Title page

Cerebral blood flow and oximetry response to blood transfusion in relation to chronological age in preterm infants

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Abstract

Preterm infants frequently receive blood transfusion during their stay in neonatal unit, and its impact on organ perfusion and oxygenation is not clear. The aim of the study was to measure the effect of blood transfusion on cerebral blood flow and oxygenation in preterm infants in relation to chronological age.

Fifty nine infants were studied: group 1=20, group 2=21 and group 3=18 infants with median age (range) at transfusion of 5 (1-7), 14 (8-27) and 45 (29-68) days respectively. Gestational age and birth weight of infants between groups were similar. Pre-transfusion vital parameters were similar between the groups except the heart rate was significantly higher in group 1 compared to group 3 ($p=0.02$). The mean BP increased significantly in all three groups and there were no significant changes in other vital parameters following transfusion. There was no change in pre and post transfusion pCO_2 and pH in blood gas. The mean pre-transfusion Anterior Cerebral artery (ACA) time averaged mean velocity (TAMV) increased significantly with chronological age ($p<0.001$), and ACA Peak Systolic Velocity (PSV) also increased with chronological age of infants but this was not significant. Pre-transfusion ACA TAMV and PSV decreased significantly ($p\leq0.04$), and there was no change in ACA Resistance Index (RI) and Pulsatility Index (PI) following transfusion in infants of all 3 groups. The pre-transfusion mean Superior Vena Cava (SVC) flow decreased significantly in Group 1 ($p=0.03$) and Group 3 ($p<0.001$) following transfusion, but the change was not significant in the Group 2 infants ($p=0.16$). The mean pre-transfusion cerebral tissue oxygenation index (cTOI) levels were significantly lower in group 3 compared to group 1 ($p=0.02$). The cerebral tissue haemoglobin level cTHI ($p<0.001$) and cTOI ($p<0.05$) increased significantly post-transfusion in all the three groups. The percentage increase in post-transfusion cTOI from baseline was 5%, 11% and 12% in group 1, group 2 and group 3

infants respectively. On comparing infants with PDA (n=14) to gestational and chronological age matched controls (n=14), there was no significant difference in these measurements.

Baseline cTOI decrease and ACA TAMV increase with increasing chronological age. Blood transfusion increased cTOI and cTHI, and decreased ACA TAMV in all age group of infants but the proportion of response is different. PDA had no impact on the baseline cerebral oximetry and blood flow as well as changes following transfusion. Future transfusion trials should take into account the impact of postnatal age on cerebral oximetry and blood flow.

Keywords: cerebral oximetry, blood transfusion, cerebral blood flow

Abbreviations:

ACA – anterior cerebral artery, APH – antepartum haemorrhage, cTOI – cerebral tissue oxygenation index, cTHI – cerebral tissue haemoglobin index, $CrSO_2$ – cerebral regional oxygen saturation, FOEC – fractional oxygen extraction capacity, GA – gestational age, Hb – haemoglobin, IUGR – intra-uterine growth restriction, PDA – patent ductus arteriosus, PET – pre-eclampsia, PI – pulsatility index, PSV – peak systolic velocity, RI – resistance index, SVC – superior vena cava, TAMV – time averaged mean velocity, VTI – velocity time integral

Introduction

In neonatal intensive care units preterm infants undergo frequent blood tests to monitor their haemodynamic and oxygenation status. As a result of regular phlebotomy losses and their inability to maintain haemoglobin levels, one of the common practices in neonatal units is to transfuse blood with an intention to maintain optimal tissue oxygenation in order to promote growth and weight gain[1]. While transfusion is beneficial in the setting of acute perinatal blood losses and severe anaemia, its advantage in moderate grades of anaemia in stable preterm infants remain uncertain[1]. Blood transfusion has been reported to be an independent risk factor of mortality[2,3] and in the first weeks of life has been linked to progression of intra-ventricular haemorrhage[4] in preterm infants. The threshold for transfusion in preterm infants remain controversial and has led to several randomised controlled trials using liberal and restrictive thresholds for blood transfusion with conflicting reports on neurological outcomes [5-7]. Whether these thresholds were not optimal or whether haemoglobin concentration in the blood does not correlate with tissue oxygenation measurements[8-10] remains to be answered. Other studies have shown conflicting reports regarding improvement of short term effects of apnoea, tachycardia and bradycardia following transfusion [1,11,12]. In current practice various clinical, laboratory and bedside biomarkers are regularly used to identify the need for transfusion in preterm infants[13]. Reducing blood tests, advanced point of care blood analysers requiring smaller volume of blood[14] and implementing restrictive transfusion guidelines have reduced the frequency of transfusions in most neonatal units[15]. Two randomised clinical trials are currently undergoing to detect whether receiving restrictive or liberal blood transfusions improve long term neurodevelopmental outcomes in preterm infants[16,17].

One of the principal objectives of blood transfusion in preterm infants is to prevent impaired tissue oxygenation in the brain and other vital organs. But the threshold at which the

demands of the tissue exceed the oxygen content of the blood remains unknown. Apart from autoregulatory mechanisms of certain organs such as brain [18], increased blood flow and increased tissue oxygen extraction are two other main processes to maintain adequate oxygenation to support metabolism in the tissues **(Reference)**. Peripheral arterial saturation, tachycardia and serum lactate [19,20] gives a realistic estimate of the haemodynamic status of the systemic circulation but fails to identify specific tissue needs. Blood flow to various organs could be measured at the bedside using Doppler ultrasound scan of contributing arteries, and systemic haemodynamic status could be measured by left or right ventricular output [21] as well as venous return from superior vena cava[22]. These are excellent non-invasive bedside measurements of assessing blood flow to vital organs but are not continuous and are a snapshot of the state of organ perfusion in specific timeframe. Other measures such as continuous measurement of cardiac output in paediatrics and neonates using transthoracic electrical bioimpedence is still in its early stage of validation and development [23]. It has been reported earlier that anaemic stable preterm infants could be at a clinically unrecognised high cardiac output state, which could lead to reduced oxygen delivery to brain [24]. This might in turn lead to cerebral haemorrhage and brain injury [25]. Near infra-red spectroscopy (NIRS) is a validated method of continuous measurement of cerebral tissue oxygenation [26] and cerebral blood volume in preterm infants[27,28]. NIRS have been used in various observational studies to measure cerebral tissue oxygenation in the past 20-30 years [29]. Recently, to highlight its efficacy in clinical practice as a tool to assess cerebral oximetry in preterm infants, NIRS have been used in various randomised clinical trials. Cerebral NIRS oximetry is currently being studied to aid in newborn resuscitation in delivery room [30], to monitor cerebral autoregulation [31] and daily monitoring in neonatal units [32], and its role in management in neonatal hypotension [33,34].

Cerebral NIRS oximetry has been used in the past to assess the effect of blood transfusion in older stable preterm infants [9,10,35]. But the effect on cerebral perfusion and oximetry in pre-

transfusion anaemic state and response to blood transfusion in different gestational and chronological ages remains unknown. The aim of this study was to measure the effect of blood transfusion on cerebral blood flow and oxygenation in preterm infants according to chronological age using Doppler ultrasound scan and NIRS.

Materials and Methods

The study was conducted at Homerton University Hospital, a tertiary level neonatal unit in London, UK. Preterm infants receiving blood transfusion for clinical indication were eligible for the study. The infants were recruited into three groups depending on their postnatal age: Group 1: day 1 to day 7 of life, Group 2: day 8 to 28 days of life and Group 3: more than 28 days of life. Major congenital anomalies and infants considered unstable for Doppler ultrasound or NIRS measurements by the attending clinical team were excluded. A pragmatic sample size of 20 infants per group was considered. Blood transfusion in our neonatal unit followed current British Committee for Standards in Haematology (BCSH) guidance [36] with 15 ml/kg of packed red blood cells were transfused over a period of 3 hours.

Cerebral blood flow measurements

The Doppler measurements were performed using an ultrasound scanner with a 7 MHz probe (Logic P6, GE Healthcare, US). The anterior cerebral artery (ACA) peak systolic and time averaged mean velocities (TAMV) as well as pulsatility and resistance indices were measured 30-60 minutes pre and post blood transfusion using a range-gated pulsed wave Doppler ultrasound scan placing the probe over the anterior fontanel in parasagittal view. Superior vena cava (SVC) flow was measured using the classical method by placing the probe in the infra-diaphragmatic view to measure the velocity time integral (VTI) and then again over the true long axis to measure the diameter of the SVC pre and post blood

transfusion. The Doppler measurements were performed by single operator (JB) to minimise intra-operator variability and utmost care was taken to minimise the angle of insonation to the direction of flow. When this was more than 30° angle correction was performed. Cardiac morphology and presence of patent ductus arteriosus (PDA) was also recorded.

Cerebral oxygenation measurements

Cerebral oxygenation was measured using a NIRS device (NIRO 300, Hamamatsu Photonics Limited, Japan) with a sample acquisition rate of 6 Hz/sec. The NIRS probe was attached to the infant's forehead and held in place by the hat used for conventional or non-invasive ventilation; utmost care was taken to minimise any movement and ambient light interference. To reduce NIRS motion artefacts the infants were minimally handled during the study period. The cerebral tissue Hb Index (cTHI) in arbitrary units and tissue oxygenation index (cTOI) in percentage were continuously measured from 15-20 min before, during and 15-20 min post blood transfusion, and downloaded into the research laptop.

Vital parameters measurements

The vital parameters such as heart rate (HR), respiratory rate (RR), blood pressure (BP) and saturation were measured using Phillips Intellivue monitor (MP50/MP70) during NIRS measurements, and downloaded continuously using ixTrend 2.0 software (ixellence GmBH, Halle, Germany) into the research laptop.

Laboratory parameters measured

The pre and post blood transfusion laboratory parameters such as hemoglobin (Hb), hematocrit (Hct) and blood gas parameters such as pH, pCO_2 and serum lactate were also

measured. Hb and Hct were measured using flow cytometry (Beckman Coulter Inc. US) in the hospital lab and the blood gas parameters were measured by blood gas machine (GEM Premier 4000, Instrumentation Laboratory, UK) in the neonatal unit.

Additional data collected

Antenatal factors: antepartum haemorrhage (APH), maternal pre-eclampsia (PET) and intra-uterine growth restriction (IUGR), chorioamnionitis; and infant characteristics: gestational age, birth weight, Hb at birth were collected. Clinical condition on the day of transfusion: ventilation status and inotropic support were recorded.

The study was approved by the National Research Ethics Committee (REC no.12/LO/0527) and was adopted as an NIHR portfolio study (NIHR Study ID 13594). Informed written parental consent was obtained.

Data analysis

A mean for 15 minute epochs of NIRS and vital parameter measurements were determined for each infant using mathematical software Mat Lab R2013b (Math works, USA) during the following time periods: T1 - 15 to 20 minutes before the start of the blood transfusion, T2 - 1 hour into blood transfusion, T3 - 2 hour into blood transfusion and T4 - 15 to 20 minutes post blood transfusion. The serial measurements of all studied variables were compared using repeated measures analysis ANOVA with Bonferroni method. The pre and post-transfusion values of all measurements and the NIRS measurements at all above time points were compared using paired two-tailed t-test, the various clinical groups as well as the

chronological age groups were compared using ANOVA and t-tests. A p value of <0.05 was considered significant. The data was analysed using SPSS 22.0 software (IBM Corp., USA).

Results

Infant characteristics

Fifty nine infants were studied; infant and maternal characteristics are presented in **Table 1**. The mean birth weight and gestational age of infants studied in the three groups were similar. The infant characteristics on the day of blood transfusion are presented in **Table 2**. 32 infants had PDA on echocardiography, of these only six were >14 days of postnatal age; otherwise normal cardiac morphology. Majority of infants in group 1, and eight each in the other two groups were receiving antibiotics for presumed sepsis; blood culture results were noted to be subsequently negative for all. Three infants in group 1, two in group 2 and one in group 3 were on single inotropic support (Dopamine) for hypotension, the dose remained unchanged for the duration of the measurements. Three infants in group 1, two in group 2 and three in group 3 had significant IVH (≥grade 4) noted before transfusion. There was no progression of these findings following transfusion in any of the three groups.

Vital signs and laboratory parameters

The mean values of pre and post blood transfusion vital parameters and laboratory parameters are presented in **Table 3**. The mean pre-transfusion RR, HR and saturation were similar between infants of three groups except the mean pre-transfusion HR was higher in group 1 compared to group 3 (p=0.02, 95% CI 1.5 to 16.6). There was no

significant change in HR, RR and saturation following blood transfusion in all three groups of infants. Systolic BP increased significantly following blood transfusion in group 1 infants, where as diastolic and mean BP increased significantly in all the three groups. The serum lactate levels decreased significantly in group 1 and 2 infants following blood transfusion. However, the mean pre and post blood transfusion pH and pCO_2 levels in the blood gas remained unaltered in infants of all three groups. The mean pre-transfusion haemoglobin was higher in group 1 compared to group 2 (p=0.03, 95% CI 0.07 to 1.56) and group 3 (p<0.001, 95% CI 1.25 to 2.88). There was significant increase in post-transfusion Hb in all three groups of infants (Table 3).

Doppler measurements

The mean pre-transfusion anterior cerebral artery (ACA) peak systolic velocity (PSV) was higher in group 2 infants compared to group 1 (p=0.06) and group 3 infants compared to group 2 (p=0.11) but this was not significant. The mean pre-transfusion ACA TAMV was significantly higher in group 3 (0.27±0.07 m/sec) compared to group 1 (0.17±0.05 m/sec; p<0.0001, CI 0.06 to 0.14), and group 3 compared to group 2 (0.19±0.06, p<0.001, CI 0.03 to 0.12). The mean pre-transfusion ACA peak systolic velocity (PSV) and time averaged mean velocity (TAMV) decreased significantly post-transfusion in all the three groups (**Table 4** and **Figure 1**). The mean pre-transfusion ACA Resistance Index (RI) and Pulsatility Index (PI) were similar in infants of all three groups, and there was no significant change in ACA RI and PI following transfusion in all three groups. The mean pre-transfusion Superior Vena cava (SVC) flow was higher in group 1 infants compared to other two groups and was higher in group 3 compared to group 2 infants but this was not significant. The mean SVC flow decreased significantly following transfusion in Group 1 and 3 infants but there was no significant change in Group 2 infants (**Table 4** and **Figure 1**).

Doppler measurements in infants with PDA

Doppler measurement of infants with PDA (n=11, mean gestational age=25 wks & Mean chronological age=16 days) compared to gestational age (mean=26 wks) and chronological age (mean=17 days) matched infants with closed PDA (n=11). The pre-transfusion mean ACA TAMV was similar in the infants with PDA (0.19±0.05 m/s) compared to those with closed PDA (0.21±0.07 m/s, p = 0.45, CI -0.07 to 0.03). The mean pre-transfusion SVC flow was similar between the PDA (102.98±42.5 ml/kg/min) and closed-PDA group (87.66±30.3 ml/kg/min, p=0.352 95% CI -16.09 to 46.7). The ACA TAMV decreased significantly following blood transfusion in both PDA group (p=0.04, CI 0.01 to 0.05) and the closed-PDA group (p=0.01, CI 0.01 to 0.06) of infants. The SVC flow also remained similar following transfusion in both PDA (p=0.99, CI -13.5 to 13.3) as well as the closed-PDA (p=0.83, CI -11.5 to 9.4) group.

NIRS measurements

Cerebral tissue haemoglobin index (cTHI)

The percentage changes in the mean pre-transfusion baseline cerebral tissue haemoglobin index (cTHI) values are shown in **Figure 2**. There was a consistent increasing trend in cTHI levels following blood transfusion in infants of all three groups except an initial drop in the first hour of transfusion in Group 1. Whereas the maximal increase in cTHI happened earlier in Group 3, the percentage increase maximised post-transfusion in all the three groups (p<0.001; **Table 5**). The cTHI increased at a higher rate following transfusion in older preterm infants in Group 3 compared to the infants in their first week of life (Group 1; **Figure 2**).

Cerebral tissue oxygenation Index (cTOI)

The mean pre-transfusion cTOI levels were significantly lower in group 3 infants compared to group 1 (p=0.02, 95% CI 2.09 to 25.44). The mean pre-transfusion cTOI increased significantly following transfusion in all the three groups. There was an increasing trend in mean cTOI over time but it did not reach statistical significance until the end of transfusion (**Table 5**; **Figure 2**). The percentage increase in post-transfusion cTOI from baseline was 5%, 11% and 12% in group 1, group 2 and group 3 infants respectively.

NIRS measurements in infants with PDA and those with closed PDA

The baseline mean pre-transfusion cTOI was higher in the PDA group (69±13.4 %) compared to the closed-PDA group (63.16±13.6%) but this was not significant (p=0.24, CI -5.01 to 18.66). Similar pattern of increase was noticed in cTOI in both the groups during blood transfusion (**Figure 3**). The cTOI increased significantly in all the time points at 1 hour, 2 hours and post-transfusion when compared to baseline pre-transfusion values in both the groups with and without PDA (**Figure 3**). The cTHI significantly increased consistently at all the time points and was similar in infants with or without PDA (**Figure 3**).

Discussion

We have shown that blood transfusion in preterm infants increased cerebral tissue oxygenation index (cTOI) as well as cerebral tissue haemoglobin index (cTHI) during the first week (group 1), 8th to 28th day (group 2) and >28 days of life. Cerebral regional tissue oxygenation ($CrSO_2$) also referred to as cTOI is a biomarker of tissue oxygenation and they represent the percentage of oxygenated Hb compared to the total Hb in the tissue traversed by the near infra-red light [37]. cTHI is another cerebral tissue oxygenation biomarker, and indicates the total concentration of Hb in the tissues and in essence the changes in red cell

volume in the tissues. For the first time we have demonstrated that the response of cerebral tissue oxygenation following transfusion is dependent on the postnatal age of the preterm infant. Similar to the present study other researchers have shown increase in $CrSO_2$ and decrease in cerebral fractional oxygen extraction (FOEC) following transfusion in stable preterm infants with a gestational age range between 25 and 34 weeks [8-10,38]. In these studies preterm infants of 5 to 93 days of age with a wide range of pre-transfusion Hb were clubbed together as a single group. In the present study infants were divided into three groups based on postnatal age to minimise the effect of postnatal maturity on haemodynamic changes measured. We have also demonstrated that as postnatal age of infant increases, the baseline pre-transfusion cTOI decreased. Similar to the present study findings, McNeill et al reported a decrease in $CrSO_2$ as infant's chronological age increased by studying 14 preterm infants between 29 to 34 weeks of gestation[39]. The pre-transfusion cTOI during the first week of life in the present study is comparable to the reported normative values of 57 to 75% [40]. The lower pre-transfusion cTOI in older preterm infants in the current study could be due to lower pre-transfusion Hb levels or physiological maturational changes. The percentage increase in cTOI as well as cTHI was also different in the chronological age groups in the current study, demonstrating variable effect of blood transfusion in different postnatal ages. The cTHI levels increased in all chronological age groups indicating an increase in cerebral blood volume following blood transfusion. Calculating from changes in cerebral total Hb concentration using NIRS in 14 preterm infants (mean gestational age 29.6±2.6 and mean birth weight 1430±332 grams), Dani et al have demonstrated an increase in cerebral blood volume following transfusion [35].

To better appreciate the effects of blood transfusion on cerebral blood flow we also measured blood flow velocity in anterior cerebral artery (ACA) and blood flow volume in superior vena cava (SVC). Previously it has been reported that blood flow velocity in the ACA [41] and pericallosal artery [38] decreases following blood transfusion, possibly due to

increased resistance in the cerebral blood flow due to increased viscosity of blood [42]. Significant increase in the diastolic blood pressure in the current study indirectly indicates increase in peripheral vascular resistance. In accordance with the findings of the previous studies we have also demonstrated decrease in the mean ACA TAMV in all the three chronological age groups. Researchers studied by merging infants of various gestational and chronological age together as one group in previous studies[35,41], where as we studied by dividing infants into three groups according to postnatal age. In the current study, the baseline pre-transfusion blood flow velocity as well as their response to blood transfusion was different in three infant groups studied. The mean ACA TAMV was lower during the first week of life (group 1 infants) and significantly higher in infants who were more than 28 days old (group 3 infants). This may be due to maturational changes but also could be attributed to group 3 infants being transfused at a lower Hb compared to the group 1 infants. We have also demonstrated for the first time that the SVC blood flow volume decreases following blood transfusion during the first week of life and in the infants who were more than 4 weeks of age. A previous study has shown that anaemic preterm infants develop a high cardiac output state and this decrease significantly on blood transfusion [24]. There was no change in the SVC flow in the Group 2 infants perhaps indicating a more stable cardiovascular state or blood being transfused at a higher baseline Hb level before any haemodynamic decompensation. We have also demonstrated for the first time that the pre-transfusion baseline SVC flow was higher in the early preterm infants compared to the older preterm group. This could be due to poor cerebral autoregulation in the earlier preterm group thereby demonstrating a higher blood flow volume to the brain.

For the first time we have demonstrated the interaction of PDA on the cerebral blood flow and oxygenation response to blood transfusion in gestational and chronological age matched preterm infants. The baseline pre-transfusion mean ACA TAMV and SVC flow were similar between the groups with open and closed PDA thereby demonstrating similar upper

body blood flow in either group. The mean baseline pre-transfusion cTOI level in the preterm infants with open PDA was also similar to those with closed PDA. This exhibits compensatory mechanism by which the upper body blood flow is maintained and so is the cerebral tissue oxygenation in the presence of PDA. The increase in cTHI and cTOI levels during transfusion was similar in both groups which indicate that PDA has no effect on the cerebral blood flow and oximetry response to blood transfusion.

There was no change in heart rate (HR) following blood transfusion in all three infant groups studied in the present study but other researchers have reported a significant decrease in heart rate[41,43]. Previous studies in older clinically stable preterm infants have reported no significant difference in mean blood pressure following blood transfusion [38,41]; we have demonstrated significant increase in the mean blood pressure following blood transfusion in all the three groups. The mean blood pressure was higher in the older preterm group compared to the earlier preterm infants, it is well recognised that mean blood pressure in preterm infants increases with postnatal age [44]. We did not notice any change in SaO_2 and respiratory rate following blood transfusion any of the three groups of infants. In older preterm infants, Fredrickson *et al* also did not notice any difference in the SaO_2, FiO_2 and oxygen consumption between two groups receiving liberal and restrictive transfusion [45]. There was a significant drop in serum lactate levels following blood transfusion in infants less than 28 days of age (Group 1 and 2) despite normal pre-transfusion levels but there was no change in older infants (group 3). Hence, use of serum lactate as a trigger for blood transfusion in preterm infants [13,20] is debatable.

Leukocyte depleted cytomegalovirus negative, Sickle cell negative, plasma reduced packed red blood cells (hematocrit 50-70%), were transfused over a period of 3 hours through an intravenous cannula which is a standard practice in most neonatal units in the UK. The

ventilatory pressures, oxygen requirement, feeding regime, the pre-transfusion pH and pCO_2 remained unchanged during the study period. The mean pre-transfusion Hb and Hct were comparable to the reported studies [6,10,43].

Decision for transfusion was made by the attending clinician based on measured Hb and clinical condition of the infant. Hence, selection bias cannot be excluded. The pragmatic estimated sample size was 20 infants per group based on chronological age. We recruited appropriate number of infants to group 1 and 2 but recruited only 18 infants to group 3 (≥28 days of life). This is unlikely to influence the study findings. Fewer babies were ventilated in group 3 compared to group 1 and 2. However, the pre and post transfusion blood gas pH and pCO_2PC_2 were similar in all three groups of infants studied, and hence ventilator status is unlikely to have impact on the study findings. Since Doppler ultrasound scan measurements are operator dependent, the measurements were performed by a single operator (JB) to minimise intra-operator variability. The cerebral oxygenation measurements of seven infants were excluded from the analysis due to motion artefacts, which is comparable to other reported NIRS studies[28,46]. One of the limitations of the study is that we measured the cerebral tissue oxygenation upto 20 minutes following transfusion. Other researchers have measured cerebral tissue oxygenation at one [35], four and 24 hours [10] post-transfusion, and reported persistence of increased tissue oxygenation state following transfusion in more stable preterm infants. Six infants were receiving Dopamine (5mcg/kg/min) and this is unlikely to influence the study findings as the dosage of Dopamine infusion remained unchanged for the duration of the measurements.

Conclusion

We have shown that the baseline cTOI decreases with increasing chronological age of preterm infants. Blood transfusion increases cTOI and cTHI in preterm infants of all chronological age groups but this is more pronounced after 28 days of life. The baseline ACA TAMV increases as infant's chronological age increase. The SVC flow decrease after 1st week of life but not consistently. The cerebral perfusion decrease following blood transfusion during the first week and after 28 days of life in preterm infants. Presence of PDA has no impact on the cerebral blood flow and oximetry response to blood transfusion; similar pre-transfusion baseline values indicate compensatory mechanisms by which the preterm body adapts to PDA to maintain cerebral blood flow and oxygenation. These findings indicate that cerebral blood flow and oximetry response to blood transfusion in preterm infants is dependent on the chronological age of the infant, and future randomised trials of blood transfusion should be planned taking into account the effect of postnatal age on cerebral blood flow and oximetry.

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The authors declare that they have no competing interests.

Authors' contributions:

JB and NA conceived of the study, JB, NA and TSL prepared the study design, protocol and ethics application and received hospital R&D approval. JB and NA consented patients; JB performed the Doppler scans and NIRS measurements and data collection. JB conducted statistical analysis supported by NA and TSL. First draft of the manuscript was prepared by JB and was reviewed and approved by all authors.

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Table 1. Infant and maternal characteristics

Characteristics	Group 1 (1 – 7 ds) n = 20	Group 2 (8 – 28 ds) n = 21	Group 3 (>28 ds) n = 18
Gestational age (completed weeks)*	26 (23 – 27)	25 (23 – 30)	26 (24 – 34)
Birth weight (grams)*	763 (600 – 1180)	740 (600 – 1240)	793 (520 – 1746)
Chronological age (days)*	5 (1 – 7)	14 (8 – 27)	45 (29 – 93)
Haemoglobin at birth (g/dl)*	14.5 (9.8 – 20.7)	14.7 (10.0 – 17.4)	15.3 (10 – 18.9)
Maternal PET†	3 (15)	5 (24)	4 (22)
IUGR†	3 (15)	5 (24)	4 (22)
Chorioamnionitis†	9 (45)	8 (38)	8 (44)
Antepartum haemorrhage†	6 (30)	8 (38)	4 (22)
Antenatal steroids†	17 (85)	20 (95)	16 (89)

† Number (percentage), * Median (Range)

Table 2. Infant characteristics at blood transfusion

Characteristics	Group 1 (1 – 7 ds) n = 20	Group 2 (8 – 28 ds) n = 21	Group 3 (>28 ds) n = 18
Weight at transfusion (grams)*	774 (700 – 1180)	805 (680 – 1250)	1125 (887 – 2045)
Pre-transfusion Hb (g/dl)*	11.0 (8.5 – 13.1)	10.3 (7.7 – 12.2)	9.2 (7 – 10.9)
Total fluids (ml/kg/d)*	150 (90 – 180)	150 (100 – 180)	165 (100 – 180)
Invasive/Non-invasive ventilation/nasal cannula oxygen or breathing in air†	13 (65)/7(35)	13 (62)/7 (33)/1 (5)	6 (33)/9 (50)/3 (17)
Presence of PDA†	19 (95)	12 (57)	1 (6)
Presumed sepsis on antibiotics†	19 (95)	8 (38)	8 (44)

† Number (percentage), * Median (Range)

Table 3. Blood transfusion (BT), vital and laboratory parameters

Vital and laboratory parameters Mean (SD)	**Group 1 (1 – 7 days) n = 20**			**Group 2 (8 – 28 days) n = 21**			**Group 3 (>28 days) n = 18**		
	Pre-BT	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**
Heart rate (bpm)	159.1 (8.8)	157.1 (15.1)	0.67	153 (13.4)	153 (14.9)	0.99	150.0 (11.7)	149.4 (13.0)	0.90
Respiratory rate (bpm)	53.2 (12.3)	50 (11.7)	0.13	48.5 (10.2)	48.4 (8.3)	0.91	52.8 (13.9)	52.1 (11.4)	0.73
Arterial saturation (SaO2)%	93.2 (2.9)	93.2 (2.5)	0.96	91.9 (3.5)	92.3 (4.0)	0.67	93.0 (3.8)	93.2 (4.1)	0.88
Systolic BP (mm of Hg)	46.7 (6.6)	51.6 (4.9)	<0.01	54.9 (9.6)	57.7 (11.7)	0.07	62.2 (14.0)	63.7 (12.1)	0.45
Diastolic BP (mm of Hg)	24.3 (3.1)	30.7 (4.7)	<0.01	31.4 (5.4)	35.8 (8.3)	<0.01	31.3 (6.0)	36.2 (6.6)	0.01
Mean BP (mm of Hg)	32.7 (3.7)	37.9 (3.7)	<0.01	39.9 (6.3)	43.4 (8.1)	0.02	43.2 (7.9)	46.2 (6.6)	0.02
Haemoglobin (g/dl)	11.2 (1.3)	13.0 (1.6)	<0.01	10.3 (1.0)	13.5 (1.1)	<0.01	9.1 (1.2)	12.2 (1.2)	<0.01
Haematocrit	0.32 (0.04)	0.40 (0.05)	<0.01	0.29 (0.03)	0.39 (0.04)	<0.001	0.25 (0.04)	0.36 (0.03)	<0.01
pH	7.3 (0.07)	7.3 (0.05)	0.50	7.3 (0.05)	7.3 (0.06)	0.57	7.3 (0.05)	7.3 (0.05)	0.30

pCO2	5.8 (1.2)	5.9 (0.9)	0.47	6.6 (1.0)	6.7 (1.3)	0.72	6.9 (1.4)	6.6 (1.5)	0.11
Lactate (mmol/l)	2.5 (1.3)	1.8 (0.5)	0.02	1.5 (0.7)	0.9 (0.5)	0.03	1.3 (0.6)	1.3 (0.4)	0.82

Table 4. Blood transfusion (BT) and cerebral Doppler blood flow parameters

Blood flow parameters **Mean (SD)**	**Group 1 (1 – 7 days)** **n = 20**			**Group 2 (8 – 28 days)** **n = 21**			**Group 3 (>28 days)** **n = 18**		
	Pre-BT	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**
ACA peak systolic velocity (m/sec)	0.32 (0.09)	0.27 (0.09)	0.04*	0.38 (0.11)	0.33 (0.09)	0.02*	0.54 (0.14)	0.44 (0.09)	0.04*
ACA time averaged mean velocity (m/sec)	0.17 (0.05)	0.14 (0.04)	0.01*	0.19 (0.04)	0.16 (0.05)	<0.01*	0.27 (0.07)	0.23 (0.05)	<0.01*
ACA RI	0.82 (0.07)	0.83 (0.05)	0.65	0.86 (0.05)	0.85 (0.07)	0.57	0.84 (0.07)	0.83 (0.07)	0.66
ACA PI	1.53 (0.25)	1.56 (0.20)	0.67	1.73 (0.26)	1.68 (0.30)	0.53	1.70 (0.32)	1.58 (0.26)	0.57
SVC flow (ml/kg/min)	110.1 (55.9)	96.6 (40.7)	0.03*	91.0 (35.1)	95.5 (39.5)	0.16	98.9 (22.6)	77.9 (23.6)	<0.01*

Table 5. Blood transfusion (BT) and cerebral NIRS parameters

Cerebral oximetry parameters **Mean (SD)**	**Group 1 (1 – 7 days)** **n = 17†**			**Group 2 (8 – 28 days)** **n = 20††**			**Group 3 (>28 days)** **n = 15†††**		
	Pre-BT	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**
Cerebral tissue haemoglobin index (cTHI) (percentage increase from baseline) %	Zeroed baseline	50.58	<0.001	Zeroed baseline	63.18	<0.001	Zeroed baseline	68.22	<0.001
Cerebral tissue oxygenation index (cTOI) %	71.0 (15.8)	74.6 (12.6)	<0.05	63.0 (12.3)	73.7 (11.8)	<0.01	57.2 (13.2)	64.1 (12.6)	<0.01

† 3 infants, †† 1 infant and ††† 3 infants excluded from this analysis due to motion artefacts

Figure 1. Blood transfusion (BT) and changes in ACA TAMV and SVC flow

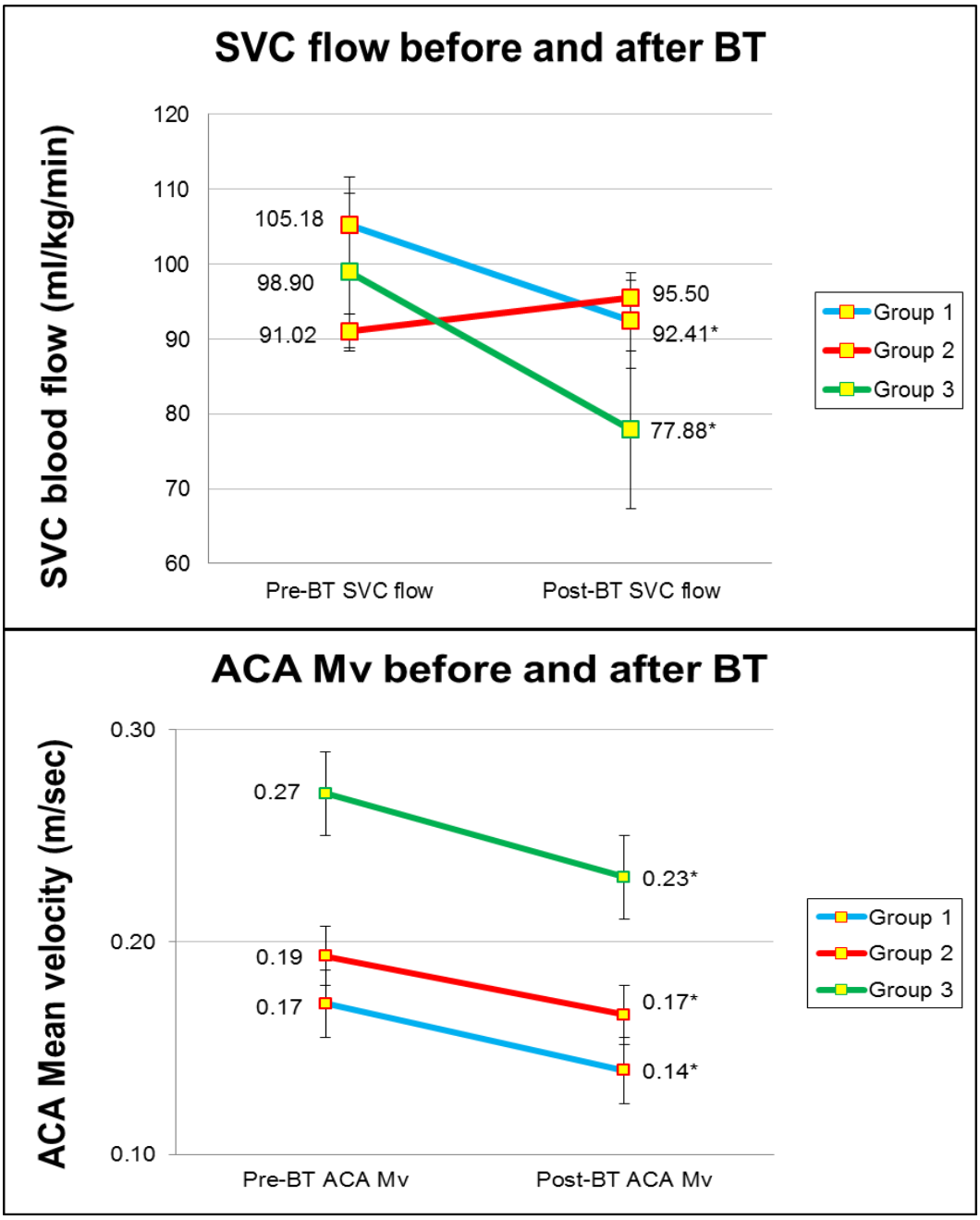
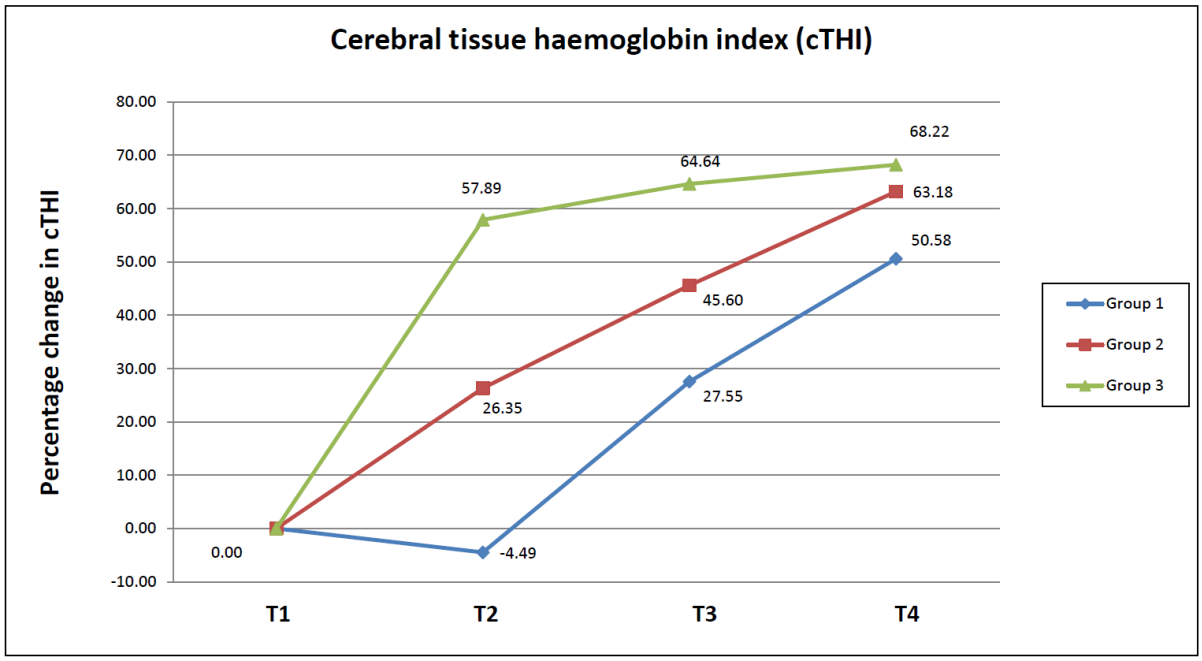


Figure 2. Blood transfusion (BT) and changes in cerebral NIRS parameters

T1 - 15 to 20 minutes before the start of the blood transfusion, T2 - 1 hour into blood transfusion, T3 - 2 hour into blood transfusion and T4 - 15 to 20 minutes post blood transfusion



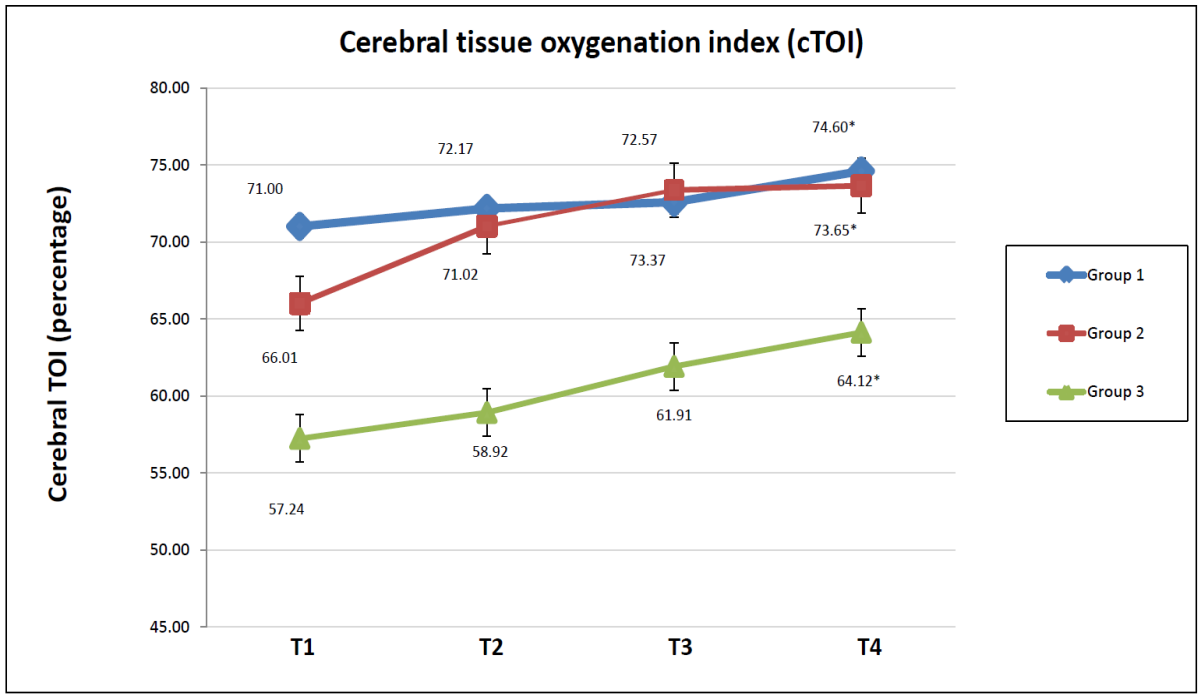


Figure 3. Changes in cTOI and cTHI (± SEM) in the infants in the PDA and closed-PDA groups following blood transfusion

T1 - 15 to 20 minutes before the start of the blood transfusion, T2 - 1 hour into blood transfusion, T3 - 2 hour into blood transfusion and T4 - 15 to 20 minutes post blood transfusion