction (cFTOE) during isolated desaturation (ID), isolated bradycardia (IB) and combination desaturation and bradycardia (DB). The central line in the boxplot is the median, the margins of the box are the 25th and the 75th percentiles and the whiskers represent 1.5 times the interquartile range (1.5*IQR). P-values for significant comparisons (generalized estimating equations) are provided.

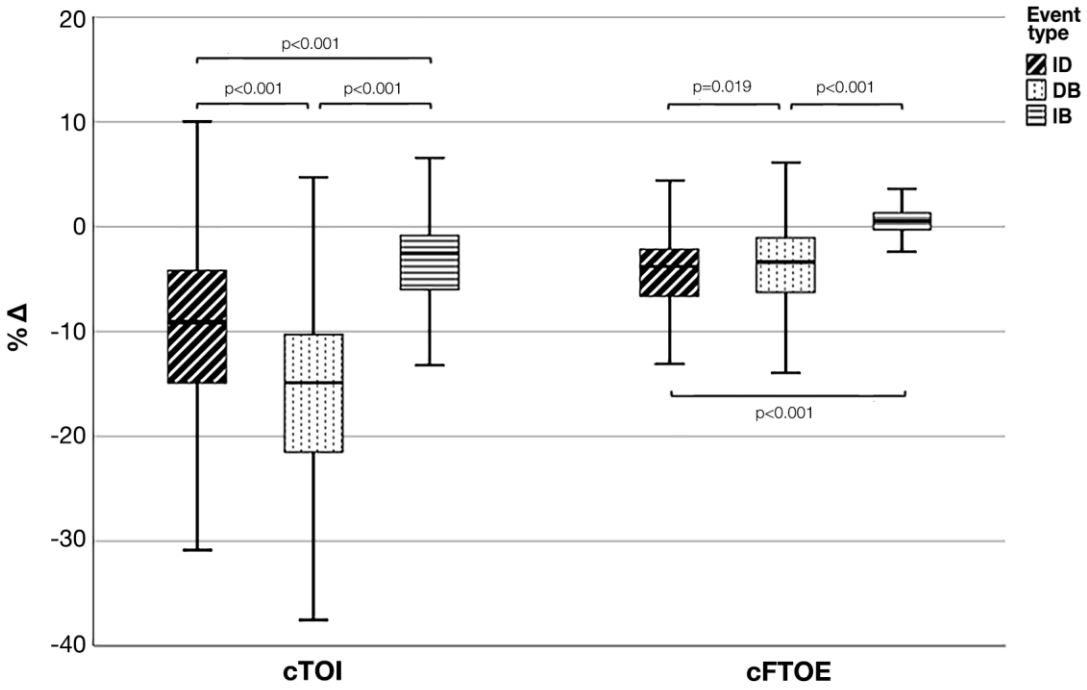


Figure 4. Percentage change (%Δ) of heart rate (HR), stroke volume (SV), cardiac output (CO), index of cardiac contractility (iCON) and systemic vascular resistance (sVR) during isolated desaturation (ID), isolated bradycardia (IB) and combination desaturation and bradycardia (DB). The central line in the boxplot is the median, the margins of the box are the 25th and the 75th percentiles and the whiskers represent 1.5 times the interquartile range (1.5*IQR). P-values for significant comparisons (generalized estimating equations) are provided.

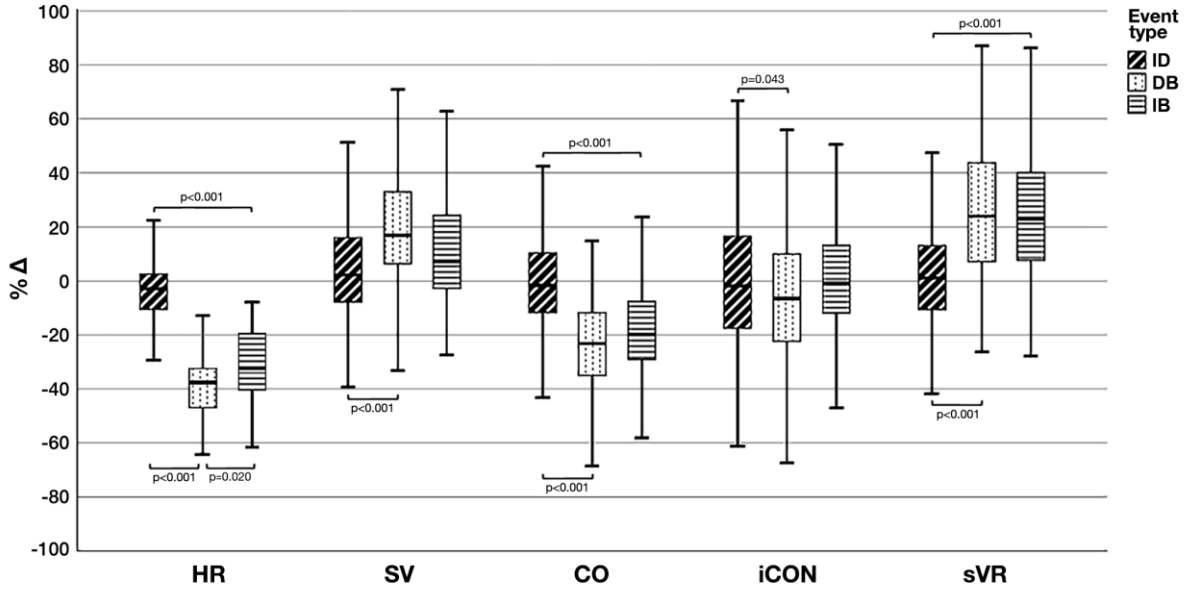
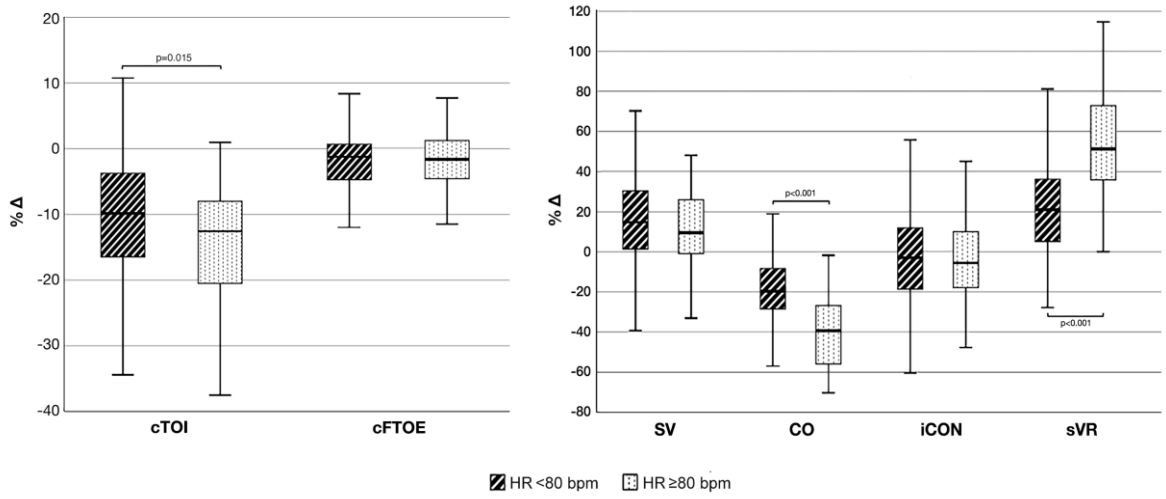


Figure 5. Percentage change (%Δ) of cerebral tissue oxygenation index (cTOI), cerebral fraction of tissue oxygen extraction (cFTOE), stroke volume (SV), cardiac output (CO), index of cardiac contractility (iCON) and systemic vascular resistance (sVR) during bradycardic events characterized by a HR nadir < or ≥80 bpm. The central line in the boxplot is the median, the margins of the box are the 25th and the 75th percentiles and the whiskers represent 1.5 times the interquartile range (1.5*IQR). P-values for significant comparisons (generalized estimating equations) are provided.



Dr Silvia Martini, MD

Silvia Martini is a neonatologist and PhD student at the University of Bologna (Italy), and an Honorary Research Fellow at the Cambridge University Hospital (UK).

Silvia obtained her medical degree with magna cum laude honors from the Bologna University in 2011. Since then, she developed a deep interest in clinical research, with particular reference to neonatal haemodynamics and near-infrared spectroscopy monitoring. Between 2016 and 2017, she spent part of her Neonatal Training at the Cambridge University Hospital, joining several research activities as a Clinical Research Fellow. During this period, in collaboration with the Brain Physics Lab of Cambridge University, she developed a research project aimed at exploring preterm infants' transitional hemodynamics using non-invasive multiparametric monitoring, which is the core of her PhD studentship, obtained in 2017.

After her PhD, Silvia is willing to pursue an academic career and to expand her research activity, investigating neonatal haemodynamics in different pathological conditions.