Gut and cerebral perfusion and oxygenation in preterm infants receiving blood transfusion

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For the degree of Doctor of Medicine (Research)

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1/25/2017

Declaration

I, Dr Jayanta Banerjee, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

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Dedicated to the memory of my father

Mr Amarnath Banerjee

Who passed away to holy abode on

13th August 2014

Who was my strongest supporter and driving force behind this work

Acknowledgements

I would like to first thank my wife Dr Mita Roy and my two children Nayanika and Nikhil who despite putting up with my inflexibility while undertaking this project have provided me with invaluable support. I would like to thank my wife Dr Mita Roy for her immense sacrifice, continuous support, encouragement and belief in me throughout the research work.

I would like to thank Dr Terence Leung, Senior lecturer, Department of Medical Physics and Bioengineering, University College London for training me with the basics of NIRS measurement using NIRO-300 double channel NIRS device, procuring the NIRS data using Mat Lab software programme and also helping me procure data for analysis from ixTrend software using Mat Lab software programme.

I would like to thank Dr Kyriakos Iliadis, Consultant Paediatric Radiologist, Homerton University Hospital, who trained me to perform Doppler blood flow measurements. I would also like to thank Mr Darius Khatibi, Medical Electronic Department, Homerton University Hospital for helping to procure the ixTrend software, and develop presets in the ultrasound scanner to measure Doppler blood flow.

I would like to thank Prof Joan Morris, Professor of Statistics, Wolfson Institute, Queen Marys University of London who guided me with the statistical aspect of the study.

I am very grateful to Mr Tanwir Mohamed, Senior haematology technician, Homerton University Hospital for his extended support in measuring pre and post-transfusion fetal haemoglobin percentage in order to estimate pre-transfusion red cell volume.

I am very grateful to the medical and nursing staff of the Neonatal Unit, Homerton University Hospital, London for their cooperation. This work could not have been possible without the cooperation of the babies and their parents and I would like to thank them for agreeing to take part in this endeavour. I also would like to thank Hamamatsu Photonics KK, Japan, HCA International and Garfield Weston Foundation for their invaluable financial support.

Finally, I would like to express my sincere gratitude to my research supervisor Dr Narendra Aladangady for his invaluable continuous guidance, encouragement and support and his belief in me throughout this research project.

Abstract

Background and Aim: Preterm infants frequently receive blood transfusion (BT) during their stay in the neonatal unit. The aim of this study was to measure the effect of BT on cerebral and gut blood flow and oxygenation in preterm infants in relation to postnatal age. Another aim of the study was also to investigate the influence of measured pre-transfusion RCV on gut perfusion in preterm infants receiving first blood transfusion for clinical indication using NIRS and Doppler ultrasound scan.

Methods: Preterm infants admitted to neonatal unit were recruited to three postnatal age groups: 1 to 7 days (group 1; n=20), 8 to 28 days (group 2; n=21) & ≥29 days of life (group 3; n=18). Pre and post-BT Anterior Cerebral artery (ACA) Time Averaged Mean Velocity (TAMV) and Superior Vena Cava (SVC) flow were measured to assess cerebral blood flow. Pre and post-BT Superior mesenteric artery (SMA) peak systolic velocity was measured to assess gut or splanchnic blood flow. Cerebral and gut Tissue Haemoglobin Index (THI), Oxygenation Index (TOI) were measured from 15-20 minutes before to 15-20 minutes post-BT using NIRS. Cerebral and gut fractional tissue oxygen extraction (FTOE) was calculated from the TOI and saturation of oxygen (SaO_2). Vital parameters and blood pressure (BP) were also measured continuously from overhead monitors. Pre-transfusion red cell volume (RCV) was measured by fetal haemoglobin (HbF) dilution method and compared with the cerebral and gut perfusion and oxygenation changes following blood transfusion. The cerebral and gut perfusion and

oxygenation were also measured over a three hour period in 12 control infants not receiving blood transfusion.

Results: There were 71 infants included in the study; of them 59 were study infants receiving blood transfusion and 12 were control infants. Amongst the vital parameters, mean BP increased significantly, and there was no significant change in heart rate (HR), respiratory rate (RR) or SaO_2 following BT.

Pre-transfusion ACA TAMV was higher in Group 2 and 3 compared to Group 1 ($p<0.001$) which remained significant after multivariate analysis ($p<0.05$). Pre-transfusion ACA TAMV decreased significantly ($p\leq0.04$) in all 3 postnatal age groups; pre-transfusion SVC flow decreased significantly in Group 1 ($p=0.03$) and Group 3 ($p<0.001$) following transfusion. Pre-transfusion cTOI was significantly lower in Group 3 compared to Group 1 ($p=0.02$) which remained significant after multivariate analysis ($p<0.011$). The cTHI ($p<0.001$) and cTOI ($p<0.05$) increased significantly post-transfusion in all three postnatal age groups. PDA had no effect on these measurements.

Pre-transfusion SMA PSV increased with postnatal age (group 3 vs. group 1: $p<0.01$; CI 0.6 to 0.1), proportion of feeds (>50% feeds: 0.91±0.4 vs. <50% feeds: 0.71±0.4 m/sec, $p<0.01$); and decreased with presence of PDA (closed PDA: 0.94±0.4 vs. open PDA: 0.68±0.3 m/sec, $p=0.006$, CI 0.07 to 0.45); but remained unaltered following transfusion. The pre-transfusion sTOI varied with postnatal age

(Group 2:44.6 vs. Group1: 36.7%; p=0.03, CI -0.6 to -15.2) on univariate analysis but was not significantly different on multivariate analysis; pre-transfusion sTOI was not influenced by feeds or presence of PDA. The sTHI and sTOI increased (p<0.01) and sFTOE decreased (p<0.01) significantly following transfusion in all postnatal age groups.

When compared between infants with patent ductus arteriosus (PDA) to gestational and postnatal age matched infants with closed PDA the pre-transfusion baseline cerebral and gut oxygenation values were similar.

Red cell volume (RCV) was measured in 14 preterm infants with indwelling arterial catheters. The median pre-transfusion RCV was 29.9 (20.6 - 38.7) ml/kg. RCV correlated well with haemoglobin (r=0.65, p<0.01) and haematocrit (r=0.60, p<0.01). Five infants had RCV <25 ml/kg. The SMA peak systolic velocity decreased significantly (p<0.03, CI 0.01 to 0.33) following transfusion in infants with RCV <25ml/kg and remained unaltered in those with RCV ≥25 ml/kg. The gut tissue oxygenation index (sTOI) increased significantly (p<0.01, CI 7.9 to 30.9) in those with RCV ≥25 ml/kg along with a subsequent decrease in the sFTOE and increase in sTHI. But the sTOI, sTHI or sFTOE did not increase in those infants with pre-transfusion RCV <25 ml/kg following transfusion.

The pre-transfusion baseline anterior cerebral artery (ACA) time averaged mean velocity (TAMV) was significantly higher in the infants with pre-transfusion

haemoglobin (Hb) <11 g/dl compared to those with Hb level ≥11 g/dl (p<0.001). The pre-transfusion baseline superior vena cava (SVC) blood flow was though higher in infants with Hb <11g/dl this difference was not statistically significant (p=0.54). The ACA TAMV decreased significantly in both the groups following blood transfusion and was similar (17%) in those with a pre-transfusion Hb <11g/dl compared to those with a pre-transfusion Hb level ≥11g/dl (18%).

When compared in relation to pre-transfusion Hb level (≥ or <11 g/dl), the pre-transfusion cTOI and sTOI levels were similar. There was a significant increase in the cerebral tissue oxygenation index (cTOI) following blood transfusion in both groups with Hb level above (p=0.005; CI 2.2, 10.1) and below (p<0.0001; CI 3.9, 8.9) 11g/dl. Similarly, the splanchnic tissue oxygenation also increased significantly in both group of infants with Hb ≥11g/dl (p=0.001; CI 6.6, 23.5) and Hb <11g/dl (p<0.0001; CI 7.3, 18.9) following blood transfusion.

The changes in ACA TAMV, SVC flow, SMA PSV as well as cTOI and sTOI following blood transfusion were not significantly different between the postnatal age groups on multivariate analysis of covariates: gestational age, birth weight, pre-transfusion Hb and mean blood pressure, presence of PDA and volume of feed.

In twelve control infants, heart rate (HR), respiratory rate (RR), systolic blood pressure (BP) and diastolic BP, mean arterial BP and SaO_2 all remained stable at

the start of the NIRS measurements (pre-oximetry measurements) and at the end of NIRS measurements (post-oximetry measurements). The cerebral and splanchnic blood flow and tissue oximetry measurements remained similar over the three hour period of measurements.

Conclusion: Baseline pre-transfusion cTOI decrease, ACA TAMV and SMA PSV increase with increasing postnatal age. Blood transfusion increased cTOI and cTHI and decreased cFTOE and ACA TAMV in all postnatal age groups. Blood transfusion improved intestinal tissue oxygenation (increase sTOI and sTHI and decrease sFTOE) without altering mesenteric blood flow velocity irrespective of postnatal age, pre-transfusion haemoglobin and presence of PDA. In infants with RCV <25ml/kg the SMA blood flow velocity decreased following blood transfusion, this could be due to an adaptive response to increased post-transfusion RCV. Unlike infants with RCV ≥25ml/kg, the gut oximetry markers did not improve following transfusion in those infants with RCV <25ml/kg. This may suggest that babies with pre-transfusion RCV <25 ml/kg may need larger volume of blood transfusion; larger studies are required to substantiate this finding.

Contents

Abbreviations

ACA – Anterior cerebral artery

ANOVA – Analysis of variance

APH – Antepartum haemorrhage

BCSH – British Committee of Standards in Haematology

BP – Blood pressure

BPD – Bronchopulmonary dysplasia

BT – Blood transfusion

BV – Blood volume

BWt – Birth weight

CBF – Cerebral blood flow

CBV – Cerebral blood volume

cFTOE – Cerebral fractional tissue oxygen extraction

CPAP – Continuous positive airway pressure

$crSO_2$ – Cerebral regional oxygen saturation

CSOR – Cerebral splanchnic tissue oxygenation ratio

$CSvO_2$ – Cerebral venous oxygen saturation

cTOI – Cerebral tissue oxygenation index

cTHI – Cerebral tissue haemoglobin index

BFU-E – Erythroid Burst forming unit

GM-CSF – Granulocyte Monocyte Colony stimulation factor

CFU-GEMM – Colony forming unit Granulocyte, Erythrocyte, Macrophage, Megakaryocyte

CSV file – comma separated version file

CtOx – Cytochrome oxidase

CW – Continuous wave

DCC – Delayed cord clamping

DP – Differential pathlength

DPF – Differential pathlength factor

ECC – Early cord clamping

EDV – End diastolic velocity

ELBW – Extremely low birth weight

ETTNO – The Effects of transfusion thresholds on neurocognitive outcome of ELBW infants

FiO2 – Fractional inspiratory oxygen

FOE – Fractional oxygen extraction

GA – Gestational age

Hb – Haemoglobin

HbF – Fetal haemoglobin

HbO_2 – Oxy haemoglobin

Hct – Haematocrit

HHb – Deoxy haemoglobin

HPLC – High performance liquid chromatography

HPRF – High pulse repetition frequency

HR – Heart rate

Hz – Hertz

ICA – Internal Carotid artery

ICC – Intra-class correlation

IQR – Inter quartile range

IUGR – Intrauterine growth restriction

IVH – Intra-ventricular haemorrhage

LVESD – Left ventricular end systolic diameter

LVEDD – Left ventricular end diastolic diameter

LVO – Left ventricular output

MBP – Mean blood pressure

MD – Mean difference

MDI – Mental developmental index

MEBM – Maternal expressed breast milk

MRI – Magnetic resonance imaging

MV – Mean velocity

NEC – Necrotising enterocolitis

NHS – National Health Service

NICU – Neonatal intensive care unit

NNU – Neonatal unit

NIRS – Near Infra-red spectroscopy

OR – Odds ratio

PCV – Packed cell volume

PDA – Patent ductus arteriosus

PET – Pre-eclamptic toxaemia

pO2 – Partial pressure of oxygen

pCO2 – Partial pressure of carbon di-oxide

PI – Pulsatility index

PINT – Premature Infants in Need of Transfusion

PINTOS – PINT Outcome study

PRBC – Packed red blood cell transfusion

$prSO_2$ – peripheral regional oxygen saturation

PRF – Pulse repetition frequency

PSV – Peak systolic velocity

PW – Pulse wave

R&D – Research and development

RBC – Red blood cell

RCV – Red cell volume

RDS – Respiratory distress syndrome

REC – Research ethics committee

RI – Resistance index

ROP – Retinopathy of prematurity

RR – Respiratory rate

RR – Relative risk

rEpo – Recombinant erythropoietin

SaO_2 – Peripheral oxygen saturation

SCF – Stem cell factor

SCOR – Splanchnic cerebral tissue oxygenation ratio

SD – Standard deviation

sFTOE – Splanchnic fractional tissue oxygen extraction

SIRS – Systemic inflammatory response syndrome

SMA – Superior mesenteric artery

$srSO_2$ – Splanchnic regional oxygen saturation

sTOI – Splanchnic tissue oxygenation index

sTHI – Cerebral tissue haemoglobin index

SV – Stroke volume

SVC – Superior vena cava

TAMV – Time averaged mean velocity

TANEC – Transfusion associated NEC

TCD – Transcutaneous Doppler

TDI – Tissue Doppler imaging

TOP – Transfusion of premature

USS – Ultrasound scan

VLBW – Very low birth weight

VTI – Velocity time integral

WHO – World Health Organisation

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1. Introduction

1.1. Blood transfusion in neonatology

1.1.1. History of blood transfusion

William Harvey, the founder of modern physiology, described the circulation of blood in the body through heart in his pioneering experiments about four hundred years back. He published his work in a 72 page booklet '*Exercitatio anatomica de motu cordis et sanguinis in animalibus*' (An Anatomical Exercise on the Motion of the Heart and Blood in Living Beings), more frequently referred to as '*De motu cordis*'. In this booklet Harvey described the two phases of cardiac movements: systole (contraction) and diastole (expansion)[1]. This was hailed by historian KF Russell as the greatest single contribution to anatomy and medicine in any century [2]. Following Harvey's pioneering research with circulation of blood; research into blood transfusion began in the 17th century. In 1665 at a meeting of the Royal Society of London Christopher Wren demonstrated animal-to-animal transfusion of blood. The first animal-to-human blood transfusion was reported as early as 1667 by Jean-Baptiste Denys of France. He transfused blood from a sheep to a 15-year-old boy, who survived the transfusion[3]. The first animal-to-human transfusion in England was performed by Lower and King five months later [4]. After multiple fatal incidents, Denys rejected the idea of animal-to-human transfusion and this led to transfusion of blood to humans falling to disrepute and was subsequently been forbidden in France and England for the next 150 years. In nineteenth century, James Blundell, an obstetrician at Guy's Hospital in London introduced human-to-human blood transfusion into medical practice. He reported satisfactory benefit of transfusion in cases of post-partum haemorrhage in 1828 [5]. Since then blood

transfusion has found its way in medical parlance and has evolved over the years to establish as a life-saving treatment.

The concept of paediatric transfusions is as old as the history of blood transfusion itself. Infants in a neonatal intensive care unit are subjected to various laboratory and bedside blood tests to inform clinicians of their physiological status which in turn allow them to make necessary ventilatory changes. But preterm newborn infants are unable to replace the blood losses quickly, hence requiring frequent blood transfusions. Advances in the field of neonatology have led to increased survival of preterm newborn infants [6]; blood transfusion patterns in the neonatal units (NNU) have changed over the last twenty to thirty years leading to more restrictive transfusion practice [7,8].

1.1.2. Current blood transfusion practice

Amongst the Extremely Low Birth Weight (ELBW) infants 90% receive packed red blood cell (PRBC) transfusions [8] and it is well recognised that patients in neonatal intensive care units (NICU) receive more PRBC transfusions than any other hospitalised group[9,10]. This is a result of frequent blood losses from phlebotomy to monitor intensive care [11] in conjunction with an immature haematopoietic system [12]. It is also compounded by the fact that preterm infants have lower haemoglobin levels at birth [13]. Over time the blood transfusion practice has been changing [14,15]; in 1980s 80 to 90% of infants <1500 grams and 100% of infants <1000 grams would have received blood transfusion [7]. Comparing the cohorts receiving blood

transfusion between 1982 and 1993 Maier et al have reported a remarkable drop in the frequency of transfusion from 7.0±7.4 to 2.3±2.7. This is primarily due to a decline in the pre-transfusion haematocrit threshold (33.6±2.8% to 29.8±5.1%) for transfusion over this period[8].

The three categories of newborn infants who receive blood transfusion include (1) infants with significant blood loss in the perinatal period, such as placental abruption, (2) infants with significant cardiopulmonary disease on high ventilatory requirement with an intention to keep haematocrit >40% and (3) infants where haemoglobin (Hb <7 g/dl) or haematocrit (Hct <23%) is below a fixed level[16]. The third category include older preterm infants with lung disease such as Broncho-pulmonary dysplasia (BPD), and stable growing infants with symptomatic anaemia (multiple apnoea, desaturation and bradycardia with a low Hb or Hct)[16]. PRBC transfusion depends not only on Hb and Hct but also on the amount of cardiorespiratory support [invasive ventilation versus continuous positive airway pressure (CPAP)], oxygen requirement and postnatal age of the infant[17]. Nearly half of the PRBC transfusions given to ELBW infants are given during the first 2 weeks of life[7,18], when these infants are sicker, needing large number of laboratory blood tests, weekly phlebotomy losses during this period can be as much as 10 - 30% of the total blood volume (10 – 25 ml/kg)[19]. Average iatrogenic blood losses could be as much as 40 – 80 ml/kg [20,21] in infants with birth weight of <1000g.

The neonatal blood transfusion guidelines rely on subjective findings and do not identify or target specific patient needs[22,23]. The various compensatory mechanisms a neonate uses to improve tissue oxygen metabolism are by

vasodilatation, increased cardiac output by increasing heart rate and by increased oxygen extraction from haemoglobin to meet metabolic demands. Anaemia impacts a patient's clinical status when the oxygen carrying capacity of the blood drops below an adequate threshold to meet the demands of oxygen consumption in the tissue. But direct measurement of oxygen consumption is technically difficult and cumbersome. Theoretically, anaemia results in a patient being symptomatic when there is an imbalance between oxygen delivery and consumption, rather than at predefined Hb levels[24].

Clinicians are reliant on clinical signs, such as tachycardia, bradycardia, apnoea or desaturation episodes, along with traditional laboratory measurements, such as Hb, Hct, reticulocyte count and serum lactate to determine when infants require a PRBC transfusion[25]. The decision for blood transfusion is made by neonatal clinicians based on national[26] and local hospital guidelines along with their clinical judgement. PRBC transfusions are administered only when clinicians predict that the benefit will outweigh the risks. However, because of its subjective nature, these judgements are dependent on local guideline and clinician's perception[27]. As a result of this multiple known and unknown physiological factors are not always considered[28]. To improve the predictability of transfusion success, many protocols in addition to Hb, consider clinical state of the infant, their clinical symptoms, and respiratory support status[29]. This approach is nonspecific, as a change in vital signs or symptoms that often prompt a PRBC transfusion in neonatal unit (NNU) can occur for many reasons other than anaemia in a premature infant. These other potential aetiologies may include minor conditions like gastro-oesophageal reflux, apnoea of prematurity, to major conditions such as respiratory distress and

sepsis[30]. As a result, too frequently, there is no significant improvement following transfusion [28].

1.1.3. Current evidence for haemoglobin thresholds

There is no firm evidence about the threshold at which an infant should receive PRBC transfusion. In the presence of an equipoise following randomised trials of the benefits of liberal and restrictive blood transfusion [31-33], majority of the guidelines advocate~~s~~ low Hb thresholds for transfusion of preterm infants primarily based on retrospective or observational studies and also relying on adult studies of blood transfusion [34]. The British Committee for Standards in Haematology (BCSH) published its revised guideline on blood transfusion in 2004[26] and the American Red Cross guideline for blood transfusion published its second edition in 2007[35]. These guidelines stressed the importance of defining poor cardiopulmonary status, such as fractional inspired oxygen (FiO_2) and its application in local practice. A comparison of some of these guidelines is shown in **Table 1**. In a new version of their blood transfusion guideline, the BCSH recommend Hb level threshold of <70 g/L in late anaemia of preterm infants. The threshold can be increased upto 85 g/L depending on the clinical situation[36].

Table 1: Comparison of British Committee for Standards in Haematology (BCSH), American, Australian and Canadian practice guidelines for PRBC transfusion in newborn infants

Clinical status	BCSH guideline [26]	American Red Cross Practice guideline [35]	Australian National Blood Authority guideline	Canadian Blood services guideline
Anaemia in the first 24 hours	Hb <12 g/dl or Hct <0.36	--	No respiratory support: Hb 10 – 12 g/dl Respiratory support Hb 11 – 13 g/dl	On ECMO and Congenital cyanotic heart disease Hb <15 g/dl
Infants receiving intensive care Severe cardiopulmonary disease (FiO_2>0.35)	Hb <12 g/dl or Hct <0.36	Hct 40 – 45%	Hb 11 – 13 g/dl	Hb <12 g/dl
Chronic oxygen dependency Moderate cardiopulmonary disease (CPAP or O_2)	Hb <11 g/dl	Hct 30 – 35%	Hb 8.5 – 11 g/dl	Hb <10 g/dl
Late anaemia, stable patient	Hb < 7g/dl	Hct 20 – 25%	Hb 7 – 10 g/dl	Hb <7 g/dl

Various randomised controlled trials used different thresholds of transfusion for the liberal and restrictive groups; although the results of the two recent trials were not unambiguous they provide valuable insight into the outcomes achieved with current transfusion practice[31,32]. The results of Premature Infants in Need of Transfusion (PINT) trial (228 liberal vs. 223 restrictive) demonstrated no difference in death or survival with broncho-pulmonary dysplasia (BPD), severe retinopathy of prematurity, or brain injury at discharge[32]. On the contrary, Bell et al in the Iowa Transfusion Trial reported higher number of severe adverse brain events (defined as grade 4 IVH, periventricular leucomalacia, or both; 6 vs. 0; p=0.012) in those infants who received restrictive transfusion (n=47) compared to liberal transfusion (n=50)[31]. An important limitation of the two randomised trials [31,32] was the trivial

mean difference of the Hb thresholds used for the liberal and restrictive groups for transfusion particularly for late anaemia. The post hoc analysis of The PINT outcome study (PINTOS) where the neurodevelopment of infants were assessed at 18 to 21 months of corrected age[37] and the 12 year follow up of Iowa Transfusion Trial where MRI brain scans were used to assess intracranial volume[38], the results have been conflicting, where the first favoured liberal and the second restrictive transfusion. Details of the thresholds of transfusion used in these two and other transfusion trials are presented in **Table 2**.

Table 2: Threshold used by different randomised trials for PRBC transfusions

Trials	Restrictive threshold	Liberal threshold
Blank et al (1984) [39]	Transfusion according to clinical indication	Transfuse if Hb <100 g/l
Ransome et al (1989) [40]	Hb levels <70 g/l or clinically symptomatic	Hb levels <100 g/l
Brooks et al (1999) [41]	PRBC transfusion when clinically symptomatic	PRBC transfusion if Hb<133 g/l
Connelly et al (1998) [42]	1st postnatal week:110 g/l 2nd postnatal week: a.FiO_2>40%: 110 g/l b.FiO_2<40%: 90g/l 3rd postnatal week: 80 g/l*	1st postnatal week: 130 g/l 2nd postnatal week: a.FiO_2>40%: 130 g/l b.FiO_2<40%: 100 g/l 3rd postnatal week: 80 g/dl*
Mukhopadhyay et al (2004) [43]	Hb levels ≤100 g/l or Hct ≤ 30%	Hb levels ≤133 g/l or Hct ≤ 40%
Bell et al (2005) [31]	Intubated: 113 g/l O_2 or CPAP: 93 g/l No respiratory support: 67 g/l	Intubated: 153 g/l O_2 or CPAP: 127 g/l No respiratory support: 73 g/l
Kirpalani et al (2006) [32]	*For infants requiring respiratory support (ventilation, CPAP or oxygen):* Postnatal week 1: 115 g/l Week 2: 100 g/l Week 3 till discharge: 85 g/l *For infants not requiring respiratory support:* Postnatal week 1: 100 g/l Week 2: 85 g/l Week 3 till discharge: 75 g/l	*For infants requiring respiratory support (ventilation, CPAP or oxygen):* Postnatal week 1: 135 g/l Week 2: 120 g/l Week 3 till discharge: 100 g/l *For infants not requiring respiratory support:* Postnatal week 1: 120 g/l Week 2: 100 g/l Week 3 till discharge: 85 g/l

Chen et al (2009) [33]	Intubated: 116 g/l CPAP: 100 g/l No respiratory support: 73 g/l	Intubated: 150 g/l CPAP: 133 g/l No respiratory support: 100 g/l

Key: * *When capillary rather than venous or arterial bloods were sampled the thresholds were 4% higher*

There are two randomised trials currently undergoing for investigation of neurodevelopmental outcome at 24 months of age in relation to liberal and restrictive transfusion practice[44,45]. The transfusion thresholds used for these two trials are presented in **Table 3**. The thresholds used in these two trials are chosen by investigators by consensus depending on various transfusion practices of various institutions. The presence of equipoise in deciding the need for transfusion in a preterm infant and the absence of current evidence is the main reason why transfusion thresholds are widely variable in practice as well as in various trials.

Table 3: Transfusion thresholds of haematocrit level used in current trials

ETTNO[44]					**TOP**[45]				
Age	***Liberal***		***Restrictive***		Age	***Liberal***		***Restrictive***	
	Critical	Non critical	Critical	Non critical		Critical	Non critical	Critical	Non critical
3-7 days	<41	<35	<34	<28	**Wk 1**	38	35	32	29
8-21 days	<37	<31	<30	<24	**Wk 2**	37	32	29	25
≥21 days	<34	<28	<27	<21	**≥Wk 3**	32	29	25	21

ETTNO trial – Effects of Transfusion Thresholds on Neurocognitive Outcome

TOP trial – Transfusion of Premature trial

1.1.4. Physiological response to anaemia and blood transfusion

There is a general perception that anaemia may lead to tachycardia, hypotension, poor perfusion and impair oxygen delivery to tissues of newborn infants. Fredrickson et al examined the physiological adaptation to anaemia in preterm infants (n=41) who were already enrolled into a trial[31] comparing two sets of haematocrit thresholds (liberal vs. restrictive) for transfusion. The vital parameters were measured before transfusion and were compared with the values following morning after transfusion. They noticed no significant change following transfusion in the oxygen consumption, mean fractional inspired oxygen (FiO_2) or mean oxygen saturation (SaO_2) in either group[46]. Kasat et al used caregivers' perception of clinical improvement as a measure of benefit following PRBC transfusion with the objective of refining transfusion guidance. Care givers of 78 neonates were requested to complete a pre and post-transfusion survey on neonates receiving PRBC transfusion. Of these neonates, 18 (23%) received transfusion based on existing guidelines, 36 (46%) based on caregivers' perception and 24 (31%) based on both. The neonates who received PRBC transfusion according to existing guidelines were more likely to be in their first week of life, had higher Hct, less symptomatic and were ventilated invasively. Those who received transfusion based on caregivers' perception were more symptomatic and were receiving non-invasive ventilation. The vital parameters were compared from before to 24 hours after blood transfusion. The characteristics of neonates who improved after PRBC transfusion were, low pre-transfusion Hct (p=0.02), presence of clinical signs (p=0.01) and undergoing non-invasive ventilation (p=0.002). Pre-transfusion tachycardia was found to be the most sensitive predictor (Odds ratio 6.48; 95% CI 1.6-26, p=0.005) which demonstrated why one of the anticipated benefit of

transfusion is reduction of heart rate[16]. Similarly, Nelle et al (n=33) who compared vital parameters between before and 4 hours after blood transfusion also noticed a significant drop in heart rate (from 161 to 149 per minute; p = 0.005) following transfusion [47]. Contrary to the findings of these studies the changes in the physiological parameters were not consistent in other studies. Dani et al (n=14) who compared with post-transfusion parameters 12 hours after blood transfusion and Alkalay et al (n=32) who recorded vital parameters at 1-2 hours, 2-4 hours, 4-7 hours and 27-34 hours after transfusion found no difference in heart rate following transfusion[48,49]. Aladangady et al reported that heart rate correlated positively with measured blood volume of preterm infants born at 24-32 weeks gestation [27]. One of the important things to consider is the timing of the measurement of heart rate following transfusion, which were not consistent in these studies and perhaps this was compounded by the clinical state of the infants studied.

Systemic blood pressure is routinely monitored to inform neonatal circulatory status and has been shown to correlate with peripheral blood flow in hypotensive preterm infants [50]. Acute perinatal haemorrhage such as placental abruption can lead to neonatal anaemia and hypotension prompting PRBC transfusion. Impact of PRBC transfusion on improving blood pressure is variable[28,47,49], this could be due to the inconsistency of methods as well as timing of blood pressure measurements. The mean arterial blood pressure does not correlate with measured blood volume in premature infants [27]. Systemic blood pressure is dependent on cardiac output as well as peripheral vascular resistance. In anaemic state the cardiac output is high in order to maintain tissue perfusion, this decreases following transfusion[49]. However, there is a subsequent increase in viscosity of

blood post-transfusion but no change in vascular hindrance (i.e. no change in vascular geometry) resulting in increased blood flow resistance or peripheral vascular resistance [47]; this may in turn result in increased diastolic and mean systemic blood pressure.

1.1.5. Benefits of blood transfusion

Transfusion is considered to be beneficial when given to replace blood loss by internal or external haemorrhage including feto-maternal haemorrhage. When given to anaemic preterm infants to replace the phlebotomy blood loss, the benefits are not clear. Blood transfusion improves systemic oxygen transport and, in case of acute blood loss, replenishes low circulating blood volume. Blood transfusion has also been reported to increase cerebral oxygenation in stable preterm infants [51-54]. Potential benefits of blood transfusion for anaemic preterm infants include decrease in the incidence of apnoea and periodic breathing [28,31,33,55,56] and promotion of weight gain[56,57]. The two large clinical trials - *the Iowa transfusion trial*[31] and *the Premature Infants in Need of Transfusion trial (PINT)* [32] who compared liberal and restrictive blood transfusion criteria failed to provide clear evidence of benefit of either transfusion criteria.

In the *Premature Infants in Need of Transfusion Outcome Study (PINTOS)*[37] which enrolled 451 infants and followed them to 18-21 months of age, the primary outcome was available in 430. The primary composite outcome was death or the presence of cerebral palsy, cognitive delay, or severe visual or hearing impairment. There was no statistically significant difference in the primary outcome

as well as the pre-planned secondary outcomes, found in 94 (45%) of 208 in the restrictive group and 82 (38%) of 213 in the liberal group. A *post hoc* analysis after redefining cognitive delay [Bayley Mental Development Index score (MDI) redefined as <85 instead of <70 as planned before] showed that the Bayley cognitive MDI scale was marginally better in infants who received liberal blood transfusion. In an observational follow-up study of premature infants receiving two different volumes of blood transfusions in two neonatal units (15 ml/kg vs. 20 ml/kg), von Lindern et al demonstrated that total transfused PRBC volume per kg body weight was not an independent predictor of the composite outcome (p = 0.96, OR 1.0, CI 0.9-1.1) defined as post discharge mortality, neuromotor developmental delay, blindness or deafness, evaluated at a mean corrected age of 24 months[58]. The *Effects of Transfusion Thresholds on Neurocognitive Outcome* of Extremely Low Birth-Weight Infants (ETTNO) study has completed recruitment in Germany, which is an observer-blinded randomised controlled clinical trial. 920 infants of 400–999 g birth weight was randomised to restrictive or liberal transfusion trigger thresholds between 48 and 72 h of life, stratified by participating centre and birth weight (400–749 g/750–999 g). The study is currently following up the recruited infants to upto 24 months of corrected age and there are further plans of following them upto 5.5 years of age, looking into the primary outcome of death and neurodevelopmental impairment [44]. *Transfusion of Prematures* study (TOP), an open, parallel group multicentre randomised controlled trial, has started its recruitment in US and is studying long term outcomes of extremely low birth weight (<1000 grams) premature infants at 22-26 months of age who received liberal compared to restrictive strategy for blood transfusion[45].

1.1.6. Risks of blood transfusion

Though PRBC transfusion can be life-saving in newborn infants with acute blood loss in the peri-partum period such as placental abruption and severe anaemia, there is an increasing unease with blood transfusion and its association with significant pathologies, such as intra-ventricular haemorrhages (IVH)[59], retinopathy of prematurity [60] and necrotising enterocolitis (NEC)[61]. Blood transfusion has also been found to be an independent risk factor of in-hospital mortality in preterm infants [62,63]. Risks associated with cross-matching are significantly lower due to rigorous screening and vigilance[64]. The complications now include a group of conditions 'oxidative diseases' which include elevated plasma non-transferrin bound iron[65] and transfusions leading to possible overloading of liver with iron[66] in very low birth weight (VLBW) infants, the clinical implications of which is unknown. Some studies have shown association between blood transfusions and risk of Broncho-pulmonary Dysplasia (BPD) [65,67,68], necrotising enterocolitis (NEC)[69-71] and Retinopathy of Prematurity (ROP)[72,73,60] but the causal relationship is yet to be demonstrated. PRBC transfusion has also been reported as an independent risk factor for mortality[62,63].

Increase in mesenteric [superior mesenteric artery (SMA)] blood flow velocity in response to feeding in anaemic preterm infants is well known [74]. In a clinical trial of 22 infants [mean gestational age 27.3 (SD 2.3) weeks; mean chronological age of transfusion of 31.2 days, range 3 to 71 days] where infants were randomised to fed and not-fed groups during the transfusion, Krimmel et al demonstrated that this increased velocity in SMA following feeding which was evident pre-transfusion was attenuated in the immediate post-transfusion state. They speculated that this

attenuation of response may contribute to transfusion-associated NEC in these infants[75]. In a case controlled study (111 preterm infants with NEC ≥ Stage 2a were compared with 222 matched controls) blood transfusion showed a temporal relationship with the onset of NEC. There was a higher risk of developing NEC within 24 h (OR=7.60, P=0.001) and 48 h (OR=5.55, P=0.001) after transfusion[70]. In a large retrospective cohort study of 2311 preterm infants, Paul et al [71] demonstrated that infants who received transfusion had increased risk (OR: 2.3; 95% confidence interval: 1.2– 4.2) of developing NEC even after adjusting for confounding factors compared with infants who did not receive a transfusion. Christensen et al reported that one-third of cases in their series (40 out of 112) developed surgical NEC following a transfusion, in comparison two-thirds (72) developed NEC without any preceding blood transfusion. Infants who developed NEC following transfusion were of an earlier gestational age [mean 27 (90% CI 26-28) weeks) vs. 30 (29-31) weeks; p<0.001], lower birth weight [mean 981 (90% CI 835-1128) grams vs. 1371 (1245-1496) grams; p<0.001], were fed larger volume of milk prior to transfusion (p=0.04) and developed NEC later on in their life [mean 23 (90% CI 20-27) days vs. 16 (13-19) days; p<0.001] [76]. A recent meta-analysis of observational data of transfusion associated NEC (TANEC) concluded that recent exposure to transfusion was associated with NEC in premature infants [77]. Three randomised controlled trials examining the benefits of transfusion using high and low haemoglobin thresholds have examined the development of NEC following blood transfusion[31-33]. Albeit *not significant*, the pooled OR (1.67; CI 0.82, 3.38) for NEC favoured high haemoglobin threshold (i.e. more common in infants in the restrictive transfusion group)[78], thereby contradicting the association between blood transfusion and NEC. After adjusting for covariates Singh et al have found that effect of low haematocrit was an independent risk factor for development of

NEC[70]. This corresponds with the reduced risk of NEC in infants in the liberal haemoglobin threshold group compared to the restricted group indicated in the randomised controlled trials [78]. Recently a prospective, multicentre cohort study of 598 VLBW infants has reported severe chronic anaemia to be associated with NEC and not blood transfusion[79]. These contradictory reports suggest that perhaps chronic severe anaemia puts the gut to risks of ischaemia and transfusion causes a reperfusion injury leading to development of conditions described as transfusion associated NEC. Another factor that remains unresolved is the effect of feeding during blood transfusion and the possible implications in the development of NEC. Perciaccante et al suggested that feeding during transfusion increases the incidence of NEC [80]. In the first phase of the study, 7 out of 18 (38.9%) infants developed NEC within 48 hours of a transfusion. In the second phase by withholding feeds, none of the infants developed NEC within 48 hours of a blood transfusion. In a case-control study El-Dib et al compared the incidence of NEC 18 months before and after implementation of strict policy of withholding feeds during blood transfusion. They reported a significant decrease in NEC from 5.3% to 1.3% ($p = 0.047$) following implementation of the new policy [81].

Early blood transfusions suppress endogenous erythropoietin production, thereby lowering serum erythropoietin levels at a critical time in neurodevelopment[82]. Darbepoetin α and erythropoietin may serve as useful adjuncts to reduce the need of blood transfusion in preterm infants[83]. However, systematic reviews of early [84] as well as late[85,86] erythropoietin therapy in preterm infants have shown no significant clinical benefit in reducing the need for transfusion, on the contrary this may have resulted in inadvertent side effects such as ROP as described below. Nopoulos et al assessed brain structure and measured the brain volume in preterm

infants, at an average age of 12 years by using MRI brain scans of 44 infants from the participants of the original *Iowa blood transfusion study* (n=100) where preterm infants were randomised to liberal or restrictive threshold for transfusion. Preterm infants who received transfusions using liberal guidelines had smaller brain volume thereby demonstrating that restrictive transfusion is beneficial towards brain growth[38]. The limitations of the study are that only 44% of the initial cohort was studied and other factors influencing brain growth was not taken into account.

1.1.7. Strategies to reduce blood transfusion

The principal strategies to reduce transfusion in preterm infants involve improving admission blood volume and haemoglobin level and reducing the phlebotomy losses from various investigations.

Delayed clamping of the umbilical cord:

Delayed clamping of the umbilical cord at birth in a preterm neonate is a subject of much debate and although a number of randomised controlled trials in term and preterm infants have evaluated the benefits of delayed cord clamping (DCC) versus early cord clamping (ECC), the ideal timing of umbilical cord clamping is yet to be established.

Studies involving term infants have demonstrated that about 80 ml of blood is transfused from placenta to the baby in the first minute, reaching upto 100 ml by 3 minutes after birth. This extra blood along with the extra iron can increase the birth weight [87] and may help prevent iron deficiency in the first year of life[88]. Aladangady

et al demonstrated in a randomised clinical trial of preterm infants (24 – 32 weeks) that DCC increases the blood volume (mean blood volume in DCC group 74.4 ml/kg compared to ECC group 62.7 ml/kg) at birth both in vaginal and caesarean section deliveries[89]. DCC increases Hb/Hct at birth in term [87] and preterm infants [90] and reduces the need for blood transfusion in very low birth weight (VLBW) preterm infants in the first 6 weeks of life [91-94].

A systematic review of randomised trials of early and delayed umbilical cord clamping in preterm infants demonstrated significant reduction in PRBC transfusion and incidence of IVH in the DCC group[95-97]. It has been reported that low Hb levels at birth is a risk factor for mortality in preterm infants <32 weeks of gestational age [98]. The WHO recommended in the care of newborn infants of all gestational age groups that DCC could be beneficial in reducing the necessity for blood transfusion[99].

Stripping of the umbilical cord:

Stripping the umbilical cord at birth from placenta to the infant significantly improves Hb/Hct at birth and reduces the need for PRBC transfusion in preterm infants; it also allays the anxiety of reduced access and delay in resuscitation during DCC in preterm infants[100]. The evidence to evaluate whether outcomes are improved by umbilical cord milking or stripping comes from a small randomized controlled trial[100,101]. In this study, premature infants (24-28 weeks gestation) were randomly assigned to immediate umbilical cord clamping (control group, n=20) or cord milking (milking group, 2-3 times over ~ 6 seconds) before clamping (n=20). The cord milking group had a decreased likelihood of receiving a transfusion

during the hospital stay (RR 0.5) and received fewer transfusions (1.7 vs. 4.0, p=0.02). In another randomised trial of 58 deliveries of premature infants (<33 weeks of GA) Rabe *et al* demonstrated that the clinical benefits of umbilical cord stripping to infants are comparable to delayed cord clamping[102].

Admission laboratory blood tests from placenta:

Routinely blood is taken from all VLBW infants soon after admission to NNU for blood culture (0.5 - 1 ml), blood gas, urea and electrolytes, blood sugar, calcium and phosphate, liver function test and full blood count (1-1.2 ml). Frequently for sicker infants other blood tests are also performed on admission (e.g. coagulation profile: 1.2 ml). In a small case controlled study (n=10) Carroll et al demonstrated the feasibility of taking blood from umbilical vein of the clamped placental end of the cord for all routine admission blood tests. Using this method, no blood was needed to be initially taken from the infant. They demonstrated that this resulted in a lower incidence of IVH (p=0.01), higher haemoglobin level at 24 h and fewer early blood transfusions (p=0.02)[103]. However, the sample size of this study is too small to substantiate the findings; this need to be confirmed by larger randomised controlled trials before routine clinical implementation.

Autologous umbilical cord blood transfusion:

The placenta has a large reservoir of residual fetal blood following delivery. Linderkamp et al demonstrated decreased residual placental blood volume from 52±8 ml/kg of neonatal body weight after early cord clamping to 15±4 ml/kg after delayed cord clamping[87]. Salvaging this blood and storing this for autologous

transfusion for sick infants could be beneficial; as this will exclude the issues of donor blood related foreign antigens or infectious agents[104-107]. The process of harvesting cord blood from term pregnancies for stem cell transplantation has been successful and popular[108]. Although this method is feasible, associated problems such as insufficient volumes collected, clotting, haemolysis, infection risk and high costs have made it not feasible for clinical application[109,110].

Reducing phlebotomy losses and using point-of-care test analysers:

Attempts have been made to decrease blood sampling by sending fewer specimens for laboratory tests, micro-sampling methods, using in-line blood gas and chemistry monitors in premature infants[19]. Bed-side blood analysers introduced in the 1990's reduced phlebotomy losses, modern inline analysers and point-of-care blood test analysers have further helped to obtain required laboratory information while drawing less blood [111,112]. Findings from a recent study indicated that using multi-parameter point-of-care analyser dropped transfusion rates for VLBW newborn infants by 48% (1.57 vs. 2.53, $p<0.01$), with fewer transfusions per transfused infant, and an 8.3% cost reduction [113]. Technological advances in recent years have allowed in newer laboratory analysers, point of care testing devices and transcutaneous measurements which has resulted in measurements to be done in smaller and smaller volumes of blood and perhaps with no blood in the future [19,112-114]. However, these technologies comes with a large price tag, need training for operators and may take longer to be commercially available to neonatology in future.

Recombinant erythropoietin (rEpo):

A Cochrane meta-analysis of *early* rEpo usage[84] in preterm infants concluded a reduction in number and volume of PRBC transfusions but this is of limited clinical importance. A recent randomised controlled study using Darbepoeitin and Erythropoietin early in life in premature infants has shown reduction in rate of transfusion[83]. Romagnoli et al suggested the association of early use of rEpo with ROP (>stage 2)[115] and this was further concurred by 6 studies enrolling 930 infants with significant rise in ROP (>stage 2). A Cochrane analysis of *late* rEpo usage concluded a reduction of transfusions (RR 0.66; 95% CI 0.59 to 0.74). However, as with studies on early rEpo usage, the clinical significance of one or two fewer late PRBC transfusions are questioned[85]. Hence rEpo is still not considered to be an important substitute of PRBC transfusion in preterm infants.

Implementing restrictive transfusion guideline:

Significant variability exists in transfusion rates, within individual NNUs and between NNUs in the same geographic area[116,117]. Parents also insist on less transfusion and want to wait until this is absolutely necessary. Restrictive transfusion practice in preterm infants also reduces their exposure to multiple donors. Christensen et al demonstrated a significant decrease in transfusion rate and financial costs (saving of $780,074 over 12 months) in 4 NNUs following implementation and strict adherence (increase in compliance from 65% to >90%) to restrictive transfusion guideline[118]. During the 12 months following implementation of new guideline, compliance remained >95% every month

accompanied by yet lower transfusion rates with no change in the infant outcomes [119].

1.1.8. Summary of current practice of blood transfusion

Blood transfusion is considered to be life-saving in an acutely bleeding infant, but the optimal timing and triggers of transfusion has remained elusive. Several randomised controlled trials have examined the effects of transfusion at different threshold levels of Hb and Hct but the benefits remained unclear. Over the years there is increasing speculation that although there are several benefits of transfusion, it might invite unintended risks. Transfusion may be associated with increased risks of IVH, ROP and BPD. There is a possible risk association with NEC but no direct causal relationship has been established so far. It is still not clear whether withholding of feeds during transfusion will reduce the incidence of TANEC.

Various strategies can be used in combination to minimise frequency of blood transfusion in preterm infants. Lately, DCC or milking of cord has been recognised as an important tool to increase blood volume and minimise neonatal transfusion. The clinical advantage of early as well as late rEpo usage is questionable. Despite ambiguity of benefits of liberal and restrictive blood transfusion criteria in the large clinical trials there is a trend towards implementing a restrictive approach towards blood transfusion.

1.2. Assessing the need for blood transfusion

1.2.1. Introduction

Blood transfusion guidelines are subjective and vary between neonatal units; it does not identify or target specific patient needs [22,23]. Clinicians rely on clinical features, such as tachycardia, apnoea or oxygen desaturation episodes, respiratory support along with traditional laboratory markers, haemoglobin (Hb) and haematocrit (Hct), to determine when their patients require a blood transfusion[25,29]. This approach is nonspecific, as a change in vital signs or symptoms that often prompts a blood transfusion in the neonatal unit (NNU) can be due to other clinical conditions [30,28]. A selective marker that reflects oxygenation at a cellular level would be ideal to assist clinicians in determining transfusion needs.

1.2.2. Laboratory measurements and blood transfusion

1.2.2.1. Serum Lactate

Serum lactate is an end product of strained anaerobic cellular metabolism and is elevated in tissue hypoxia, hypoperfusion or injury. Serum lactate has been investigated by researchers as a marker of tissue perfusion and its changes following blood transfusion in newborn infants [46,120,121] (**Table 4**).

Table 4: Serum lactate and blood transfusion

Study reference	Patient characteristics	Measurements	Findings
Ross et al (1989)[56]	16 preterm infants (≤32 weeks of gestational age, chronological age 1 to 3 months) with Hct ≤0.29 scheduled to be transfused, were randomised to blood transfusion vs. no transfusion	Clinical: HR, apnoea/bradycardia Laboratory: lactate, serum erythropoietin	PRBC transfusion significantly reduced HR (mean 6±2 bpm). Mean (SD) lactate significantly decreased from 1.6 (0.4) to 0.9 (0.3) µmol/g following blood transfusion.
Moller et al (1996)[120]	Studied 56 anaemic preterm infants (mean gestational age 31.8 weeks and chronological age 29.9 days) Group 1: asymptomatic anaemic infants (n=37) Group 2: symptomatic anaemic infants who received blood transfusion (n=19)	Clinical: HR, capillary refill, SaO_2 Laboratory: Hb, lactate Ultrasound: Cardiac output, oxygen delivery	There was no correlation between Hb and serum lactate in 56 infants studied Mean serum lactate significantly reduced from 3.23 mmol/l to 1.71 following blood transfusion Serum lactate negatively correlated with oxygen delivery in infants who received transfusion
Frey et al (2001)[122]	Studied 18 anaemic preterm infants [median (range) gestational age 29.7 (24 – 38) weeks and	Clinical: HR, RR, weight gain (g/week) Laboratory: Hct, pH, lactate, reticulocyte count	Mean (SD) lactate significantly decreased from 2.5 (1.0) to 1.7 (0.5) mmol/l following PRBC transfusion

	chronological age 35 (10 – 74) days]		No correlation between pre-transfusion lactate and pre-transfusion Hct, heart rate, respiratory rate and weight gain
Takahashi et al (2009) [121]	Retrospective study of 12 VLBW infants [mean(SD) gestational age 26 (2) weeks, chronological age 68 (35) days]	Clinical: HR and SaO_2 Laboratory: Hb, lactate	There was no significant correlation between pre-transfusion lactate and Hb Mean (SD) lactate significantly decreased from 2.9 (1.1) to 2.1 (0.9) mmol/l following PRBC transfusion

HR: Heart rate; SaO_2: saturation of oxygen; Hb: Haemoglobin; Hct: Haematocrit; PRBC: packed red blood cell; SD: standard deviation; SIRS: Sudden Inflammatory response syndrome

In an adult series of 29 patients with Systematic Inflammatory Response Syndrome (SIRS), Mazza et al[123] demonstrated an increase in haemoglobin levels following blood transfusion, this was not accompanied by a significant change in lactate levels, [mean (SD) 1.87 (1.22) pre-transfusion to 1.56 (0.28) mmol/l post-transfusion, p=0.28]. In a randomised study of 16 preterm infants Ross et al demonstrated that blood lactate may be useful to identify preterm infants who will benefit from blood transfusion[56]. It has been demonstrated that pre-transfusion serum lactate level decreases significantly following blood transfusion [120-122]. The site of blood sampling was not consistent in these studies. However, Frey et al demonstrated that capillary whole blood lactate agrees well with arterial values in newborn infants [122]. There was no correlation between pre-transfusion Hb and serum lactate in any of these studies [120,122]. The mean pre-transfusion serum lactate levels ranged from 2-4 mmol/l in these studies, which on its own was not clinically significant. Capillary lactate levels could also vary depending on tissue perfusion factors such as cardiac output and stroke volume in addition to haematological parameters such as haemoglobin (Hb) and haematocrit (Hct).

Serum lactate is a non-specific marker of cellular biochemistry and metabolism, which depends on tissue perfusion and oxygenation. Clinical conditions like congenital cardiac lesions, neonatal sepsis, poor ventilatory status and use of inotropes such as Adrenaline results in elevated serum lactate. Serum lactate may also be elevated during the first 24 to 48 hours of life in infants who suffered acute perinatal hypoxia as well as those with chronic placental insufficiency. Serum lactate is a non-specific marker of anaemia and on its own may not provide any added information to decide the requirement of blood transfusion in the preterm neonates.

1.2.2.2. Haemoglobin, Haematocrit and Reticulocyte count

Neonatal blood transfusion guidelines[26,35] rely heavily on Hb or Hct as an index of transfusion. Using Near Infra-Red Spectroscopy (NIRS) Van Hoften *et al* demonstrated that in premature infants pre-transfusion Hb correlated reasonably well with cerebral oxygenation (Spearman rank order test: $r=0.414$, $p<0.005$), and infants with Hb <9.7 g/dl showed significant increase in cerebral oxygenation following blood transfusion[51]. However, other researchers failed to demonstrate relationship between pre-transfusion Hb or Hct and cerebral, gut or peripheral tissue oxygenation. Bailey et al reported no correlation between pre-transfusion Hb and cerebral ($r = - 0.01$, $p=0.98$) or gut ($r = - 0.26$, $p=0.17$) tissue oxygenation [53]. In another study by Seidel et al pre-transfusion Hct level did not correlate with either the pre-transfusion cerebral ($r = - 0.09$; $p=0.45$) or peripheral regional tissue oxygenation ($r = - 1.14$; $p = 0.22$)[54]. Wardle et al demonstrated a very weak correlation between pre-transfusion Hb and peripheral fractional oxygen extraction (pFOE) in preterm infants [124]. This indicate that tissue oxygenation is not entirely dependent on Hb or Hct. Various other factors such as blood flow, blood pressure, fetal Hb percentage, acid base status, pCO_2 levels, vascular resistance, vascularity of tissue and metabolism can all influence tissue oxygenation. Hence, relying only on Hb or Hct level as a surrogate of tissue oxygenation may not be accurate.

Pre-transfusion Hct has a poor correlation with echocardiographic parameters such as stroke volume (SV), left ventricular output (LVO), left ventricular end systolic diameter (LVESD) and left ventricular end diastolic diameter (LVEDD) [49]. None of these studies demonstrated a threshold of Hb or Hct at which tissue perfusion or

oxygenation is compromised. The findings of these studies demonstrate that the current pre-transfusion Hb and Hct do not necessarily reflect tissue perfusion and oxygenation.

Reticulocyte count is a marker of bone marrow response to anaemia, and this alongwith Hb or Hct may be useful in deciding the need for PRBC transfusion. Some transfusion guidelines such as Canadian Paediatric Society recommend reticulocyte count <100 /µl (<5%) alongwith low Hb or Hct as a trigger for blood transfusion[16,125,126]. However, evidence on using reticulocyte count as an indicator of blood transfusion is lacking.

1.3. Doppler Ultrasound Scan

1.3.1. Introduction

In recent years, the capabilities of ultrasound flow imaging in assessing neonatal haemodynamic status have increased enormously. Bedside colour flow imaging is currently used in majority of tertiary neonatal units and due to its versatility and convenience its application has been extended to more demanding measurements in order to try and examine increasingly subtle changes in the fetal and neonatal circulations. It is also important to be aware of the various factors that affect the Doppler signal in a colour flow image or a Doppler sonogram.

1.3.2. Basic principles

In this section, some basic concepts of ultrasound scan are defined and explained[127].

The Nature of Ultrasound

A sound wave of upto 20 kHz is audible to human ear. Ultrasound is a form of mechanical vibration or sound wave where the frequencies are more than the human audible range. For medical diagnostics, the frequency of ultrasound scanners typically range from 1 to 30 MHz and it follows the same physical laws as a sound wave as discussed below[127].

Velocity

Velocity of a wave is one of its basic properties and is dependent on the density and temperature of the medium it propagates through. The velocity of a sound wave in the human body is considered to be 1540 m/s[127].

The relation between velocity and frequency is expressed in the equation below:

Frequency

Frequency of a wave is described as the number of times (cycles) the wave returns to the baseline per second, expressed as cycles per second or Hertz (Hz).

Wavelength

Wavelength is another basic property of sound wave which is the total distance traversed by the wave on returning to the same relative position (**Figure 1**). Since the velocity of the waveform in a medium is constant, as the wavelength increases, the frequency will decrease.

Amplitude

Amplitude is described as the extent of maximum deviation of the waveform from the baseline (**Figure 1**).

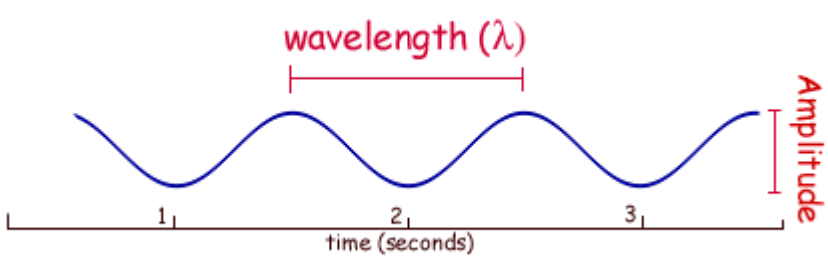


Figure 1. Basic properties of sound wave: wavelength, frequency and amplitude

1.3.3. Modes of Ultrasound scan [127]

M-mode

The M-mode (motion mode, one dimension image) was the first ultrasound modality to record moving noises or echoes from the heart, and thus the movement or the motion of the image could be interpreted in relation to myocardial and valvular function. This is regularly used to measure size of cardiac chambers and diameter of blood vessels which is an important component for measurement of blood flow volume such as stroke volume and right ventricular output.

2-dimensional (2D) imaging

A 2D image is built up by firing an ultrasound wave (beam) vertically, waiting for the return echoes, maintaining the information and then firing a new line from a neighbouring transducer along the line of the ultrasound probe.

Doppler Ultrasound Scan

In ultrasound imaging, echoes received from most tissues will be at the same frequency as the transmitted beam. However, if echoes received are from tissues or blood cells that are moving, the transmitted and received frequencies will not be the same. This “shifted” frequency can be used to determine the relative velocity and the direction of these moving tissues. This effect is known as the ***Doppler Principle***. The movement toward the transducer results in a higher received frequency (conventionally shown as red) and movement away in a lower received frequency (conventionally shown as blue).

The size of the Doppler signal is dependent on:

(1) *Velocity of blood:* as velocity increases, so does the Doppler frequency

(2) *Ultrasound frequency:* higher ultrasound frequencies give increased Doppler frequency. As in 2D-mode, lower ultrasound frequencies have better penetration. The choice of frequency is a compromise between better sensitivity to flow or better penetration.

(3) *The angle of insonation:* the Doppler frequency increases as the Doppler ultrasound beam becomes more in line with the flow direction

Pulse Wave Doppler

In Pulse Wave Doppler, a single ultrasound wavefront is repeatedly fired. Echoes reflected from moving structure, including blood cells, experience a Doppler shift in frequency. The echo information obtained within the ***sample volume*** is analysed using the Doppler equation. From this information, the blood velocity can be determined. This allows for measurement or sampling to be done at the correct position of the flow. This is especially important where the blood flow velocity is not uniform and is limited by change in direction, motion of other structures such as valves and shunts such as patent ductus arteriosus. The frequency data is converted to velocity, and displayed in a moving strip format on the monitor. The highest detectable velocity is half of the rate at which the ultrasound lines are fired; this is known as ***Nyquist Limit***.

Continuous Wave (CW) Doppler

With CW Doppler, the waves are transmitted and information is received simultaneously from the probe. This overcomes the maximum velocity limit, but the

exact place along the ultrasound line from where the velocity data is obtained cannot be determined (no range resolution). CW Doppler is used in diagnosing abnormalities in which range resolution is not important such as aortic outflow tract or when the sonographer is interested in the quantification of high velocity jets such as tricuspid regurgitant jets.

Aliasing

Pulsed wave systems suffer from a fundamental limitation known as aliasing. When pulsed waves are transmitted at a given sampling frequency (known as the pulse repetition frequency); the maximum Doppler frequency that can be truly measured is half the pulse repetition frequency. If the blood velocity and beam/flow angle being measured combine to give a value greater than half of the pulse repetition frequency, ambiguity in the Doppler signal occurs. This ambiguity is known as aliasing.

1.3.4. Validation of Doppler ultrasound scans

1.3.4.1. Cerebral Doppler scan measurements

Other than near infra-red spectroscopy (NIRS), ultrasound being a bedside tool is the most convenient form of cerebral hemodynamic monitoring in critically ill neonates. Cerebral blood flow velocity (CBFV) can be measured in newborn infants

by monitoring the frequency shift of acoustic waves that scatter from moving red blood cells in the large cerebral arteries using trans-cranial Doppler (TCD) [128].

In 42 preterm infants (28-33 weeks of gestational age) Greisen et al measured mean cerebral blood flow using continuous wave and range-gated Doppler ultrasound scan examination of anterior cerebral and internal carotid artery and compared this with ^{133}Xe clearance. The pulsatility index, mean blood flow velocity and the end diastolic velocities were measured from the Doppler recordings. The correlation coefficients between the Doppler and ^{133}Xe measurements ranged from 0.41 to 0.82. The correlation coefficients were consistently higher for the range gated compared to the continuous wave Doppler, and was lower for the pulsatility index than for the mean flow velocity and end-diastolic flow velocity[129]. This indicates that range-gated Dopplers are more reliable than continuous waves in measuring mean and end-diastolic blood flow velocities in small cerebral arteries. Miles et al investigated six ultrasound measures of blood flow: Pourcelot's pulsatility index, Gosling's pulsatility index, area under curve, systolic, diastolic and mean amplitude. The measurements of an in vitro arterial model were compared with measurements from anterior cerebral arteries in 33 newborn infants with a diagnosis of either asphyxia or intra-ventricular haemorrhage or were normal stable infants. All the measures in the neonates showed excellent correlation with blood flow in the arterial model. The highest accuracy was obtained for pulsatility indices[130]. It can be inferred that pulsatility index measured by Doppler ultrasound scan correlates well with in-vitro models.

Traditionally, perfusion in the middle cerebral, anterior cerebral, and/or internal carotid arteries is monitored to assess global brain perfusion. The parameters measured with trans-cranial Doppler (TCD) include peak systolic, mean, and end diastolic velocities (i.e., PSV, MV, and EDV, respectively). The angle of insonation used to measure velocities with TCD may affect the values of PSV, MV and EDV [131]. The resistance index is defined as:

$$Resistance\ index\ (RI) = \frac{\text{Vmax systole} - \text{Vmin diastole}}{\text{Vmax systole}}$$

The pulsatility index is defined as:

$$Pulsatility\ index\ (PI) = \frac{\text{Vmax systole} - \text{Vmax diastole}}{\text{V mean}}$$

RI is a ratio that reflects cerebrovascular resistance. The sequence of changes in PSV, EDV, MV, and RI after birth in healthy infants has been well documented[132-134].

With additional information about the cross-sectional area (A) of the insonated vessel, cerebral blood flow velocity (CBFV) permits calculations of arterial cerebral blood flow (CBF) using the formula:

CBF = CBFV x A.

But, cerebral vessels are small in size, making their diameter difficult to measure[135]. To minimise this source of error relative changes in flow could be measured. In addition, these blood vessels often change calibre over time, leading to large errors in calculations of relative change, which in turn cause errors in estimates of the amount of oxygen and nutrients delivered to the surrounding tissue[136-138]. Although,

TCD measures cerebral blood flow velocity in the large vessels that supply perfusion to the brain; measuring regional variations in cerebral blood flow using TCD is not possible.

1.3.4.2. Superior vena cava Doppler scan measurements

One of the major problems in estimating systemic blood flow in neonates is the presence of intra-cardiac (patent foramen ovale) and extra-cardiac (patent ductus arteriosus) shunts at birth. This impairs use of left ventricular output (stroke volume) and right ventricular output as a marker of systemic blood flow and organ perfusion as it can lead to overestimation of the real systemic blood flow by up to 100%. Systemic blood pressure on the other hand does not correlate with systemic or organ perfusion[139-141]. Blood from the upper body drains to the superior vena cava (SVC), 80% of this flow is from brain[137]. This venous flow is unaffected by shunting that occurs both at ductal and atrial levels in preterm infants in the first few days of life and is a direct estimate of cerebral perfusion.

The use of Doppler ultrasound to measure cardiac output and flow in vessels is a well-established and validated technique in neonates[142-144]. Doppler echocardiography of the SVC has been used previously to assess the function of the right heart in adults[145-147] and children[148,149], and to understand the haemodynamics of bidirectional cavo-pulmonary anastomosis[150]. Using thermo-dilution or dye-dilution method to measure SVC flow in neonates is difficult. But a validation of SVC flow was possible with good correlation to left ventricular output in

neonates with a closed duct, which is a true representation of systemic blood flow in those babies[144]. Using cine magnetic resonance mapping in healthy adults Mohiaddin et al have reported that SVC flow was 35% of the cardiac output[151] which is similar to the proportion of mean SVC flow to LV output measured by Doppler in preterm infants with a closed duct [152]. Salim et al reported the changing pattern of the SVC flow in proportion to cardiac output in children[150] and this was again similar to the flow found in newborn term infants by Evans et al[135].

But there are certain limitations of SVC flow measurement. Kluckow et al[152] reported median intra-observer variability 8% and inter-observer variability 14%, which is comparable to other studies measuring reproducibility of Doppler techniques[153]. One of the major pitfalls of the measurement is the variability in the diameter of the SVC through the cardiac cycle, the average variability reported is 22% compared to 8-15% for the main arteries[152]. Another limitation is the diameter of the vessel is not uniform as noted in MRI studies of SVC; the cross section of the SVC assumes the shape of a sickle wrapped around the ascending aorta and hence at times could be difficult to estimate the diameter of the vessel[154]. Also in infants who are ventilated may have a distended lung field it might be difficult to visualise the SVC in the true para-sternal view resulting in difficulty to measure the diameter. Another potential problem is the normal variation of persistent left sided SVC which drains into the coronary sinus (0.3% of general population) which can amount to an underestimation of the SVC flow by 50%[155].

1.3.4.3. Splanchnic circulation Doppler scan measurements

The superior mesenteric artery (SMA) supplies the majority of the intestine from the second part of duodenum to the junction between the right two-third and left one-third of the transverse colon [156]. Hence, this is the major area of the intestine which is important in terms of absorption and abdominal pathology in a premature neonate. The majority of the cases of necrotising enterocolitis (NEC) a serious condition in preterm infant happens in this area of the intestine[157]. Measuring the SMA blood flow velocity can be helpful in assessing the haemodynamic status of small gut, and thereby may help in identifying early signs of reduced blood flow and vascular insufficiency of the intestine. Goldberg et al used transcutaneous Doppler to measure the aorto-mesenteric angle and size of the artery[158]. Animal studies and human studies using dye dilution techniques [159] have been used in the past to understand the flow of blood in the SMA. Later on SMA blood flow was also measured in an animal study using the spill-over angiographic reflux method [160] and the video-dilution technique[161]. Both of these required arterial catheterisation and injection of dye. The use of Doppler ultrasound scan in measuring arteries in the splanchnic circulation was attempted in 1982 in humans[162]. Qamar et al used a Duplex scanner to measure SMA blood flow velocity in 70 healthy human volunteers and found the mean coefficients of variability of the measurements as 6.8% over short term and 8.2% over long term[163]. Animal models such as a canine bleed-out model compared the difference between directly measured abdominal aorta blood flow and the same measured by a locally implanted pulsed Doppler flowmeter, where Doppler seems to overestimate by only 2%[164]. In another in vivo study of a pig model, abdominal aorta blood flow velocity measured by transcutaneous Doppler was well correlated with simultaneous electromagnetic measurements

(r=0.91)[165]. Canine SMA blood flow velocity measurements using an electromagnetic method was comparable to the transcutaneous Doppler method and had a similar variability as obtained by spill over technique[166]. The animal as well as the human studies indicates that the measurement of SMA blood flow velocity using Doppler ultrasound scan is feasible and reliable. However, various factors such as invasive ventilation, CPAP, feeds, presence of PDA, and coarctation of aorta or left ventricular outflow tract obstruction, antenatal redistribution of flow in umbilical artery or IUGR and medications such as Ibuprofen may all have important influence on the SMA blood flow velocity.

1.3.5 Doppler USS and blood transfusion

Doppler ultrasound scan can reliably measure changes in blood flow to various organs following blood transfusion and its effect on systemic and regional haemodynamics. However, the current Doppler measurements cannot be applied as a trigger of blood transfusion in clinical practice. Details of studies that investigated organ perfusion following blood transfusion using Doppler ultrasound scan are presented in **Table 5**.

Table 5. Blood transfusion and organ perfusion measured by ultrasound scan

Study	Infant characteristics	Measurements	Findings
Alkalay et al (2003)[49]	Studied 32 stable anaemic preterm infants [median (IQR) GA 29 (28, 30), PNA 33.3 (31.9, 34.9)] Group 1: Low Hct (≤21%) Group 2: Mid Hct (22-26%) Group 3: High Hct (≥27%) Blood was transfused in two aliquots of 10 ml/kg each 12 hours apart	SV, LVESD, LVEDD and CO measured on 4 occasions: 1-3 hours before, 2-4 hours after 1st aliquot, 4-7 hours and 24 -37 hours after the 2nd aliquot Hct	There were no difference in the measured echo findings before and after blood transfusion in aggregate as well as Hct subgroup analysis Infants with low and mid-range Hct had significantly high SV (p=0.03), LVESD and LVEDD (p=0.003) compared to those with high range Hct
Dani et al (2002)[48]	Studied 14 anaemic preterm infants [mean (SD) GA 29.6 (22.6), PNA 29 (14) days]	Cerebral Doppler USS of pericallosal artery: PSV, DV and RI before and after blood transfusion	Diastolic velocity decreased and RI increased following blood transfusion PSV and V_{mean} did not change following blood transfusion
Nelle et al (1994)[47]	Studied 33 anaemic preterm infants [mean (SD) GA 29 (5)	Hct, blood viscosity (capillary viscometer) SV, CO	SV decreased from 2.28 (0.57) ml/kg to 2.14 (0.46) (p=0.05) post-transfusion alongwith a decrease in HR

	weeks and PNA 48 (21) days	ICA, ACA and coeliac trunk flow velocities Systemic flow resistance: blood pressure to cardiac output ratio	resulting in a significant reduction in CO (p=0.005) Blood flow velocities decreased in ACA by 23%, ICA by 8% and coeliac trunk by 12% (p=0.05) following transfusion Blood viscosity increased by 33% and systemic flow resistance by 23% following transfusion
Krimmel et al (2009)[75]	Studied 22 anaemic preterm infants [mean (SD) GA 27.3 (2.3) weeks and PNA 31.2] Stratified by weight on the day of transfusion>/<1250 g and randomised to fed or unfed during transfusion	SMA flow velocities measured 4 times: Pre-transfusion: pre and post-prandial Post-transfusion: pre and post-prandial	Pre-transfusion SMA PSV (p=0.02) and V_{mean} (p=0.01) increased significantly in response to feeding but this was not noticed in the post-transfusion state. Similar finding was noticed in subgroup of infants weighing >1250 g but not in infants <1250 g. The above response was similar in infants randomised to fed or unfed during transfusion. SMA PSV and V_{mean} changes in response to feeding were similar between formula (n=7) and breast milk (n=15) fed infants

SV: stroke volume; LVESD: left ventricular end systolic diameter; LVEDD: left ventricular end diastolic diameter; CO: cardiac output; BT: Blood transfusion; RI: resistance index; ICA: internal carotid artery; ACA: anterior cerebral artery; BA: basilar artery; SMA: superior mesenteric artery; V_{mean}: mean velocity; PSV: peak systolic velocity; DV: diastolic velocity

1.4 Near Infra-Red Spectroscopy (NIRS)

1.4.1 The principles of Near Infra-Red Spectroscopy

NIRS operates on two basic principles:

a) The biological tissue is relatively transparent to light in the near infra-red region of the light spectrum (700-1000 nm), *and*

b) In tissue, there are compounds known as chromophores whose absorption of light is oxygenation status dependent

Amongst the various electromagnetic waves, light within the visible spectrum (wavelength 450-700 nm) is not able to penetrate biological tissue to a depth greater than 1 cm because of attenuation as a result of powerful absorption and scattering by the tissue constituents. Studies have reported that at near infra-red (NIR) wavelengths (700-1000 nm) the absorption of light is significantly lower in biological tissues and it can penetrate tissues upto a depth of 8 cm [167]. Biological tissues e.g. bone appear transparent allowing examination of substantial region of the tissue.

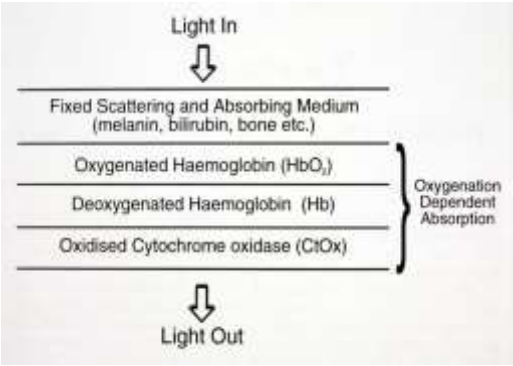


Figure 2: **Different light absorbing and scattering compartments within tissue** *(With permission from C. Elwell: 1995 The physical principles of tissue spectroscopy. A practical users' guide to near infrared spectroscopy)*[168]

Thereby, NIRS can monitor the changes in concentration of those compounds which are present in tissue in significant concentration and whose absorption characteristics in the NIR region are well defined (**Figure 2**).

The two main properties of light when travelling through tissues are scatter and absorption, the amount of each of these two properties depend on the wavelength of the light and the nature of tissue illuminated [169]. These and other properties of light waves are discussed in more details below.

1.4.2 Light waves

The only electromagnetic waves that are visible to human are the visible light spectrum (400 – 700 nm). Red has the longest wavelength and violet the shortest (**Figure 3**). The sun as well as the light bulb is a source of visible light waves.

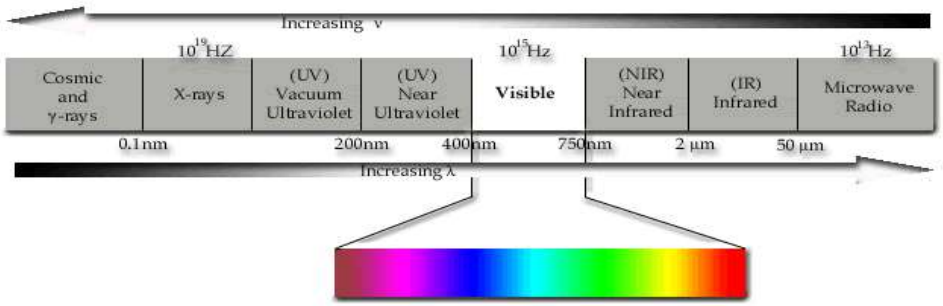


Figure 3. Various electromagnetic waves and visible spectrum

<u>Infra-red and NIR waves:</u> Infra-red light lies between the visible and microwave portions of the electromagnetic spectrum. The infrared wavelengths closer to the visible spectrum are called the 'Near Infra-red (NIR)' light and closer to the

microwave region are called the 'Far Infra-red' light. Infra-red radiation had wavelengths between about 700 to 1000 nm.

1.4.3 Absorption of light

When incident on a particular tissue; light undergoes a process of absorption, attenuation, reflection, refraction, diffraction and scattering. If we consider a laboratory model of a cuvette containing a non-absorbing medium and dissolve an absorbing compound with **concentration c** (**Figure 4**), the amount of light this compound absorbs will depend upon the wavelength of the incident light. This wavelength dependent absorption is described by the absorption spectrum of the compound, in which the **specific extinction coefficient of the compound (α)** is expressed as a function of wavelength[168].

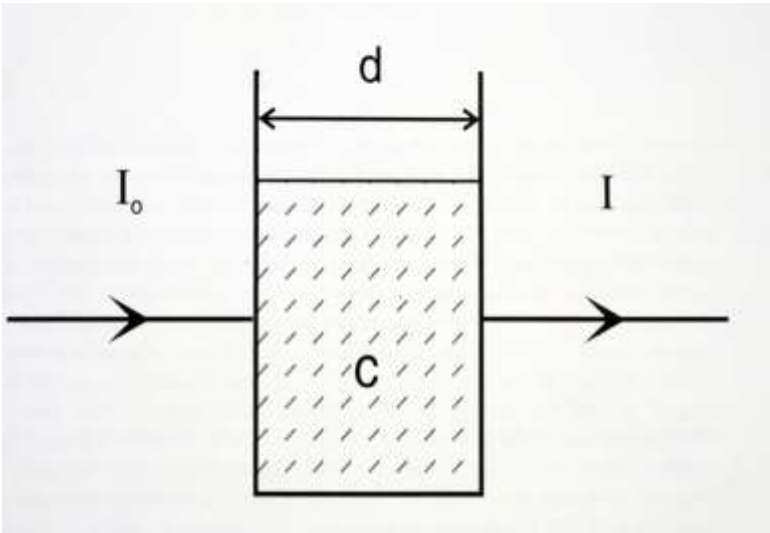


Figure 4: The cuvette model for absorption.
An absorbing compound 'c' is dissolved in a non-absorbing medium.
(With permission from C. Elwell: 1995 The physical principles of tissue spectroscopy. A practical users' guide to near infrared spectroscopy)[168]

Since the solution does not scatter any light, the light travels in a straight line covering a **distance** otherwise known as **optical pathlength (d)**. The intensity of the light incident on the solution is $\mathbf{I_0}$ and the intensity of light transmitted through the solution is **I**. **I** is less than $\mathbf{I_0}$ because some of the light has been absorbed or attenuated by the compound in the solution. This loss of light intensity (**attenuation**) is usually measured in units of **optical density (OD)** and can be described using the **Beer-Lambert Law**.

The Beer Lambert law states that for a light absorbing compound dissolved in a non-absorbing medium, the attenuation of light incident is proportional to the concentration of the compound in the solution (c), the specific extinction coefficient of the compound (α) and the optical pathlength (d), and their relationship can be demonstrated using the following equation[168,170]:

$$A = \log \left[\frac{I_0}{I}\right] = \alpha.c.d$$

Where A = attenuation measured in OD

I_0 = the light intensity incident on the medium

I = the light intensity transmitted through the medium

α = specific extinction coefficient of the absorbing compound measured in $\mu molar^{-1}.cm^{-1}$

c = the concentration of the absorbing compound in the solution measured in µmolar

d = distance between the points the light enters and leaves the solution measured in cm

N.B. Optical density is a logarithmic unit (base 10) indicating the measure of number of orders of magnitude the light intensity is reduced when traversing the medium.

The product of α.c is known as the absorption coefficient of the absorbing medium (μ_a). Therefore, the above equation can also be expressed as:

$$A_n = ln[\frac{I_0}{I}] = \mu_a.d$$

Where A_n is the natural attenuation since μ_a is expressed in natural logarithmic units.

The specific extinction coefficient indicates the level of absorption per µmol of the compound per litre of solution per cm. μ_a is used to represent absorption coefficient per centimetre (cm^{-1}). The term extinction coefficient is expressed using base 10 logarithmic units (*log*) and absorption coefficient using natural logarithmic units (*ln*).

In a solution or tissue with multiple absorbing compounds, the overall extinction coefficient is simply the sum of the contributions of each compound.

$$A = [\alpha_1.c_1 + \alpha_2.c_2 + \alpha_3.c_3 + \ldots\ldots\alpha_n.c_n].d$$

1.4.4 Absorbers in tissue

A chromophore is a compound which absorbs light in a certain spectral region. Each chromophore has got its own particular absorption spectrum (extinction coefficient against wavelength). The chromophores of interest for NIRS measurements are

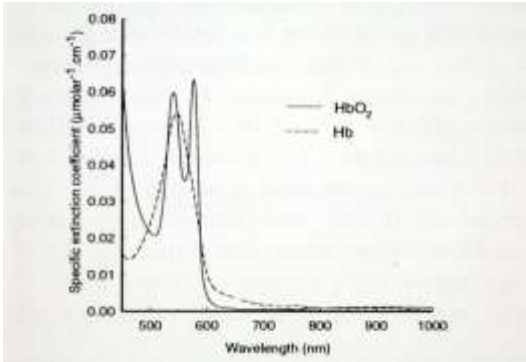
those whose absorption varies with oxygenation status. It is also important to be aware of the role of other absorbers whose concentration is likely to remain fixed but still contributes to the total loss of light in tissue.

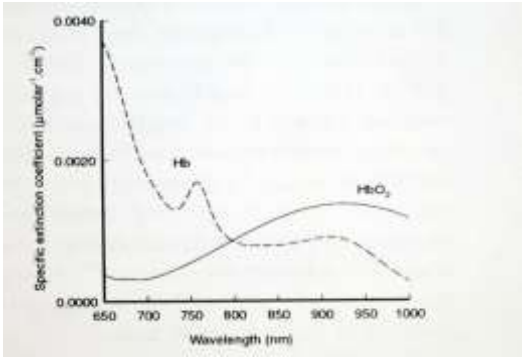
Water:

The absorption of light by water is relatively low between 200-900 nm. Above 900 nm, absorption of light starts to rise with increasing wavelength, reaching a spectral peak at 970 nm. The living tissue has high water content, such as 80% in adult brain tissue [171]. As water acts as a fixed constant absorber, for the purpose of clinical measurements water concentration in the tissue can be considered to be constant.

Haemoglobin:

The most important absorbers within the 'window' of transparency are the haemoglobin group. The absorption spectra of HbO_2 and HHb in the wavelength range 450-1000nm have been extensively studied (**Figure 5**). The difference in the absorption levels between the two compounds in the visible range is noticeable; which explains the well-recognised phenomenon of arterial blood being bright red and venous or deoxygenated blood being more purple or blue. Though the absorption of both chromophores (HbO_2 and Hb) decreases significantly in the NIR spectrum compared to the visible spectrum, it still remains significantly different allowing spectroscopic separation of the compounds to be possible using only a few sample wavelengths. At 800 nm also known as **isobestic point**, the extinction coefficients of the two compounds are identical, which can be used to calculate the haemoglobin concentration independent of oxygen saturation [172].





(a) (b)

Figure 5: The absorption spectra of oxy-haemoglobin and deoxy-haemoglobin
(a) in the whole spectrum and (b) in the near infra-red region
(With permission from C. Elwell: 1995 The physical principles of tissue spectroscopy. A practical users' guide to near infrared spectroscopy)[168]

1.4.5 Scattering of light

Scattering of light is an important phenomenon caused by the unpredictable variation in refractive index at a microscopic and macroscopic scale. The particle's ability to scatter light is dependent on effective surface area and is called the total scattering cross section $\boldsymbol{\sigma_s}$ expressed in mm^2. The density of the scattering particles within a solution is expressed in (number.ml) and called the number density $\boldsymbol{\rho}$. The scattering coefficient, $\boldsymbol{\mu_s}$, for a medium containing a single type of scattering particle is expressed by the equation[168]:

$$\mu_s = \rho.\sigma_s$$

μ_s is expressed in mm^{-1} and is a measure of likelihood that a photon would be scattered in a given medium.

1.4.6 Spectroscopic measurements of tissue

The fibres which carry the NIR light to and from tissues are referred as the **optical fibres;** these are small cylindrical optodes containing prisms which direct the light on to the surface of the tissue. The distance between the two optodes known as **inter-optode spacing (IOS)** is the chord (straight line distance) rather than the arc between the two points. This assumption is based upon the fact that the light essentially becomes diffuse after a few millimetres of entering the tissue [173].

The chromophores of interest within the tissue, whose concentration vary with oxygenation, are HbO_2, HHb and CtOx. Once d, α and DP are known the change in concentration of the chromophores can be easily computed from the measured change in attenuation (modified Beer Lambert Law)[168].

The absolute concentration of a chromophore cannot be determined due to the effects of light scattering within the tissue. All NIRS measurements due to the effects of light scattering are expressed as absolute concentration changes of a chromophore from an arbitrary zero at the start of the measurement period (baseline). Thus using the NIRS technique the quantified changes in a tissue chromophore/oxygenation can be monitored non-invasively [170,172].

The spectroscopic measurements required for my research are of brain and gut/splanchnic circulation. These will be detailed below:

1.4.7 Spectroscopic measurements of the brain

The quantified changes in the concentration of oxy-haemoglobin (HbO_2) and deoxy-haemoglobin (HHb) in micro molar unit can be measured non-invasively by NIRS techniques. Other parameters such as tissue oxygenation index (TOI: ratio of oxygenated to total haemoglobin in tissue) and tissue haemoglobin index (THI: sum of oxygenated and de-oxygenated haemoglobin in the tissue) can be measured from the changes in HbO_2 and HHb. Using oxygen saturation of arterial haemoglobin, fractional tissue oxygen extraction (FTOE) can be calculated which can give a direct estimate of the oxygen availability and extraction balance. Cerebral blood flow [174] and cerebral blood volume [175] are some of the haemodynamic parameters which can be estimated using these measurements. **Figure 6** shows the experimental set up for spectroscopy measurement across head.

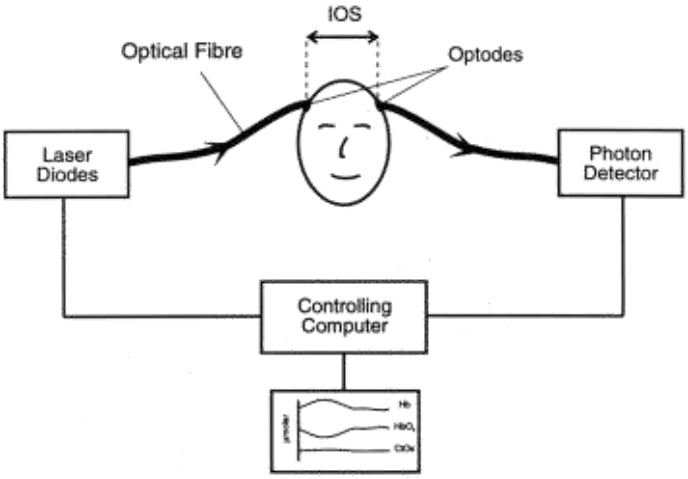


Figure 6: Schematic experimental set up for NIRS measurement across the head

(with permission from C. Elwell: 1995 The physical principles of tissue spectroscopy. A practical users' guide to near infrared spectroscopy)[168]

1.4.8 Spectroscopic measurements of intestine

Similar to brain, the changes in the intestinal HbO_2 and HHb could be measured using NIRS; these measurements can then be used to measure Tissue oxygenation Index (TOI), tissue haemoglobin index (THI) and fractional tissue oxygen extraction (FTOE). The ratio of TOI of intestinal and brain oxygenation (SCOR = splanchnic cerebral oxygenation ratio) has been used by researchers to monitor the need of blood transfusion[176,177]. **Figure 7** shows the experimental set up for measurements of intestinal oxygenation.

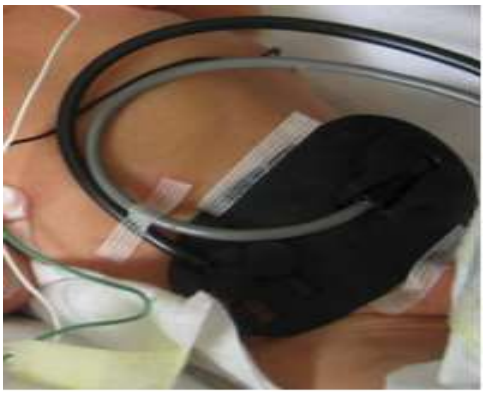


Figure 7. Experimental set up for NIRS measurements of the gut/splanchnic circulation

1.4.9 Types of NIRS devices

Continuous wave NIRS device:

This was the first and the most frequently used NIRS device [168], which uses lasers to generate near infra-red (NIR) light at different wavelengths. One major problem with this technique is that the precise pathlength of NIR light is unknown; it only measures absolute change in the concentration of chromophores and requires

biochemical or haemodynamic changes to occur in tissues for a measurement to be made[178-180].

Intensity modulated NIRS device:

This device also calculates coefficients for absorption and scattering, which are required to make absolute measurements of chromophores [178].

Time resolved NIRS device:

Time resolved spectroscopy (TRS) or time domain spectroscopy has the benefit of measuring the actual pathlength of light, and capable of measuring absolute concentrations of chromophore. This system is expensive, cumbersome and hence not suitable for bedside measurement in a Neonatal Unit[178-180].

Spatially resolved NIRS device:

Spatially resolved spectroscopy (SRS) also known as multi-distance spectroscopy is a NIRS device based on the principle that light intensity is measured at several different source-detector distances thereby enabling better quantification of the chromophores [181]. The SRS system cancels out the chromophore changes in the superficial layers of the tissue illuminated by NIR light, and allows to measure changes in deeper tissue layers[182]. The SRS device is able to measure the ratio of HbO_2 to total haemoglobin (HbO_2 + HHb) and thus tissue oxygen saturation [179] ; this has been reported in the literature as regional saturation (rSO_2) [53] or tissue oxygenation index (TOI) [183]. NIRO 300 is a spatially resolved NIRS device which can be used to measure TOI expressed as a ratio of oxygenated to total haemoglobin in percentage and Tissue Haemoglobin Index (THI) which is an index of a sum of

oxygenated and de-oxygenated haemoglobin in the tissue illuminated by the NIR light. NIRO 300 has a high degree of sensitivity and specificity to intracranial and extra cranial changes[184]. SRS has also got the advantage over TRS of being more portable and user friendly and provides measurements with a high time resolution[185]).

1.4.10 Validation of cerebral NIRS measurements

In practice, validation of NIRS measurements is difficult and limited, because of lack of availability of clinically applicable gold standards. It is also important to understand the ethical principles of research on neonates; validation between non-invasively measured cerebral blood volume (CBV) by NIRS and CBV measured by CT scan or radiolabelled red blood cell dilution is neither practical nor ethical in sick ventilated newborn infants. Similarly, validating non-invasively measured cerebral venous oxygen saturation using NIRS with cerebral venous blood (sagittal sinus or jugular bulb) co-oximeter is not feasible for newborn infants; albeit the various NIRS techniques have been compared with other methods of assessment of tissue oxygenation and haemodynamics.

1.4.10.1 Newborn animal studies

Cerebral tissue oxygenation measurements using NIRS in newborn piglets was found to correlate strongly with weighted mean arterial and sagittal sinus blood haemoglobin saturation ($r=0.9$, $p<0.0001$) [186]. The CBV and CBF measured using NIRS and Indocyanine green (ICG) correlated well with measurements made in newborn piglets using CT scan [187]. The CBV measured in newborn rats [188] and

immature lambs [189] using NIRS was almost identical to measurements by laser Doppler flowmetry and radio labelled indicators (^{125}I-labelled serum albumin and ^{51}Cr-labelled red blood cells) respectively. Excellent agreement has been demonstrated between cerebral metabolic rate of oxygen ($CMRO_2$) measured by the NIRS technique and simultaneous measurements obtained from arterial and sagittal sinus blood samples on piglets [190]. The cerebral venous oxygen saturation measured by NIRS method agreed well with measurements made using superior sagittal sinus blood in newborn lambs[191].

1.4.10.2 Newborn infant and children studies

The cerebral blood flow (CBF) measurement in newborn infants using NIRS technique has been validated with measurement using ^{133}Xenon clearance[192,193]. Measurements of cerebral venous oxyhaemoglobin saturation in children and newborn infants using NIRS correlated well with co-oximetry of jugular venous bulb blood sample[194-197]. The peripheral venous oxyhaemoglobin saturation measured non-invasively using NIRS correlated well with direct measurement performed using peripheral [198] and central [199] venous blood sample.

1.4.11 NIRS measurements of abdomen

Animal studies

A good correlation was demonstrated between the blood flow in the distal ileum and the mid-gut of the small bowel using Doppler ultrasound scan and the tissue

oxygenation of the liver using NIRS [200]. In a study in rabbits, where the mesenteric artery was clamped for 30 minutes, a significant decrease in liver tissue oxygenation index (TOI) was seen after 90 minutes of occlusion of the SMA and is likely to be the consequence of bowel ischemia[201].

Human studies

Teller *et al*[202,203] was the first to use the measurement of tissue oxygenation index (TOI) of the liver as a possible parameter of intestinal flow. They found a decrease of TOI of the liver after feeding a bolus of breast milk. It can be inferred that changes in tissue oxygenation of the liver may reflect changes in the whole splanchnic bed and also, most importantly, changes in oxygen consumption of the liver itself.

Other researchers have studied splanchnic oxygenation and perfusion by measuring splanchnic or abdominal oxygenation using NIRS. They have placed the NIRS probe on the abdominal wall in the hypogastrium to measure splanchnic tissue oxygenation changes. Petros and Fortune were the first to report this method demonstrating a decrease in splanchnic oxygenation in ischaemic conditions such as necrotising enterocolitis (NEC) and hypoxic ischaemic encephalopathy (HIE) in a small case series; they also reported that the ratio between cerebral and splanchnic oxygenation index known as the cerebro-splanchnic oxygen ratio (CSOR) can be used to identify early signs of ischaemia to the gut [177,204]. They reported that CSOR had a 90% (56-100%) sensitivity to detect splanchnic ischemia, indicating that this might be a non-invasive way to detect necrotising enterocolitis early. Dave *et al* looked at ~~the~~ splanchnic tissue oxygenation during feeding and found that splanchnic tissue oxygenation increased during feeding in stable infants, but there

was no associated change in cerebral oxygenation[205]. Bailey *et al* also reported splanchnic cerebral oxygenation ratio (SCOR) to be an important index to inform the decision to PRBC transfusion[176]. It is important to note that all these studies were observational, with small sample size and were not validated against robust standardised techniques. One of the major limitations of measuring splanchnic tissue oxygenation is the structure and properties of the intestine itself. Intestine is a hollow structure, it is constantly mobile with peristalsis and presence of meconium or transitional stool make measurement of splanchnic or intestinal oxygenation very challenging. However, newer techniques and algorithms (such as stool interference algorithm) have shown significant promises [206].

1.4.12 Limitations of NIRS technique

The various limitations of NIRS technique has been detailed below:

1) Measurement of NIRS signals requires ambient light to be blocked from optodes to prevent contamination of the signals that are being measured.
2) An incorrect attachment of the sensor might lead to light escaping and consequent large errors.
3) Heterogeneous tissue cannot be measured if the physical model assumes a homogenous tissue.
4) The different NIRS methodologies show a different degree of susceptibility to movement artefacts; single distance measurements are highly sensitive while multi-distance geometrics are not.
5) The most serious limitation of NIRS recordings is the difficulty in establishing the DPF[178-180].

6) Peristalsis, meconium and transitional stool within the bowel make the NIRS measurements of bowel tricky but not impossible[206].
7) By principle NIRS measurements are relative to the initial baseline and are not absolute measurements. Using ratios such as tissue oxygenation index (TOI) might be a way to avoid this particular problem[168].

1.4.13 NIRS and blood transfusion

The regional tissue oxygen saturation (rSO_2) or tissue oxygenation index (TOI) of various organs and the balance between tissue oxygen supply and demand can be measured using Near-Infrared Spectroscopy (NIRS)[207,208]. Several researchers used NIRS to study the effect of blood transfusion on various tissues to identify a trigger for blood transfusion in newborn infants (**Table 6**).

Fractional oxygen extraction (FOE) reflects the balance between oxygen delivery and consumption. In an observational study of 33 anaemic preterm infants Van Hoften *et al* found PRBC transfusion significantly improves cerebral tissue oxygenation ($crSO_2$) and reduced fractional tissue oxygen extraction (FTOE)[51]. Similarly, Dani *et al* also noticed improvement in cerebral, renal and splanchnic tissue oxygenation and reduction in FTOE following transfusion in symptomatic anaemic preterm infants [52]. Wardle *et al* reported that peripheral fractional oxygen extraction (pFOE) was significantly higher in the symptomatic anaemic preterm infants (0.43±0.06) compared to asymptomatic (0.33±0.05) and control (0.35±0.06) infants. The authors suggested that the elevated pFOE in symptomatic anaemic infants may be used as a marker of the need for blood transfusion[124,209,210]. The

same group later used FOE values as a trigger to transfuse in a pilot randomised trial. Infants less than 1500g birth weight who were stable and less than 2 weeks old were randomised to conventional or a NIRS based transfusion protocol.

Table 6. NIRS measurements to predict the need for blood transfusion

Study reference	Infant characteristics	Measurements	Findings
van Hoften et al (2010)[51]	Studied 33 preterm infants [median (range) gestational age 27.3 (25 – 34) weeks and chronological age 17 (1 – 93) days]	$crSO_2$, $tcSaO_2$ and FTOE measured 1 hour pre-transfusion, 1 hour and 24 hour post-transfusion FTOE=($tcSaO_2$ – $crSO_2$)/$tcSaO_2$	crSO2 and FTOE correlated strongly with pre-transfusion Hb tcSaO2 did not correlate with pre-transfusion Hb crSO2 increased and FTOE decreased following transfusion Increase in crSO2 levels and decrease in FTOE were most prominent in the those infants with Hb<9.7 g/dl
Dani et al (2010)[52]	Studied 15 symptomatic anaemic preterm infants [mean (SD) gestational age 27 (2.4) weeks and chronological age 32 (23) days]	crSO2, srSO2 and rrSO2 and FOE, CSOR, CROR CBF, RBF and SBF measured using Doppler ultrasound scan	crSO2, srSO2, rrSO2, CSOR and CROR increased and FOE decreased during and 1 hour post-transfusion
Wardle et al (1998)[124]	Studied 94 preterm infants Group 1 – stable control infants not	HbF fraction, RCV (HbF dilution method)	Pre-transfusion mean (SD) pFOE was significantly higher in symptomatic 0.43 (0.06) but

	transfused (n=52) [Median (range) GA 29 (28 – 31), PNA of measurement 18 (9 – 36) Group 2 – asymptomatic anaemic infants transfused (n=24) [Median (range) GA 26 (25 – 28), PNA of measurement 21 (11 - 35) Group 3 – symptomatic anaemic infants transfused (n=18) [Median (range) GA 28 (26 – 29), PNA of measurement 23 (16 - 37)	Oxygen consumption, delivery and peripheral FOE (pFOE)	not asymptomatic 0.33 (0.05) infants compared with control subjects 0.35 (0.06) Post-transfusion mean (SD) pFOE decreased significantly in symptomatic anaemic infants from 0.43 (0.06) to 0.367 (0.06) p<0.001 Pre-transfusion HbF (r=0.49, p<0.001) and RCV (r= - 0.48, p=0.04) correlated well with pFOE Pre-transfusion Hb had a weak correlation (r=-0.21 p=0.04)
Wardle et al (2002)[211]	Randomised trial: 74 anaemic infants studied NIRS group (n=37) transfusion if FOE >0.47: [median (range) GA 29 (27 – 31), PNA 5 (3 – 8) Conventional group (n=37) transfused according to standard clinical practice: [median (range) GA 30 (27 – 32), PNA 5 (3 – 8)	Hb and pFOE	Infants transfused according to the NIRS protocol were more likely to be transfused later and at a lower Hb than those transfused using conventional protocol Infants in the NIRS group spent a significantly longer period than those in the conventional group with Hb <10 g/dl In the NIRS group 66% (37/56) of transfusions

			were given because of clinical reasons before reaching the treatment FOE threshold
Bailey et al (2010)[53]	Studied 30 symptomatic anaemic preterm infants [Mean (SD) gestational age 28.4 (3) and chronological age 31.7 (16.2) days	Hb levels; $crSO_2$ and $srSO_2$ measured for 20 minute duration immediately before, during, immediately after and 12 hours after blood transfusion	Mean $crSO_2$ and $srSO_2$ values increased significantly following transfusion and this remained elevated 12 hours after transfusion Pre-transfusion Hb did not correlate with $crSO_2$ (r=-0.01 p = 0.98) or srSO2 (r=-0.26 p = 0.17)
Bailey et al (2012)[176]	Studied 52 premature anaemic infants (mean GA 28.6 weeks): Group 1: transfused (n=34) and Group 2: asymptomatic control (n=18)	Hb, $crSO_2$, $srSO_2$ and SCOR	Mean (SD) pre-transfusion SCOR values were significantly lower in infants who improved [0.61(0.22)] with transfusion compared to those without improvement [0.75 (0.16)] and asymptomatic control [0.77 (0.16)] Based on a ROC curve, symptomatic infants with a pre-transfusion SCOR ≤0.73 had the highest sensitivity and specificity for predicting improvement of symptoms of anaemia
Seidel et al (2012)[54]	Studied 76 infants [mean (SD) GA 27 (3) weeks and PNA 38 (22) days] Group 1: $crSO_2$ ≥ 55% (n=51)	$crSO_2$, $prSO_2$, SaO_2before, during, immediately after and 24 hours after transfusion	$crSO_2$ and $prSO_2$ increased significantly in all infants following transfusion

	Group 2: $crSO_2$<55% (n=25)		Increase in $crSO_2$ following transfusion was significantly ($p<0.005$) higher in those infants with a pre-transfusion $crSO_2$<55% Infants with pre-transfusion $crSO_2<0.55$ had significantly higher episodes of desaturations <80% ($p<0.05$) compared to infants with $crSO_2$ ≥55%

PNA: postnatal age; GA: gestational age; $crSO_2$: cerebral regional O_2 saturation; $prSO_2$: peripheral regional O_2 saturation; FTOE: fractional tissue oxygen extraction; $tcSaO_2$: transcutaneous saturation of oxygen; $srSO_2$: splanchnic regional O_2 saturation; $rrSO_2$: renal regional O_2 saturation; CSOR: cerebro-splanchnic oxygenation ratio; CROR: cerebro-renal oxygenation ratio; HbF: foetal Haemoglobin; pFOE: peripheral fractional oxygen extraction; CBF: cerebral blood flow; RBF: renal blood flow; SBF: splanchnic blood flow; RCV: red cell volume

Infants transfused using conventional protocol received transfusion earlier and at a higher Hb compared to infants transfused based on NIRS protocol. Infants in the conventional group spent significantly shorter period with Hb <10 g/dl compared to the NIRS group. However, there was no difference in the number of transfusions received between the two groups. The postnatal age at discharge, weight at discharge, rate of weight gain, and rate of linear growth were not significantly different between the groups. In the NIRS group, 66% of transfusions were given because of clinical concerns before reaching the threshold of transfusion i.e. FOE >0.47. This may have been because clinicians relied on conventional indicators of transfusion or a peripheral FOE of 0.47 alone may not be a sensitive enough predictor of the need for transfusion[211].

Bailey *et al* studied 30 anaemic preterm infants and demonstrated improvement in gut and cerebral oxygenation following blood transfusion [53]. In a separate study, the same group showed that the ratio between splanchnic rSO_2 and cerebral rSO_2 (splanchnic cerebral oxygenation ratio – SCOR) can be a useful marker for PRBC transfusion. Infants with a low pre-transfusion SCOR (≤0.73) were more likely to improve after transfusion (likelihood ratio, 2.8; 95% confidence interval 1.1-6.7)[176]. Seidel *et al* measured $crSO_2$ and $prSO_2$ before, during, immediately after and 24 hours after blood transfusion and noticed a significant improvement in cerebral and peripheral tissue oxygenation and perfusion as well as improvement in symptoms of anaemia on transfusing for anaemic infants with $crSO_2$<55% compared to infants with $crSO_2$ ≥55% [54].

1.4.14 Summary for NIRS

Near Infra-red Spectroscopy (NIRS) can be used as a bedside non-invasive tool to monitor tissue oxygenation changes continuously. NIRS has been validated to measure cerebral tissue oxygenation and has been used in research extensively in measuring tissue oxygenation in splanchnic, renal and peripheral perfusion in preterm infants. It may help to monitor the requirement of oxygen delivery, routine monitoring in intensive care, management of hypotension and perhaps as a tool for neurodevelopmental prognosis in preterm infants. There is increasing evidence that NIRS can be a reliable non-invasive tool to measure tissue oxygenation which might help clinicians to identify the need for transfusion.

1.5 Red Cell Volume (RCV)

1.5.1 Introduction

The overall survival and outcomes of low birth weight preterm infants have progressively improved with advances in the fetal and newborn infant care[212,213]. Advances in neonatal resuscitation techniques and management[214], use of managed clinical networks in UK[215] and centralised neonatal transportation [216] have all resulted in improvement of preterm survival and outcomes. However, morbidity amongst the survivors still remains high[213,217,218]. One of the important factors that have an important role in the pathogenesis of morbidity and mortality is the instability of haemodynamic state in these infants in the first few hours of life[219-222].

It is important to maintain an optimal circulating blood volume (BV) in newborn infants undergoing intensive care. Delayed umbilical cord clamping in preterm infants can result in better cardiovascular stability by improving blood volume by 30% and red cell volume by 60% compared to early umbilical cord clamping [223]. Due to the changes in plasma volume in the first few days of life, blood haematocrit (Hct) values do not accurately reflect the true red cell volume (RCV) [224]. In order to accurately reflect the effect of placental transfusion by delayed umbilical cord clamping, red cell volume measurements need to be precise and results must be related to specific clinical endpoints [225]. However, measurements of RCV are laborious, cumbersome and expensive and require a dedicated biomedical lab for processing[226]. Albeit, rapid measurement of RCV can help clinicians understand haemodynamic changes to management and may help to decide the requirement of certain management such as blood transfusion[208].

1.5.2 Importance of Red cell volume (RCV)

The various haematological variables involved in oxygen delivery to the tissues include haemoglobin (Hb) concentration, red cell volume (RCV) and blood volume (BV)[227-229]. Low Hb levels at birth are known to be associated with poor short term outcomes and mortality in preterm infants[98,230]. Maintaining optimum blood volume is essential for cardiovascular stability in preterm infants. Hypovolaemia in critically ill patients may produce serious and unexpected physiological consequences and is reported to be associated with morbidity and mortality in critically ill adults[231,232]. Shoemaker et al studied 98 critically ill adult patients by comparing haemodynamic measurements of survivors (n=67) with non-survivors (n=31) and found lower stroke volume and BV in the non-survivors. This could be due to reduced cardiac output, oxygen availability and oxygen transport [232].

The pre-transfusion RCV may be helpful in assessing the need for blood transfusion. Measuring pre-transfusion RCV in 73 sick newborn infants using HbF dilution method, Kinmond et al demonstrated that lower RCV at birth was associated with longer duration of assisted ventilation as well as time to discharge from hospital [233]. Using ^{50}Cr tagged red blood cell dilution; Faxelius et al measured RCV and BV in 259 newborn infants (gestational age 28–41 weeks; birth weight 787-5386g) within 0.8-71 hours of birth, and found that 31% (n=14 out of 45) infants with RCV <30ml/kg and BV >70ml/kg died. 51% (n=49 out of 99) of those with RCV <30ml/kg and BV <70ml/kg died. The percentage mortality of those two groups was significantly high compared to the infants with BV>70ml/kg and RCV >30ml/kg[234]. These two studies indicate that a lower RCV can result in worse outcome.

Using Biotin labeled red blood cell dilution Hudson and colleagues measured RCV in 42 preterm infants (gestational age 24-34 weeks) on the first day of life and

compared their clinical outcomes. They reported that overall clinical outcomes improved ($p < 0.05$) with increasing RCV: intracranial haemorrhage, blood pressure, severity of respiratory illness (duration of ventilation, peak inspiratory pressure and oxygen requirement), transfusion needs, time to regain birth weight and survival. From regression analysis, gestational age was the most important variable; the best outcome was seen with RCV value of >40ml/kg [235]. Linderkamp et al measured RCV in 128 preterm infants (gestational age 26-36 weeks) using ^{125}I labeled human serum albumin within 2 hours of birth, and compared with the incidence of respiratory distress syndrome (RDS). Infants with RCV <35ml/kg showed a significantly higher incidence of RDS and mortality in spite of similar Apgar scores [236].

Excessive expansion of blood volume was also associated with worsening morbidity and mortality in critically ill adults [237-239] and newborn infants [240]. In a multicentre study (n=3534; 146 Western European Adult Intensive Care Units), Vincent et al reported that there was a significant association between blood transfusion and mortality in adults. Authors also found that for similar degrees of organ dysfunction, patients who had a transfusion (categorical variable) had a higher mortality rate[237]. Ewer et al studied anonymised regional case notes (n=22 infants) of Project 27/28, a national case controlled study run by the Confidential Enquiry of Stillbirth and Deaths in Infancy in UK. This was compared to matched regional control infants (survivors; n=29). The primary outcome was death within 28 days. The infants who died received more than twice the volume expansion compared with controls in the first 48 hours of life (38.2 vs. 18.2ml/kg; $p=0.007$). There was no significant difference between the groups in lowest blood pressure or base excess within the first 12 hours of life. Newborn infants who received ≥30ml/kg volume expansion in the first 48 hours of life were more likely to die than those who received <30ml/kg (OR 4.5; 95% CI 1.2, 17.2) [240]. Blood transfusion has been reported to be an

independent risk factor of mortality in preterm infants born at <32 weeks of gestational age [62]. Others have also reported blood transfusion as an independent risk factor of in hospital mortality in preterm infants[63].

It is a common practice to give 10 to 20ml/kg of fluid bolus to a very low birth weight infant in the first few hours of life. A questionnaire study from Canada reported that 97% of responded neonatologists with at least 10 year experience from level two and three neonatal intensive care units used a fluid bolus for blood pressure less than gestational age in weeks for infants born with birth weight less than 1500gms during the initial 72 hours of life, irrespective of their clinical condition. Normal saline was the predominant (95%) volume administered [241]. In a National Survey of the level II and level III neonatal units in UK it has been shown that normal saline is the most commonly administered treatment for neonatal hypotension [242]. Many neonatologists are keen to improve peripheral perfusion and blood pressure fairly quickly after birth as hypotension has been related to development of intra-ventricular haemorrhage. However, routine administration of fresh frozen plasma (FFP) or colloid on admission and again 24 hours later failed to demonstrate any advantage for preterm infants born at less than 32 weeks gestation [243,244].

1.5.3 Red Cell Volume (RCV) of infants

Red Cell Volume (RCV)

RCV of 17-59ml/kg has been reported for newborn infants within the first 72 hrs of birth[234,245-249]. The majority of the studies have reported RCV on average from 30 to 40ml/kg. The total RCV may vary depending on the presence of perinatal blood loss, gestational age at birth, mode of delivery, time of umbilical cord clamping, and the method used for RCV measurement **(Table 7).**

Measurement of Red Cell Volume

RCV can be measured using various techniques including ^{50}Cr, ^{51}Cr and ^{99m}Tc labelled autologous RBC dilution techniques (**Table 7**). Radioactive labelling of red cells are not ethically acceptable in preterm infants due to high radiation exposure; so current techniques used are Biotin labelling [226,250-252] and HbF (fetal Hb) dilution technique following blood transfusion using flow cytometry or high performance liquid chromatography (HPLC)[27,89,253,254]. Recent studies using four different doses of Biotin in sheep have shown that all four densities can be used simultaneously and independently to determine RCV [250]. Biotin labelling and fetal haemoglobin dilution methods are found to be safe, feasible and comparable [255].

Table 7. Measured Red Cell Volume (RCV) within 72 hours of birth

Study reference	**Measurement Method**	**Age at Measurement**	**Comment**	**RCV - ml/kg mean (range)**
Bratteby, 1968[245]	^{51}Cr-labelled autologous RBC dilution	Within 72 hrs after birth	No of infants - 27 Gestation: 31-42wks Vaginal delivery DCC – 5 min	42.4 (27.4-54.8)
Faxelius et al, 1977[234]	^{50}Cr-labelled autologous RBC dilution	Within 71 hrs after birth	No of infants – 144 Gestation: 28-41wks No perinatal blood loss Variable cord clamping time	32.4 (range not reported)

Faxelius et al, 1977	^{50}Cr-labelled autologous RBC dilution	Within 71 hrs after birth	No of infants – 105 Gestation: 28-41wks With perinatal blood loss Variable cord clamping time	26.3 (range not reported)
Robinson et al., 1978[248]	Adult Hb dilution	Within 0.5 to 30 hrs after birth	No of infants – 23 Gestation: 29.2 ± 2.4wks Variable cord clamping time	32.9 (17-59)
Quaife et al., 1981[247]	Tc-99m labelled autologous RBC dilution	Exact time not reported	No of infants – 62 Gestation: term + preterm Variable cord clamping time	32.2 ± 9.2 (range not reported)
Strauss et al, 2003[249]	Biotin labelled autologous RBC dilution	Within 24 hrs after birth	No of infants – 24 Gestation: ≤36wks Caesarean + Vaginal delivery Early cord clamping (≤15 sec)	36.8 ± 6.3 (range not reported)
Aladangady et al 2004[27]	HbF dilution (n=6) and Biotin labelled RBC dilution (n=32)	Within 24 hours of birth	No of infants – 38 Gestation:24-32 wk Blood volume estimated from measured RCV	35.5 ± 6 (26.5 – 52.5)

1.5.4 Summary for RCV

Aladangady et al have demonstrated that clinicians' ability to predict the actual circulating blood volume using clinical and laboratory assessments is poor[27]. The correlation between measured RCV as well as BV with haematocrit (Hct) of newborn infants is poor[27,253]. Mock et al reported a reasonable correlation between haemoglobin and RCV in preterm infants[256]. Hudson et al demonstrated that the pre-transfusion RCV correlated well with changes in cardiac output following transfusion; infants with a pre-transfusion RCV of <25ml/kg showed a significant fall in cardiac output compared to those with >25ml/kg[257]. Aladangady et al[27,89,258] and others[253,256] have demonstrated the feasibility of measuring reliable RCV using foetal haemoglobin dilution method in babies receiving first blood transfusion.

Pre-transfusion RCV or BV could be a useful biomarker in deciding blood transfusion in newborn infants. Nevertheless, measurement of RCV and BV is time consuming; require either blood transfusion or injecting contrast (e.g. biotin) and the results may not be readily available before blood transfusion. These arguments preclude using measured RCV or BV as a trigger to decide blood transfusion in newborn infants.

1.6 Summary of Introduction

Current blood transfusion guidelines are based on pre-transfusion Hb level or haematocrit and blood transfusion is recommended in chronic oxygen dependent preterm infants with a pre-transfusion Hb of <11 g/dl. It is not clear whether restrictive or liberal transfusion practice is beneficial for preterm infants. On the other hand both chronic anaemia and blood transfusion has been reported to be associated with gut injury and NEC. Severe anaemia is associated with a high cardiac output state and this decreases following blood transfusion. Pre-transfusion Red Cell Volume (RCV) <25 ml/kg is associated with significant reduction of cardiac output following blood transfusion. Hb concentration in the blood does not necessarily correlate with tissue oxygenation.

Doppler ultrasound scan is a reliable bedside tool and can be used to assess blood flow to major organs such as brain and gut. NIRS is a reliable bedside tool to measure tissue oxygenation continuously in the brain and has recently been used to assess tissue oxygenation in the gut. Researchers have assessed tissue oxygenation changes using NIRS and blood flow changes using Doppler USS in preterm infants by combining infants of variable gestational and postnatal ages together as a single group. The haemodynamic changes in the transitional period are different from the stable preterm infant of later postnatal ages.

The evidence for applying the current clinical and laboratory parameters as a trigger for blood transfusion is lacking. Further research is needed to understand the basic haemodynamics, oxygenation status and the effect of blood transfusion in these preterm infants in relation to gestational age, postnatal age, ventilation status, pre-

transfusion Hb level, feeds and presence of intra and extra-cardiac shunts such as patent foramen ovale (PFO) and patent ductus arteriosus (PDA).

2 Study aims, objectives and hypothesis

2.1 Aims

To investigate the effect of blood transfusion on gut and cerebral perfusion in preterm infants

2.2 Objectives

1. To measure gut and cerebral perfusion in preterm infants receiving blood transfusion for clinical indication using Near Infra-red Spectroscopy (NIRS) and Doppler ultrasound scan.
2. To measure the pre-transfusion baseline cerebral and gut perfusion and oxygenation levels and compare them between three postnatal age groups.
3. To compare the cerebral and gut blood flow and oxygenation changes following blood transfusion between gestational and postnatal age matched infants with and without patent ductus arteriosus (PDA).
4. To compare the cerebral and gut blood flow and oxygenation changes following blood transfusion in preterm infants receiving varying amount of feeds.
5. To continuously measure and compare vital parameters before and after blood transfusion.
6. To study changes in the pre-transfusion laboratory parameters following blood transfusion.
7. To study the effect of measured red blood cell volume (RCV) on gut and cerebral perfusion in preterm infants receiving first blood transfusion for clinical indication using NIRS and Doppler ultrasound scan.

8. To measure cerebral and gut blood flow and oxygenation in control infants and compare them with measurements of infants who received blood transfusion.

2.3 Hypotheses

a) Blood transfusion does not improve gut and cerebral perfusion in babies with Hb more than 11g/dl.

b) Blood transfusion improves cerebral perfusion more than gut perfusion in babies with Hb less than 11 g/dl.

c) Blood transfusion to babies with RCV >25ml/kg does not improve gut or cerebral perfusion

3 Study Methodology

This was a prospective single-centre observational study.

3.1 Inclusion criteria

Preterm infants born at 23 to 36+6 weeks admitted to neonatal unit of Homerton University Hospital receiving blood transfusion for clinical indication/s were eligible for the study.

3.2 Exclusion criteria

Preterm infants with below conditions were excluded from the study:

1. Major congenital brain malformations (e.g. anencephaly, holoprosencephaly), intestinal malformations (gastroschisis, omphalocoele), chromosomal and genetic abnormalities (e.g. Trisomy 18, Trisomy 13)
2. Significant abdominal pathology (e.g. proven NEC)
3. Pre-existing cutaneous disease (e.g. epidermolysis bullosa, congenital icthyosis)
4. Babies on HFOV or considered unstable for NIRS and Doppler measurement by the attending clinical team

3.3 Sample Size

Study infants:

A pragmatic sample size of 60 preterm (gestational age at birth 23 – 36^{+6} weeks) was selected. Infants were planned to be recruited to three postnatal age groups as shown below:

Group 1 20 preterm infants between 1 to 7 days of postnatal age

Group 2 20 preterm infants between 8 days to 28 days of postnatal age

Group 3 20 preterm infants of ≥29 days of postnatal age

The rationale for dividing the study population in three postnatal age groups was:

1. Transitional circulation, intra and extra cardiac shunts and cardiorespiratory support is different between the groups.
2. Haemoglobin threshold for transfusion is also different between the groups.

Measurement of pre-transfusion Red cell volume (RCV) was planned in 20 infants with indwelling arterial catheter receiving their first blood transfusion among the above groups.

Control infants:

Measurement was planned in 12 control infants who were stable preterm infants, receiving invasive or non-invasive ventilation but was not receiving blood transfusion. This group was selected to measure intra-operator variability of Doppler measurements and to compare NIRS measurements with the infants in the transfused group.

Subgroups:

I also planned to divide the study population into four subgroups:

1. PDA groups: Gestational and postnatal age matched infants with open and closed PDA
2. Feeding groups: Infants who received ≥ and <50% of enteral feeds
3. Haemoglobin groups: Infants with pre-transfusion Hb level ≥ and <11 g/dl.
4. Red cell volume groups: Infants with a pre-transfusion RCV ≥ and <25 ml/kg

3.4 Overview of the study measurements

The study measurements were performed in the following order. First pre-transfusion Hb, Hct and blood gas parameters (pH, pCO_2 and Lactate) were measured. This was followed by Doppler cerebral and gut blood flow measurements 30-60 minutes before blood transfusion. After the Doppler measurements, the NIRS and continuous vital parameters measurements were started simultaneously. Blood transfusion was started 15-20 minutes after starting NIRS measurements. Blood was transfused over 3 hours and the NIRS and vital parameter measurements were continued during this period. These measurements were stopped 15-20 minutes post blood transfusion. The post-transfusion Doppler measurements were performed soon after the NIRS measurements were stopped (within 30-60 minutes post-transfusion). The post-transfusion laboratory blood tests (Hb, Hct and blood gas parameters) were performed after the Doppler measurements (**Figure 8**).

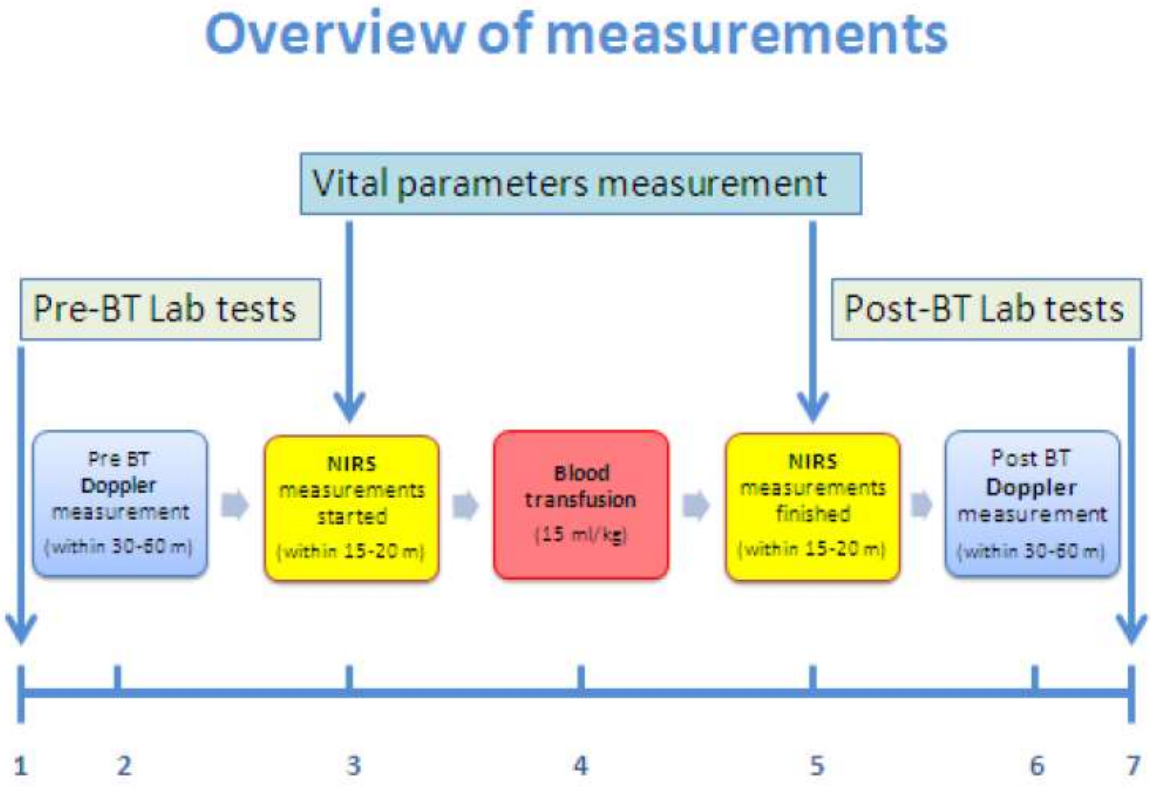


Figure 8: Overview of measurement steps

Further details of the study measurements are described in the subsequent section.

3.4.1 Measurements done before transfusion

Cerebral and splanchnic blood flow was measured using Doppler ultrasound scan and the cerebral and splanchnic oximetry measurements were done using NIRO 300 NIRS device. Apart from cerebral and splanchnic Doppler blood flow measurements the cardiac morphology was examined using 2D and Doppler echocardiography and presence of PDA was recorded. The ultrasound scan was performed in the following order: anterior cerebral artery Doppler, SVC VTI measurement, structural echocardiography, SVC diameter measurement, SMA Doppler measurements. Blood gas (pH, pCO_2 and lactate) and haemoglobin and haematocrit were also measured pre-transfusion. Vital parameters were continuously recorded along with NIRS measurements.

3.4.1.1 Measurement of cerebral blood flow using Doppler Ultrasound scan

Cerebral blood flow was assessed by measuring blood flow velocity in the anterior cerebral artery (ACA) and blood flow volume in the superior vena cava (SVC). A Logic P6 (GE Healthcare, USA) USS machine (**Figure 9**) with 7 MHz ultrasound probe was used for measurement of blood flows in anterior cerebral artery (ACA) and superior vena cava (SVC) within 30 minutes pre-blood transfusion.

Figure 9. Logic P6 ultrasound scan machine (GE Healthcare, USA) on the left and blood flow measurement of a baby on the right.

The USS probe was placed on the anterior fontanel in a parasagittal view, the ACA was identified and then the pulsed wave Doppler gate was placed in a straight segment of the artery to get the Doppler flow on the screen of the ultrasound scanner. The cursor was then placed on the Doppler waveform to measure the maximum (peak) systolic velocity and minimum (trough) diastolic velocity. Then by tracing the Doppler waveform the USS machine software estimated the time averaged mean velocity, resistance index (RI) and pulsatility index (PI) (**Figure 10**).

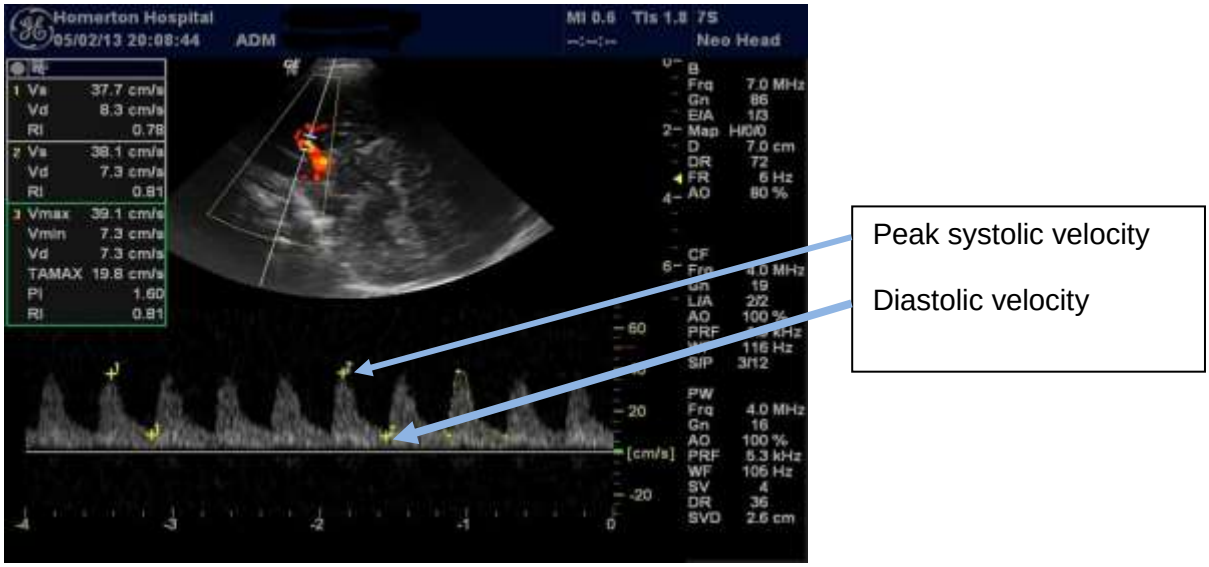


Figure 10. The ACA velocity measurements

For measuring the SVC flow the Doppler probe was placed in the infra-diaphragmatic region in the bi-caval view. The pulsed wave Doppler gate was placed over the SVC flow to measure the SVC Doppler velocity time integral (VTI) (**Figure 11**). The heart rate was recorded at the same time. Then the Doppler probe was placed over the chest in the true long axis and negotiated behind the aorta to get a view of the SVC. Following this the diameter of the SVC was measured using an M-mode view of the SVC in systole and diastole over 5-6 cardiac cycles to get a mean diameter. Finally the Doppler flow of the SVC was calculated by using the formula below in ml/kg/min[152].

$$SVC\ flow = \frac{\left\{VTI \times \pi\left(\frac{d^2}{4}\right)\right\} \times HR}{Body\ weight}$$

Where, SVC=superior vena cava, VTI=velocity time integral, d=diameter of the SVC, HR=heart rate

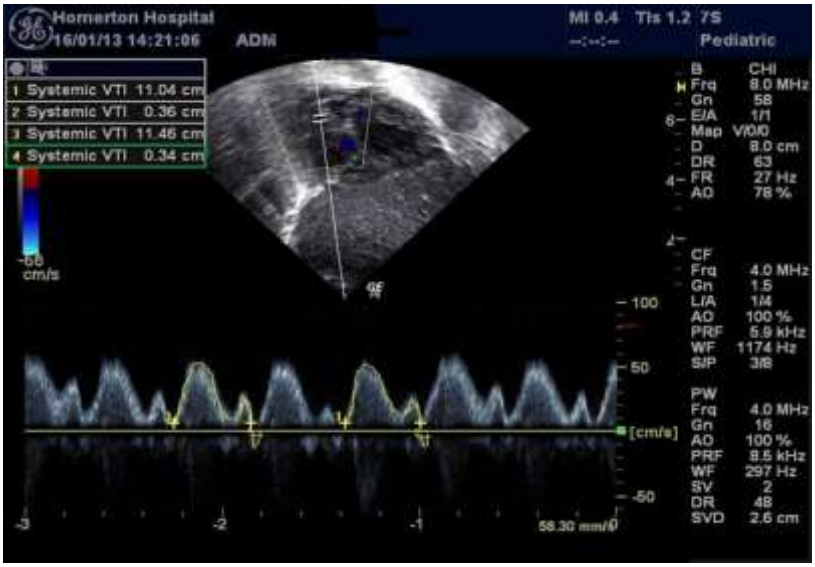


Figure 11. SVC VTI measurement

3.4.1.2 Measurement of intestinal/splanchnic blood flow using Doppler Ultrasound scan

The 7 MHz multi-frequency (5-7 MHz) Doppler probe was placed in a long axis view over the infra-diaphragmatic region, then the abdominal aorta and the superior mesenteric artery (SMA) was identified using 2D imaging and colour Doppler imaging. Then the pulsed wave Doppler gate was placed over the SMA in the direction of the flow and the Doppler waveforms were obtained (**Figure 12**). The peak systolic and trough (diastolic) velocity of the SMA was measured using this method and the values were averaged over 5-6 cardiac cycles.

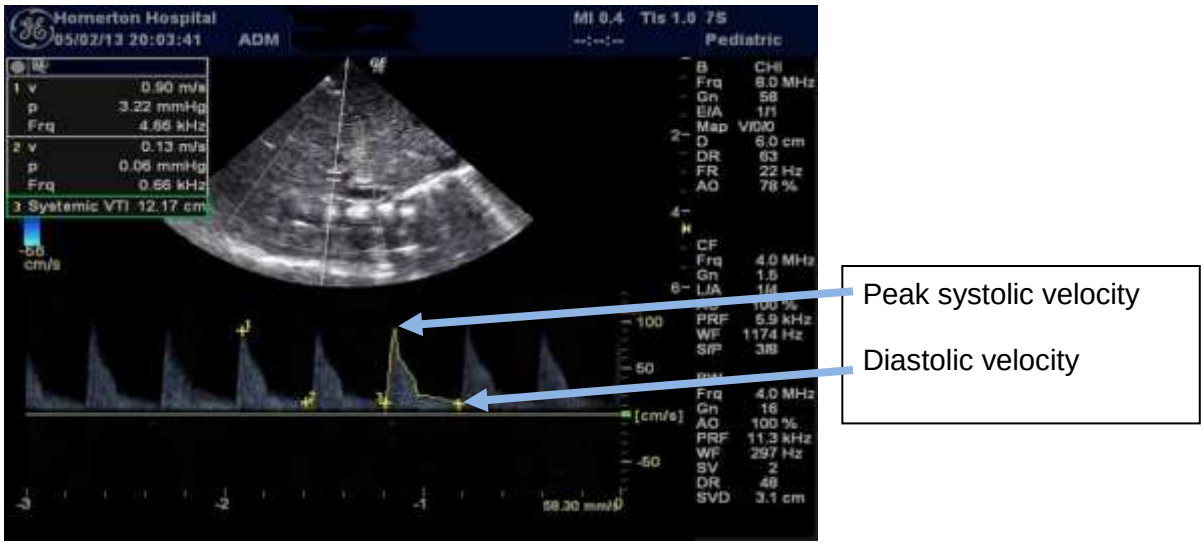


Figure 12. SMA flow measurements

3.4.1.3 Measurement of vital parameters

In order to download real-time vital parameters from overhead monitors, I searched for various options and identified ixTrend (ixellence, GmbH, Germany) software as the most feasible option for downloading data (six data points per second) from Phillips Intellivue MP70 monitors. I applied and received a grant from Garfield Weston Foundation, which enabled me to buy this software. With the help of the IT department at the Homerton University Hospital and the software support team at ixellence in Germany I managed to install the software into the study laptop and created a pathway for direct downloading of numeric parameters such as heart rate, saturation, respiratory rate and blood pressure from overhead monitors to a secured Homerton hospital network shared drive folder. Using ixTrend (ixellence GmBH, Germany) software continuous data from the overhead monitors (Philips Intellivue MP50 or MP70) **(Figure 13a)** was downloaded into the study laptop. The USB end of the connector cable (**Figure 13b**) was connected with the laptop and the other end (RJ45) was connected to the overhead monitors (**Figure 13c**).

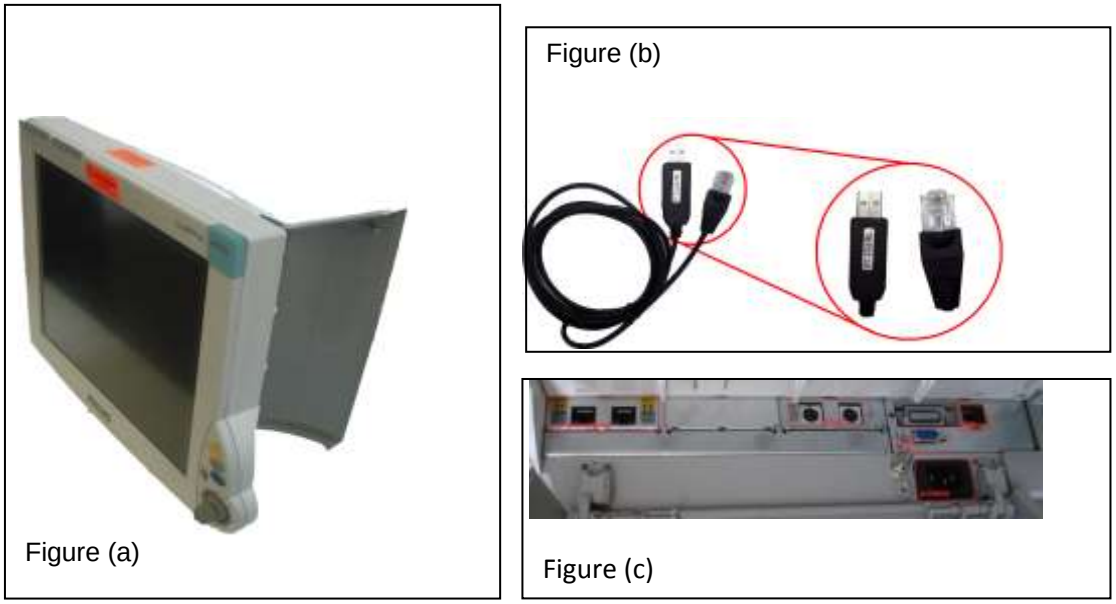


Figure 13. (a) Phillips Intellivue MP70 monitor, **(b)** RJ45 to USB cable and **(c)** RJ45 and other ports available for Phillips Intellivue monitor.

The software was then initiated in the laptop (**Figure 14**) in order to start continuous downloading alongwith the NIRS measurements.

Figure 14. Data downloading from overhead monitor using ixTrend software (*Figure presented with permission from ixTrend, ixellence, GmbH, Germany*)

3.4.1.4 Measurement of cerebral perfusion using NIRS

A NIRO 300 (Hamamatsu Photonics KK Japan), NIRS device was obtained for this study. I received training for initialising, performing measurements and downloading data from the NIRO 300 device from Dr Terence Leung, Department of Medical Physics and Biomedical Engineering at the University College London over a period of 2 weeks. Dr Leung also helped me in developing a Mat lab programme for downloading raw NIRS data in order to analyse them at specific time points in various epochs of measurements. The NIRS measurements were performed using the double channel NIRO 300 device (Hamamatsu Photonics KK, Japan) with a sample acquisition rate of 6Hz (**Figure 15**). Detailed NIRS measurement steps are shown in **Appendix 1**.

Figure 15. NIRO 300 NIRS device (Hamamatsu Photonics KK, Japan) on the left and a study infant with NIRS probe over forehead and lower abdomen (right)

After the initial probe testing the NIRO probes were placed over the forehead and fixed under a hat. The probe was then initialized and was then ready to measure cerebral tissue oximetry changes of cerebral oxy-haemoglobin ($cHbO_2$) and deoxy-haemoglobin (cHHb). The cerebral tissue haemoglobin index (cTHI) and cerebral tissue oxygenation (cTOI) were measured continuously. The NIRO 300 was connected to the laptop through a USB port (RS232 to USB) for continuous downloading of these measurements. The cerebral NIRS measurements were started from 15-20 min before transfusion, and continuously measured until 15-20 min post blood transfusion. Simultaneously oxygen saturation (SaO_2) was also measured. Cerebral fractional tissue oxygenation extraction (cFTOE) was calculated from peripheral arterial saturation (SaO_2) and cTOI using the formula below[52]:

$$cFTOE = 100 \times \left(\frac{SaO_2 - cTOI}{SaO_2}\right)$$

Where, cFTOE=cerebral fractional tissue oxygen extraction, SaO_2=peripheral arterial saturation in percentage and cTOI=cerebral tissue oxygenation index

3.4.1.5 Measurement of gut/splanchnic perfusion using NIRS

The NIRO 300 probe was placed over the hypogastrium in the midline and held in place with a single use tourniquet (Vygon 'Vene K' Quick Release, Vygon UK Ltd.). The intestinal or splanchnic tissue Hb Index (sTHI) in arbitrary units and tissue oxygenation index (sTOI) in percentage were measured using the NIRO 300, Hamamatsu Photonics K.K., Japan.

The gut or splanchnic NIRS measurements were started from 15-20 min before transfusion, and continuously measured until 15-20 min post blood transfusion. Simultaneously oxygen saturation (SaO_2) was also measured. Splanchnic FTOE (sFTOE) was calculated using measured SaO_2 and sTOI using the formula below[52]:

$$sFTOE = 100 \times \left(\frac{SaO_2 - sTOI}{SaO_2}\right)$$

Where, sFTOE=splanchnic fractional tissue oxygen extraction, SaO_2=peripheral arterial saturation, sTOI=splanchnic tissue oxygenation index

3.4.2 Blood transfusion and measurements

Blood transfusion was started 15-20 minutes after commencing NIRS and vital parameter measurements (**Figure 8**). Blood transfusion was given as per the current British Committee for Standards in Haematology (BCSH) guidance [26] with 15 ml/kg of leukocyte depleted, cytomegalovirus negative, Sickle cell negative, plasma reduced packed red blood cells (hematocrit 50-70%) over a period of 3 hours. The decision for blood transfusion was made by the attending neonatal team, and this

was based on the Hb level and clinical condition of the baby in line with the BCSH guideline[26].

Measurements done during blood transfusion

Throughout the period of blood transfusion NIRS measurements and vital parameters were continuously recorded and downloaded into the study laptop. The infants were minimally handled during this period, infants who were receiving feeds continued to receive nasogastric feeds during blood transfusion. None of the infants who were studied were receiving oral suck (bottle/breast) feeds during the measurements.

3.4.3 Post blood transfusion measurements

3.4.3.1 Measurements of cerebral and gut perfusion using NIRS

Cerebral and gut oxygenation was measured continuously till 15-20 minutes post-transfusion (**Figure 8**) and these measurements were downloaded to the study laptop which was connected to Homerton trust network drive (**Figure 16 & 17**). These measurements were stored under each individual filename for each infant in the Homerton network drive in the raw format (**Figure 16**). This was then converted using mathematical software Mat lab 2013b (Math works, USA) to an .OD format. Following this conversion, these data were analysed according to pre-planned time points in the Mat lab programme (**Appendix 2**). The pre-planned time point epochs were: T1 - 15 to 20 minutes before the start of blood transfusion, T2 - 1 hour into blood transfusion, T3 - 2 hour into blood transfusion and T4 - 15 to 20 minutes post blood transfusion.

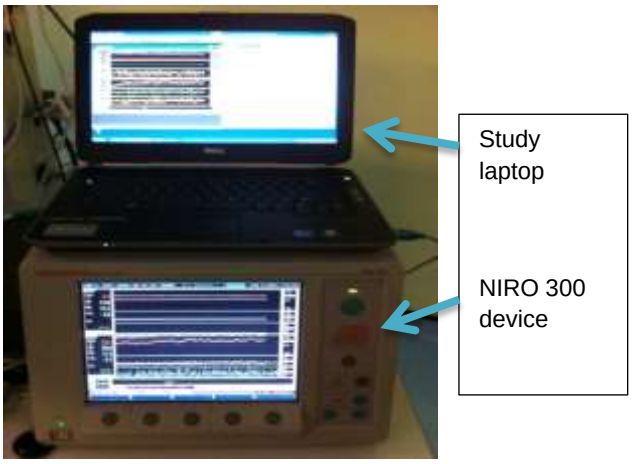


Figure 17. NIRS data continuously downloaded to the NIRO 300 device and then to the study laptop.

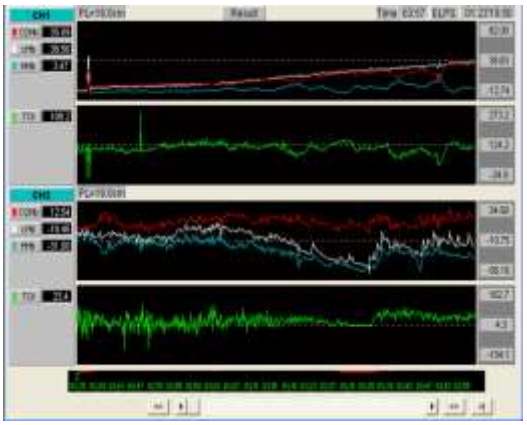


Figure 16. Continuous NIRS recordings of a study infant

3.4.3.2 Measurement of vital parameters

The measurement of vital parameters was continued throughout the blood transfusion and was stopped at the same time as the NIRS measurement. The numeric data were then downloaded to the Homerton network drive under each infant individual filename after being converted to a comma separated version (.csv) Excel file (**Figure 18**).

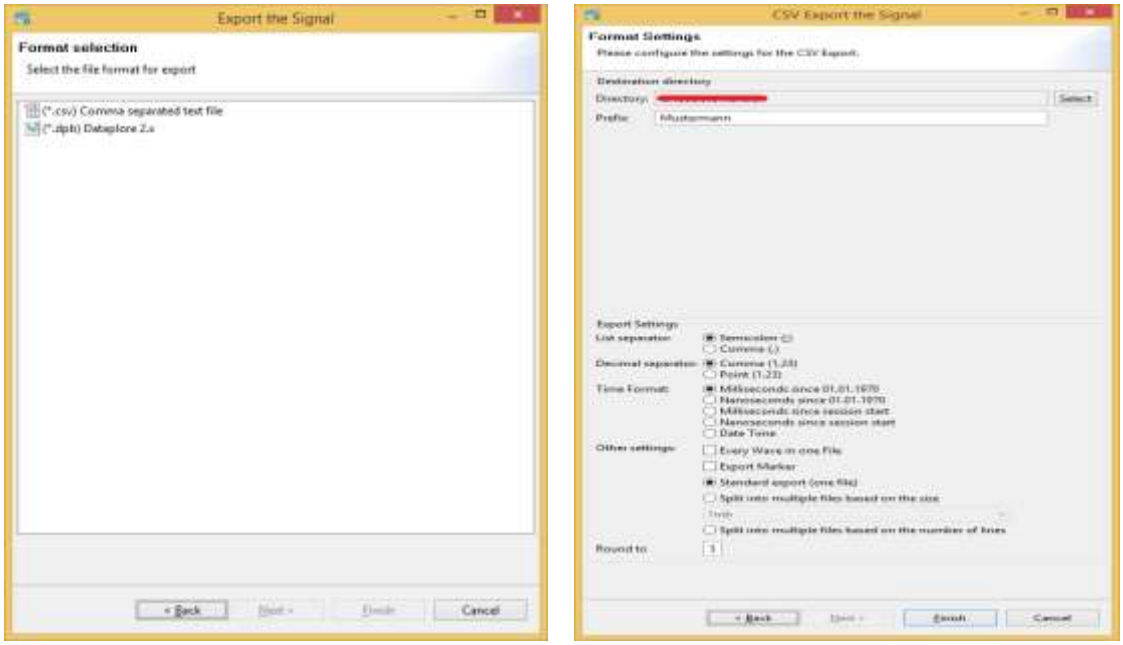


Figure 18. Exporting vital parameter data to csv file

(Presented with permission from ixTrend, ixellence, GmbH, Germany)

3.4.3.3 Measurement of cerebral blood flow using Doppler USS:

The ACA and the SVC Doppler flow was measured following post-transfusion NIRS measurements. Practical Doppler ultrasound scan measurement steps were similar to pre-transfusion Doppler measurements (see section 3.4.1.1).

3.4.3.4 Measurement of intestinal/splanchnic blood flow using Doppler USS:

The SMA blood flow was measured along with cerebral blood flow measurements following post-transfusion NIRS measurements. Practical Doppler ultrasound scan measurement steps were similar to pre-transfusion Doppler measurements (see section 3.4.1.2).

Similar to pre-blood transfusion measurements blood gas (pH, pCO_2 and lactate) and haemoglobin and haematocrit were also measured post-transfusion.

3.4.4 Measurement in control infants

Stable preterm infants not receiving blood transfusion were selected as control infants. Cerebral and splanchnic oximetry changes were measured over 3 hours using NIRO 300. Continuous vital parameters were also measured simultaneously with NIRS measurements using ixTrend software. Practical measurement steps of NIRS and vital parameters were similar to study infants as explained in section 3.4.1.4, 3.4.1.5 and section 3.4.1.3 respectively.

Anterior cerebral artery (ACA), superior vena cava (SVC) and superior mesenteric artery (SMA) blood flows were measured just before starting NIRS measurement and again after completion of NIRS measurement. Practical measurement steps of ACA, SVC and SMA blood flows were similar to study infants as explained in section 3.4.1.1 and 3.4.1.2.

3.4.5 Measurement of red cell volume (RCV) by Fetal haemoglobin dilution method:

Red cell volume was measured in infants with indwelling arterial catheters using the fetal haemoglobin (HbF) dilution method. 0.3ml of infant's blood was collected just before (pre-transfusion sample) and 10-15 minutes after the completion of the blood transfusion (post-transfusion sample) from indwelling arterial catheter to measure fetal haemoglobin (HbF) percentage. HbF was measured by High Performance Liquid Chromatography (HPLC) using Bio-Rad Variant 1 Haemoglobin testing machine (Bio-Rad Laboratory Inc. Atlanta, USA). 0.3ml of donor blood sample was collected to measure the donor blood haematocrit (Hct). The exact amount of donor

blood transfused to the infant was noted in order to estimate the amount of donor red blood cells transfused (V) using measured donor blood Hct:

$$V\ (ml) = donor\ blood\ volume\ transfused\ (ml) \times donor\ blood\ haematocrit$$

Pre-transfusion RCV was then calculated using the following equation [253]:

$$RCV\ (ml) = \frac{V \times (Post - BT\ HbF\%)}{(Pre - BT\ HbF\%) - (Post - BT\ HbF\%)}$$

Where, *V* = Total donor red cell volume transfused

Post-BT HbF% = Post-blood transfusion HbF percentage

Pre-BT HbF% = Pre-blood transfusion HbF percentage

3.4.6 Other data collected:

Demographic details: gestational age, birth weight, sex, ethnicity, age of the infant on the day of gut and cerebral perfusion measurements

Maternal characteristics: pregnancy complications such as pre-eclampsia (PET), intra-uterine growth restriction (IUGR), antepartum haemorrhage (APH), chorioamnionitis and use of antenatal steroid

PET was defined as pregnancy induced hypertension and proteinuria after 20 weeks of pregnancy.

IUGR was defined as fetal abdominal circumference (AC) or estimated fetal weight (EFW) <10th centile and showing reduced growth velocity on consecutive scans three weeks apart.

APH was defined as bleeding from or in to the genital tract occurring from 24^{+0} weeks of pregnancy and prior to the birth of the baby.

Chorioamnionitis was defined as presence of at least two of the following factors: preterm pre-labour rupture of membranes alongwith maternal pyrexia, tachycardia, leucocytosis, uterine tenderness, offensive vaginal discharge and fetal tachycardia.

Use of *antenatal steroid* was defined as receiving two doses of antenatal betamethasone 12 hours apart prior to delivery.

Condition of the infant at birth: Admission haemoglobin (Hb) and haematocrit (Hct)
Clinical parameters: respiratory and inotropic support; type, amount and frequency of enteral feed; Hb at birth and at transfusion; weight at birth and at transfusion; Ultrasound head findings and blood gas parameters such as pH, pCO_2 and Lactate on the day of gut and cerebral perfusion measurements
Indication/s for blood transfusion

3.5 Statistical analysis:

To understand the quality of Doppler ultrasound scan blood flow measurements intra-operator variability was determined. The Doppler ultrasound scan measurements of control infants were repeated within 3 hours and were analysed for intra-operator variability, reliability and repeatability using mean difference (MD) and Bland Altman method.

Changes in Doppler measurements before and after blood transfusion and NIRS measurements before, during and after blood transfusion were analysed using a paired student t-test and repeated measures ANOVA. The pre-transfusion measurements between the three postnatal age groups were compared using

unpaired t-test and ANOVA. The cerebral and gut oximetry NIRS data was analysed at specific time points of 15 minute epochs: 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 mean of these epochs were then compared using repeated measures ANOVA with Bonferroni correction. The pre and post-transfusion values of all other measurements were compared using paired (*two-tailed*) t-test.

The infants were further subdivided to gestational age and postnatal age matched groups with or without PDA and the changes following transfusion was compared between the two groups. The infants were also subdivided into those receiving majority feeds (>50% feeds) with those with <50% feeds and the cerebral and gut perfusion between those infants were also compared.

The effect of Hb more than 11 g/dl and RCV >25 ml/kg on the changes in Doppler and NIRS values was also investigated using repeated measures analysis of variance. An analysis of correlation was performed to ascertain the relationship between the haemoglobin (Hb) and cerebral and gut perfusion and oxygenation measurements.

A multivariate analysis of variance (MANOVA) with covariates (MANCOVA) was performed using the following outcome (dependent) variables: pre-transfusion anterior cerebral artery time averaged mean velocity (ACA TAMV), superior vena cava (SVC) flow, superior mesenteric artery peak systolic velocity (SMA PSV), cerebral tissue oxygenation index (cTOI), cerebral tissue haemoglobin index (cTHI), splanchnic tissue oxygenation index (sTOI) and splanchnic tissue haemoglobin index (sTHI); changes following blood transfusion in ACA TAMV, SVC flow, SMA PSV, cTOI, cTHI, sTOI and sTHI. The covariates included in all these analyses were

gestational age, birth weight, pre-transfusion haemoglobin, blood pressure, presence of PDA and volume of feed. The outcomes (stated above) were analysed by categorising them to the following groups: postnatal age groups (Group 1: 1 to 7 days; Group 2: 8 to 28 days and Group 3: ≥29 days), feeding groups (received <50% and ≥50% feeds) and Hb groups (pre-transfusion Hb <11g/dl and ≥11g/dl).

Multivariate analysis of covariance (MANCOVA) is a statistical technique that is the extension of analysis of covariance (ANCOVA). The MANCOVA is used to investigate the statistical differences on multiple continuous dependent variables, assessed by an independent grouping or categorical variable, while controlling for a third list of variables known as covariates. Covariates are known as confounding factors, which are added to reduce error and that the analysis eliminates the covariates' effect on the relationship between the independent grouping variable and the continuous dependent variables.

The various assumptions for multivariate analysis were checked for each of the variables included: normality, homogeneity, independent random sampling, level and measurements of the variables, absence of multicollinearity and the relationship between covariates and dependent variables assessed by correlation analysis. A descriptive analysis of the outcome variables indicated that they were minimally skewed with small inter-quartile range (IQR). A descriptive analysis of the covariates indicated that they were normally distributed. Pre-transfusion RCV was not taken as a covariate and multivariate analysis of RCV groups (RCV <25ml/kg, n=5 and ≥25ml/kg, n=9) was not possible due to very small number[259].

Using SPSS 22.0 software as the analysis tool for the multivariate analysis (MANCOVA) the dependent variables and the covariates were added to the various

required section for analysis and interpretation. All tests were performed at 5% level of statistical significance.

3.6 Ethical issues

3.6.1 Research Ethics

The study received research ethics approval from Charing Cross Research Ethics Committee (REC no.12/LO/0527); the study was also registered with the Homerton R&D department (**Appendix 3 and 4**). The study was subsequently adopted as a Portfolio study by National Institute of Health and Research (NIHR Study ID 13594). A major amendment of the research protocol was sought on April 2014 to study control infants which was granted by the Charing Cross REC. Informed written parental consent was obtained from parents before recruiting infants to the study (**Appendix 5**). The original signed consent form was kept the infants' medical record, copy was given to the parents and one was stored in the study file.

3.6.2 Data Storage

The consent form and Doppler ultrasound findings (print outs) were stored in folders which were kept within locked filing cabinet in the Chief Investigator's office at the Homerton University Hospital. The raw NIRS data and the continuous vital parameter data were stored in secured password protected Homerton Hospital network drive. The processing of both NIRS and vital parameter data were performed in the same drive and the post-processed data was kept as anonymised data with study ID.

3.6.3 Research funding

The study received funding from Hamamatsu Photonics KK Japan, Garfield Weston Foundation and HCA International.

4 Results

4.1 Infant and maternal characteristics

4.1.1 Infant characteristics at birth

Fifty nine infants were studied; infant and maternal characteristics of the study population who received transfusion are presented in **Table 8**. The median birth weight and gestational age of infants studied in the three groups were similar. The median haemoglobin (Hb) level at birth was also similar between the three groups as well as the control group. The ranges of Hb level at birth were wide although similar amongst the three groups. Amongst the total study population 20% of infants studied had maternal PET and IUGR. Maternal chorioamnionitis and antepartum haemorrhage were noticed in 42% and 30% of infants respectively. Mothers of 89% of infants received two doses of antenatal steroids prior to delivery.

Table 8. Infant and maternal characteristics

Characteristics	**Group 1 (1 – 7 days) n = 20**	**Group 2 (8 – 28 days) n = 21**	**Group 3 (≥29 days) n = 18**	**Control group** (1-7days=4, 8-28d=5, ≥29days=3 **n = 12**
Gestational age (completed weeks)*	26 (23 – 27)	25 (23 – 30)	26 (24 – 34)	27 (24 – 33)
Birth weight (grams)*	763 (600 – 1180)	740 (600 – 1240)	793 (520 – 1746)	804 (528 – 2372)
Haemoglobin at birth (g/dl)*	14.5 (9.8 – 20.7)	14.7 (10.0 – 17.4)	15.3 (10 – 18.9)	13.3 (10.5 – 16.1)
Maternal PET†	3 (15)	5 (24)	4 (22)	2 (17)
IUGR†	3 (15)	5 (24)	4 (22)	3 (25)
Chorioamnionitis†	9 (45)	8 (38)	8 (44)	5 (42)
Antepartum haemorrhage†	6 (30)	8 (38)	4 (22)	4 (33)
Antenatal steroids†	17 (85)	20 (95)	16 (89)	10 (83)

† Number (percentage), * Median (Range)

4.1.2 Infant characteristics at blood transfusion

The infant characteristics on the day of blood transfusion are presented in Table 10. Patent ductus arteriosus (PDA) was noted in 32 (54%) infants on echocardiography, of these only six (15%) were >14 days of postnatal age; otherwise normal cardiac morphology. Majority of infants in Group 1 (infants received transfusion on day 1 to day 7), and eight each in the other two groups (Group 2: day 8 to day 28 and Group 3: ≥day 29) 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 @5mcg/kg/min in all infants) for hypotension, the dose remained unchanged for the duration of the measurements. Three infants in Group 1, one in Group 2 and three in Group 3 had significant (≥Grade 4) intra-ventricular haemorrhage (IVH) before transfusion. Further details of IVH amongst the study infants are shown in **Table 9**. There was no progression of these findings on repeat cranial ultrasound scan following transfusion in any of the infants in the three groups.

Table 9. Infants with various grades of intra-ventricular haemorrhage (IVH)

Grades of IVH (Papille staging)[260]	**Group 1 (1 – 7 ds) n = 20**	**Group 2 (8 – 28 ds) n = 21**	**Group 3 (≥29 ds) n = 18**
No IVH	9	16	11
Grade 1	6	4	3
Grade 2	2	0	1
Grade 3	0	0	1
Grade 4 or PVL	3	1	3

The majority of the infants in Groups 1 and 2 were ventilated by invasive conventional ventilation. A high proportion of infants in Group 3 were receiving non-invasive ventilation or breathing in air (**Table 10**).

Table 10. Infant characteristics at blood transfusion

Characteristics	**Group 1 (1 – 7 ds) n = 20**	**Group 2 (8 – 28 ds) n = 21**	**Group 3 (≥29 ds) n = 18**
Chronological age (days)*	5 (1 – 7)	14 (8 – 27)	45 (29 – 93)
Weight at transfusion (grams)*	774 (700 – 1180)	805 (680 – 1250)	1125 (887 – 2045)
Invasive/Non-invasive ventilation/nasal cannula oxygen or breathing in air†	13 (65)/7(35)/0 (0)	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)
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)
Total feeds (ml/kg/d)*	18 (0 – 70)	120 (0 – 180)	155 (0 – 180)

† Number (percentage), * Median (Range)

The proportion of feeds in the study population increased with postnatal age. In Group 1 ten infants were not receiving any feeds, the other ten were receiving hourly bolus nasogastric feeds, one infant was on preterm formula and the rest were on maternal expressed breast milk (MEBM). In contrast, in Group 2 two infants were unfed, two were receiving 2 hourly and the rest were on hourly bolus feeds with MEBM. Only two infants in Group 3 were unfed, while 15 infants were fed with MEBM, one was fed formula, and all infants were on 1 to 2 hourly feed. None of the

infants studied developed feed intolerance, clinical or radiological signs of NEC following blood transfusion.

4.2 Vital Parameters

4.2.1 Baseline vital parameters

The mean values of baseline pre-transfusion vital parameters are shown in **Table 11**. The mean pre-transfusion respiratory rate (RR), heart rate (HR) and peripheral arterial saturation (SaO2) were similar between infants of the three groups apart from the mean pre-transfusion HR which was significantly higher in Group 1 compared to Group 3 (p=0.02, 95% CI 1.5 to 16.6). The mean pre-transfusion systolic, mean and diastolic blood pressure (BP) increased with postnatal age and was significantly higher in the later postnatal age group (Group 3) compared to the earlier groups (Group 1 and Group 2) of infants.

4.2.2 Changes in vital parameters following transfusion

The mean values of the post-transfusion vital parameters are shown in **Table 11**. There was no significant difference in HR, RR and SaO_2 following blood transfusion in all the three groups of infants. There was a significant increase in the systolic BP following blood transfusion in infants of Group 1; this was not evident in the older postnatal age groups. The diastolic and mean BP increased significantly following blood transfusion in infants of all the three postnatal age groups.

Table 11. 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 (≥29 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 (SaO_2)%	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

4.3 Laboratory parameters

4.3.1 Baseline laboratory parameters

The baseline laboratory parameters measured are shown in **Table 11**. The mean pre-transfusion haemoglobin decreased with postnatal age and 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). The pre-transfusion pH and pCO_2 were similar between the three postnatal age groups of infants. Though the pre-transfusion baseline serum lactate levels were higher in the infants of the early group (Group 1: infants transfused between 1st to 7th day of life) compared to the other two groups, but this was not statistically significant.

4.3.2 Changes in laboratory parameters following transfusion

The pre and post-transfusion laboratory parameters are shown in **Table 11**. There was significant increase in post-transfusion Hb and Hct in all three groups of infants. There was a significant drop in serum lactate levels in Group 1 and 2 infants following blood transfusion. There was no significant difference between the mean pre and post blood transfusion pH and pCO_2 levels in the blood gas in infants of all three groups.

4.4 Measurement of blood flow

4.4.1 Intra-operator variability of Doppler measurements

The Doppler ultrasound scan measurements of the blood flow to the brain and gut were analysed for intra-operator variability. The Doppler measurements include anterior cerebral artery (ACA) peak systolic velocity; ACA time averaged mean velocity (TAMV), superior vena cava (SVC) flow, superior mesenteric artery (SMA) peak systolic velocity (PSV) and superior mesenteric artery (SMA) diastolic velocity. The Doppler measurements were performed on 12 control infants by me and the measurements were repeated at 3 hours. These infants did not receive blood transfusion and there were no alterations of management during the intervening period.

The mean and standard deviations (SD) of the Doppler flow measurements are shown in **Table 12** below. The mean difference of these parameters varied between 0.01 to 0.02 m/sec in blood flow velocities and 9.1 ml/kg/min in blood flow volume measurements.

Table 12. Mean and standard deviations of the Doppler measurements on two consecutive occasions

Measurements (Mean±SD)	**1st measurement**	**2nd measurement**	**Mean difference**
ACA peak velocity (m/sec)	0.39±0.08	0.38±0.07	0.01
ACA time averaged mean velocity (m/sec)	0.21±0.04	0.20±0.02	0.01
SVC flow (ml/kg/min)	53.8±19.4	44.7±22.3	9.1
SMA peak velocity (m/sec)	0.97±0.31	0.95±0.44	0.02
SMA diastolic velocity (m/sec)	0.11±0.06	0.13±0.03	0.02

The findings of intra-operator variability of Doppler ultrasound scan measurements analysed using Bland-Altman method of comparison showed significant agreement between the two sets of measurements; this is demonstrated in the **Table 13** below.

Table 13. Bland Altman analysis of Doppler measurements

Measurement	Limits of agreement	Mean difference (CI)	Pitman's test of difference in variance
ACA peak systolic velocity	-0.092 to 0.106	0.007 (-0.026 to 0.040)	r=-0.335, p=0.314
ACA time averaged mean velocity	-0.035 to 0.058	0.012 (-0.004 to 0.027)	r=0.012, p=0.969
SVC flow	-14.194 to 14.454	0.130 (-4.993 to 5.253)	r=-0.142, p=0.696
SMA peak systolic velocity	-0.293 to 0.284	-0.005 (-0.101 to 0.092)	r=-0.071, p=0.835
SMA diastolic velocity	-0.041 to 0.062	0.010 (-0.007 to 0.028)	r=-0.418, p=0.201

There was significant correlation between the two consecutive measurements thereby demonstrating repeatability of measurements. The consecutive Doppler measurements clearly show consistency and reliability of the individual measurements. Amongst the cerebral blood flow measurements, the ACA peak systolic velocity and time averaged mean velocity showed 85% and 72% repeatability respectively, while the SVC flow was repeatable in 79%. Amongst the gut blood flow measurements the SMA peak systolic velocity was repeatable in 77% and the diastolic velocity in 85% cases.

4.4.2 Blood flow to brain

4.4.2.1 ACA blood flow measurements

The mean pre-transfusion Anterior Cerebral Artery (ACA) peak systolic velocity (PSV) and time averaged mean velocity (TAMV) showed an increasing trend with postnatal age (**Table 14** and **Figure 19**). The mean pre-transfusion ACA 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 2 (0.19±0.06, $p<0.001$, CI 0.03 to 0.12).

The pre-transfusion ACA TAMV remained significantly higher in Group 3 compared to Group 1 ($p=0.016$, CI 0.014 to 0.128) and Group 2 ($p=0.009$, CI 0.014 to 0.094) after a multivariate analysis including the covariates such as gestational age, birth weight, pre-transfusion Hb, blood pressure, presence of PDA and volume of feed.

The mean pre-transfusion ACA PSV and TAMV decreased significantly post-transfusion in all the three Groups (**Table 14** and **Figure 19**). 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.

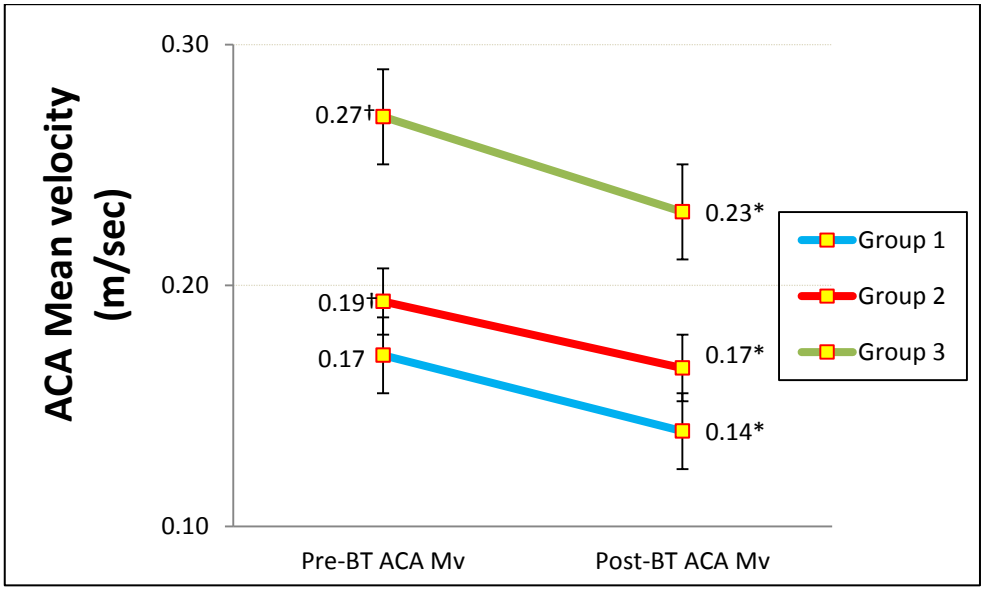


Figure 19. Blood transfusion (BT) and changes in ACA TAMV

Group 1: 1 to 7 days, Group 2: 8 – 28 days and Group 3: ≥29 days of postnatal age

Key: † p<0.05 comparison between the pre-blood transfusion ACA mean velocity between the groups and * p<0.05 comparison between pre and post-transfusion

Table 14. 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 (≥29 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)	105.2 (55.9)	92.4 (40.7)	0.03	91.0 (35.1)	95.5 (39.5)	0.16	98.9 (22.6)	77.9 (23.6)	<0.01

4.4.2.2 SVC blood flow measurements

The mean pre-transfusion Superior Vena Cava (SVC) flow was higher in Group 1 infants compared to other two groups. The pre-transfusion SVC flow was noted to be higher in older Group 3 compared to relatively younger Group 2 infants but this was not significant. The pre-transfusion SVC flow showed no significant differences between the postnatal age groups (Group 1 vs. Group 2: p=0.16, CI 0.54 to 3.23 and Group 2 vs. Group 3: p=0.45, CI 0.04 to 13.11) following multivariate analysis including all the covariates mentioned earlier.

The mean SVC flow decreased significantly following blood transfusion in Group 1 and 3 infants but there was no significant change in Group 2 infants where there was an increasing trend noted (**Table 14** and **Figure 20**).

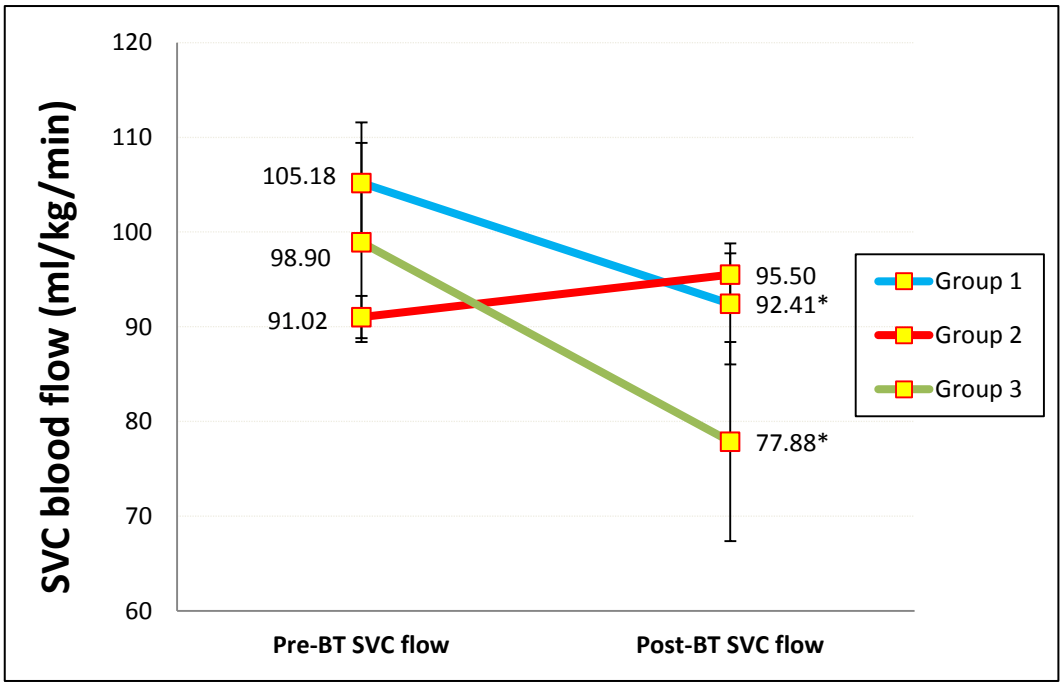


Figure 20. Blood transfusion (BT) and changes in SVC flow

Group 1: 1 to 7 days, Group 2: 8 – 28 days, Group 3: ≥29 days of postnatal age

* p<0.05 comparison between pre and post-transfusion

4.4.2.3 ACA and SVC blood flow in infants with and without PDA

Doppler measurement of infants with PDA (n=11, mean gestational age=25 wk & mean postnatal age=16 days) were compared to gestational age (mean=26 wk) and postnatal age (mean=17 days) matched infants with closed PDA (n=11). The basic characteristics of these infants are presented in **Table 15**.

Table 15: Basic characteristics of matched infants with or without PDA

Characteristics	**Infants with PDA** **n=11**	**Infants without PDA** **n=11**	**Difference between groups**
Gestational age (completed weeks)*	25 (23 – 29)	26 (23 – 29)	NS
Birth weight (grams)*	730 (600 – 1014)	740 (592 – 1240)	NS
Haemoglobin at birth (g/dl)*	14.7 (10.0 – 15.9)	14.7 (10.5 – 17.4)	NS
Chronological age (days)*	16 (8 – 42)	17 (6 – 40)	NS
Maternal PET†	4 (36)	6 (54)	NS
IUGR†	4 (36)	6 (54)	NS
Chorioamnionitis†	5 (45)	3 (27)	0.04
Antepartum haemorrhage†	5 (45)	3 (27)	0.04
Antenatal steroids†	10 (90)	11 (100)	NS
Weight at transfusion (grams)*	814 (680 – 1043)	900 (1250)	NS
Pre-transfusion Hb (g/dl)*	10.3 (9.6 – 12.2)	9.7 (8.9 – 11.1)	0.07
Total fluids (ml/kg/d)*	135 (100 – 150)	150 (120 – 180)	NS
Total feeds (ml/kg/d)*	40 (0 – 165)	150 (0 – 180)	<0.001
Invasive/Non-invasive ventilation/nasal cannula oxygen or breathing in air†	10/1/0	8/2/1	NS
Presumed sepsis on antibiotics†	6 (55)	2 (19)	<0.001

* Median (Range), † Number (percentage)

The ACA TAMV and SVC blood flow results are demonstrated in **Figure 21**. The mean pre-transfusion 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 higher in those with open PDA (102.98±42.5 ml/kg/min) compared to closed-PDA Group but this was not statistically significant (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 remained similar following transfusion in both PDA (p=0.99, CI -13.5 to 13.3) as well as the closed-PDA Group (p=0.83, CI -11.5 to 9.4).

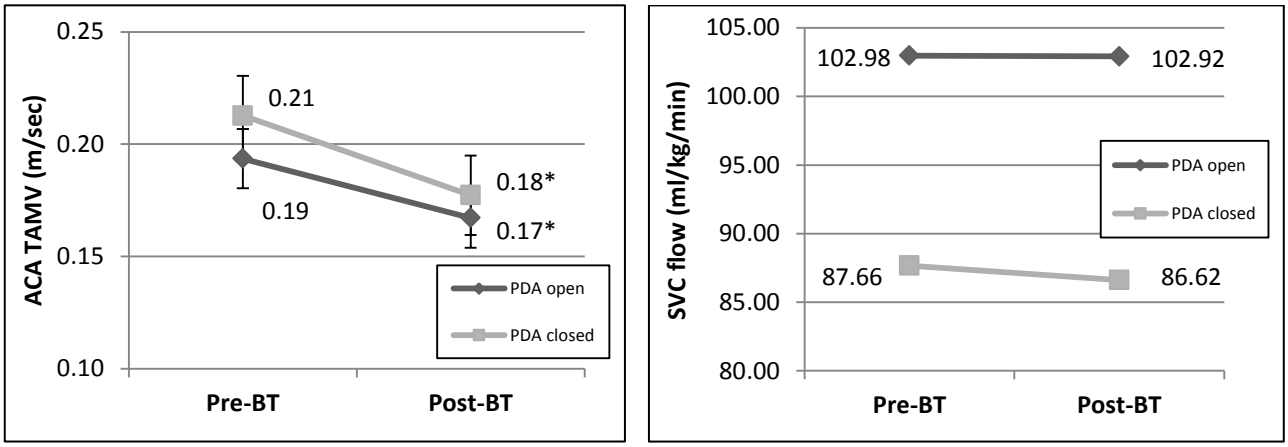


Figure 21. Blood transfusion, cerebral blood flow and PDA

* p<0.05 comparison between pre and post-transfusion

4.4.3 Blood flow to gut

4.4.3.1 SMA blood flow measurements

The mean baseline pre-transfusion superior mesenteric artery (SMA) peak systolic velocity (PSV) showed an increasing trend with postnatal age (Group 2 vs. Group 1, p=0.09 and Group 3 vs. Group 2, p=0.14) and was significantly higher in older postnatal age (Group 3: infants transfused at ≥29 days of age) infants compared to younger (Group 1: infants transfused between day 1 to day 7 of life) infants (p<0.01; CI 0.6, 0.1).

The pre-transfusion SMA PSV remained significantly higher in Group 3 compared to Group 1 (p=0.024, CI 0.012 to 0.054) infants after multivariate analysis including the covariates such as gestational age, birth weight, pre-transfusion Hb, blood pressure, presence of PDA and volume of feed.

The SMA diastolic velocity was similar between the three postnatal age groups. The mean pre-transfusion SMA PSV showed a decreasing trend following transfusion in all the three groups but this was not statistically significant (**Table 16** and **Figure 22**). The SMA diastolic velocity remained unaltered following transfusion in all the three postnatal age groups. The ultrasound software did not allow measuring the SMA TAMV.

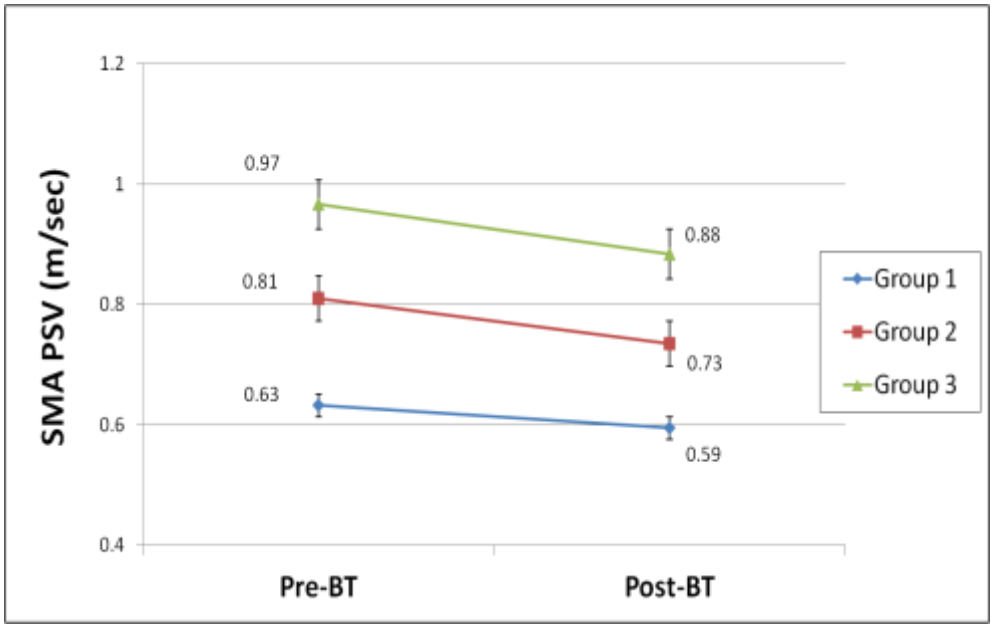


Figure 22. Blood transfusion (BT) and Superior mesenteric artery (SMA) peak systolic velocity (PSV)

Group 1: 1 to 7 days, Group 2: 8 – 28 days, Group 3: ≥29 days of postnatal age

Table 16. Blood transfusion (BT) and Superior Mesenteric Artery (SMA) 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 (≥29 days) n = 18**		
	Pre-BT	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**
SMA peak systolic velocity (m/sec)	0.63 (0.32)	0.59 (0.23)	0.51	0.81 (0.33)	0.73 (0.24)	0.22	0.97 (0.40)	0.88 (0.32)	0.32
SMA diastolic velocity (m/sec)	0.12 (0.05)	0.12 (0.04)	0.65	0.13 (0.04)	0.12 (0.04)	0.45	0.13 (0.04)	0.12 (0.02)	0.37

4.4.3.2 SMA blood flow in fed and unfed infants

The whole study population was divided into infants who received majority feeds (≥50%) and those who received <50% feeds. The basic characteristics of the infants are presented in **Table 17**.

Table 17. Basic characteristics of infants who received feeds ≥ or <50% of total fluids

Characteristics	**Feeds ≥50%** **n=27**	**Feeds <50%** **n=32**	**Difference between groups**
Gestational age (completed weeks)*	25 (24 - 34)	26 (23 – 27)	NS
Birth weight (grams)*	745 (520 – 1746)	763 (592 – 1180)	NS
Haemoglobin at birth (g/dl)*	14.7 (9.8 – 17.1)	15.5 (10 – 19.2)	NS
Chronological age (days)*	28 (13 – 93)	6 (1 – 62)	<0.001
Maternal PET†	7 (26)	5 (16)	NS
IUGR†	5 (18)	5 (16)	NS
Chorioamnionitis†	11 (41)	10 (31)	NS
Antepartum haemorrhage†	9 (33)	6 (19)	NS
Antenatal steroids†	23 (85)	31 (97)	0.075
Weight at transfusion (grams)*	1000 (790 – 1760)	794 (540 – 1520)	NS
Pre-transfusion Hb (g/dl)*	10.0 (7.0 – 11.4)	10.9 (8.5 – 12.6)	NS
Total fluids (ml/kg/d)*	150 (120 -180)	150 (90 – 180)	NS
Total feeds (ml/kg/d)*	150 (60 - 180)	15 (0 – 70)	<0.001
Invasive/Non-invasive ventilation/nasal cannula oxygen or breathing in air†	16/11/0	16/12/4	NS
Presence of PDA†	12 (44)	20 (62)	<0.001
Presumed sepsis on antibiotics†	14 (52)	21 (65)	<0.001

* Median (Range), † Number (percentage)

The mean pre-transfusion SMA PSV was significantly higher in infants who were mostly fed (receiving >50% feeds, n=32) compared to those who were receiving lesser amount (receiving <50% feeds, n=27) of feeds (0.91±0.35 vs. 0.71±0.35 m/sec; p<0.01); this remained significant after multivariate analysis of the covariates (p=0.02, CI 1.2 to 2.2). The SMA PSV showed a decreasing trend following transfusion but was not significant in either of the groups (**Figure 23**). The pre-transfusion baseline SMA diastolic velocity was similar in both feeding groups (p=0.89) and showed no significant change post-transfusion (p=0.79).

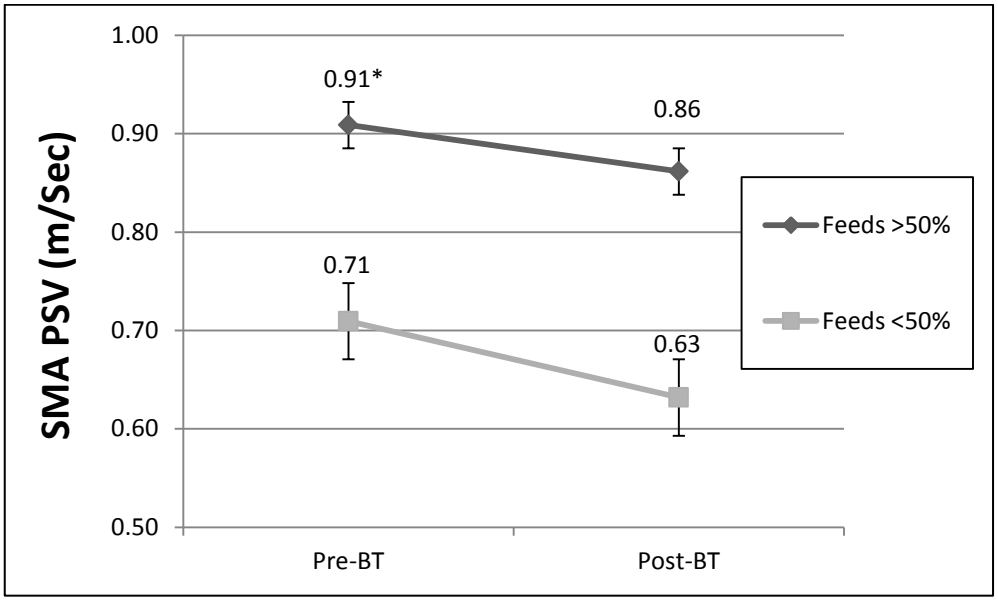


Figure 23. Blood transfusion (BT) and changes in SMA peak systolic velocity (PSV) in relation to percentage of feeds

* p<0.05 comparison between baseline pre-transfusion measurements

4.4.3.3 SMA blood flow in infants with and without PDA

SMA Doppler measurements of infants with PDA (n=11, mean gestational age=25 wk & mean postnatal age=16 days) were compared to gestational age (mean=26 wk) and postnatal age (mean=17 days) matched infants with closed PDA (n=11). The mean baseline pre-transfusion SMA PSV was significantly higher in those

infants with closed PDA (0.84±0.4 vs. 0.77±0.3 m/s, p = 0.006, CI 0.07, 0.45) (**Figure 24**). The pre-transfusion SMA diastolic velocity was similar between the two PDA groups. The SMA PSV reduced following blood transfusion but was not significant in infants with both PDA (p=0.29, CI -0.05, 0.15) and closed-PDA Group (p=0.19, CI -0.04, 0.20) (**Figure 24**). The SMA diastolic velocity also remained unchanged post-transfusion.

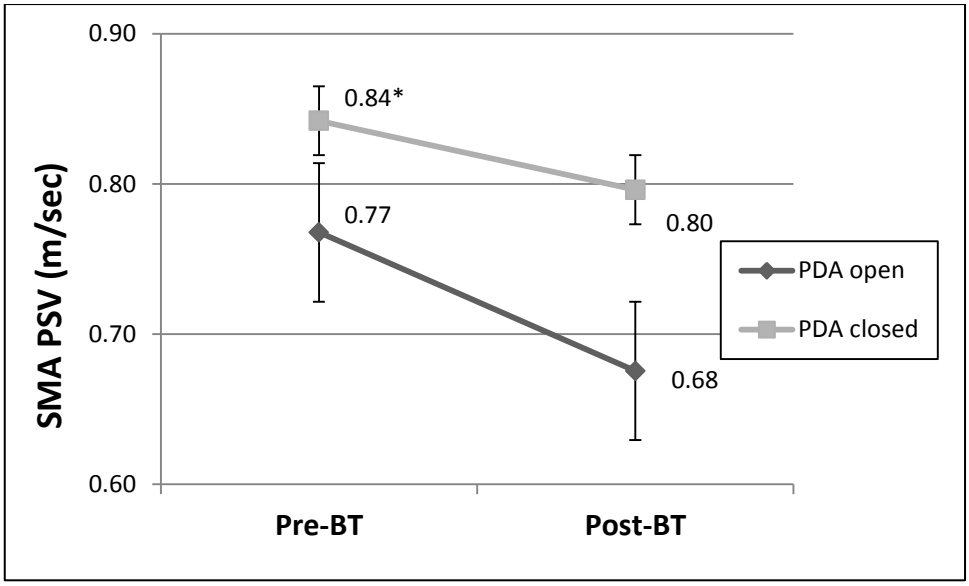


Figure 24. Blood transfusion (BT) and changes in SMA PSV in relation to PDA

* p<0.05 comparison between baseline pre-transfusion measurements

4.5 Measurement of tissue oximetry

4.5.1 Cerebral tissue oximetry

4.5.1.1 Baseline cerebral tissue oximetry

Cerebral tissue haemoglobin index (cTHI)

The mean pre-transfusion baseline cerebral tissue haemoglobin index (cTHI) values in the postnatal age groups are shown in **Figure 25**. The pre-transfusion baseline cTHI values were significantly lower in Group 2 infants (8-28 days of postnatal age) compared to Group 1 (1 to 7 days of postnatal age), however, this difference was not significant on multivariate analysis (p=0.14) including the covariates.

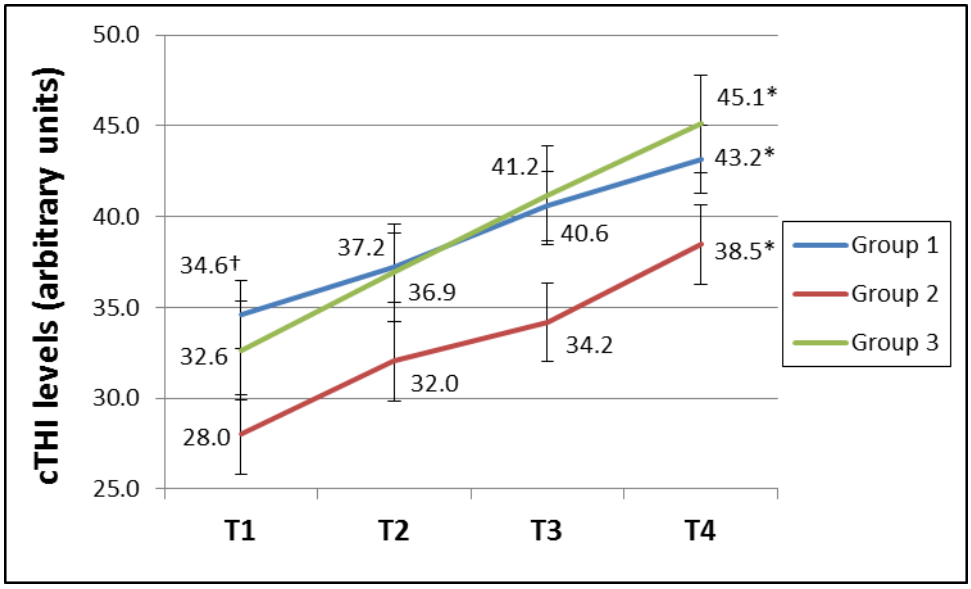


Figure 25. Blood transfusion and changes in cerebral tissue haemoglobin index (cTHI)

Group 1: birth to 7th day, Group 2: 8 – 28 days, Group 3: ≥29 days of postnatal age
*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; * p<0.05 comparison between pre and post-transfusion*
† p<0.05 comparison between Group 1 and Group 2

Cerebral tissue oxygenation Index (cTOI)

There was a decreasing trend in the mean baseline pre-transfusion cTOI levels with increasing postnatal age (**Figure 26**). 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 pre-transfusion cTOI was significantly higher in Group 1 (p=0.011, CI 5.3 to 38.02) compared to Group 3 on multivariate analysis including the covariates mentioned earlier.

4.5.1.2 Cerebral tissue oximetry and blood transfusion

Cerebral tissue haemoglobin index (cTHI)

The absolute cTHI level (in arbitrary units) changes over time are also described in percentage change (taking pre-transfusion level as baseline) to further help understanding the alteration over time. The percentage changes in the mean cerebral tissue haemoglobin index (cTHI) values are shown in **Figure 26**. There was a consistent increasing trend in cTHI levels following blood transfusion in infants of all three groups except in the first hour of transfusion in Group 1. While 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 17**). 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 26**).

Cerebral tissue oxygenation Index (cTOI)

The mean pre-transfusion cTOI increased significantly following transfusion in all the three postnatal age Groups. There was an increasing trend in mean cTOI over time but it did not reach statistical significance until the end of transfusion (**Table 17**;

Figure 26). The percentage increase in post-transfusion cTOI from baseline was 5%, 11% and 12% in Group 1, Group 2 and Group 3 infants respectively.

Table 17. Blood transfusion (BT) and cerebral NIRS parameters according to postnatal age groups

Cerebral oximetry parameters Mean (SD)	**Group 1 (1 – 7 days) n = 17†**			**Group 2 (8 – 28 days) n = 20††**			**Group 3 (≥29 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) %	34.6 (11.5)	50.6 (14.9)	<0.001	28.0 (10.4)	63.2 (19.9)	<0.001	32.6 (12.5)	68.2 (17.6)	<0.001
Cerebral tissue oxygenation index (cTOI) %	71.0 (15.8)	74.6 (12.6)	<0.05	66.0 (12.3)	73.7 (11.8)	<0.01	57.2 (13.2)	64.1 (12.6)	<0.01
Cerebral fractional tissue oxygen extraction (cFTOE)	33.1 (10.9)	25.7 (11.4)	0.003	33.3 (12.3)	22.8 (11.0)	0.002	40.6 (10.3)	32.6 (11.5)	0.005

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

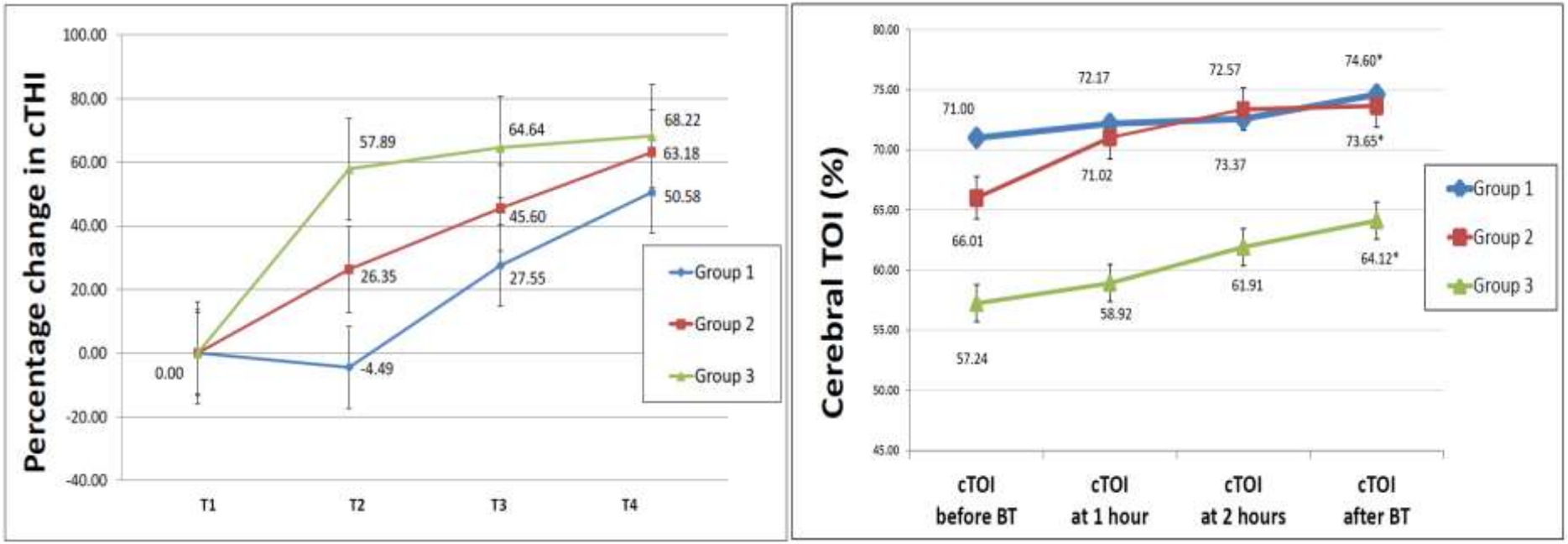


Figure 26. Blood transfusion (BT) and changes in cerebral NIRS parameters

Group 1: birth to 7th day, Group 2: 8 – 28 days, Group 3: ≥29 days of postnatal age

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

Cerebral fractional tissue oxygen extraction (cFTOE)

The baseline pre-transfusion cFTOE was similar in Group 1 and Group 2 infants (**Figure 27**). The baseline pre-transfusion cFTOE was significantly higher (p<0.01) in older age group infants (Group 3) compared to younger preterm infants (Group 1 and Group 2). The mean pre-transfusion cFTOE decreased significantly in all the three postnatal age group infants (**Figure 27** and **Table 17**).

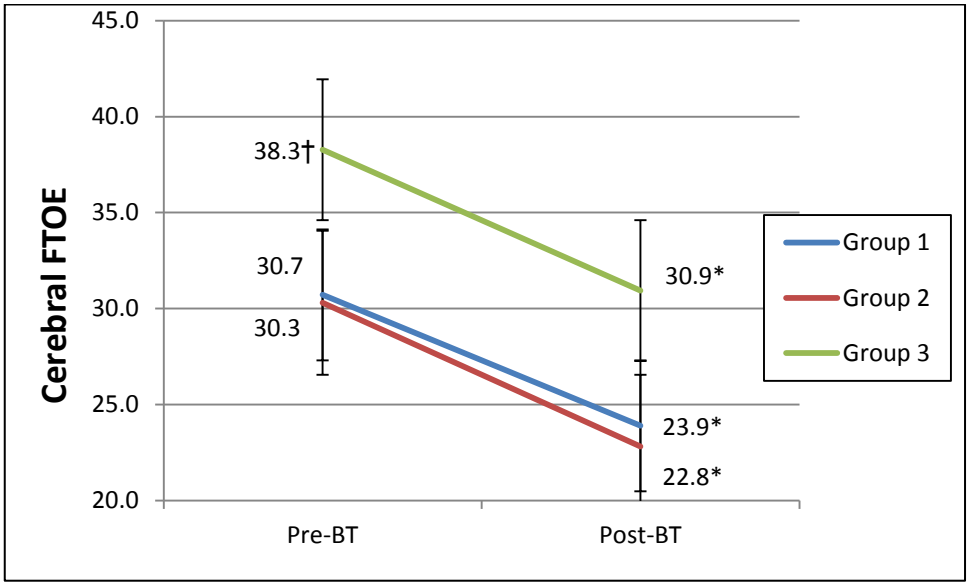


Figure 27. Cerebral fractional tissue oxygen extraction (cFTOE) and blood transfusion

Group 1: 1 to 7 days, Group 2: 8 – 28 days, Group 3: ≥29 days of postnatal age

† Comparison between Group 3 and Group 1 and 2

* p<0.05 comparison between pre and post-transfusion

4.5.1.3 Cerebral tissue oximetry and PDA

Cerebral tissue oximetry measurements were also compared between gestational and postnatal age matched groups with open and closed PDA (**Table 15**). The baseline mean pre-transfusion cTOI was higher in the PDA Group (69.9±13.4 %) compared to the closed-PDA Group (63.2±13.6%) but this was not significant (p=0.24, CI -5.0 to 18.7).

There was a similar pattern of increase noticed in cTOI in both the groups during blood transfusion (**Figure 28**). The cTOI increased significantly in all the time points at 1 hour (T2), 2 hours (T3) and post-transfusion (T4) when compared to baseline pre-transfusion values in both the groups with and without PDA (**Figure 28**). The cTHI increased consistently at all the time points and was similar in infants with or without PDA (**Figure 28**).

4.5.1.4 Multivariate analysis of changes in cerebral blood flow and tissue oximetry

On multivariate analysis, the changes in the ACA TAMV, SVC flow and cTOI post-transfusion were not significantly different between the postnatal age group infants and were independent of the covariates: gestational age, birth weight, pre-transfusion Hb, mean BP, presence of PDA and feeding volume.

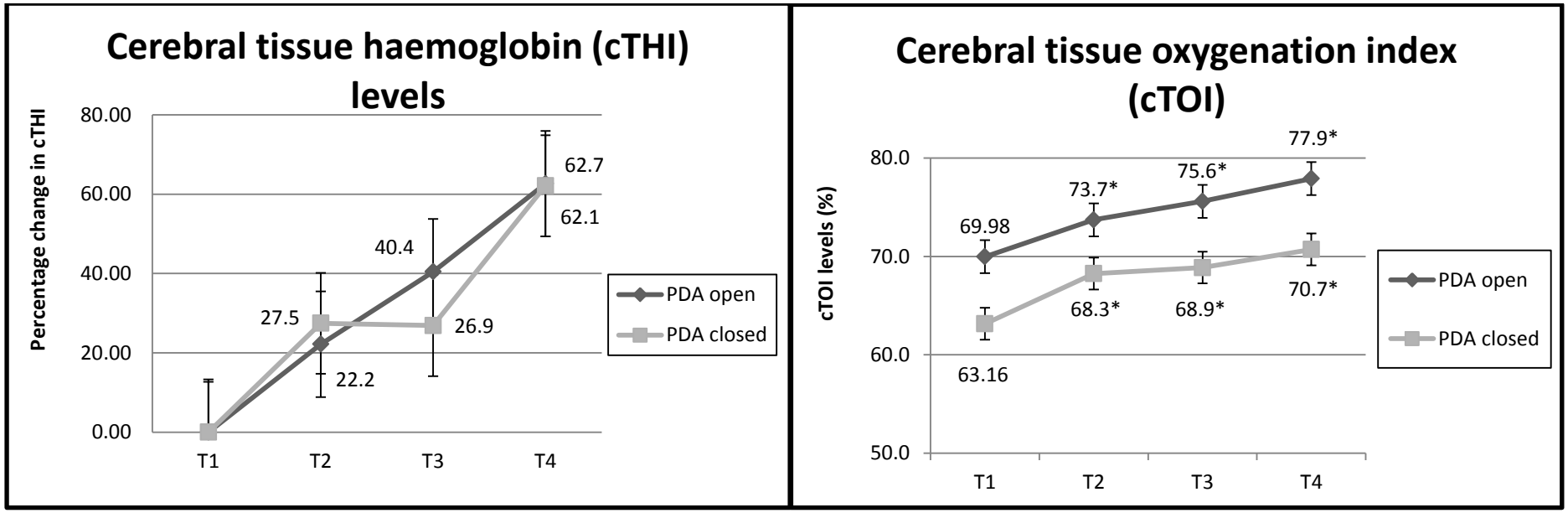


Figure 28. Blood transfusion and changes in cTOI and cTHI in PDA and closed-PDA group of infants

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

* $p<0.05$ comparison within groups at specific time points

4.5.2 Gut tissue oximetry

4.5.2.1 Baseline gut tissue oximetry

Intestinal or splanchnic tissue haemoglobin index (sTHI)

The mean pre-transfusion baseline sTHI levels in the postnatal age groups are shown in **Figure 29**. The baseline sTHI levels in Group 3 (≥29 days of postnatal age) infants were significantly lower compared to Group 1 and Group 2 infants. However, there was no significant difference noted of the pre-transfusion sTHI levels between the postnatal groups after multivariate analysis.

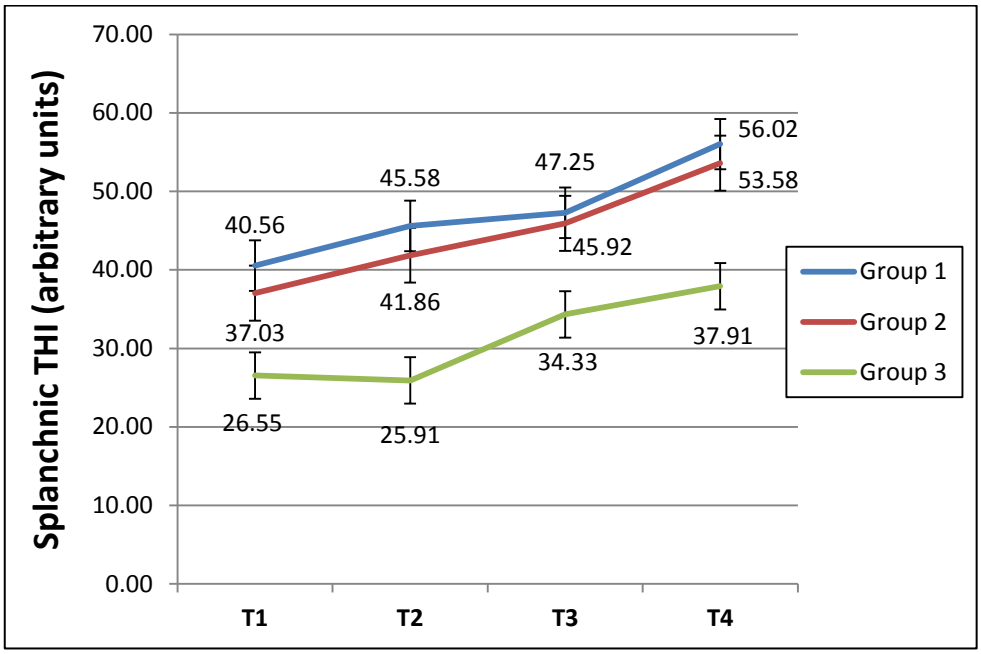


Figure 29. Mean sTHI levels in the different postnatal age group infants.

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

Intestinal or splanchnic tissue oxygenation Index (sTOI)

The mean pre-transfusion baseline sTOI levels in the postnatal age groups are shown in **Table 18** and **Figure 31**. The mean pre-transfusion sTOI was significantly higher in Group 2 infants compared to Group 1 (44.6 vs. 36.7 %; p=0.03, 95% CI -

0.6, -15.2). The mean pre-transfusion sTOI was similar between the postnatal age groups on multivariate analysis (Group 2 vs. Group 1: p=0.14, CI -2.7 to 18.9) and was independent of all the confounding factors.

4.5.2.2 Gut tissue oximetry and blood transfusion

Intestinal or splanchnic tissue haemoglobin index (sTHI)

The sTHI levels increased consistently during transfusion in all three postnatal age groups and the pattern of increase was identical except in the first hour of transfusion in Group 3 (**Figure 30**). While the maximal increase in sTHI happened later in Group 3, the percentage increase maximised post-transfusion in all the three groups (p<0.001; **Table 18**). The sTHI increased by 39%, 45% and 47% in Group 1, Group 2 and Group 3 respectively.

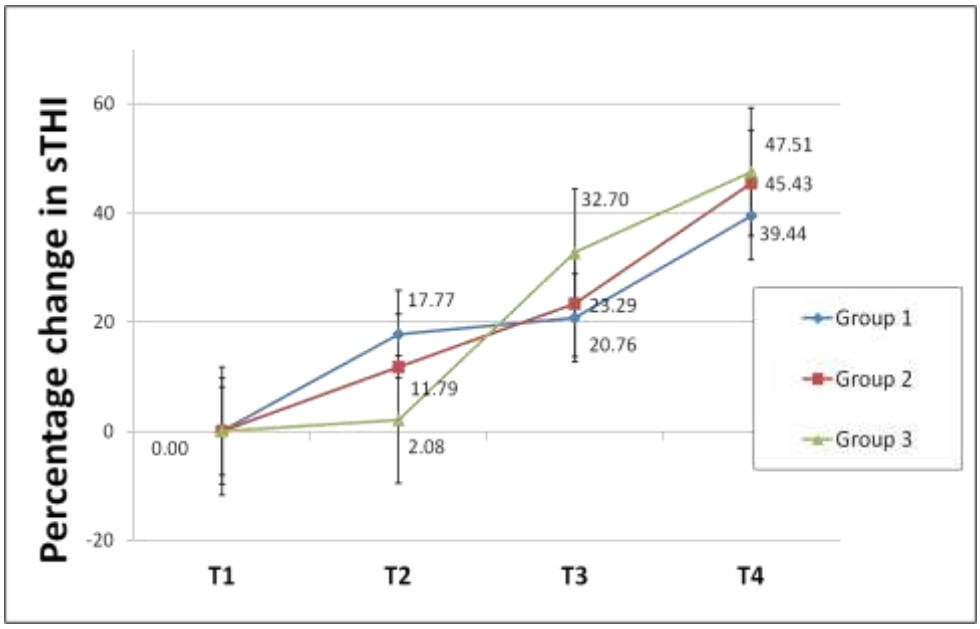


Figure 30. Blood transfusion and changes in splanchnic tissue haemoglobin index (sTHI).

Group 1: 1 - 7 days, Group 2: 8 – 28 days, Group 3: ≥29 days of postnatal age

Intestinal or splanchnic tissue oxygenation Index (sTOI)

The mean pre-transfusion sTOI showed an increasing trend over time but it did not reach statistical significance until the end of transfusion (**Table 18, Figure 31**). The baseline sTOI increased by 42%, 29% and 30% following transfusion in Group 1, Group 2 and Group 3 infants respectively.

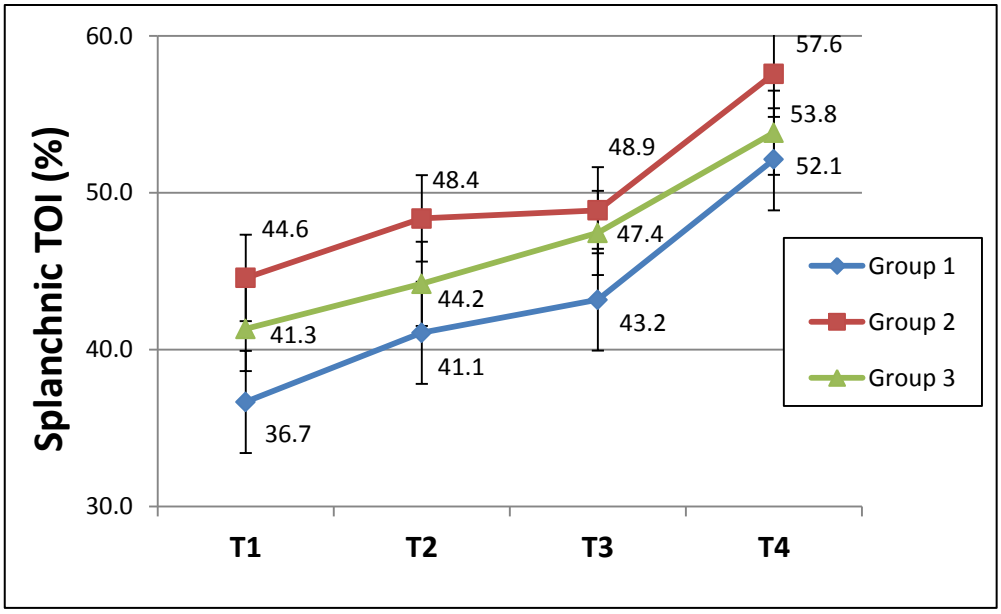


Figure 31. Blood transfusion and changes in splanchnic tissue oxygenation (sTOI)

Group 1: 1 - 7 days, Group 2: 8 – 28 days, Group 3: ≥29 days of postnatal age

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

Intestinal or splanchnic fractional tissue oxygen extraction (sFTOE)

The mean pre-transfusion sFTOE was significantly lower in Group 2 infants compared to Group 1 (64.7 % vs. 51.4%, p=0.02, CI 1.1, 17.6). The mean pre-transfusion sFTOE decreased significantly post-transfusion in all the three groups (**Table 18**).

Table 18. Blood transfusion (BT) and splanchnic NIRS parameters according to postnatal age groups

Splanchnic oximetry parameters Mean (SD)	**Group 1 (1 – 7 days) n = 17†**			**Group 2 (8 – 28 days) n = 20††**			**Group 3 (≥29 days) n = 15†††**		
	Pre-BT	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**	**Pre-BT**	**Post-BT**	**p value**
Splanchnic tissue haemoglobin index (sTHI) (percentage increase from baseline) %	Zeroed baseline	39.4	0.001	Zeroed baseline	45.4	0.001	Zeroed baseline	47.5	0.001
Splanchnic tissue oxygenation index (sTOI) %	36.7 (19.3)	52.1 (20.8)	0.01	44.6 (10.4)	57.6 (14.3)	0.01	41.3 (10.4)	53.8 (16.5)	0.01
Splanchnic fractional tissue oxygen extraction (sFTOE)%	64.7 (13.4)	44.4 (20.3)	0.004	51.4 (11.5)	37.0 (14.9)	0.005	55.6 (11.8)	42.7 (15.1)	0.0004

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

4.5.2.3 Gut tissue oximetry and feeds

The pre-transfusion baseline splanchnic tissue Haemoglobin index (sTHI) was higher in majority fed (>50% feeds) infants compared to those receiving <50% feeds, but this was not statistically significant (38.2±13.8 vs. 31.8±7.9, CI -0.2, 12.8; p=0.06). Similarly, the pre-transfusion baseline sTOI was comparable between the two groups of infants (43.1±8.7% vs. 39.3±13%, p=0.23, CI -2.5, 10.1).

The sTOI and sTHI increased and sFTOE decreased significantly post-transfusion in both feeding groups (**Table 19**).

Table 19. Gut tissue oximetry and blood transfusion in feeding groups

Gut tissue oximetry measurements (Mean ± SD)	**Feeds (>50%)**			**Feeds (<50%)**		
	Pre-BT	Post-BT	P value	Pre-BT	Post-BT	P value
Splanchnic tissue oxygenation index (sTOI: %)	43.1 (8.7)	59.5 (14)	<0.001	39.3 (13)	50.6 (16)	<0.001
Splanchnic tissue haemoglobin index (sTHI: arbitrary units)	38.2 (13.8)	46.2 (13)	<0.001	31.8 (7.9)	53.2 (17)	<0.001
Splanchnic fractional tissue oxygen extraction (sFTOE: %)	53.4 (10)	36.1 (14)	<0.001	57.6 (14)	45.7 (17)	<0.001

4.5.2.4 Gut tissue oximetry and PDA

The baseline mean pre-transfusion sTOI was similar in the PDA Group (44.7±10.2 %) and the closed-PDA Group (43.1±10.1%; p=0.73, CI -7.6, 10.7). The sTOI and sTHI increased significantly in all the time points at 1 hour (T2), 2 hours (T3) and

post-transfusion (T4) when compared to baseline pre-transfusion values in both the groups with and without PDA (**Figure 32**). The sFTOE decreased in both groups post-transfusion (p<0.001).

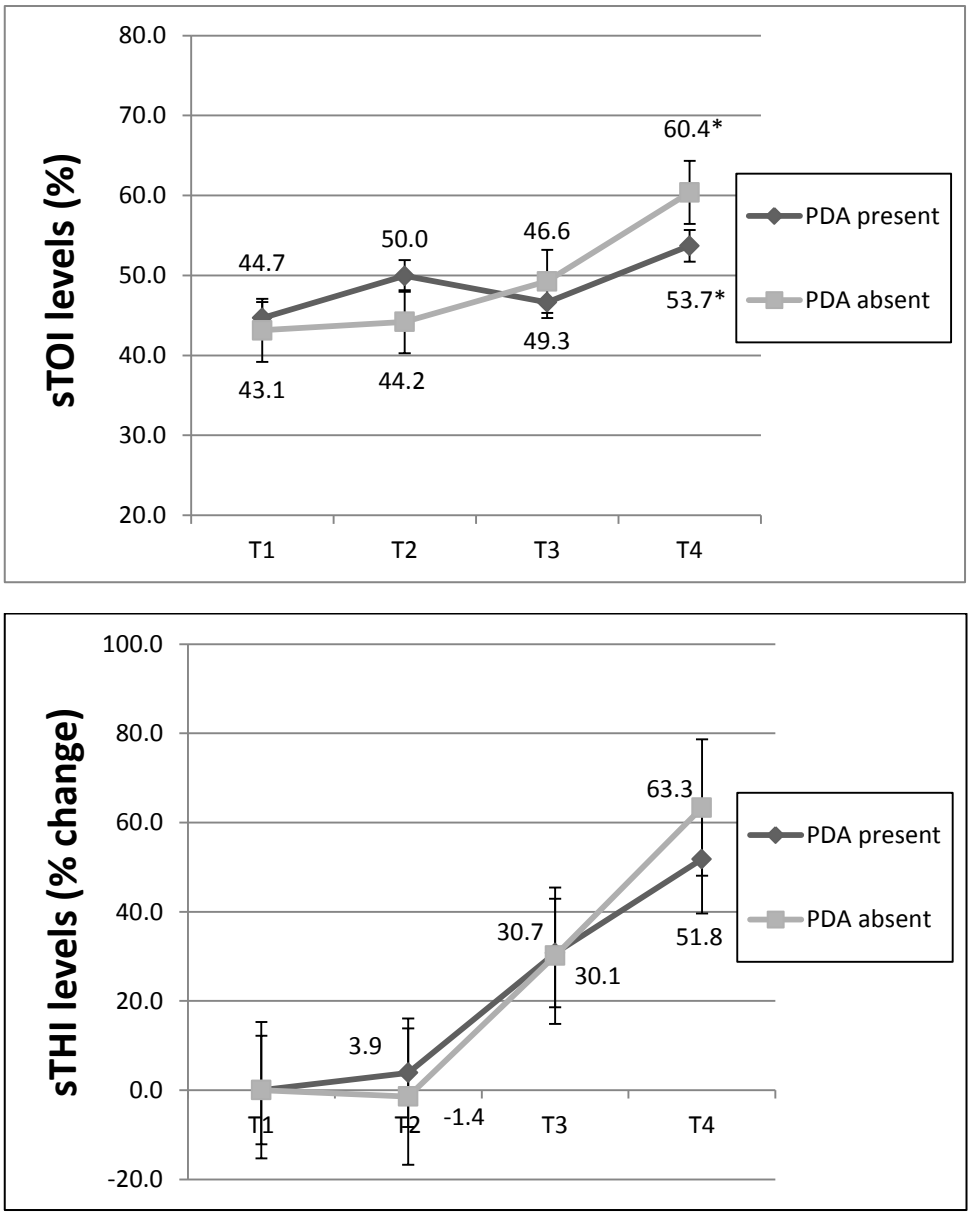


Figure 32. Blood transfusion and Splanchnic Tissue Oxygenation Index (sTOI) and Tissue Haemoglobin Index (sTHI) in relation to PDA

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

4.5.2.5 Multivariate analysis of changes in splanchnic blood flow and tissue oximetry

The changes in SMA PSV and sTOI following blood transfusion were not significantly different between the postnatal age group infants and were not influenced by covariates: gestational age, birth weight, pre-transfusion Hb, mean blood pressure, presence of PDA and feeding volume on multivariate analysis.

4.5.3 Measurements of control infants

4.5.3.1 Basic characteristics of control infants

There were twelve infants in the control group. Of them four were studied in the first week of life, five between day 8 to day 28 of life and three were ≥29 days of postnatal age. The mean gestational age at birth was 29±5 weeks and birth weight was 1400±972 grams. The mean postnatal age of measurement was 20±15 days. The mean haemoglobin (Hb) level at birth was 13.3±2.8mg/dl and the pre-measurement Hb was 10.3±2.8mg/dl. Two infants were undergoing invasive ventilation, five each were undergoing non-invasive ventilation or breathing in air. None of the babies were on inotropic support or receiving treatment for suspected or proven sepsis. Eight infants had no IVH and four had Grade 1 haemorrhage. These infants were receiving a total mean fluid volume of 144±25 ml/kg and a total volume of feeds of 110±64 ml/kg.

The heart rate (HR), respiratory rate (RR), systolic blood pressure (BP) and diastolic BP, mean arterial BP and SaO2 all remained stable at the start of the NIRS measurements (pre-oximetry measurements) and at the end of NIRS measurements (post-oximetry measurements) as shown in **Table 20**.

Table 20. Vital parameters in control infants

Vital parameters	Pre-oximetry measurements	Post-oximetry measurements	Difference between groups
Heart rate (bpm)	157.3±12.6	158.2±9.3	NS
Respiratory rate (bpm)	52.6±12.6	49.2±11.7	NS
Systolic BP (mm Hg)	46.8±7.0	47.4±5.8	NS
Diastolic BP (mm Hg)	24.5±3.3	25.7±2.9	NS
Mean BP (mm Hg)	36.0±3.8	37.3±3.2	NS
SaO_2	93.2±2.7	93.0±1.9	NS

4.5.3.2 Tissue oximetry of control infants

The mean cerebral tissue oximetry measurements in each epoch remained unchanged over the three hour period of measurement. This was same for the splanchnic tissue oxygenation measurements as well (**Table 21**).

Table 21. Tissue oxygenation of control infants

Tissue oxygenation measurements Mean (SD)	T1	T2	T3	T4
cTOI	71.1 (18.3)	71.9 (19)	71.2 (16.9)	72.0 (15)
cTHI	38.0 (7.3)	38.1 (7.6)	39.6 (7.6)	39.8 (7.8)
sTOI	42.1 (8.1)	43.1 (8.2)	43.8 (8.3)	43.5 (7.7)
sTHI	50 (10.1)	48.3 (11.9)	50.7 (12.2)	50.9 (10.1)

4.6 Measurement of red cell volume

4.6.1 Infant characteristics

Red cell volume was measured using fetal haemoglobin (HbF) dilution method in 17 preterm infants with indwelling arterial catheters. The characteristics of those infants

who had red cell volume (RCV) measurements are detailed in **Table 22**. Of the 17 infants studied the RCV measurement was unavailable for three infants: one pre-transfusion sample was clotted, one post-transfusion sample was insufficient and one post-transfusion blood sample was lost in the lab; the RCV measurement was available for the rest of the 14 infants.

Table 22. Infant characteristics of those who had RCV measured

Infant characteristics	**Median (Range)**
Gestational age (weeks)	26 (23 – 27)
Birth weight (grams)	830 (700 – 1240)
Chronological age at RCV measurement (days)	2 (1 – 14)
Total volume of fluids (ml/kg/day)	150 (90 – 180)
Total volume of feeds (ml/kg/day)	15 (0 – 180)
Pre-transfusion haemoglobin (g/dl)	11.2 (8.7 – 12.7)
Pre-transfusion haematocrit (%)	32 (26 – 38)
Weight of baby on RCV measurement day (grams)	810 (700 – 1180)
Pre-transfusion RCV (ml/kg)	29.9 (20.6 – 38.7)

There were 5 infants whose pre-transfusion RCV was <25 ml/kg and 9 whose RCV was >25 ml/kg. The basic characteristics of these subgroups are presented in **Table 23**. As planned earlier the cerebral and gut blood flow and oximetry were further analysed between those subgroups.

Table 23: Basic characteristics of infants with pre-transfusion RCV <25 or ≥25 ml/kg

Characteristics	RCV <25 ml/kg n=5	RCV ≥25 ml/kg n=9	Difference between groups
Gestational age (completed weeks)*	25 (24 – 28)	26 (24 – 27)	NS
Birth weight (grams)*	790 (600 – 1240)	830 (715 – 1000)	NS
Haemoglobin at birth (g/dl)*	14.5 (10.0 – 16.1)	14.5 (10.5 – 16.5)	NS
Chronological age (days)*	6 (1 – 14)	2 (1 – 6)	0.03
Maternal PET†	2 (40)	3 (33)	NS
IUGR†	2 (40)	3 (33)	NS
Chorioamnionitis†	3 (60)	4 (44)	NS
Antepartum haemorrhage†	2 (40)	3 (33)	NS
Antenatal steroids†	4 (80)	7 (78)	NS
Weight at transfusion (grams)*	805 (540 – 1150)	810 (715 – 1000)	NS
Pre-transfusion Hb (g/dl)*	9.8 (8.7 – 10.6)	12.6 (10.7 – 13.1)	<0.001
Total fluids (ml/kg/d)*	150 (120 – 180)	120 (90 – 180)	0.04
Total feeds (ml/kg/d)*	50 (15 -180)	0 (0 – 20)	<0.001
Invasive/Non-invasive ventilation/nasal cannula oxygen or breathing in air†	3/2/0	5/4/0	NS
Presence of PDA†	3 (60)	6 (67)	NS
Presumed sepsis on antibiotics†	2 (40)	4 (44)	NS

* Median (Range), † Number (percentage)

4.6.2 Red cell volume measurements

Details of the pre-transfusion red cell volume measurements in all the infants are shown in **Appendix 8**. The donor haematocrit ranged from 0.53 to 0.69 and the pre-

transfusion red cell volume ranged from 20.6 to 38.7 ml/kg. There were five infants whose pre-transfusion RCV was <25 ml/kg and nine had ≥25 ml/kg.

4.6.3 Haemoglobin and red cell volume

Pre-transfusion haemoglobin showed good correlation (r=0.65, p<0.01) with pre-transfusion red cell volume on Pearson correlation statistics **(Figure 33)**. Similarly, pre-transfusion haematocrit also showed good correlation (r=0.60, p<0.01) with pre-transfusion RCV **(Figure 33)**.

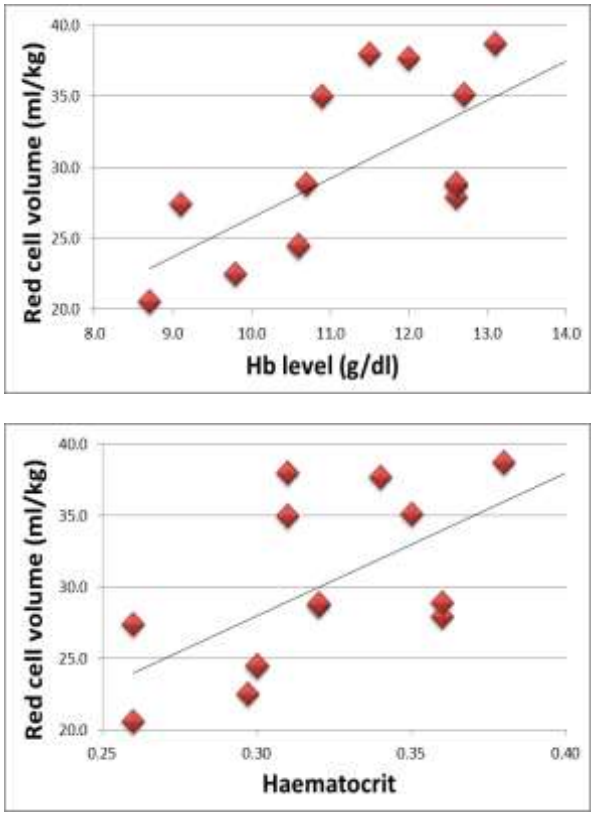


Figure 33. Relationship between Hb and Hct level and red cell volume

4.6.4 Red cell volume and cerebral blood flow and oximetry

Five infants had RCV <25 ml/kg while nine had ≥25 ml/kg. The baseline pre-transfusion ACA PSV (0.42±0.11 m/sec) and ACA TAMV (0.22±0.05 m/sec) were higher in those with pre-transfusion RCV <25 ml/kg compared to those with RCV ≥25 ml/kg (0.30±0.07 m/sec and 0.15±0.05 m/sec respectively). The ACA peak

systolic velocity decreased significantly following blood transfusion in those with RCV ≥25 ml/kg; although there was decreasing trend in those with RCV <25 ml/kg this was not statistically significant (**Table 24**). There was no significant change in either ACA time averaged mean velocity or SVC flow in either of these two groups of infants. The baseline pre-transfusion cTOI was higher in infants with RCV <25 ml/kg (77.9±17.4%) compared to those with RCV ≥25 ml/kg (66.4±19.1%) but this was not statistically significant. The pre-transfusion cTHI was similar between the two groups (**Table 24**). There was an increasing trend in cerebral TOI following blood transfusion in those with RCV ≥25 ml/kg, and a significant increase in the cerebral THI were noticed post-transfusion in both groups.

4.6.5 Red cell volume and gut blood flow and oximetry

Though there was no difference in the pre-transfusion peak systolic velocity (PSV) of the SMA following transfusion in the group with RCV ≥25 ml/kg; there was a significant decrease in the SMA PSV following transfusion (p=0.03, CI 0.01, 0.33) in the group with RCV <25 ml/kg (**Table 24**). The splanchnic tissue oxygenation index (sTOI) increased significantly in those with RCV ≥25 ml/kg (p<0.01, CI 7.9, 30.9) along with a subsequent decrease in the sFTOE and increase in sTHI. There was no change in the sTOI, sTHI or sFTOE in those infants with pre-transfusion RCV <25 ml/kg (**Table 24**).

A similar multivariate analysis using MANCOVA could not be performed for RCV groups in view of the small sample size (n=14) and even smaller sample size in each of the groups (RCV <25ml/kg=5 and ≥25ml/kg=9).

Table 24. Changes in measurement parameters in relation to red cell volume (RCV) values

(n=14, 17 infants were attempted)

Parameters measured *Mean (SD)*	**RCV <25 (n=5)**			**RCV ≥25 (n=9)**		
	Pre-BT	**Post-BT**	**P value; CI**	**Pre-BT**	**Post-BT**	**P value; CI**
ACA PSV (m/sec)	0.42 (0.11)	0.32 (0.08)	0.28; -0.14 to 0.33	0.30 (0.07)	0.23 (0.07)	0.01; 0.02 to 0.11
ACA TAMV (m/sec)	0.22 (0.05)	0.16 (0.04)	0.31; -0.08 to 0.19	0.15 (0.05)	0.13 (0.03)	0.15; -0.01 to 0.07
SVC flow (ml/kg/min)	160.4 (51.5)	130.7 (29.1)	0.16; -21.8 to 81.0	79.4 (16.9)	76.4 (14.9)	0.65; -11.9 to 18.1
SMA PSV (m/sec)	0.77 (0.11)	0.59 (0.07)	0.03; 0.01 to 0.33	0.62 (0.28)	0.56 (0.16)	0.57; -0.17 to 0.28
cTOI (%)	77.9 (17.4)	80.5 (11.6)	0.55; -18.2 to 13.0	66.4 (19.1)	71.4 (13.9)	0.06; -10.4 to 0.5
cFTOE (%)	27.9 (0.9)	21.4 (0.6)	0.10; -6.9 to 20.0	41.1 (8.1)	31.8 (7.4)	0.006; 3.9 to 31.8
cTHI (arbitrary units)	32.2 (3.8)	39.8 (5.3)	0.01; -11.7 to -3.5	34.7 (6.5)	44.8 (8.6)	0.0001; -13.3 to -6.9
sTOI (%)	45.4 (22.6)	45.0 (10.5)	0.97; -35.6 to 36.1	37.4 (11.1)	56.8 (21.5)	0.0046; -30.9 to -7.9
sFTOE (%)	51.7	51.7	0.85; -10.6 to 23.1	59.3	38.9	0.002; 8.5 to 28.4
sTHI (arbitrary units)	35.3 (7.9)	51.3 (15.9)	0.83; -36.9 to 5.1	48.7 (14.4)	67.2 (17.0)	0.0012; -26.3 to -10.7

4.7 Relationship between Hb and tissue perfusion

The study population was divided into two groups depending on the pre-transfusion Hb level of 11g/dl as previously decided. There were 15 infants whose Hb at transfusion was ≥11g/dl, and 44 infants who were transfused when Hb was <11g/dl. Further details of the infant and maternal characteristics of these two groups are detailed in **Table 25**. The two groups had similar gestational age, birth weight and Hb level at birth. The groups with pre-transfusion Hb of <11g/dl had higher incidence of antepartum haemorrhage (APH). They were also older (postnatal age), higher weight at transfusion and were on higher volume of feeds compared to those where blood was transfused with pre-transfusion Hb ≥11g/dl.

Table 25. Infant and maternal characteristics of the two groups with Hb≥11 and <11g/dl

Characteristics	Hb≥11g/dl n=15	Hb<11g/dl n=44	Difference between groups
Gestational age (completed weeks)*	26 (23 – 27)	25 (23 – 31)	NS
Birth weight (grams)*	768 (592 – 1180)	745 (540 – 1746)	NS
Haemoglobin at birth (g/dl)*	14.1 (10.0 – 19.2)	14.7 (9.8 – 20.7)	NS
Chronological age (days)*	5 (1 – 26)	18 (1 – 73)	<0.001
Maternal PET†	4 (27)	11 (25)	NS
IUGR†	4 (27)	8 (18)	0.04
Chorioamnionitis†	7 (47)	18 (41)	NS
Antepartum haemorrhage†	3 (20)	15 (34)	0.03
Antenatal steroids†	15 (100)	38 (86)	0.03
Weight at transfusion (grams)*	780 (630 – 1180)	914 (660 – 2045)	NS
Pre-transfusion Hb (g/dl)*	12.0 (11.0 – 13.1)	9.8 (7.0 – 10.9)	0.01
Total fluids (ml/kg/d)*	135 (90 – 180)	150 (100 – 180)	NS
Total feeds (ml/kg/d)*	8 (0 – 160)	115 (0 – 180)	<0.001
Invasive/Non-invasive ventilation/nasal cannula oxygen or breathing in air†	2 (13)/8 (53)/6 (40)	2 (5)/14 (32)/26 (59)	NS
Presence of PDA†	1 (7)	31 (71)	<0.001
Presumed sepsis on antibiotics†	6 (40)	29 (66)	0.04

* Median (Range), † Number (percentage)

4.7.1 Haemoglobin and blood flow

The effect of pre-transfusion Haemoglobin level on the degree of cerebral and gut blood flow changes following blood transfusion was analysed by dividing the study infants into two groups with Hb ≥11g/dl and <11g/dl. The pre-transfusion baseline anterior cerebral artery (ACA) time averaged mean velocity (TAMV) was significantly higher in the infants with pre-transfusion Hb <11 g/dl (0.23±0.07 m/sec) compared to those with Hb level ≥11 g/dl (0.16±0.04 m/sec, p<0.001) (**Figure 34**); this remained significant after multivariate analysis (p=0.01, CI 1.1 to 2.4) including all the covariates: gestational age, birth weight, blood pressure, presence of PDA and volume of feed. The pre-transfusion baseline superior vena cava (SVC) blood flow was also higher in infants with Hb <11g/dl but this was not statistically significant (100.1±36.8 compared to 93.2±39.1 ml/kg/min, p=0.54). The ACA TAMV decreased significantly in both the groups following blood transfusion (**Figure 34**) and the degree of decrease was similar (17%) in those with a pre-transfusion Hb <11g/dl (from 0.23±0.07 to 0.19±0.06 m/sec, p<0.001) compared to those with a pre-transfusion Hb level ≥11g/dl (from 0.16±0.04 to 0.13±0.03 m/sec; p=0.03; 18%). The baseline SVC flow decreased in both Hb groups post-transfusion but this was not statistically significant (p=0.07).

The baseline pre-transfusion superior mesenteric artery (SMA) peak systolic velocity (PSV) was higher in infants with a pre-transfusion Hb <11g/dl (0.85±0.35 m/sec) compared to those with a pre-transfusion Hb of ≥11g/dl (0.66±0.39 m/sec) but this was not statistically significant (p=0.08).

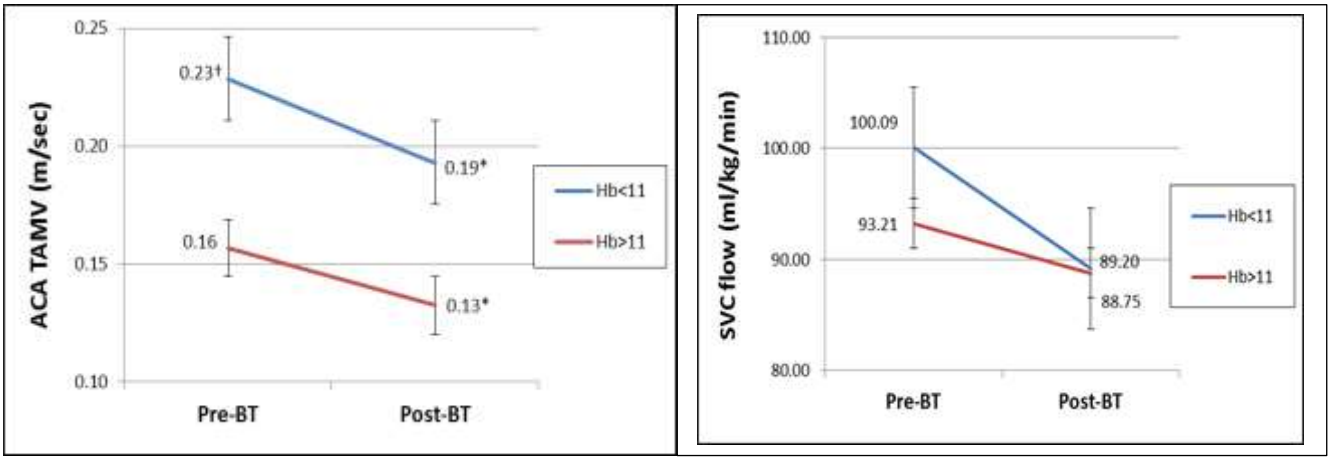


Figure 34. Anterior cerebral artery (ACA) time averaged mean velocity (TAMV) and superior vena cava (SVC) flow in the groups with pre-transfusion Hb levels ≥ and <11g/dl. * p<0.05 comparison between pre and post-transfusion; † p<0.05 comparison between groups with Hb <11 and Hb ≥11g/dl

The pre-transfusion SMA PSV decreased in both group of infants with Hb level <11g/dl (from 0.85±0.35 to 0.78±0.29 m/sec; p=0.1) and ≥11g/dl (from 0.66±0.39 to 0.62±0.21 m/sec; p=0.4) but these changes were not significant (**Figure 35**).

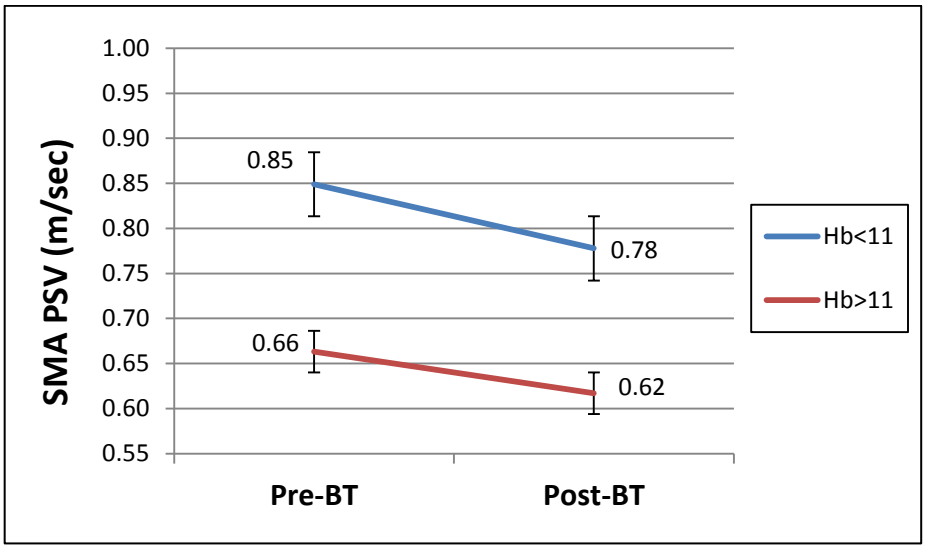


Figure 35. Superior mesenteric artery peak systolic velocity in the groups with pre-transfusion Hb levels ≥ and <11g/dl.

4.7.2 Haemoglobin and tissue oximetry

4.7.2.1 Changes in tissue oxygenation in relation to Hb level

In order to find the effect of pre-transfusion Hb level on the degree of cerebral and gut tissue oxygenation changes, the total study population was divided into infants with Hb <11g/dl and Hb ≥11g/dl (**Table 25**). The pre-transfusion cTOI levels were similar between the two groups (**Figure 36**). A repeated measures ANOVA showed that there was a significant increase in the cerebral tissue oxygenation index (cTOI) following blood transfusion in both groups with Hb level above (p=0.005; CI 2.2,10.1) and below (p<0.0001; CI 3.9,8.9) 11g/dl (**Figure 36**).

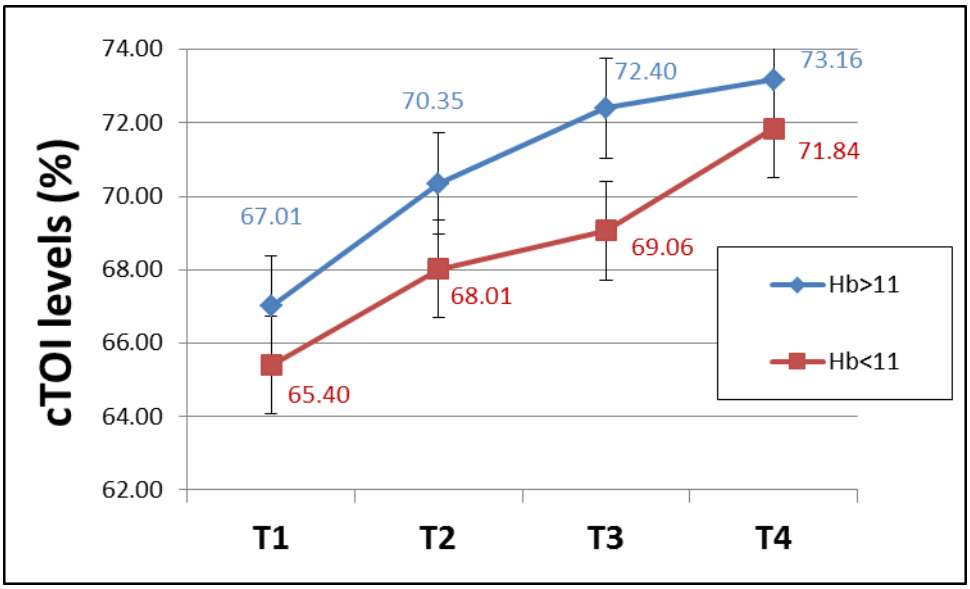


Figure 36. Pre-transfusion Haemoglobin and cerebral tissue oxygenation index (cTOI) and changes following blood transfusion

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

Similarly, the pre-transfusion sTOI levels were similar between the two groups (**Figure 37**). The splanchnic tissue oxygenation also increased significantly in both group of infants with Hb ≥11g/dl (p=0.001; CI 6.6, 23.5) and Hb <11g/dl (p<0.0001; CI 7.3, 18.9) following blood transfusion (**Figure 37**).

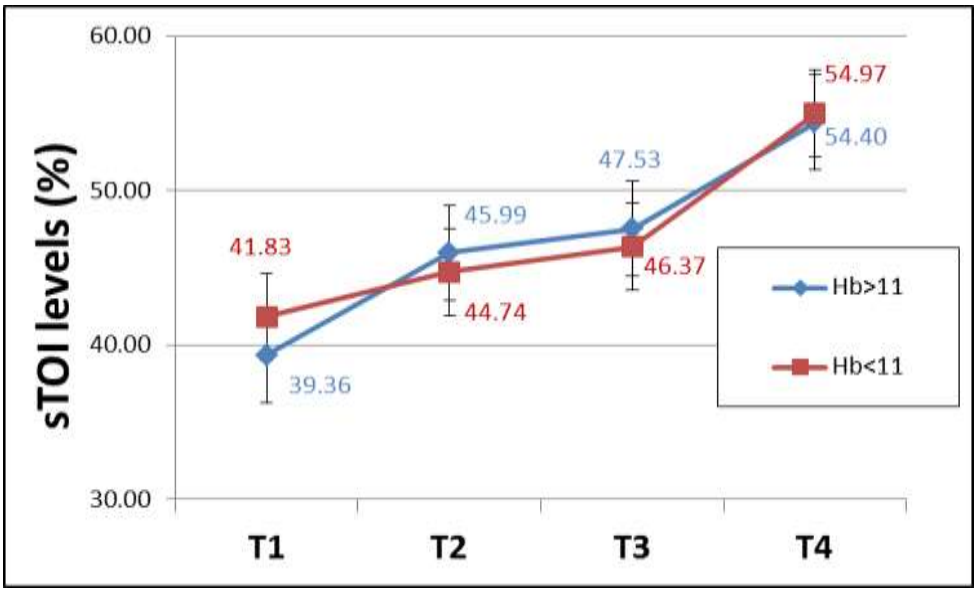


Figure 37. Pre-transfusion haemoglobin and splanchnic tissue oxygenation (sTOI) level and changes following blood transfusion

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

In infants with Hb <11g/dl the cTOI increased by 9.8% and the sTOI increased by 31.4%. In the infants with Hb ≥11g/dl cTOI increased by 9.2% and the sTOI increased by 38.2%.

5 Discussion

5.1 Overall summary of results

The study has demonstrated that pre-transfusion baseline cerebral blood flow increases with increasing postnatal age; the pre-transfusion intestinal or splanchnic blood flow increases with increasing postnatal age. The pre-transfusion baseline cerebral tissue oxygenation decreases while the intestinal or splanchnic oxygenation does not significantly increase with increasing postnatal age. Overall blood transfusion improves cerebral and splanchnic tissue oxygenation extraction balance (i.e. increase in oxygenation and decrease in FTOE) in preterm infants irrespective of postnatal age. The cerebral blood flow velocities as well as venous return from upper body decreases following blood transfusion, whereas there was no appreciable change in intestinal blood flow velocity post-transfusion. The blood pressure increases but there is no immediate change in other vital parameters such as heart rate, respiratory rate and oxygen saturation following blood transfusion in preterm infants. The degree of change in blood pressure following blood transfusion decreases with increasing postnatal ages. Both cerebral and splanchnic oxygenation improved following blood transfusion in infants with pre-transfusion RCV ≥25 ml/kg. In infants with pre-transfusion red cell volume (RCV) <25ml/kg the intestinal blood flow velocity decreased following blood transfusion. The gut oxygenation extraction balance improved following transfusion in those infants with RCV ≥25ml/kg, but this improvement was not noticed in those with pre-transfusion RCV <25ml/kg. A multivariate analysis using MANCOVA could not be performed because of the small sample size of the RCV groups[259].

Blood transfusion improved cerebral and splanchnic tissue oxygenation irrespective of pre-transfusion haemoglobin levels of ≥ or <11 g/dl. Though blood transfusion reduced cerebral blood flow there was no significant change in intestinal blood flow post-transfusion irrespective of the pre-transfusion Hb levels. Blood transfusion improved gut oxygenation more than cerebral oxygenation in infants with pre-transfusion haemoglobin <11 g/dl.

Pre-transfusion baseline blood flow velocity of the anterior cerebral artery and the SVC flow was similar in infants between the open and closed PDA groups, while the SMA velocity was higher in the closed PDA group. The blood flow velocity decreased significantly in the ACA following transfusion but there were no changes in the SVC flow or SMA blood flow velocity in both groups. The pre-transfusion baseline cerebral and gut tissue oxygenation was similar between the two matched PDA groups and increased significantly following blood transfusion in both groups.

In the following sections I will discuss these findings in detail and compare with the current literature. I will also discuss the strengths and limitations of this study, the generalisability and future directions in research in line with the current study.

5.2 Infants studied

It is well known that majority of the preterm infants receive at least one blood transfusion during their stay in the neonatal unit[225]. In this study I aimed to recruit 60

infants receiving blood transfusion divided into three post-natal age groups (Group 1: 1-7 days, Group 2: 8-28 days and Group 3: ≥29 days). The main reason for recruiting to three postnatal age groups is the difference in haemodynamics due to presence of intra-cardiac shunts such as patent foramen ovale (PFO) and extra-cardiac shunts such as patent ductus arteriosus (PDA)[152], incremental feeding volumes and different haemoglobin (Hb) or haematocrit (Hct) threshold for transfusion in infants of different postnatal age groups [26]. The previous researchers who demonstrated tissue oxygenation[52-54] or changes in blood flow following blood transfusion[49,52], studied infants of wide postnatal ages together as a single group. In the current study, in addition, majority of infants in Group 1 (1-7 days of age) were receiving some form of ventilatory support while those in older postnatal age group (Group 3 ≥29 days) were receiving less amount of invasive ventilatory support. These physiological and haemodynamic variability as well as the variable management according to their gestational and postnatal maturity can result in a variable effect on the degree of changes caused by blood transfusion on blood flow and tissue oxygenation of brain and gut.

For this study a pragmatic sample size of 20 infants in each study group and 12 infants in the control group (stable infant not receiving blood transfusion) were chosen. Previous researchers have demonstrated that a sample size of 10 infants are required to identify a 10% increase in cerebral or splanchnic tissue oxygenation index at the end of transfusion with 80% power at 0.05 level[52,261]. This indicates that the sample size chosen in the current study is appropriate to demonstrate the degree of change in tissue oxygenation.

Out of the 97 infants consented for the study; measurements were performed in 59 infants who received blood transfusion and 12 control infants. 97 (94%) out of 103 parents of study infants approached consented for their baby to be studied. 12 (70%) out of 17 parents of control infants also agreed for their infant to participate in the study. Amongst the infants whose parents declined to participate in the study, the majority of those who were receiving transfusion were sick and the effect of transfusion may have been different in them. In many cases the consent was taken prior to possibility of transfusion and was further discussed with parents when the transfusion was imminent. However, many transfusions happened during out of hours (6pm – 8am), over weekends, whilst I was on-call with clinical commitments or on annual leave. The clinical team made the decision to transfuse based on transfusion guidance[26], cardiorespiratory deterioration and increase in ventilatory or oxygen requirement. Informed written consent was taken for every infant and the study protocol and a copy of the consent form were attached to the clinical records.

Apart from the three groups who received transfusion the study recruited 12 infants of similar gestational and postnatal age groups and similar antenatal and infant characteristics as controls. The measured changes in the cerebral and splanchnic blood flow and tissue oximetry in the study group and the control group were compared and it is one of the strengths of this study. This enabled me to differentiate between physiological variability over time and the true changes in cerebral and splanchnic tissue blood flow and oxygenation following transfusion.

Bailey[53], Dani[52] and Seidel[54] have measured tissue oxygenation changes following blood transfusion in preterm infants of 5 to 93 days of postnatal age with a wide range of pre-transfusion Hb and Hct together as a single group. Bailey et al studied 35 preterm infants (28.4±3.0 weeks) with a mean birth weight 1115±426 grams, pre-transfusion Hb of 9.3±1.2g/dl and transfused at a mean postnatal age of 31.7±16.2 days. Of the 30 patients who were included in the final analysis 5 were on conventional ventilatory support, 7 were on CPAP, 10 were on nasal cannula and rest of the 8 babies were on no respiratory support. None of the infants studied by Bailey et al were in the first week of postnatal age where they are most sick and require blood transfusions, which is in contrast to the present study. Dani et al measured cerebral, splanchnic and renal perfusion before and after blood transfusion in 15 preterm infants (mean gestational age of 27.0±2.4 weeks, birth weight 904±235 g) at a mean postnatal age of 32±23 days of life. Dani et al studied infants of a wide range of postnatal age, who would surely have variable physiological and haemodynamic status, but this was not analysed separately; this is in contrast to the current study. Seidel et al measured cerebral and peripheral regional oxygen saturation before and after transfusion in 93 preterm infants (mean gestational age of 27±3 weeks) who were transfused at a mean age of 38±22 days. Twelve infants were excluded due to incomplete NIRS data. Eight of the infants studied were on conventional ventilator, 41 were on CPAP and the 27 were on no respiratory support. In contrast to the current study Seidel at al did not measure infants receiving transfusion in the first week of life and the majority of them were receiving non-invasive ventilatory support or on no respiratory support at all. Sandal et al[261] studied 23 symptomatic patients with anaemia who were <30 weeks of gestational age, who were ≥1 months of postnatal age and had a pre-transfusion Hct of ≤27%. Mintzer et al [262] studied 10 infants (gestational age 26±0 weeks)

receiving transfusion in the first week of postnatal age. However, both studies did not measure blood flow to the brain and gut concurrently in the same infants during blood transfusion which is in contrast to the current study where both blood flow and tissue oxygenation was measured in the brain and gut during transfusion. Alakalay et al measured the effect of blood transfusion on cardiac output and other echocardiography measurements such as left ventricular diameter in systole and diastole and blood flow in the aorta in 32 preterm infants with a median gestational age of 29 (IQR 28,30) weeks and postnatal age 33.3 (IQR 31.9,34.9) weeks[49]. It is evident from all these observational studies that the study population was of a wide spectrum of gestational and postnatal ages receiving variable degrees of ventilatory support. In contrast, in the present study infants were divided into three groups based on postnatal age to minimise the effect of postnatal maturity on haemodynamic and oximetry changes measured.

At the outset we have planned to recruit infants to three postnatal age study groups in order to investigate their variable adaptation to postnatal haemodynamic changes as well as the effect following transfusion, this is one of the important strengths of the study.

5.3 Vital parameters

One of the strengths of this study is the continuous recording of vital parameters before, during and post-transfusion. The previous studies have only reported vital parameter recordings from observational charts that recorded random hourly values

pre and post-transfusion. There was no change in heart rate (HR) following blood transfusion in all three infant groups in the present study but other researchers have reported a significant decrease in heart rate[16,47]. Kasat et al noted tachycardia to be the most notable and sensitive predictor of a benefit from blood transfusion (OR 6.48, p=0.005)[16]. Nelle et al measured physiological parameters before and four hours after blood transfusion in 33 preterm infants (mean gestational age 29±5 weeks, birth weight 1153±390 grams and a postnatal age of 48±21 days) and reported a significant reduction in heart rate (from 161±14 to 149±12 bpm, p=0.005) along with stoke volume[47]. On the contrary, in keeping with the current study, Dani et al did not notice significant change in heart rate (133±8 pre-transfusion to 122±8 one hour post-transfusion, p=NS) following blood transfusion[52]. Bailey[53] and Seidel[54] also did not notice changes in heart rate following blood transfusion. Pre-transfusion tachycardia is an adaptive mechanism by which preterm infants maintain a high cardiac output in response to the declining haematocrit to maintain tissue perfusion [49]. It is quite possible that the pre-transfusion tachycardia takes considerable amount of time (at least 12 hours) to settle and adapt to the increased haematocrit and viscosity following blood transfusion[16,47], whereas most of the observational studies including the current study recorded heart rate upto one hour post-transfusion.

Some of the previous studies in older clinically stable preterm infants have reported no significant difference in mean blood pressure (MBP) following blood transfusion[47,52]. Nelle et al measured mean blood pressure using Dinamap and noticed no change in MBP following blood transfusion (56.4±7.9 to 58.1±7.9 mmHg)[47]. Similarly Dani et al did not notice any change in MBP following blood

transfusion (56±7 to 56±6 mmHg)[52]. Contrary to these findings, in the current study there was a significant increase in the MBP following blood transfusion in all the three postnatal age groups studied. These findings could be due to the different time points these vital parameters were measured in these studies. Whereas, Nelle et al measured MBP at 4 hours following transfusion, Bailey, Dani and the current study recorded MBP upto 1 hour post-transfusion. In the current study, the pre-transfusion 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 [263]. None of the previously reported observational studies have described findings according to postnatal age groups and hence it is difficult to compare the findings of the current study with them.

While blood pressure in the majority of the infants in the early group (Group 1: 1-7 days) was measured using invasive indwelling arterial catheter (11 out of 20), blood pressure was measured using non-invasive oscillometric technique (Dinamap) in the majority of the infants in the other two groups (Group 2: 18 out of 21 and Group 3: all 18 infants). It is possible that analysing together the blood pressure measured by two different methods would have affected the findings of the present study. However, systolic and diastolic blood pressures were also measured simultaneously, showing a similar trend to mean blood pressure post-transfusion. In a study of 398 infants between 24 to 32 weeks of gestational age, serial measurement of systolic blood pressure using three different methods have shown good agreement between invasive and Doppler measurement; but there was wider variation between oscillometric (Dinamap) and Doppler measurement methods of blood pressure[264].

There was no noticeable change in oxygen saturation (SaO_2) and respiratory rate following blood transfusion in any of the three groups of infants in the current study. Dani et al similarly did not notice any changes in SaO_2 following blood transfusion. 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 (n=22) and restrictive (n=19) transfusion [46]. Whereas other researchers[16,46] have compared random recording from observational charts from few hours to days before, and few hours to 1-2 days after blood transfusion, we have compared continuous recordings of vital parameters from 15-20 minutes pre to post-transfusion.

5.4 Laboratory parameters

As expected the pre-transfusion haemoglobin (Hb) and haematocrit (Hct) levels increased significantly post-transfusion in this study. Leukocyte depleted, cytomegalovirus negative, Sickle cell negative, plasma reduced and cross-matched 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. Overall the mean pre-transfusion Hb and Hct were comparable to the reported studies[16,32,54]. Bailey et al reported mean pre-transfusion Hb level of 9.3±1.2g/dl which subsequently increased by 3.1±1.3g/dl following transfusion[53]. Dani et al reported an increase of mean pre-transfusion Hct level of 27.1±2.1% to 43.3±2.7% post-transfusion[52]. Nelle et al transfused infants at a mean pre-transfusion Hb level of 8.8±1.5g/dl which subsequently increased to 12.2±1.7g/dl post-transfusion[47]. Transfusion of infants in the current study was decided by the

attending clinical team based on British Committee for Standards in Haematology (BCSH) blood transfusion guidance[26], hence the pre-transfusion Hb or Hct levels were significantly different between the three postnatal age group of infants studied.

One of the strengths of this study is the measurement of pre and post-transfusion blood gas parameters such as pH, pCO_2 and serum lactate levels in all infant transfused, which has significant effects on cerebral[265], splanchnic[266] and pulmonary[267,268] blood flow . Cerebral vascular vasodilatation [269,270] and pulmonary[268] and intestinal vascular vasoconstriction[266] with increasing pCO_2 has been well documented. Blood flow volume depends on the fourth power of the radius of the vessels; which would explain a small change in the diameter of the vessels resulting in significant changes in the blood flow. Such an important factor of blood flow has not been taken into account in previous studies demonstrating changes in the blood flow following blood transfusion[47,48,52,271]. In the current study the pre and post-transfusion blood gas pH and pCO_2 levels were not different thereby minimising their effects on the changes in cerebral and splanchnic blood flow. Mintzer et al measured laboratory parameter changes in preterm infants receiving transfusion in the first week of life and noted no change in pH, base deficit, lactate and creatinine following blood transfusion. The pre-transfusion mean haematocrit level was 35.2±1.2% which increased to 38.5±1.2% post-transfusion[262]. In the current study the Group 1 infants (postnatal age 1-7 days) showed no difference in pH and pCO_2 post-transfusion but showed a significant reduction in the serum lactate levels; this could be due to a lower pre-transfusion Hct of 32±4% in the current study compared to Mintzer et al, which increased to 40±5% post-transfusion.

But serum lactate level has its own limitations; Frey et al[122] using multiple regression noticed that pre-transfusion serum lactate did not correlate with pre-transfusion Hct, heart rate, respiratory rate, number of apnoea/bradycardia and weight gain. From these findings they concluded that since serum lactate does not correlate with other conventional parameters of compromised tissue oxygenation it adds very little to inform the decision to transfuse infants. Similarly, other researchers reported no correlation between pre-transfusion Hb and serum lactate levels [120-122] thereby demonstrating the lack of significance of serum lactate in deciding the requirement of blood transfusion[56,208]. Whereas Fredrickson[46], Moller[120], Frey[122] and Takahashi[121] showed a significant drop in serum lactate levels, Mintzer[262] reported no decrease in serum lactate levels post-transfusion. Fredrickson et al noticed a significant drop in serum lactate level following transfusion in both liberal (from 1.1±0.2 to 0.7±0.1 mmol/L, $p<0.0001$) and restrictive (from 1.1±0.2 to 0.9±0.2 mmol/L, $p=0.032$) transfusion groups thereby demonstrating a shift in the aerobic metabolism of the tissue following transfusion[46]. In the current study, 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 appreciable change in older infants (Group 3). One can speculate that preterm babies are more susceptible to low haemoglobin and oxidative stress in the first 4 postnatal weeks compared to the older preterm infants which is reflected in the findings of this study. This could also be related to maturational changes in the preterm haemodynamics, changes in peripheral vascular tone and resistance resulting in better peripheral tissue perfusion and changes in the composition of haemoglobin from fetal to adult

haemoglobin over time allowing easier dissociation of oxygen to the tissues to meet the metabolic demand.

5.5 Doppler measurements

The ultrasound scan measurements included a sequential echocardiography of cardiac morphology and superior vena cava (SVC) flow measurement. It also involved performing a cranial ultrasound scan to identify cranial abnormalities, intracranial bleeds and measuring anterior cerebral artery (ACA) blood flow velocities. Finally the ultrasound scan involved measuring superior mesenteric artery (SMA) blood flow velocities.

Doppler ultrasound scan is operator dependent and depends on the technique of the operator. Hence in 12 control infants I performed paired measurements in each infant 3 hours apart and compared the intra-operator variability and repeatability which was satisfactory and similar to the reported literature[74,152,153]. Groves et al have reported using MRI scans that the cross section of the SVC is crescent shaped as it wraps around the ascending aorta, this results in faulty measurements of the diameter of the SVC which is an obvious important component of the SVC flow measurement[154]. However, the SVC measurements in the current study are comparable to the echocardiographic measurements in the reported literature[152,153,272].

Noori et al have demonstrated in haemodynamically stable term neonates the perceived positive linear relationship between cerebral blood flow and pCO_2 may not be present on postnatal day one; further, on postnatal day three and possibly day two a pCO_2 threshold exists for this relationship, above which the cerebral blood flow response to increasing pCO_2 levels may result in reperfusion injury resulting in IVH[269]. However, in the current study the pCO_2 levels remained similar before and after transfusion and none of the infants developed new or worsening of IVH following transfusion.

It has been reported previously that mean blood flow velocity in the anterior cerebral artery (ACA) [47] and pericallosal artery [52] decreases following blood transfusion, possibly due to increased resistance in the cerebral blood flow due to increased viscosity of blood [273]. 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 there was significant decrease in the mean ACA time averaged mean velocity (TAMV) in all the three postnatal age group of infants in the current study. Infants of various gestational and postnatal age groups were merged together as a single group in previous studies[47,48]. In the current study, the baseline pre-transfusion blood flow velocity as well as the degree of their response to blood transfusion was different in the three infant groups studied. The pre-transfusion baseline mean ACA TAMV was lower during the first week of life (Group 1) and significantly higher in infants who were more than 28 days old (Group 3). This may be due to maturational changes but also could be attributed to older (Group 3) infants being transfused at a lower Hb threshold compared to the Group 1 infants. This finding can be supported from a study by

Deeg et al in a group of 121 healthy premature and full term infants, who noticed an exponential increase in ACA flow velocity with increasing conceptional age and also noted a linear increase in ACA TAMV with increasing postnatal age[274].

Superior Vena Cava (SVC) flow volume is a more reliable marker of preterm neonatal cerebral blood flow compared to left or right ventricular output in the presence of intra-cardiac shunts such as patent foramen ovale (PFO) and extra-cardiac shunts such as patent ductus arteriosus (PDA)[152]. Low SVC flow has been associated with development of IVH in preterm infants[272]. Using SVC flow as a surrogate of cerebral blood flow volume in order to assess changes following blood transfusion is strength of this study. Kluckow et al have demonstrated an increase in median (range) SVC blood flow from 76 (34–143) ml/kg/min on day one to 93 (55-111) ml/kg/min at around 48 hours of life in stable preterm infants[152], these values were comparable to the present study infants in the first week of their postnatal age. In the current study the SVC blood flow volume decreased following blood transfusion during the first week of life and in the infants who were more than 4 weeks of age. Alkalay et al have shown that anaemic preterm infants develop a high cardiac output state and this decrease significantly following blood transfusion [49]. There was no statistically significant change in the SVC flow in the Group 2 (8-28 days of age) infants perhaps indicating a more stable cardiovascular state or blood being transfused at a higher baseline Hb level before haemodynamic decompensation. It has been reported in the past by using NIRS technique that cerebral blood volume (CBV) decreases following blood transfusion in anaemic preterm infants. Koyano et al[275] have demonstrated significant decrease in CBV following transfusion (number of infants studied was 19); this was thought to be an

improvement from a compensatory rise in CBV in the anaemic state. Dani et al using NIRS techniques have also demonstrated a decrease in cerebral blood volume following transfusion. Koyano et al conducted their study in 19 preterm infants (median gestational age 27.1 weeks, range 23.5 – 30.4 weeks) of wide postnatal age range (2 – 85 days, median 39 days); Dani et al performed measurements in 14 infants (mean gestational age 29.6±2.6 weeks) of 29±14 days of mean postnatal age. Because of the wide postnatal age range of the infants included in these studies, this information should be interpreted cautiously. The maturational changes in the cerebral circulation and absence of cerebral autoregulation in the early postnatal age and development of autoregulation later on in life may play an important role in the changes in cerebral blood volume following transfusion which was not taken into account in these studies. In contrast, the infants were recruited to postnatal age groups in the current study and showed significant decrease in the SVC flow following blood transfusion in the earlier (1-7 days) and late (≥29 days) infants but was not noted in the infants between 8-28 days of postnatal age. The decrease in CBV[275] in conjunction with the significant decrease in cardiac output[49] following transfusion correspond to a decrease in the upper body flow and venous return i.e. SVC flow following blood transfusion as was shown in the current study.

PDA is an important determinant of cerebral blood flow in preterm infants[269]. One of the strengths of the current study is the demonstration of the interaction of PDA on the cerebral blood flow and oxygenation response to blood transfusion in gestational and postnatal age matched preterm infants. The baseline pre-transfusion mean ACA TAMV and SVC flow were not statistically different between the groups with open

and closed PDA thereby demonstrating similar upper body blood flow in either group. However, this may be confounded by the small study size and perhaps larger studies may be able to show significant differences between the two groups. Martin et al [276] have reported reversed diastolic blood flow in the cerebral arteries, with pulsatility index (PI) being significantly higher in infants with large PDA ($p<0.0001$) compared to those with a small PDA and controls with no PDA. In the current study the size of the PDA was not measured, but there was no noted reversed flow in the descending aorta in any of these infants studied. Measuring spectral analysed EEG and EEG response to photic stimulation, Kurtis et al [277] have demonstrated that the degree of decreased cerebral blood flow in infants with a significant PDA is not sufficient to cause any significant alteration in electrocortical activity. From the findings of Kurtis et al one can speculate that cerebral blood flow alteration caused by a significant PDA may not have any impact on the cerebral activity.

The pre-transfusion superior mesenteric artery (SMA) peak systolic velocity (PSV) was significantly higher in older postnatal age group infants in the current study and this may reflect maturational change, but this could also be complicated by other factors such as presence of PDA, different modes of ventilatory support and higher amount of feed intake as demonstrated in stable preterm infants by Havranek et al[278]. In 20 preterm infants (gestational age 28±2 weeks, birth weight 1002±173g) by measuring SMA flow and relative vascular resistance (RVR: calculated as mean arterial blood pressure divided by mean blood flow velocity) upto 14 days of life Yanowitz et al[279] noticed no change in the SMA relative vascular resistance (RVR) over time in the first two weeks of life. They also noticed that the baseline pre-prandial SMA mean blood flow velocity (BFV) was significantly higher in those

infants where feeds were started early rather than late but the response to feeds was similar in both the groups[279]. This is in keeping with the current study where increase in the baseline pre-transfusion peak systolic velocity of SMA was noticed over increasing postnatal age and feeding volume. The baseline pre-prandial SMA PSV and mean velocity may also be dependent on ventilation and CPAP as suggested by Havranek et al[280]. In eighteen stable preterm infants (gestation 32.1±1.1 weeks, birth weight 1793±350g) at a mean age of 2.5±0.8 days they noticed significantly lower baseline SMA mean velocity and PSV in infants who were on CPAP compared to those where CPAP was taken off. In 38 preterm infants with various grades (size) of PDA Havranek et al reported that the baseline pre-prandial SMA blood flow velocity was lower in the large PDA group, with marked significance in the end-diastolic phase ($p=0.002$) [281]. The findings of Havranek et al cannot be compared with the current study as the size of the PDA was not measured.

Presence of a large haemodynamically significant PDA can lead to ductal steal phenomenon; this was first described in preterm infants by Cassels et al [282]. Van Bel et al described the association between necrotising enterocolitis (NEC) and PDA [283]. Shimada et al reported that pulsatility index (PI) in the abdominal aorta was significantly higher in infants with severe respiratory distress syndrome (RDS), and it decreased to control levels after closure of the PDA[284]. Freeman-Ladd compared the ratio of the pulsatility indices of left pulmonary artery to aorta with pulsatility index of the SMA and reported significant negative correlation ($r=-0.47$, $p<0.008$). They concluded that hypoperfusion and hypoxia associated with large PDA may contribute to the developmental of NEC[285]. In the current study the measurement of the PDA were recorded alongwith the Doppler measurements of the SMA blood

flow; but none of the infants had reversal of flow or ductal steal noted in descending aorta or SMA.

In the present study there was a decreasing tend but no significant change in SMA flow velocity post-transfusion in all the three groups of infants studied, which is similar to the findings of Dani et al [52]. Though not comparable to our measurements, Nelle et al reported a 12% decrease in coeliac artery flow velocity following blood transfusion in 33 clinically stable preterm infants (mean gestational age 29 ± 5 weeks and mean postnatal age 48 ± 21 days)[47]. They also noticed a 21% increase in the red cell transport (blood flow velocity x packed cell volume) in the coeliac artery following transfusion indicating improved oxygen delivery despite reduced blood flow to the gut.

Pitzele et al [286] measured pre and post-prandial SMA blood flow velocity of 21 VLBW preterm infants (gestation 26±1.6 weeks, birth weight 819±240g), who were older than 14 days and were tolerating bolus enteral feeds three hourly. The measurements were performed pre, immediately post and 24 and 48 hours after blood transfusion. Post-feed SMA blood flow velocities which increased significantly pre-transfusion ($p<0.001$) were attenuated in the immediate post-transfusion period ($p=0.22$) but normalised 24 hour post-transfusion ($p=0.004$), this was irrespective of the presence of PDA. Similar blunting of response to feeds immediately post-transfusion with normalisation of response at 48 and 96 hours post-transfusion was noticed by Krimmel et al[75]; these two studies suggest that though there might be a blunting of post-feed response to the SMA blood flow velocities immediately post-transfusion this normalises within 24 hour after transfusion. These studies may have clinical implications of feeds initiation post-transfusion amongst preterm infants.

Though there was no significant change in SMA blood flow velocities post-transfusion in three study groups in the current study, there was a significant decrease in the SMA PSV in a small number of infants with pre-transfusion red cell volume of <25ml/kg. These measurements were performed immediately post-transfusion, the number of infants studied was small (n=5) and none of the infant developed any signs of feed intolerance or signs of NEC post-transfusion. But this may have clinical implications if this finding is replicated in a larger study group. It is well known that transfusion associated NEC is seen in extreme preterm infants, who are severely anaemic and are of older postnatal age[287,288]. At present the evidence is still not strong to suggest withholding of feeds during or post-transfusion in preterm infants receiving blood transfusion to prevent NEC.

5.6 NIRS measurements

NIRO 300 (Hamamatsu Photonics KK, Japan) is one of various commercially available near infra-red spectroscopy (NIRS) devices. Studies have shown the reproducibility and mean variability of the commercially available devices vary with each other. Recently by measuring and comparing regional saturations using NIRO 200 (Hamamatsu Photonics K.K, Japan), the INVOS 5100c (Somanetics, USA), the Fore-Sight (CAS Med Inc., California, USA) and the SenSmart X-100 (NONIN, Minnesota, USA), Schneider et al reported that they showed highly significant variation in local cerebral tissue oxygenation levels and hence concluded NIRS should only be used for measurements of trend rather than absolute values in preterm infants [289]. Hence, the results of spot tissue oxygenation measurements using NIRS devices should be interpreted with caution. The NIRS device in the

present study was mainly used to measure tissue oxygenation changes following blood transfusion.

Use of NIRS for measuring cerebral oximetry is well known and validated as described earlier (please see section 6.4.19). However, measuring other organ/tissue oximetry using NIRS has been reported in increasing numbers, such as splanchnic/gut oximetry[52,53,176], peripheral[50,54,209] and renal[52,290] oximetry. NIRS is a validated method of continuous measurement of cerebral tissue oxygenation in animal models [291] and measurement of cerebral blood volume in preterm infants[258,292]. NIRS have been used in various observational studies to measure cerebral tissue oxygenation in the past 20-30 years [293]. Greisen et al argued that to use NIRS as a clinical tool, NIRS oximetry measurements must demonstrate an added benefit in a randomised controlled clinical trial in newborn infants. They suggested that cerebral oximetry measurements must be used to reduce the risk of a clinically relevant endpoint such as death or neurodevelopmental delay[294]. A series of randomised controlled trials were developed in the last few years to try and ascertain the usefulness of NIRS as a clinical aid. Cerebral NIRS oximetry is currently being studied to aid newborn resuscitation in delivery room [295], to monitor cerebral autoregulation [296] and daily monitoring in neonatal units [297], and its role in management of neonatal hypotension [298,299].

The present study has demonstrated 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 ≥29 days (Group 3) of life. Other devices such as INVOS 5100c uses regional tissue oxygen saturation (rSO_2) as a marker of tissue oximetry which is similar to the tissue oxygenation index (TOI) used by Hamamatsu NIRO 300 device. Cerebral regional tissue oxygenation ($CrSO_2$) as well as cTOI is a marker 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 [300]. The cerebral tissue haemoglobin index (cTHI) is another cerebral tissue oxygenation measurement, and indicates the total concentration of Hb in the tissues and is in essence proportional to red cell volume in the tissue. The current study has demonstrated that the pre-transfusion baseline cerebral tissue oxygenation as well as 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[51-54]. In these studies preterm infants of 5 to 93 days of age with variable haemodynamic status and a wide range of pre-transfusion Hb were examined 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. The present study also demonstrated that as postnatal age of infants increased, 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[290]. 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% [301]. The lower pre-transfusion cTOI in older (≥29 days) preterm infants compared to the earlier infants (1-7 days) in the current study could be due to physiological

maturational changes or lower pre-transfusion Hb levels. The percentage increase in cTOI as well as cTHI was also different in the postnatal 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 tissue haemoglobin level following blood transfusion. This merely indicates an increase in tissue haemoglobin concentration in the cerebral tissue following blood transfusion and does not necessarily mean there was an increase in the cerebral blood volume. Calculating from changes in cerebral total Hb concentration (HbT) 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 a decrease in cerebral blood volume following transfusion [48], similar changes were noticed by Koyano et al[275]. In the current study the cerebral blood volume was not measured.

The mean baseline pre-transfusion cTOI level in the preterm infants with open PDA was also similar to gestational age and postnatal age matched infants with closed PDA in the current study. 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. Using Doppler measurements of cerebral blood flow velocities and spectral EEG it has been demonstrated that PDA does not cause any significant change in the cerebral functional activity despite having reversal of flow in aorta[277]. The increase in cTHI and cTOI levels during and post-transfusion in the current study was similar in both groups with open and closed PDA which indicated that PDA had no effect on the cerebral blood flow and oximetry response to blood transfusion in this study group.

Splanchnic tissue oximetry measurement using NIRS is feasible and has been reported in literature in the context of ischaemia[177,204], PDA[302], feeding[205] and blood transfusion[52-54,262]. In a small case series (transfusion related NEC group: n=4 and non-NEC group: n=4) Marin et al have demonstrated that blood transfusion resulted in a greater fluctuation of the rSO_2 above and below pre-transfusion baseline levels in those infants who went on to develop NEC post-transfusion compared to the non-NEC infants receiving blood transfusion. She speculated that the sharp decline from baseline could be due to development of pneumoperitoneum, mesenteric ischaemia or reduced perfusion while the wide fluctuation of rSO_2 may be due to ischaemia reperfusion injury[303]. The splanchnic regional saturation has been reported to change with postnatal age over the first three weeks of postnatal age in stable preterm infants[290]. In 12 preterm infants (29 to 33 weeks of gestation) McNeill et al demonstrated that the median splanchnic regional oxygenation decreased over the first week and then started to increase over the next two weeks. The day to reach the lowest value (median nadir) was variable between 4.5 to 7 days depending on the gestational age; the earlier nadir (at 4.5 days) was noted in the higher gestational age group (32-33 weeks) infants compared to 29-30 week infants[290]. This clearly indicates the variability of splanchnic regional saturation in stable preterm infants across postnatal age, resulting in variable pre-transfusion baseline tissue oxygenation which has not been taken onto account in previous studies of blood transfusion. Gillam-Krakauer et al measured abdominal regional saturation by placing NIRS probe on the abdomen in the midline below the umbilicus for a period of three days in 18 stable 25-31 week (median birth weight 1203 g, median age 5 days) infants and compared the findings with changes in SMA velocity from

immediately before to 10, 60 and 120 minutes after feeding. The changes in abdominal rSO_2 was significantly associated with SMA velocity changes from fasting to 60 to 120 minutes after feeding (p=0.016) [304]. Marin[303] and Fortune[177] have noted wider fluctuation from the baseline and a suppressed baseline splanchnic regional oxygen saturation in preterm infants with NEC. These studies indicate that abdominal NIRS measurements are feasible and can record true changes in splanchnic oxygenation in various clinical scenarios.

Pre-transfusion splanchnic tissue oxygenation (sTOI) and tissue haemoglobin index (sTHI) increased and splanchnic fractional tissue oxygen extraction (sFTOE) decreased with increasing postnatal age in the current study, however, these changes were not significant on multivariate analysis. The pre-transfusion sTOI and sTHI increased and sFTOE decreased significantly post-transfusion in all the postnatal age groups thereby demonstrating a true improvement in the balance between tissue oxygen delivery and extraction following blood transfusion.

In the current study blood transfusion in preterm infants increased sTOI and sTHI and decreased sFTOE during the first week (Group 1), 8th to 28th day (Group 2) and ≥29 days (Group 3) of life thereby demonstrating a true improvement in the balance between tissue oxygen delivery and extraction post-transfusion. Bailey et al also reported a significant increase in splanchnic regional oxygen saturation ($srSO_2$) in preterm infants more than seven days of age (n=30; mean postnatal age 31.7±16.2 days) from a baseline 41.3±2.2% to 48.2±2.5% following transfusion[53]. Dani et al studied $srSO_2$ changes in preterm infants (n=15; mean postnatal age 32±23 days) and noted similar changes (pre-transfusion 54±12% to 70±8% post-transfusion)[52]. Mintzer et al reported an increase in $srSO_2$ and decrease in fractional tissue oxygen

extraction (FTOE) following transfusion by studying 10 preterm infants (mean gestational age 26±0 weeks) during the first week of life, and speculated that these NIRS parameters could be used to evaluate the relationship between oxygen delivery and consumption[262].

Infants who received more than 50% feeds in the current study had higher trend of pre-transfusion sTOI and sFTOE indicating better balance of tissue oxygen delivery and extraction than those with less amounts of feeds. In concurrence with previous reports[205,278] the current study has shown that pre-transfusion baseline blood flow in the splanchnic circulation was higher in the predominantly fed infants. There was a significant increase in sTOI and decrease in sFTOE following transfusion in both the groups of infants in this study irrespective of the amount of feeds; this indicates improvement in the balance between tissue oxygen delivery and extraction following blood transfusion irrespective of the amount of feeds. None of these infants developed NEC or demonstrated decline in the oxygen delivery and extraction balance during blood transfusion.

This study also demonstrated that splanchnic tissue oxygenation (sTOI) as well as splanchnic tissue haemoglobin index (sTHI) increased and fractional tissue oxygen extraction (sFTOE) decreased following blood transfusion irrespective of the presence of PDA by comparing infants with PDA to gestational and postnatal age matched infants with closed PDA. The pre-transfusion baseline gut tissue oximetry was similar in infants with open and closed PDA indicating that either PDA had no effect on splanchnic oxygenation levels or that the PDA in these infants was not significant enough to demonstrate a difference in the oximetry levels.

Though results from the current study indicate that blood transfusion improves oxygen delivery to the splanchnic tissues its clinical implications should be interpreted carefully. None of these infants were severely anaemic for a prolonged period and hence the splanchnic tissue may not have been hypoxic pre-transfusion protecting them from NEC as suggested in the literature[288]. The median (range) pre-transfusion Hb was 11.0 (8.5 – 13.1) g/dl in Group 1 (1-7 days), 10.3 (7.7 – 12.2) g/dl in Group 2 (8 – 28 days) and 9.2 (7 – 10.9) in Group 3 (≥29 days) in the current study. Pooled data from the retrospective studies have shown that the infants who developed NEC following transfusion compared to those with classical NEC were younger (27 weeks vs. 28 weeks), had lower birth weight (864 g vs. 1120 g) and had lower haematocrit pre-transfusion (26% vs. 32%) [288]. Eighteen out of 59 infants transfused in the current study had a pre-transfusion haematocrit (Hct) ≤26%. But none of them had abnormal splanchnic tissue oxygenation changes post-transfusion. After adjusting for covariates Singh et al have found that effect of low haematocrit was an independent risk factor for the development of NEC[70]. This corresponds with the reduced risk of NEC in infants in the liberal haemoglobin threshold group compared to the restricted group indicated in the randomised controlled trials [78]. A recent retrospective study has shown significantly reduced risk of developing NEC in those infants who had more exposure to blood transfusion[287]. To substantiate the conclusion that anaemia may lead to tissue hypoxia and early blood transfusion to maintain a higher haematocrit may be protective towards developing NEC, factors such as exogenous erythropoietin has been reported to lower the risk of developing NEC[305] in very low birth weight infants; but whether this is by limiting anaemia, protecting against hypoxic injury or by directly reducing oxidant activity in the tissues remains to be explored [305]. In our neonatal unit

Erythropoietin is not routinely used to treat anaemia; none of the infants studied received erythropoietin as part of their management.

5.7 RCV, blood transfusion and organ perfusion

We are aware that red cell volume (RCV) may not correlate consistently with Hb or Hct levels in neonates [208] and measuring RCV is not straightforward, needs labelling of RBC (Biotin)[27,306] in a biomedical lab or can only be measured after blood transfusion (Fetal haemoglobin dilution method)[27,253]. For the purpose of this study I used fetal Hb dilution technique to measure pre-transfusion RCV. Despite a strict approach we failed to measure fetal Hb blood samples for 3 infants. One pre-transfusion sample was deemed insufficient, one post-transfusion sample was clotted and one post-transfusion sample was lost after reaching the lab.

The median pre-transfusion red cell volume in the current study was 29.9 (20.6 – 38.7) ml/kg, these infants were all receiving transfusion within the first two weeks of life (median 2 days, range 1 – 14 days) while the median pre-transfusion haematocrit was 32 (range 26 – 38)%. The RCV measured using fetal haemoglobin (HbF) dilution method in the current study is comparable to previously reported measured RCV using various methods such as HbF dilution, Biotin labelled red cell dilution and Indocyanine green (ICG) dilution in preterm infants[27,234,249,307]. Aladangady et al measured red cell volume to determine total blood volume on the first day of life in 38 infants; they used fetal haemoglobin (HbF) dilution method in 6 infants and biotinylated red blood cells (RBC) dilution in 32 infants. The mean RCV

reported by Aladangady et al was 35.4 ml/kg (range 18.0 – 48.3 ml/kg)[27] which is slightly higher than the measured RCV in the current study. Leipala et al measured blood volume (BV) simultaneously using HbF dilution and Indocyanine green (ICG) dilution techniques in 8 preterm infants receiving blood transfusion for clinical indication. The mean RCV was 33.6±12 ml/kg using ICG method and 32.1±5.2 ml/kg using HbF dilution technique[307] which is comparable to the present study findings. Mock et al measured RCV using Biotin labelling in 26 infants with mean gestational age at birth of 28±2 weeks and a mean postnatal age of 37±16 days at the time of measurement and noticed the mean circulating RCV was 22.9±7.6 ml/kg; the range was 11.9 to 43.9 ml/kg[256]. Strauss et al compared the effects of early versus delayed umbilical cord clamping using traditional haematocrit and red cell volume determination using biotinylated RBCs. The red cell volume measured following delayed cord clamping was 42.1±7.8 ml/kg compared to early cord clamping of 36.8±6.3 ml/kg, this difference was not appreciated by traditional measurement of haematocrit[249]. Hudson et al measured RCV by simultaneously using HbF and Biotinylated RBCs in 13 preterm infants (gestational age 25 -34 weeks) and noted strong correlation (r=0.989) between the two methods[235]. The infants in the current study population were all sick preterm infants in their first two weeks of life; they were receiving invasive or non-invasive ventilatory support thereby resulting in regular blood tests to optimise ventilation. Nevertheless, the RCV measured in the current study were comparable to the existing reports.

The effect of blood transfusion on cerebral and gut blood flow and perfusion was also analysed in relation to the RCV of the preterm infants. This is the first study to explore the effect of pre-transfusion baseline RCV on cerebral and gut blood flow

and oxygenation changes following blood transfusion. There was a significant decrease in the pre-transfusion anterior cerebral artery peak systolic velocity following transfusion in infants with pre-transfusion RCV ≥25 ml/kg but no change in the time averaged mean velocity. The Superior Vena Cava flow also remained unaltered indicating no significant increase in the cerebral blood flow post-transfusion. There was no change in Anterior Cerebral Artery velocities or Superior Vena Cava flows post-transfusion in infants with RCV<25ml/kg. This indicates that cerebral blood flow remains unaltered following transfusion in both infant groups with RCV ≥ or <25ml/kg. There is no similar published data to directly compare with these findings. Progressive haemodilution using 6% hetastarch (haematocrit diluted from 45% to 20%) in rat models have shown significant increase in forebrain cerebral blood flow [308]; these changes would be similar to the effect of anaemia in neonates and the opposite response to blood transfusion with increasing haematocrit levels. Using radiolabelled method of measuring cerebral blood flow in rat models, Todd et al have demonstrated that in a normal brain cerebral blood flow does not change following volume expansion despite an increase in cardiac output measured by thermodilution technique [309]. These animal experiment findings supports the present study findings that cerebral blood flow is protected to some extent irrespective of RCV in preterm infants. The number of infants with pre-transfusion RCV (n=12) studied was small, particularly the infants with RCV <25ml/kg (n=5) and this may have influenced the present study findings.

The Superior Mesenteric Artery (SMA) peak systolic velocity remained unaltered in infants where RCV was ≥25ml/kg, whereas in infants with RCV<25ml/kg the SMA PSV decreased significantly post-transfusion. It has been previously reported that

the risk of necrotising enterocolitis is higher in infants with lower pre-transfusion haemoglobin[70,288,310]. However, a drop in SMA PSV does not necessarily indicate a decrease in tissue oxygenation or perfusion, it merely reflect an adaptive response to increased red cell volume following blood transfusion. Studies with larger number of infants may be able to further substantiate the findings taking into account the effect of the covariates in a multivariate analysis[259].

Hudson et al [257] compared whether haemoglobin concentration or RCV was a better predictor of outcome of red cell transfusion. They measured haemoglobin and haematocrit levels and RCV simultaneously in 24 preterm infants (24-34 weeks of gestational age) and measured their cardiac output (CO) pre and post-transfusion as a measure of benefit following transfusion. After excluding those infants with PDA (n=4), they noted that CO did not decrease significantly following transfusion in those infants with a RCV ≥25 ml/kg but showed a significant drop if the RCV was <25 ml/kg[257]. In the current study, there was no change in cerebral blood flow but there was a significant drop in the SMA PSV following blood transfusion in those infants with a pre-transfusion RCV <25 ml/kg, this could be due to a fall in cardiac output noticed by Hudson et al[257] and in relation to significant anaemia as noted by Alkalay et al[49]. Supported by the findings of these studies one can speculate that in light of the findings of the current study blood should always be transfused prior to RCV dropping to levels of <25 ml/kg. Randomised controlled trials using RCV and other measures of tissue perfusion could help to substantiate this threshold of blood transfusion.

The cerebral tissue oxygenation index (cTOI) increased and cerebral fractional tissue oxygen extraction (cFTOE) decreased in the current study following blood transfusion in infants with RCV ≥25ml/kg but this was not statistically significant. These changes were similar in infants with RCV <25ml/kg. There was a significant increase in the cerebral tissue haemoglobin index (cTHI) post-transfusion in both the two groups. These findings indicate that cerebral oxygenation changes following blood transfusion was similar in infants who had a pre-transfusion RCV ≥ or <25 ml/kg. There are no published reports to compare these findings.

The intestinal tissue oxygenation index did not demonstrate any change following transfusion in the infants with pre-transfusion RCV <25ml/kg, which is in contrast to those with pre-transfusion RCV ≥25ml/kg, who demonstrated a significant increase in the intestinal oxygenation (sTOI) post-transfusion. One can speculate from these findings that in infants with a pre-transfusion red cell volume of <25 ml/kg the blood flow velocity decreases and there was no change in splanchnic tissue oxygenation, which may link low pre-transfusion haemoglobin as a predisposing factor and blood transfusion as a contributory factor to development of intestinal tissue ischaemia and subsequent development of necrotising enterocolitis. The present study findings may also imply that the amount of donor blood cells transfused was not sufficient enough to improve intestinal oxygenation in infants with lower pre-transfusion RCV (<25 ml/kg). But the clinical implication of these results should be interpreted with caution. These measurements were performed in a small number of infants who were all <14 days of postnatal age when transfusion associated NEC is rare, so the clinical relevance of the findings of the current study cannot be substantiated.

Blood transfusion and cerebral and gut perfusion in relation to red cell volume

The current study has demonstrated that blood transfusion improves cerebral and gut oxygenation infants with RCV >25ml/kg but there was no change in gut oxygenation following blood transfusion in those infants with pre-transfusion RCV<25 ml/kg. There was a significant drop in SMA peak systolic velocity following transfusion in those infants with pre-transfusion RCV<25 ml/kg. Pre-transfusion RCV of 25 ml/kg was used as a cut off in the current study in relation to the findings of Hudson et al who demonstrated a significant drop in cardiac output following blood transfusion in infants with a pre-transfusion baseline RCV of <25 ml/kg[257]. The current study has demonstrated that the cerebral tissue oxygenation increases irrespective of the pre-transfusion RCV.

5.8 Haemoglobin, blood transfusion and organ perfusion

The current study has demonstrated that blood transfusion results in reduced blood flow to the brain and simultaneously increased cerebral tissue oxygenation in preterm infants of all postnatal age groups irrespective of pre-transfusion Hb level of ≥ or <11 g/dl. In infants with Hb <11g/dl the cTOI increased by 9.8% and the sTOI increased by 31.4%. In the infants with Hb ≥11g/dl cTOI increased by 9.2% and the sTOI increased by 38.2%. These findings indicated that in both groups of infants with Hb ≥ or <11g/dl the sTOI improved significantly more compared to the cTOI

levels post-transfusion. It also demonstrated that the percentage increase in cerebral or splanchnic tissue oxygenation post transfusion in both the Hb groups (≥ and <11 g/dl) was similar. While some of the reported literature have shown no correlation between Hb level and cerebral[52] or gut tissue[52,53] oxygenation, others have demonstrated a reasonable correlation with peripheral[209], cerebral[51,54,311] and splanchnic[290] tissue oxygenation. Other factors that determine cerebral and splanchnic perfusion and tissue oximetry are percentage of fetal haemoglobin, acid-base status and autoregulation of perfusion of organs such as brain.

The current study has also demonstrated that the degree of increase in gut oxygenation was more compared to the changes in cerebral oxygenation post-transfusion. Whether this is due to protective autoregulatory mechanism in the preterm brain or a normal physiological phenomenon in preterm infants remains to be explored.

5.9 Limitations

Study infants all received 15 ml/kg of cross matched donor red blood cells with varied haematocrit (Hct) concentration (50-70%). The effect of donor blood haematocrit on cerebral or gut perfusion as well as oxygenation changes noticed following transfusion cannot be excluded. Infants due to receive blood transfusion for clinical indication were studied and decision to give a blood transfusion was made by the attending clinical team based on infants' clinical condition and haemoglobin (Hb) as per departmental transfusion and BCSH guidance[26], hence selection bias cannot be excluded. Infants who were felt unstable for NIRS and Doppler measurements by the clinical team were excluded (n=5). Infants who received blood transfusion during standard working hours (08:00 to 18:00 hours) were studied and those who received blood transfusion during out of hours were excluded, there is a possibility that this might have excluded anaemic and very sick infants. I aimed to recruit 20 infants to each group; managed to recruit 20 to Group 1 (1-7 days) and 21 infants to Group 2 (8-28 days) but 18 infants to group 3 (≥29 days of life). This is unlikely to influence the study findings. Previous researchers have demonstrated that a sample size of 10 infants are required to identify a 10% increase in cerebral or splanchnic tissue oxygenation index at the end of transfusion with 80% power at 0.05 level[52,261]. Compared to other reported studies that combined infants of various postnatal ages together and examined them as a single group [52,53] in the current study the infants were recruited into three postnatal age groups to avoid confounders such as adaptive physiological haemodynamic changes and 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_2 were similar in all three groups of infants studied, and hence ventilation status is unlikely

to have impact on the study findings. 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.

To improve the quality of the study Doppler blood flow to the brain and gut were measured alongwith NIRS measurements. Doppler measurements have their own limitations of intra and inter-operator variability and repeatability. All Doppler measurements were performed by me with good intra-operator variability and repeatability and were comparable to the reported literature[74,152,153]. Utmost care was taken to minimise the angle of insonation to the direction of blood flow to get a true measurement.

NIRS measurement has its own inherent limitations and extreme care was taken to minimise movements, handling of the baby and ambient light sources during NIRS measurements. Splanchnic or intestinal tissue oxygenation measurement is known to be associated with some assumptions. Intestine is not a solid organ, it is not static and the venous to capillary proportion in the intestinal tissue is not clearly known. The basic properties of intestine such as hollow tube like structure, peristaltic movements, presence of faeces and meconium make it difficult to measure splanchnic tissue oxygenation. Hence the splanchnic tissue oxygenation measurements have not been extensively validated. However, the present study has shown steady splanchnic oxygenation over 3 hours in control infants (not received blood transfusion) and a significant improvement in oxygenation in study infants following blood transfusion. This implies that measurement of intestinal oxygenation

by NIRS device is feasible and reliable. The splanchnic and cerebral oxygenation measurements of seven infants were excluded from the analysis due to motion artefacts, which is comparable to other reported NIRS studies[254,258].

One of the limitations of the study is the measurement of cerebral tissue oxygenation upto 20 minutes following transfusion. Other researchers have measured cerebral tissue oxygenation at one [48], four and 24 hours [54] post-transfusion, and reported persistence of increased tissue oxygenation state following transfusion in more stable preterm infants. Similarly researchers have measured splanchnic tissue oxygenation up to one[52] and 12[53] hours post-transfusion, and reported persistence of increased tissue oxygenation state following transfusion in more stable preterm infants.

Fetal haemoglobin (HbF) dilution method was used for measurement of red cell volume. This method can only be used reliably upto the second blood transfusion, because of recurrent dilution of HbF at each transfusion. Another major limitation of this technique is that a post-transfusion sample is required to measure post-transfusion HbF level which is required to calculate the red cell volume. Thus by using this method red cell volume cannot be measured pre-transfusion and so cannot be used to identify the requirement of transfusion. However, other methods such as Biotin labelling are cumbersome and require dedicated biomedical lab for measurements which was beyond the scope of this study. Despite strict adherence to protocol I failed to measure fetal Hb in 3 infants where blood samples were sent to the lab. One pre-transfusion sample was deemed insufficient, one post-

transfusion sample was clotted and one post-transfusion sample was lost in the lab. The number of infants where red cell volume was measured was small, The reliability of a multivariate analysis depends on the number of infants studied, and the ratio of sample size to the number of variables and matrices of the covariates involved[259]; this prevented performing a multivariate analysis of covariates for the RCV groups.

5.10 Conclusion

This study has demonstrated that the pre-transfusion baseline cerebral tissue oxygenation index (cTOI) decreases with increasing chronological age of preterm infants. Blood transfusion increases cTOI and cerebral tissue haemoglobin index (cTHI) in preterm infants of all postnatal age groups but this is more pronounced in older age group (≥29 days of age). The baseline anterior cerebral artery (ACA) time averaged mean velocity (TAMV) increases and the baseline pre-transfusion superior vena cava (SVC) flow decreases as infant's postnatal age increase. The ACA TAMV decreases significantly following transfusion in all postnatal age group infants. The pre-transfusion superior vena cava (SVC) flow decreases post-transfusion after 1st week of life but not consistently, cerebral autoregulation may play an important role in this. The cerebral perfusion decreases following blood transfusion during the first week and after 28 days of life in preterm infants. Similar pre-transfusion baseline values in infants with patent ductus arteriosus (PDA) to gestational and postnatal age matched infants with closed PDA indicate compensatory mechanisms of preterm circulation by which it adapts to anemia in the presence of PDA. These findings indicate that cerebral blood flow and oximetry response to blood transfusion

in preterm infants is dependent on the postnatal age, and future randomised trials of blood transfusion should be planned taking into account the effect of postnatal age on cerebral blood flow and oximetry.

The study demonstrated the feasibility and reliability of using NIRS device to measure splanchnic oxygenation changes by comparing measurements between study and control groups of infants. It has also demonstrated that pre-transfusion mesenteric blood flow increases with postnatal age. Pre-transfusion splanchnic blood flow also varies with feeding status and presence of PDA. The study has also demonstrated that blood transfusion improves intestinal tissue perfusion without altering mesenteric blood flow velocity following transfusion irrespective of postnatal age, feeding status and presence of PDA. NIRS may be a useful non-invasive bedside monitoring tool to detect early signs of compromised oxygenation-extraction balance in splanchnic tissue. The effect of postnatal age on splanchnic tissue oxygenation should be taken into account in future clinical trials related to gut circulation in preterm infants.

Amongst the vital parameters recorded, blood pressure increases significantly following blood transfusion in infants of all postnatal age groups following blood transfusion. There was no immediate effect on heart rate and oxygen saturation following blood transfusion.

The cerebral oximetry markers increased significantly following blood transfusion irrespective of the pre-transfusion baseline RCV. In infants with pre-transfusion red cell volume (RCV) <25ml/kg the splanchnic blood flow velocity decreased following blood transfusion. The gut oximetry markers improved significantly following transfusion in those infants with RCV ≥25ml/kg, but this improvement was not noticed in those with RCV <25ml/kg. This may suggest that babies with pre-transfusion RCV <25 ml/kg may need larger volume of blood transfusion; larger studies are required to substantiate this finding in a multivariate analysis.

The percentage increase in cerebral or splanchnic tissue oxygenation post transfusion in both the Hb groups (≥ and <11 g/dl) was similar. In both groups of infants with Hb ≥ or <11g/dl the splanchnic oxygenation improved significantly more compared to the cerebral oxygenation levels post-transfusion.

5.11 Future directions

The relationship between haemoglobin (Hb) and tissue oxygenation is not clear. Hence using the standard laboratory and bedside markers to decide blood transfusion in preterm infants has the inevitability of unintended outcomes. There is a need to establish the pre-transfusion Hb and Hct threshold that significantly improves cerebral and splanchnic oxygenation following blood transfusion without causing harm in preterm infants. The organ perfusion and oxygenation can be studied non-invasively using Doppler USS and NIRS respectively. These markers of tissue perfusion and oxygenation could then be used in randomised controlled trials to identify the threshold of blood transfusion in preterm infants at which level the maximum benefit of transfusion could be achieved. Current ongoing large multicentre trials receiving liberal and restrictive transfusion may help clinicians in future to identify the threshold of Hb or Hct at which blood transfusion will be most effective. Some of these trials are also measuring the long-term neurodevelopmental outcomes of blood transfusion. Future studies of the effect of blood transfusion should also take into consideration the effect of donor blood haematocrit.

The relationship between development of transfusion associated NEC in older extreme preterm infants with low pre-transfusion haemoglobin and intestinal tissue hypoxia remains uncertain[312]. Retrospective and observational studies have examined the association and temporal relationship between blood transfusion and development of NEC[61]. Whereas retrospective studies implicated blood transfusion as an important association for development of NEC, randomised controlled trials

favoured blood transfusion for reduced incidence of NEC[78]. Subsequently studies have linked pre-transfusion anemia to development of NEC following blood transfusion[70]. Though this theory is plausible the evidence is not clear about this association. Future studies should investigate the tissue markers of hypoxia or reduced perfusion associated with anemia in preterm infants. It has been demonstrated that in response to hypoxia in the tissues, a tissue marker called hypoxia-inducible factor (HIF) increases the expression of erythropoietin and vascular endothelial growth factor (VEGF)[313]. Relatively recently, it has been reported that VEGF could be an important biomarker to help clinicians decide the requirement of blood transfusion[314]. In an effort to identify early signs of NEC researchers have demonstrated a higher expression of faecal volatile organic compounds (VOC)[315,316] in those infants who subsequently developed NEC. Other factors such as liver fatty acid binding protein (L-FABP), intestinal fatty acid binding protein (I-FABP) and trefoil factor 3 (TFF3) has been investigated as well. In pilot studies in preterm infants who developed radiologic and surgically confirmed NEC these are found in significantly higher levels when compared with those that did not show confirmed signs of NEC[317-319]. There are strong arguments that these markers have shown substantial link with tissue ischaemia and may be used to identify early signs of NEC. Measuring these factors of tissue hypoxia or damage may help us identify early signs of intestinal ischaemia caused by anaemia. Future studies of measurement of markers of tissue hypoxia may help clinicians to decide the requirement of blood transfusion and achieve the maximal benefits of transfusion.

The question of whether or not to hold feeds during and after blood transfusion still remain unanswered. Future randomised trials should be directed to measure

intestinal tissue oxygenation in infants receiving blood transfusion where feeds were withheld compared to those where feeds were continued during transfusion. Tissue markers of hypoxia as noted above could be used in conjunction to assess early signs of intestinal tissue hypoxia and cytokine release prior to development of NEC. The response of the splanchnic vasculature to blood transfusion depends on the balance between mesenteric vasoconstriction and relaxation induced by intestinal endothelial production of nitric oxide. A recent study has shown blood transfusion in enterally fed preterm lambs (n=16) promotes mesenteric vasoconstriction and impairs vaso-relaxation by reducing mesenteric arterial endothelial nitric-oxide synthase [320]. Animal research aiming to identify the response to inhaled nitric oxide (iNO) during blood transfusion should be conducted to identify mesenteric tissue hypoxia; findings of such studies may help to design randomised controlled trials using iNO during transfusion to reduce or prevent the risk of transfusion associated NEC in preterm infants.

Further studies should also be aimed to minimise blood loss by regular phlebotomy losses and minimising the amount of blood needed for regular blood tests using bedside kits and further improvements in non-invasive techniques of measuring serum bilirubin, haemoglobin, electrolytes, blood gas parameters such as pCO_2 and pO_2. Studies should also be aimed at developing better storage techniques and using fresher blood for neonates at the same time strike a balance of reducing the number of donors used in the lifetime of the preterm neonate. Further studies should also look into identifying non-infective factors transfused such as unknown proteins and other factors which might have an effect on the response to blood transfusion.

6 References

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7 Appendices

7.1 Appendix 1: Measurement of cerebral and splanchnic oximetry using NIRO-300

Near Infrared Spectroscopy (NIRS) monitor

The NIRS device (**Figure 38**) used in the current study was NIRO-300 (Hamamatsu Photonics, Hamamatsu KK, Japan). NIRO-300 has both functional capabilities of difference spectroscopy and Spatially Resolved Spectroscopy (Hamamatsu Photonics, Hamamatsu KK, Japan).

Figure 38: NIRO-300 (Hamamatsu Photonics, Hamamatsu KK, Japan)

There are two main parts in NIRO-300:

1) Measurement Unit and

2) Display Unit (Hamamatsu Photonics K.K., 2000).

The Measurement Unit of the device has the following sections (**Figure 39**):

a) Main body

b) Measurement Unit – Display Unit cable

c) Detection Probe (Figure 39)

d) Emission Probe (Figure 39)

e) Probe Holder (Figure 39)

f) Light attenuator: The light attenuator is used to reduce the amount of irradiated light where the light absorption by the measured tissue is very small.

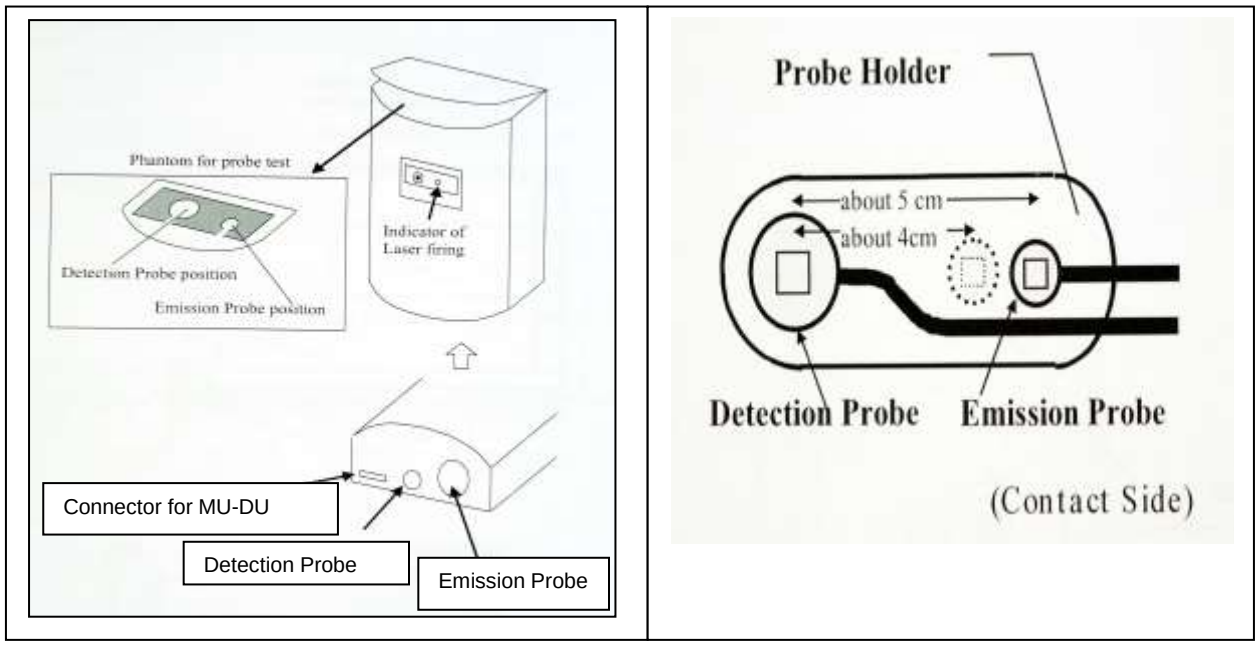

Figure 39: Measurement Unit of NIRO-300 and Detection Probe and Probe Holder (Hamamatsu Photonics, Hamamatsu KK, Japan)

The Display Unit has the following parts (**Figure 40** and **41**):

a) Main body

b) Power supply cable

c) Detection Probe

d) Emission Probe

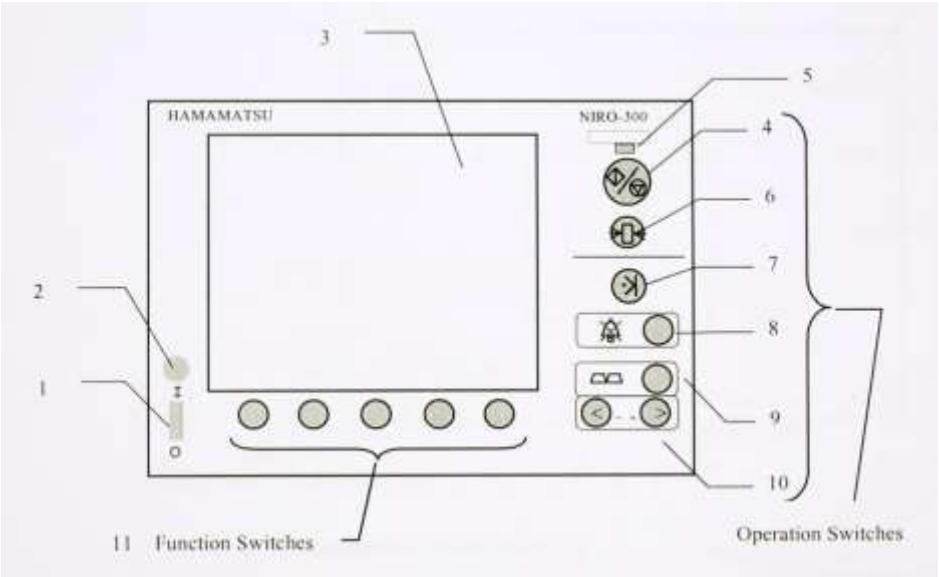

Figure 40: Display Unit (DU) (Hamamatsu Photonics, Hamamatsu KK, Japan)

1. Power switch: The switch to turn ON/OFF the main power.

2. Power lamp: The light to indicate the ON/OFF status of the power supply.

3. Display: Measured data are graphically displayed together with the numeric values. Measurement parameters are also displayed. At the bottom of the screen, the Function menus and functions are displayed, which are controlled by the Function switches (11) just below the screen.

4. Start/Stop switch: The switch to start/stop the measurement.

5. Measurement lamp: The light to indicate the measurement (started/stopped) state.

6. Zero set switch: The switch to reset the concentration change data back to zero (base line).

7. Event switch: The switch to place an Event marker on the graph (events are numbered sequentially).

8. Alarm stop switch: The switch to temporarily silence the alarm.

9. Menu select switch: The switch to select the function menus.

10. [<] / [>] switch: The switch to scroll the graphs forward/ backward or to increase/decrease the parameter values in the currently selected function.

11. Function switches: The switches to perform the functions displayed on the screen. Functions are changed automatically depending on the function menu selected by the 'Menu select' switch (9).

Safety of NIRO-300:

Electricity: Class 1 (IEC601-1-1988) degree of protection against electrical shock.

Laser: Class 1 (IEC 825-1-1993) irradiation to patient.

The laser calibration is automatically done by performing the Initialisation before the measurement.

Measurement of cerebral and splanchnic tissue oximetry

Step 1. NIRO-300 device and probe testing

1. Cleaning the NIRO-300 monitor:

The NIRO-300 monitor (including the probes and cables) was cleaned with sterile alcohol wipes for the cleaning of medical devices (Alco wipe, Seton Healthcare Group, England) just before and soon after BV measurement.

2. Cable connection:

Emission probe (Figure 41): The screw at the upper left corner of the Measurement Unit released and the guard plate removed. The connector end of the Emission probe inserted to laser emitor slot and fixed by turning the lock ring clockwise.

The guard plate is fixed by the screw after fixing the Emission Probe.

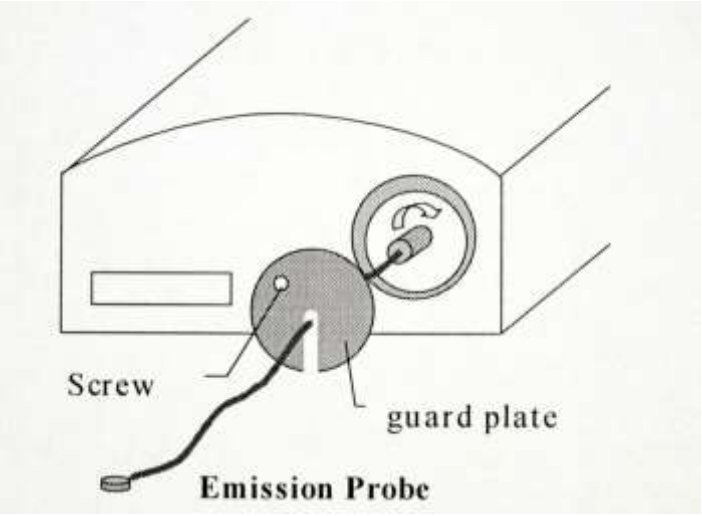

Figure 41: Emission Probe connection

*Detection Probe (**Figure 42**)*: The connector end of the Detection Probe connected to the detection probe slot in the Measurement Unit. The Detection Probe connected properly by positioning the connector marker (white spot) correctly. The Detection Probe connector end inserted into the receptacle until it is locked properly (clicked).

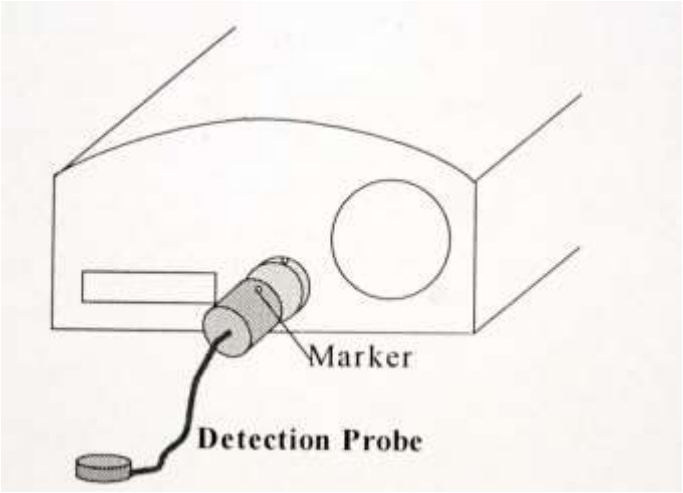

Figure 42: Detection Probe connection

Measurement Unit – Display Unit (MU-DU) Cable: The MU-DU cable connected to Measurement Unit and Display Unit by inserting the cable into the connector until it is locked (clicked).

3. <u>NIRO–300 monitor power turned on</u>:

The previous data stored in the Data Memory is loaded and displayed after turning on the power switch.

Caution: the lasers start to fire when the power switch is turned on.

4. Enter into the measurement screen:

The ‘Start Here’ key pressed to enter into the measurement screen (**Figure 40**). The ‘Data Clear’ window appears on pressing ‘Start Here’ key. The previous data stored in the Data Memory deleted by selecting Yes on Data Clear window using the [<]/ [>] switches. On pressing the Enter key, the previous stored data is cleared and the Measurement Screen asking for probe test will appear.

5. Probe test:

To perform the Probe Test, the ‘Yes’ key selected on the probe test window using the [<]/ [>] switches. The probes attached to the test phantom in the Measurement Unit (**Figure 40**).

The Probe Test is performed by pressing the Enter key. After successful Probe Test, the ‘Probe Test OK’ message appears on the Display Unit screen. All the above procedures repeated where the Probe Test failed (“Probe Test Error”).

Note: The probe test is performed to test the total probe sensitivity and the sensitivity difference between the three sensors in the detection probe.

The most common reason for Probe Test Error is dirt on the detection probe. When Probe Test Error message appeared, the surface of the probes gently wiped with sterile alcohol wipes (Alco wipe, Seton Health Group, England) and the Probe Test repeated. The probe test needs to be correct in order to start the process.

Step 2. Initialisation of probe following probe test

1. Placing probe to holder:

The emission probe and detection probe placed into the black probe holder as shown in the **figure 39**.

2. Placement of probe onto baby:

Cerebral oximetry measurement: The NIRO-300 optical probe was attached to the baby's forehead. This is fixed in place under the hat used for invasive ventilation or CPAP to minimise movement and ambient light interference.

Splanchnic oximetry measurement: The second NIRO-300 optical probe was placed over the hypogastrium (area below umbilicus) in the midline and held in place using a single use tourniquet (Vygon 'Vene K' Quick Release, Vygon UK Ltd.).

Gentle pressure was applied to the probe to improve contact.

3. Initialisation:

The initialisation window appears on pressing the Initialisation key on the Display Unit (**Figure 40**). The Yes key selected on the Initialisation window using [<]/ [>] switches.

The Enter switch on the Display Unit was pressed to start the Initialisation procedure. When the Initialisation was successfully completed, Initialisation OK message was displayed on the Display Unit screen.

Note: In some babies the message "Signal Overflow" appeared during the Initialisation procedure because the detected light was too large for the measurement. This may be due to very small absorption of light by the measured

tissue. In such babies, the **Light Attenuator** was used to reduce the irradiated light (**Figure 43**), and successful Initialisation achieved.

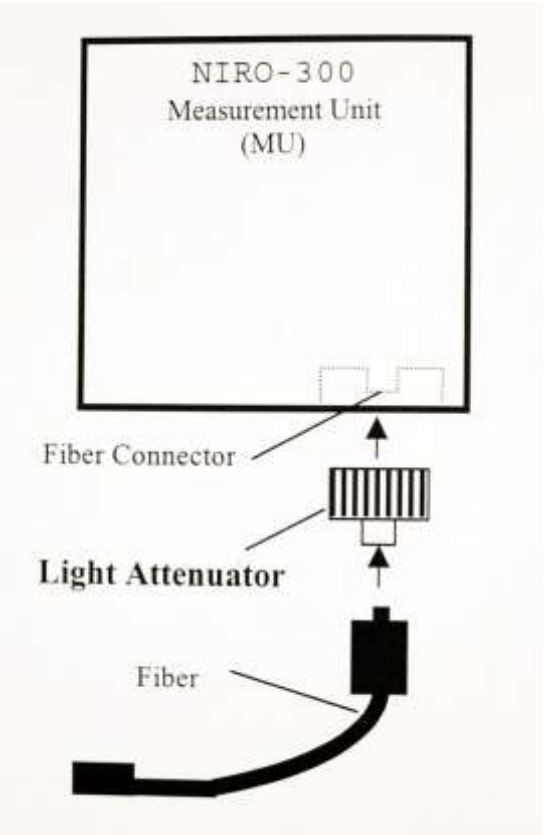

Figure 43: Light Attenuator helping in initialisation

4. Measurement parameter setting:

The distance between the Emission Probe and Detection Probe entered; the probe distance was 5cm. The DPF used in this study was 5.13.

Step 3. NIRS measurement of cerebral and splanchnic tissue oximetry

1. Connection of NIRO-300 device with study laptop for continuous measurement: the NIRO-300 device was connected to the study laptop using RS232C to USB cable and the NIRO-300 software was opened in the laptop. The laptop was now ready for continuous downloading of NIRS measurements.

2. Measurement start:

The Start/Stop switch pressed to start measurement. The NIRS data was continuously recorded for 15-20 minutes before the blood transfusion was started.

3. Measurement stop:

After the blood transfusion was completed, the NIRS measurements were continued for a period of another 15-20 minutes and then the Start/Stop switch pressed again to stop the measurement.

The probes attached to the baby removed.

The ON/OFF power switch pressed to turn off the main power supply.

The probes and cables disconnected carefully to avoid the damage to fragile optical fibres.

Step 4. Data recording in the laptop and further analysis:

Each baby measurement data stored in laptop was then transferred and stored in the secured password protected network drive of Homerton hospital.

First the NIRS data is stored in .NI3* format as individual case files. This was then converted to an .OD format using a mathematical software called Mat lab (Mat Lab 2013a, Math works, US).

Using the same software programme the data was then analysed for four epochs (each of 15 minutes): T1 – 15-20 minutes pre blood transfusion, T2 – 1 hour of blood transfusion, T3 – 2 hour of blood transfusion and T4 – 15-20 minutes post blood transfusion.

The mean of these epochs were recorded to statistical software SPSS 22.0 and further analysed.

7.2 Appendix 2: Vital parameter measurement steps using ixTrend

For continuous measurement of vital parameters Phillips Intellivue MP70/MP50 monitor was used and the data was continuously downloaded from the overhead monitor to the study laptop using ixTrend software (ixellence GmBH, Germany). The license for the software was purchased and the software downloaded to the study laptop.

Steps of downloading and analysing the vital parameters are described below:

Step 1. Connection to the Phillips Intellivue monitor

An RJ45 to USB cable was used to connect the Phillips Intellivue monitor with the study laptop (**Figure 44**). The RJ45 cable was inserted to the overhead monitor and the USB cable to the laptop.

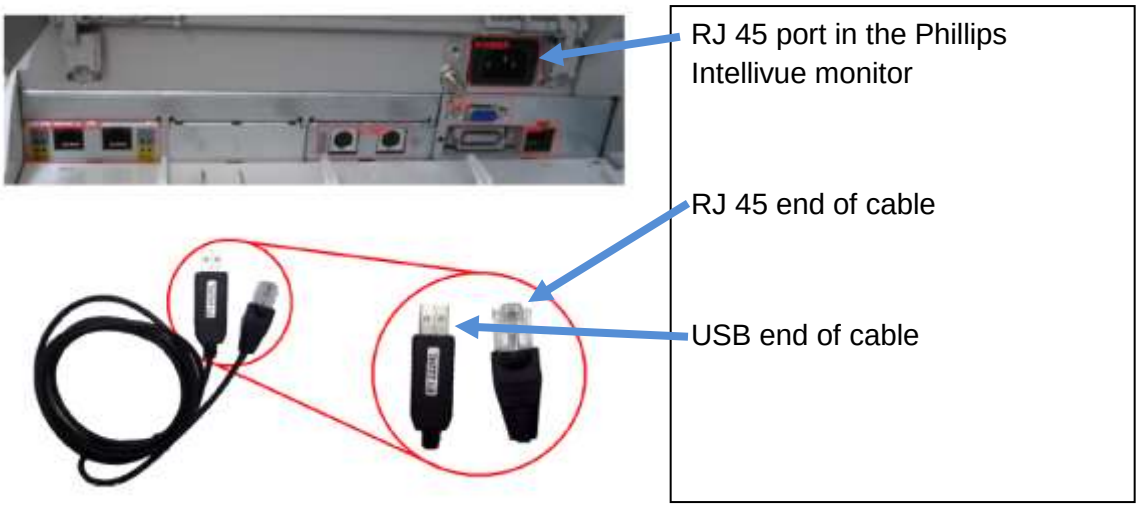

Figure 44. Demonstrating the connections for the Phillips Intellivue monitor and the connector cable

Step 2. Initiating the software at the laptop and connect with the overhead monitor

After the cable connection between the laptop and the overhead monitor the software (ixTrend 2.0) was initialised in the laptop.

1. Linking the Phillips Intellivue patient monitor to the Laptop

Under *Resources* all the available monitors are seen, the required monitor can then be connected to the laptop. Select the required monitor by right-clicking on it and click 'Connect' (**Figure 45**).

Figure 45. Connecting the monitor with the laptop using the software

2. Choosing the patient

Now either a new patient can be created or an existing patient can be loaded.

To create a new patient an empty file will come up. It needs to be filled up with the patient's data. The star-marked data fields are mandatory.

In order to select an existing patient the patient file the required file can be clicked to open it.

Step 3. Signal settings

Confirm the patient choice by clicking ‘Next’ or ‘Finish’. Finish links the laptop to the patient monitor and the data is now ready to be transferred. When clicking finish a diagram profile will need to be created or an already created profile (e.g. ECG and Heart rate) needs to be chosen. After the profile is chosen then click ‘Finish’ and the data will start downloading.

Step 4. Stopping the recording

When the blood transfusion is finished the data still continues to be downloaded for another 15-20 minutes and then the ‘Stop’ button is clicked in the software. The recording will stop immediately.

Step 5. Storing the recordings

The session file (e.g. 21/03/13 12.00 hrs to 21/03/13 16.00 hours) from the resource menu is now double clicked (**Figure 46**) and the numeric values (heart rate, respiratory rate, saturation, systolic, mean and diastolic blood pressure) are clicked.

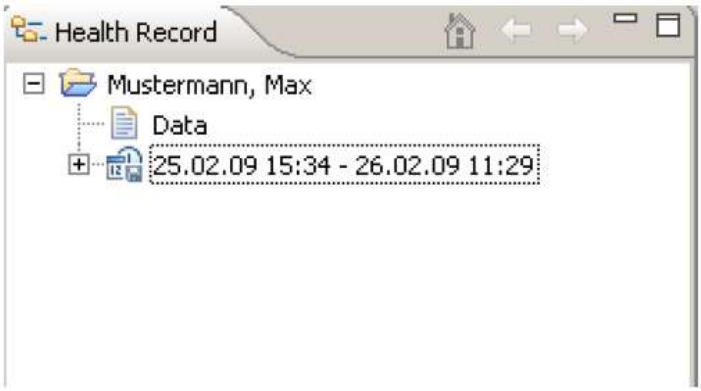

Figure 46. Identifying the session file in the software

After this click ‘File’ and go to ‘Data Export’. The data is then exported from the session file of the particular patient to a comma separated version (.csv) file. In the format settings click ‘semicolon’, ‘comma (1.2.3)’, ‘milliseconds since 01.01.1970’ and ‘standard export (one file)’, and then click ‘Finish’. In that way all numeric values will be recorded to a single file.

Step 6. Analysing the data

The data was the analysed using a Mat Lab programme (Mat Lab 2013a, Math works, USA). The epochs of 15 minutes before and after blood transfusion were analysed using this programme and the mean of these epochs were then analysed using statistical software SPSS 22.0.

7.3 Appendix 3: NHS Research ethics approval

NHS
Health Research Authority
NRES Committee London - Surrey Borders
Charing Cross Hospital
Research Ethics Committee (REC) Centre Charing Cross
Room 12, 4th Floor West
Fulham Palace Road,
London
W6 8RF

Telephone: 020 331 17294

04 May 2012

Dr Jayanta Banerjee
Neonatal Unit
Homerton University Hospital NHS Foundation Trust
Homerton Row, London
E9 6SR

Dear Dr Banerjee

Study title:	**Measurement of gut and cerebral perfusion of newborn infants requiring blood transfusion for clinical indication**
REC reference:	**12/LO/0527**

Thank you for your letter of 23 April 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Investigator CV		07 March 2012
Letter from Sponsor		07 March 2012
Other: Dr Narendra Aladangady (Academic Supervisor CV)		07 March 2012
Participant Consent Form	3	13 March 2012
Participant Information Sheet	6	23 April 2012
Protocol	V8	09 March 2012
REC application		30 April 2012
Referees or other scientific critique report		04 March 2012
Response to Request for Further Information		23 April 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/LO/0527	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Canon Chris Vallins
Vice-Chair

Email: Rachelbell3@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Dr Narendra Aladangady, Homerton University Hospital NHS Foundation Trust*
Ms Linda Legrand, R&D Department

7.4 Appendix 4: Homerton R&D approval

Homerton University Hospital NHS
NHS Foundation Trust

Research & Development Committee
Chair: Dr Narendra Aladangady

Linda Legrand
Research & Development Manager
Linda.legrand@homerton.nhs.uk

Homerton University Hospital
Research and Development
Yellow Roof Office
Homerton Row
London
E9 6SR

Tel: 020 8510 5134
Fax: 020 8510 7850
www.homerton.nhs.uk

Dr Jayanta Banerjee
Clinical Research Fellow
Neonatal Unit
Homerton University Hospital NHS Trust
Homerton Row
London
E9 6SR

09 October 2012

Dear Dr Banerjee,

Re: Research Study "Measurement of Gut and Cerebral Perfusion of Newborn Infants".

R&D No: PA1201 ***CSP: 92945*** ***Ethics: 12/LO/0527***

Thank you for sending all the relevant documents for Homerton University Hospital Trust Research and Development Approval of the above research study. As part of the Research and Development approval process we have conducted a site specific assessment for this study. I am happy to inform you that the Trust will sponsor this study and has approved the conduct of the study and that the Trust will indemnify against negligent harm that might occur during the course of this project.

The following main document/s has been received by R&D department as part of the approval process;

Protocol Version 8	Dated: 9th March 2012
Patient Information Sheet Version 6	Dated: 23rd April 2012
Consent Form Version 3	Dated: 13th March 2012

All other documents that you are required to submit as part of the process have been received.

I would like to draw your attention to the following conditions of the approval of this research project with which you must comply. **Failure to do so may result in the Trust withdrawing R&D approval which allows you to conduct this research project at Homerton University Hospital NHS Foundation Trust.**

Untoward events - Should any untoward event occur it is **essential** that you complete a clinical incident form and write on the form '**R&D**'. Contact the R&D Office immediately and if patients or staff are involved in an incident you must also contact the Risk Manager on 020 8510 7649.

Status of Research - Inform us if your project is amended or if your project terminates early/requires an extension as well as informing the Research Ethics Committee. This is

Incorporating hospital and community health services, teaching and research

necessary to ensure that your indemnity cover is valid and also helps the office to maintain up-to-date records. A copy of any publications arising from the research should be sent to the R&D Office for use in the R&D Annual Report. Please be reminded that this hospital should be acknowledged in any publication.

First Patient Recruited Within 30 Days - The Department of Health (DoH) expects the first patient should be recruited within 30 days from the date of this letter. The Trust has to submit quarterly report to the DoH on this key performance Indicator (KPI). Failure to meet this KPI will result in the DoH withdrawing the funding Trust receives to support research at Homerton. R&D will contact you shortly to see if you have met this KPI.

Research Information - You will be required to complete a project update as required by the R&D Office to ensure that we have up to date information so that we can send accurate reports to the DoH and research networks. The project update form will be emailed or sent to you by the R&D Office.

Research Governance - As part of research governance, all investigators accessing identifiable personal information are required to comply with current data protection requirements.

Intellectual Property - If you believe that protectable intellectual property may arise from your research, please contact the Linda Legrand R&D Manager on ext 5134 who will advise you on the proper course of action.

Monitoring of Studies – You must comply with the Trust's legal responsibility as host of this research project to monitor and audit the research to ensure that the Research Governance Framework and Good Clinical Practice (GCP) if applicable is being adhered too. Monitoring questionnaires will be sent to you and random audit visits will also take place across the trust and will be conducted following at least a seven day notice period. **Failure to respond to any of these monitoring or auditing requests may result in the Trust withdrawing your**

R&D approval to conduct this research at Homerton University Hospital NHS Foundation Trust.

Please note that all NHS and social care research is subject to the DoH *Research Governance Framework*. If you are unfamiliar with the standards contained in this document, you may obtain details from the Trust R&D Office or from the DoH website (www.dh.gov.uk).

Please do not hesitate to contact Linda Legrand, Research and Development Manager or me if you have any further questions.

Yours sincerely,

Jo Farrar
Finance Director

Cc: CI - Dr Narendra Aladangady, Neonatal Consultant, Homerton University Hospital
Charlie Sheldon, Chief Nurse & Director of Governance, Homerton University Hospital

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7.5 Appendix 5: Consent form for study infants

Homerton University Hospital NHS
NHS Foundation Trust

Version: V3

Date: 13th March 2012

Homerton University Hospital
Neonatal Unit
Homerton Row
London
E9 6SR

Tel: 0208 510 7952/7049
Fax: 0208 510 7448

Measurement of Gut and Cerebral Perfusion of newborn infants

CONSENT FORM

Study number ☐☐☐

Baby's First Name

Baby's Last Name (Surname)

Please initial each box

1. I confirm that I have read and understand the information letter for the above study. This has also been explained to me and I have had the opportunity to ask questions ☐
2. I understand that my baby's participation is voluntary and that I am free to withdraw him/her at any time, without giving any reason and without his/her medical care or legal rights been affected. ☐
3. I understand that sections of any of my baby's medical and nursing notes may be looked at by staff involved in the study or from regulatory authorities where it is relevant to his/her taking part in research. I give permission to these individuals to have access to those records. ☐
4. I agree to my child taking part in the above study. ☐

Name of person taking consent

Signature

Name of the parent/guardian

Signature

7.6 Appendix 5: Consent form for control infants

Homerton University Hospital NHS
NHS Foundation Trust

Version: V1

Date: 3rd February 2014

Homerton University Hospital
Neonatal Unit
Homerton Row
London
E9 6SR

Tel: 0208 510 7952/7049
Fax: 0208 510 7448

Measurement of Gut and Cerebral Perfusion of newborn infants

CONSENT FORM (Control Group)

Study number ☐☐☐

Baby's First Name

Baby's Last Name (Surname)

Please initial each box

1. I confirm that I have read and understand the information letter for the above study. This has also been explained to me and I have had the opportunity to ask questions ☐
2. I understand that my baby's participation is voluntary and that I am free to withdraw him/her at any time, without giving any reason and without his/her medical care or legal rights been affected. ☐
3. I understand that sections of any of my baby's medical and nursing notes may be looked at by staff involved in the study or from regulatory authorities where it is relevant to his/her taking part in research. I give permission to these individuals to have access to those records. ☐
4. I agree to my child taking part in the above study. ☐

Name of person taking consent

Signature

Name of the parent/guardian

Signature

7.7 Appendix 6: Parent information leaflet for study infants

Homerton University Hospital NHS
NHS Foundation Trust

Version: V6

Date: 23rd April 2012

Homerton University Hospital
Neonatal Unit
Homerton Row
London
E9 6SR

Tel: 0208 510 7952/7049
Fax: 0208 510 7448

Measurement of gut and cerebral perfusion of newborn infants requiring blood transfusion for clinical indication

PARENT INFORMATION LEAFLET

Current blood transfusion practices in neonatal units:

Many preterm and sick babies admitted to the neonatal unit receive blood transfusions to improve the oxygen delivery to vital organs such as the brain, heart and gut. 90% of infants born with a birth weight less than 1000g receive a blood transfusion. The majority of these blood transfusions are given during the first two weeks of life while the baby is receiving intensive care. Some babies also receive blood transfusions after two weeks of life for symptomatic anaemia (low level of haemoglobin, the pigment within the red blood cells which carries oxygen to the various parts of the body).

Current guidelines for blood transfusion in babies vary from hospital to hospital and are based on opinion of experienced doctors. Most neonatal units make a decision on blood transfusion based on the amount of haemoglobin in the baby's blood, the baby's age and how unwell they are.

There have been few previous studies on the benefits of blood transfusion for babies and their findings are unclear. Some studies showed an improvement in symptoms of anaemia but others did not. Babies with low Red Cell Volume (amount of red blood cells in the body) show a good response to blood transfusion. A few studies have raised the concern that frequent blood transfusions to premature babies may be linked with prolonged lung disease (continued oxygen need at around the time the baby should have been born), necrotising enterocolitis (inflammation of the gut) and retinopathy of prematurity (eye disease).

What is the purpose of the study?

The purpose of this study is to measure the changes in blood flow and oxygen delivery to the brain and gut after a blood transfusion. The measurements will be

1

performed without causing any pain or distress to the baby using Doppler Ultrasound (used for routine antenatal scans and brain scans of babies on the unit) and Near Infra-red Spectroscopy [NIRS; similar to the way oxygen saturation (amount of oxygen in the blood) is monitored in babies].

The changes in brain and gut blood flow after blood transfusion will also be compared with measured Red Cell Volume.

Why has my baby been chosen?

Doctors responsible for the care of your baby have decided to give a blood transfusion to improve his/her overall condition. All newborn babies receiving a blood transfusion can be included in this study.

What will happen to my baby if I agree to take part?

If you agree for your baby to take part in this study, brain and gut blood flow and oxygen delivery will be measured (without causing any pain or distress to baby) before, during and after the blood transfusion.

The measurements will not be performed if the doctor or nurse providing care to your baby feels that your baby may not tolerate minimal additional handling.

Measurement of blood flow to the brain and gut: a Doppler ultrasound probe will be placed over the soft spot on the head, upper part of the chest and on the tummy to measure blood flow to the brain and gut. The measurements will be performed 15 minutes before and within 1 hour after blood transfusion, and this may take approximately 15 minutes.

Measurement of oxygen delivery to the brain and gut: a Near Infra-Red Spectroscopy (NIRS) probe (similar to an oxygen saturation probe) will be fixed over the forehead and the upper part of tummy to measure oxygen delivery of the brain and gut. The probes will remain attached to the baby approximately 15 minutes before, throughout the duration of blood transfusion (normally 3 to 4 hours), and approximately 15 minutes after the blood transfusion.

The above measurements may be repeated once during 2-4 weeks of age, and once more after 4 weeks of age if your baby receives blood transfusion.

Measurement of Red Cell Volume: a few drops (0.3ml) of blood will be collected through an existing arterial line (which allows easy blood sampling from the baby without causing any pain or distress) before and after the blood transfusion to estimate red cell volume. An additional 0.6ml of donor blood (the blood given in the transfusion) is added to the volume of blood to be transfused, to replace the sample taken for research purpose.

Measurement of Red Cell Volume will be restricted to babies receiving their first blood transfusion and an existing arterial line.

What information will be collected about me and my baby?

We will need to collect standard clinical information about your pregnancy, the condition of your baby at birth and on the day of blood transfusion and progress throughout the hospital stay. This information will be collected from the baby's written and electronic case record.

Does my baby have to take part?

You do not have to agree to your baby taking part in this study. If you decide not to take part, your baby will receive the usual care of the neonatal unit without the extra measurements taken as described above. If you do decide for any reason that you would like your baby to take part and then change your mind later your baby can be taken out of the study at any time. Your baby's healthcare will not be compromised. Your decision whether or not to take part will not affect the normal high level of care given in the neonatal unit.

What are the possible disadvantages and benefits of taking part?

There are no disadvantages or benefits to your baby by taking part in this study. The findings of the study may help in understanding brain and gut perfusion changes following blood transfusion and improving the blood transfusion practices in neonatal units.

What if something goes wrong?

The chance of anything going wrong as a result of taking part in this study is very small. However, we are required to tell you the following:

If your baby is harmed and this is due to someone's negligence, then you may have grounds for legal action for compensation against the Homerton University hospital in respect of any harm arising out of the participation in this study.

Will my baby taking part in this study be kept confidential?

All information collected for this study will be kept securely and will only be seen by the study organisers and people from the regulatory authorities e.g. from the NHS Trusts' Research and Development Office who ensure that studies such as these are carried out safely. They may also look at your baby's notes to check that the study is being carried out in a correct manner. Information about your baby will not be used for any purpose other than to answer this research question.

What will happen to the results of the study?

At the end of the study, the results will be analysed, presented and published in peer reviewed international medical journal/s. A copy of the full journal article can be

requested from the local co-ordinator/chief investigator. You and your baby will not be identified in any report or publication about the study.

Who is organising and funding the study?

The study is run and funded by the Homerton University Hospital NHS Foundation Trust.

Who has reviewed the study?

All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by a NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However approval means that the Committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits, and that you have been given sufficient information on which to make an informed decision to take part or not. The X Research Ethics Committee has reviewed and approved the study.

What if I have any concerns?

If you have any concerns or other questions about this study or the way it has been carried out, you should contact the Investigators [their name and contact details are below], or you may contact the hospital complaints department.

Local contacts:

Dr Narendra Aladangady
Consultant Neonatologist and Chief Investigator
Homerton University Hospital
Tel - 02085107360

Dr Jayanta Banerjee
Clinical Research Fellow and Principal Investigator
Homerton University Hospital
Tel – 07771826045

Thank you for reading this information leaflet. The doctor or nurse who gave you this leaflet will be pleased to discuss the study in more detail and provide further information if this would be helpful. Alternatively, the contact details of the study's Chief and Principal Investigators are provided on this page.

7.8 Appendix 7: Parent information leaflet for control infants

Homerton University Hospital NHS
NHS Foundation Trust

Homerton University Hospital
Neonatal Unit
Homerton Row
London
E9 6SR

Tel: 0208 510 7952/7049
Fax: 0208 510 7448

Version: V1

Date: 3rd February 2014

Measurement of gut and cerebral perfusion of preterm newborn infants (control group)

PARENT INFORMATION LEAFLET

Background:

Almost 90% of preterm and sick babies admitted to the neonatal unit receive blood transfusions to improve the oxygen delivery to vital organs such as the brain, heart and gut. The majority of these blood transfusions are given during the first two weeks of life while the baby is receiving intensive care. Some babies also receive blood transfusions after two weeks of life for symptomatic anaemia (low level of haemoglobin, the pigment within the red blood cells which carries oxygen to the various parts of the body). Current guidelines for blood transfusion in babies vary from hospital to hospital and are based on opinion of experienced doctors. Most neonatal units make a decision on blood transfusion based on the amount of haemoglobin in the baby's blood, the baby's age and how unwell they are.

What is the purpose of the study?

The purpose of this study is to compare the changes in brain and gut blood flow and oxygen delivery between the anaemic preterm infants receiving blood transfusion for clinical indication and stable preterm infants.

Why has my baby been chosen?

Your baby is preterm, clinically stable and not anaemic (control group).

What will happen to my baby if I agree to take part?

If you agree for your baby to take part in this study, brain and gut blood flow and oxygen delivery will be measured (without causing any pain or distress to baby) over a 3-4 hour period.

Measurement of oxygen delivery to the brain and gut: a Near Infra-Red Spectroscopy (NIRS) probe (similar to an oxygen saturation probe) will be fixed over the skin of the forehead and the lower part of tummy to measure oxygen delivery of the brain and gut respectively. The

1

probes will remain attached to the baby over a 3-4 hour period. This is non-invasive monitoring without causing any discomfort to the baby.

Measurement of blood flow to the brain and gut: by placing an ultrasound probe over the head (over soft spot of head), chest and on the tummy, blood flow to the brain and gut will be measured without causing any discomfort to the baby. The measurements will be performed once before the start and once after the NIRS measurements are completed.

What information will be collected about me and my baby?

We will need to collect standard clinical information about your pregnancy, the condition of your baby at birth and on the day of measurement. This information will be collected from the baby's written and electronic case record.

Does my baby have to take part?

You do not have to agree to your baby taking part in this study. If you decide not to take part, your baby will receive the usual care of the neonatal unit without the extra measurements taken as described above. If you do decide for any reason that you would like your baby to take part and then change your mind later your baby can be taken out of the study at any time. Your baby's healthcare will not be compromised. Your decision whether or not to take part will not affect the normal high level of care given in the neonatal unit.

What are the possible disadvantages and benefits of taking part?

There are no disadvantages or benefits to your baby by taking part in this study. The findings of the study may help in understanding brain and gut perfusion changes following blood transfusion and improving the blood transfusion practices in neonatal units.

What if something goes wrong?

The chance of anything going wrong as a result of taking part in this study is very small. However, we are required to tell you the following:

If your baby is harmed and this is due to someone's negligence, then you may have grounds for legal action for compensation against the Homerton University hospital in respect of any harm arising out of the participation in this study.

Will my baby taking part in this study be kept confidential?

All information collected for this study will be kept securely and will only be seen by the study organisers and people from the regulatory authorities e.g. from the NHS Trusts' Research and Development Office who ensure that studies such as these are carried out safely. They may also look at your baby's notes to check that the study is being carried out in a correct manner. Information about your baby will not be used for any purpose other than to answer this research question.

What will happen to the results of the study?

At the end of the study, the results will be analysed, presented and published in peer reviewed international medical journal/s. A copy of the full journal article can be requested

from the local co-ordinator/chief investigator. You and your baby will not be identified in any report or publication about the study.

Who is organising and funding the study?

The study is run and funded by the Homerton University Hospital NHS Foundation Trust.

Who has reviewed the study?

All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by a NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However approval means that the Committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits, and that you have been given sufficient information on which to make an informed decision to take part or not. The NRES Committee London - Surrey Borders, Charing Cross, Research Ethics Committee has reviewed and approved the study.

What if I have any concerns?

If you have any concerns or other questions about this study or the way it has been carried out, you should contact the Investigators [their name and contact details are below], or you may contact the hospital complaints department.

Local contacts:

Dr Narendra Aladangady
Consultant Neonatologist and Chief Investigator
Homerton University Hospital
Tel - 02085107360

Dr Jayanta Banerjee
Clinical Research Fellow and Principal Investigator
Homerton University Hospital
Tel – 07771826045

Thank you for reading this information leaflet. The doctor or nurse who gave you this leaflet will be pleased to discuss the study in more detail and provide further information if this would be helpful. Alternatively, the contact details of the study's Chief and Principal Investigators are provided on this page.

7.9 Appendix 8. Details of pre-transfusion RCV measurements

Infant	Pre-BT HbF	Post-BT HbF	Donor blood (ml)	Donor Hct	RCV (ml)	RCV (ml/kg)
1	57.8	42.9	15.6	0.69	30.8	38.0
2	57.5	45.4	11.0	0.63	26.2	35.1
3	61.6	47.3	16.0	0.61	32.3	38.7
4	80.1	61.0	14.0	0.58	25.9	28.8
5	80.8	61.6	12.0	0.54	20.8	28.7
6	80.4	61.5	15.0	0.56	27.3	27.9
7	80.3	59.5	12.0	0.55	18.9	24.5
8	48.5	37.6	11.0	0.66	25.0	35.0
9	56.5	39.1	9.0	0.55	11.1	20.6
10	70.8	57.0	12.0	0.57	28.3	37.7
11	67.5	51.2	15.0	0.53	21.9	24.3
12	59.9	47.1	12.6	0.65	30.1	37.4
13	73.2	51.6	17.0	0.61	24.8	21.5
14	68.5	48.5	12.0	0.55	17.9	23.6

8Publications

8.1 List of abstracts

1. Banerjee J., Leung T.S., Illiadis K., Morris J. and Aladangady N. Effect of blood transfusion on cerebral and intestinal blood flow during the first week of life in extreme preterm infants. Available at p153 of https://www.eiseverywhere.com/file_uploads/9206db9fe962868d47f709b38365ec5e_9349_abstract_book_-_25sett13-it-it.pdf. European Society for Pediatric Research; 2013; Oporto, Portugal

2. Banerjee J., Asamoah F., Leung T.S. and Aladangady N. Blood Transfusion and Superior Mesenteric Artery Blood Flow in Extreme Premature Infants http://www.abstracts2view.com/pas/view.php?nu=PAS14L1_4103.74. E-PAS2014:4103.74

3. Banerjee J., Asamoah F., Leung T.S. and Aladangady N. Blood Transfusion and Cerebral Blood Flow and Oxygenation in Extreme Premature Infants http://www.abstracts2view.com/pas/view.php?nu=PAS14L1_4109.219. E-PAS2014:4109.219

4. Banerjee J., Asamoah F., Leung T.S. and Aladangady N. Effect of Blood Transfusion on Intestinal Blood Flow and Oxygenation during the First Week of Life in Extreme Premature Infants http://www.neonatalsociety.ac.uk/abstracts/banerjeej_2013_transfusionflowoxygenation.shtml. Neonatal society autumn meeting 2013

5. Banerjee J, Leung TS, Aladangady N.
   Cerebral blood flow and oxygenation changes following blood transfusion in preterm infants
   Arch Dis Child. doi: 10.1136/archdischild-2014-307384.1130

6. Banerjee J, Leung TS, Aladangady N.
   Cerebral haemodynamic response to blood transfusion varies with chronological age in preterm infants
   Arch Dis Child. doi: 10.1136/archdischild-2014-307384.1131

7. Banerjee J, Leung TS, Aladangady N.
Blood transfusion in preterm infants improves intestinal tissue oxygenation without alteration in blood flow
http://www.abstracts2view.com/pas/view.php?nu=PAS16L1_1380.8
E-PAS2016:1380.8

8. Banerjee J, Leung TS, Aladangady N.
Pre-transfusion Red Cell Volume (RCV) and its effect on gut perfusion in infants receiving blood transfusion
http://www.abstracts2view.com/pas/view.php?nu=PAS16L1_1380.6
E-PAS2016:1380.6

8.2 List of journal articles

1. Banerjee J, Aladangady N. Biomarkers to decide red blood cell transfusion in newborn infants. *Transfusion 2014; 54(10):2574-82. doi: 10.1111/trf.12670.*

2. Banerjee J, Leung TS and Aladangady N. Effect of blood transfusion on intestinal blood flow and oxygenation in extremely preterm infants during first week of life. *Transfusion 2016 Apr; 56(4):808-15. doi: 10.1111/trf.13434.*

3. Banerjee J, Leung TS, Aladangady N. Cerebral blood flow and oximetry response to blood transfusion in relation to chronological age in preterm infants. *Early Human Development 2016 Jun; 97:1-8. doi: 10.1016/j.earlhumdev.2015.10.017.*

4. Banerjee J, Leung TS, Aladangady N. Blood transfusion in preterm infants improves intestinal tissue oxygenation without alteration in blood flow. *Vox Sang, 111: 399–408. doi:10.1111/vox.12436.*