Australian Placental Transfusion Study (APTS) secondary analysis

Supplementary Appendix One

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Australian Placental Transfusion Study

Should very preterm babies receive a placental transfusion at birth? A randomised controlled trial

Protocol number
Version 1
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Signature Date

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SYNOPSIS

Primary clinical hypothesis:
An autologous transfusion of placental blood at birth will improve health outcomes in preterm babies. Specifically, placental transfusion by deferred cord clamping is associated with an 8% absolute risk difference, from 30% to 22% in mortality and/or major morbidity, at 36 weeks post menstrual age.

Study objective:
To establish if placental transfusion in a population of preterm babies less than 30 weeks gestation will have improved health outcomes compared with the standard practice of early cord clamping.

Study design:
This is a multicentre 2-arm parallel open label randomised controlled trial

Study interventions:
The two arms of the trial comprise:
(a) early cord clamping (which is the control arm)
(b) deferred cord clamping (for 60 seconds or more)

Duration: 48 months for recruitment with a further 24 months for follow-up i.e. total study duration of 72 months.

Study population: babies less than 30 weeks gestation

Primary outcome

Death and/or major morbidity at 36 weeks post menstrual age

Secondary outcomes include

1. Death at 36 weeks postmenstrual age
2. Major morbidity at 36 weeks post menstrual age
3. Death and major morbidity in infants of 27^{0} weeks gestation or more
4. Death and major morbidity in infants of 26^{6} weeks gestation or less
5. Death or severe disability to (i) 24 months and (ii) 3 years corrected age

Tertiary (hypothesis generating) outcomes include
Birth weight, haematocrit on admission, peak serum bilirubin, number of exchange transfusions, number of partial exchange transfusions

Pre-defined subgroups:

i. Gender
ii. Infants of 26^{6} weeks gestation or less
iii. Infants of 27^{0} weeks gestation or more

Power and Sample Size

Controls (early clamping) are expected to have a 30% rate of the primary combined outcome of mortality (10%) and major morbidity (20%) at 36 weeks post menstrual age. A sample size of 1600 would have 90% power to detect a reduction in absolute risk of 8% (27% relative risk reduction) in the primary outcome, allowing for 10% non-compliance at 2p = 0.05. If the non-compliance rate was 20%, this sample size would have more than 80% power to detect a reduction in absolute risk of 8%.

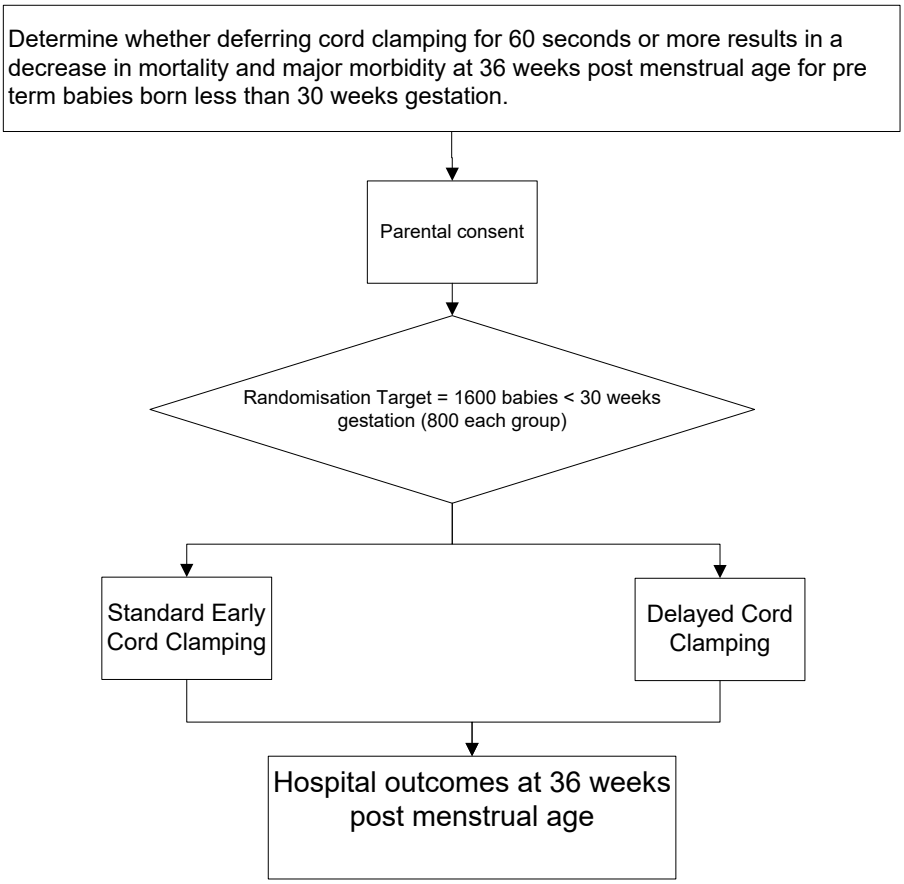

Figure 1. Australian Placental Transfusion Study schema.

1 BACKGROUND INFORMATION

Babies born less than 30 weeks of gestation have increased incidence of mortality, brain damage and other morbidities.[1] Death and disability often results from inflammatory damage mediated by hypoxia, hyperoxia, cardiovascular compromise, ischaemia, reperfusion injury, infection or a combination of these factors. Placental transfusion is a low cost, simple haemodynamic intervention that may reduce these morbidities and mortality in very preterm babies.

Summary of the clinical scenario

Placental transfusion at birth is achieved by deferring clamping of the umbilical cord or 'milking' residual cord blood into the baby after clamping. Table 1 summarises an unpublished updated meta-analysis of 11 RCTs of placental transfusion in 494 babies (Reynolds unpublished data). The majority of these data are derived from studies undertaking delayed cord clamping with only one study involving the milking strategy.

Table 1: Effects of placental transfusion versus early clamping in 11 RCTs, 494 babies < 37 weeks gestation

Outcome	*No. of RCTs*	*No. of babies*	*Effect type*	*Estimate (95% confidence interval)*
Potential benefits			**1.1.1**	
• Haematocrit* 1 hr after birth	6	281	WMD[T]	3.00 [1.49, 4.51]
• Haematocrit* 4 hr after birth	4	134	WMD	5.40 [3.52, 7.28]
• Number of blood transfusions	5	212	WMD	-1.32 [-1.93, -0.70]
• Transfused for low blood pressure	2	58	RR ^	0.38 [0.18, 0.84]
• Intraventricular haemorrhage (IVH)	10	436	RR	0.54 [0.37, 0.78]
• Necrotising enterocolitis (NEC)	6	281	RR	0.61 [0.43, 0.88]
• Late onset sepsis (LOS)	1	72	RR	0.1 [0.01, 0.85]
• Mortality	11	480	RR	0.84 [0.43, 1.63]
• Temperature on admission (°C)[#]	3	143	WMD	0.14 [-0.03, 0.31]
Potential adverse effects				
• Peak serum bilirubin (micromol/L)	7	320	WMD	11.13 [1.75, 20.51]

* packed cell volume; [T] WMD weighted mean difference; ^ Relative risk;
[#] Rabe et al., 2008[2]

The evidence suggests that placental transfusion in babies <37 weeks gestation is safe, well tolerated and reduces the risks of intraventricular haemorrhage, necrotising enterocolitis and late onset sepsis by 40% or more. However, power was inadequate to exclude a moderate difference in mortality. Furthermore the results, especially for sepsis, are based on small numbers and must be viewed with caution and IVH reported above is for all grades of IVH and there is very little data on severe IVH. We were also unable to exclude from the analyses babies of 31-36 weeks gestation, who outnumber very preterm babies by more than 10:1 and who are at much less risk of adverse outcome. Nevertheless, the reductions in intraventricular haemorrhage, necrotising enterocolitis and late onset sepsis are important outcomes, and each is associated with mortality or later disability. If even a moderate decrease in these morbidities were confirmed in babies <30 weeks gestation, placental transfusion would have major implications for their survival and healthy development.

The main determinants of placental transfusion are time of cord clamping, uterine contraction and the position of the baby. Placental transfusion increases the blood volume by 35-60% in term babies if cord clamping is delayed for three minutes. If the infant is kept at or below the level of the placenta, about 25-30% of this transfusion occurs in the first 10-15 seconds. Umbilical arterial constriction is

present by 45 seconds. Placental transfusion is completed by 1 minute if ergometrine is given early in the third stage of labour, and by 30 seconds if the baby is lowered to 40 cm below the introitus, illustrating the importance of hydrostatic pressure.[3,4] Saigal *et al.* found that a complete placental transfusion produced a 47% and 50% increase in blood volume in preterm and term babies, respectively. Over half the full transfusion occurred by 1 minute in the preterm babies. Babies held 15 cm above the introitus for 1 minute received 60% of the transfusion received by babies held below it.[5]

The increase in blood volume provided by placental transfusion may be physiologically appropriate, and necessary, because the central haemodynamic event at birth is the rapid opening up of new vascular beds in the lungs.[4] Looked at another way, early clamping may deprive the baby of a substantial volume of blood. This causes volume depletion and systemic and peripheral hypoperfusion as pulmonary vascular beds must be filled with blood from the systemic circulation instead. This is well compensated for in normally delivered term babies, but may not be after Caesarean section (when the uterus is often not contracting) or in preterm babies. The preterm fetus responds to volume expansion by intrauterine blood transfusion with a rapid increase in plasma concentrations of natriuretic peptide.[6] Transudation or diuresis after placental transfusion could help explain why the mean haematocrit rises 1 and 4 hours after birth [Table 1].

Haematocrit correlates closely with red cell volume,[7] which is inversely associated with risk of respiratory distress syndrome.[8] In RCTs, placental transfusion increased initial haematocrit, blood pressure and oxygenation, and reduced use of surfactant and duration of ventilation or added oxygen.[9,10,11] De Halleux *et al.* reported that decreased oxygen affinity after transfusion with adult blood increases the oxygen available to the tissues of pre-term babies.[12] This may increase oxygen toxicity to brain, eye[13] and lung. By reducing exposure to adult donor blood transfusions, [Table 1] placental transfusion may reduce this oxygen toxicity. Placental transfusion may also increase total transfer of immunoglobulin to the infant. Lastly, preterm cord blood is rich in haemopoietic precursor and stem cells. As well as enhancing bone marrow function and immunocompetence against infection, these may have anti-inflammatory, neurotrophic and neuro-protective effects. [14]

Although our meta analysis [Table 1] showed no statistically significant negative impact on safety from placental transfusion in babies < 37 weeks gestation overall,[2, 15] some preterm babies < 30 or <27 weeks gestation may be at higher risk of hypothermia, low Apgar scores, IVH , NEC or death if resuscitation is delayed until the cord is clamped after 60 seconds or more, especially the most immature or compromised, in whom myocardial dysfunction on echocardiography is common. Placental transfusion may increase serum bilirubin (a breakdown product of haemoglobin) and volume expansion with placental blood may be poorly tolerated in shock, asphyxia, or placental vascular disease.[16, 17, 18] On the other hand, continuing perfusion with warm, oxygenated placental blood is well tolerated during the EXIT procedure, in which fetuses with complex airway problems are partially delivered by Caesarean section and supported by the placental circulation for 20 minutes or more while the airway is stabilized. Continuing placental transfusion might improve hypothermia, hypoglycaemia, gas exchange, hypovolaemia and shock.

Results from the APTS pilot study comparing delayed cord clamping, cord milking and both delayed cord clamping and milking combined with immediate cord clamping (unpublished) indicated that compliance with the 4 intervention arms was acceptable. There was no difference in haemoglobin at 6 hours after birth between the 4 treatment arms in the first 40 babies studied and no safety issues with any of the arms. It was thus recommended by the TMC to commence the main study comparing immediate cord clamping with deferred cord clamping, for which there is most data currently available.

This two arm study is proposed to establish if placental transfusion in a population of preterm babies less than 30 weeks gestation will have improved health outcomes compared with the standard practice of early cord clamping.

2 TRIAL OBJECTIVES

To establish if placental transfusion, using deferred cord clamping for 60 seconds or more, in pre term babies less than 30 weeks of gestation will have improved health outcomes compared with standard early cord clamping.

3 TRIAL DESIGN

3.1 Design

This is a multi centre, two arm parallel, open label randomised controlled clinical trial

3.2 Consent

All mothers considered by the obstetric team to have a reasonable chance of delivering before 30 weeks gestation are eligible. Consent will be sought by members of the delivery team or appropriate research staff at any time during pregnancy. For example, if it is anticipated some weeks in advance that delivery will be necessary before 30 weeks gestation, consent may be obtained well in advance of randomisation.

3.3 Randomisation

Timing of randomisation
While consent can be attained at any time, randomisation should only take place when delivery is considered inevitable, for example when labour is considered irreversible or when the operating theatre is booked for Caesarean Section, and the baby is due to be born before 30 weeks gestation. Randomisation should be performed allowing sufficient time to use the Interactive Voice Response System i.e. at least ten minutes before the expected birth, or longer than this for multiple births.

Method of randomisation
Randomisation can be done by any member of the obstetric, midwifery, or neonatal team using a phone to call a computerised interactive voice response system.

The computer generated randomisation lists used with the interactive voice response system will be prepared by an independent statistician at the NHMRC Clinical Trials Centre, University of Sydney. The randomisation code will be stored securely by the statistical group at the centre.

Randomisation will be by the method of minimisation with two strata: Institution and Gestational age (<27 weeks; >=27 weeks):

3.4 Endpoints

3.4.1 Primary outcome

Death and/or major morbidity at 36 weeks post menstrual age

Morbidity is defined by one or more of the following:

- Brain injury on ultrasound
- Chronic lung disease
- Severe retinopathy
- Necrotising enterocolitis
- Late onset sepsis

3.4.2 Definitions of primary outcome for study

Morbidity Measures

Based on definitions used by the Australian and New Zealand Neonatal Network. [19]

- Brain injury on ultrasound

Grade of 3 and 4 IVH (major intraventricular haemorrhage) seen on ultrasound according to the system of grading defined below:

1. Subependymal germinal matrix bleed
2. IVH without ventricular distension
3. IVH with ventricular distension with blood
4. Intraparenchymal haemorrhage

Or echodense intraparenchymal lesions, periventricular leukomalacia, porencephalic cysts or ventriculomegaly (97 percentile plus 4mm), or

- Chronic lung disease (also known as bronchopulmonary dysplasia) defined as receiving supplemental oxygen or any form of assisted ventilation at 36 weeks post menstrual age for 4 consecutive hours in a 24 hour period, or
- Severe retinopathy warranting treatment with laser surgery, cryotherapy or monoclonal antibody therapy or
- Necrotising enterocolitis diagnosis of proven Necrotising Enterocolitis with the following signs:
  1. at least one systemic sign: temperature instability, apnoea, bradycardia or lethargy and at least one intestinal sign: residual of 25% of the previous feed on 2 consecutive occasions, or abdominal distension, or vomiting or faecal blood
  2. profile consistent with definite NEC including at least one of the following: abdominal wall cellulitis and palpable abdominal mass, or pneumatosis intestinalis , or portal vein gas, or a persistent dilated loop on serial X rays, or a surgical or post mortem diagnosis.
  3. warranted treatment for NEC, which included nil by mouth and antibiotics
- Late onset Sepsis

  Systemic sepsis is defined as a clinical picture consistent with sepsis, and either a positive bacterial or fungal culture of blood and/or cerebrospinal fluid, or a positive urine culture by sterile collection only. At least one episode of systemic sepsis with initial symptoms from 48 hours after birth. Isolation of organisms from one blood culture and, after considering clinical/laboratory evidence, decision made to give antibiotics with therapeutic intent against this organism. Infections with coagulase − negative staphylococci, and other potential contaminants, or group β streptococcal antigen detected in urine should be included only if the baby is considered clinically septic and there is supporting evidence such as raised white cell count or thrombocytopenia. Viral infections must be proven by culture and/ or haematological results consistent with infection. The following must not apply: mixed CNS or other skin flora contaminant; same blood organisms isolated from blood during the previous 14 days – repeat isolate.

3.4.3 Secondary outcomes

1. Death at 36 weeks post menstrual age
2. Major morbidity (see above for components of combined morbidity) at 36 weeks post menstrual age
3. Death or severe disability to (i) 24 months and (ii) 3 years corrected age
4. Death to
   (i) 18 or 24 months corrected age
   (ii) 3 years corrected age
5. Severe disability to
   (i) 18 or 24 months corrected age
   (ii) 3 years corrected age
6. Brain injury on ultrasound at 36 weeks post menstrual age
7. IVH at 36 weeks post menstrual age

I. IVH all grades

II. grades 3 and 4
III. grade 4
8. Chronic lung disease at 36 weeks post menstrual age
9. Severe retinopathy at 36 weeks post menstrual age
10. Necrotizing enterocolitis and surgery for at 36 weeks post menstrual age
11. Late onset sepsis at 36 weeks post menstrual age

3.4.4 Descriptions of secondary outcomes

18 - 24 month assessment of death or severe disability

Evaluation of severe disability will be assessed by a short paediatric questionnaire that can be filled out from routine hospital assessments and the Ages and Stages questionnaire. [20]

One of the following is required in order to determine severe disability:

1. Ages and stages score indicative of developmental delay
2. Cerebral palsy
3. Blindness
4. Deafness
5. Problems with language or speech
6. Any other major medical problems or diagnoses

3.4.5 Tertiary outcomes

1. birth weight
2. blood transfusions (number of transfusions up to 36 weeks corrected for gestation)
3. haematocrit on admission
4. peak serum bilirubin (to 36 weeks gestation)
5. admission temperature
6. admission blood glucose concentration
7. length of hospital admission (to discharge)
8. maternal post partum haemorrhage requiring blood transfusion
9. number of exchange transfusions and partial exchange transfusions up to 36 weeks gestation

3.4.6 Predefined subgroups

1. Gender
2. Infants of 26 ^{6} weeks gestation or less
3. Infants of 27 ^{0} weeks gestation or more

4 PARTICIPANT POPULATION

4.1 Participant Population

Male or female pre term babies less than 30 weeks gestational age.

4.2 Inclusion criteria

- mother imminently delivering < 30 weeks of gestation
- informed consent has been received from a parent

4.3 Exclusion criteria

No indication or contraindication to placental transfusion, in view of mother or doctor. For example, contraindications may include

- fetal haemolytic disease
- fetal hydrops
- major malformations considered incompatible with survival

4.4 Withdrawal criteria

Any family that wishes to withdraw from the trial may do so, without giving a reason and without any change in any other aspect of treatment. Parents of any baby who is withdrawn from the study after randomisation and before or after the intervention is administered will be asked to allow collection of outcome data. Information on hospital outcomes for any baby who is withdrawn after the baby is discharged from hospital will be used in the study.

5 TREATMENT OF PARTICPANTS

(a) Immediate cord clamping (< 10 seconds after birth)

- The cord is clamped 6 cm from the umbilicus within ten seconds of delivery of the baby.

(b) Deferred cord clamping

- A birth attendant holds the baby as low as possible below the level of the introitus or placenta for 60 seconds or more, then clamps the cord about 6 cm from the umbilicus.

It is recommended that clamping be deferred for 60 seconds or more even if the fetus appears in extremis because adequate blood volume may assist subsequent resuscitation. However, if the cord is collapsed no blood can be transferred from the placenta, perhaps because of placental abruption, the cord may be clamped early at the discretion of the attending physician.

There is some concern that palpating the cord may restrict blood flow from the placenta to the infant, therefore, palpating the cord is discouraged subject to the clinician's discretion.

5.1 Compliance

As compliance with this intervention can vary, the following parameters will be recorded and monitored centrally:

- Duration in seconds of deferred cord clamping
- Height at which baby was held in relation to placenta

5.2 Follow-up

The primary measure of outcome is the composite of death and major morbidity up to 36 weeks, as defined in the study hypotheses above. However, consent will also be sought at recruitment for continuing follow up to 3 years of age, when a definitive assessment of neuro-sensory and cognitive function will be performed, using the most up to date and appropriate instrument available at that time. This will be the subject of a separate proposal later in the project.

To facilitate good follow up at 3 years, regular contact by phone and post will be maintained with the families, who will complete a short questionnaire at 18 or 24 months (depending on hospital policy) to screen for severe disability.

6 EFFICACY AND TOLERABILITY

6.1 Assessment of Efficacy

Efficacy will be assessed through routinely collected hospital measures including demographic data, admission data such as blood glucose and outcomes data such as incidents of necrotising enterocolitis and/or brain injury which are all contained within the medical record.

At 18 to 24 months of age, depending on hospital policy, children will also have 2 follow up assessments: an Ages and Stages questionnaire to assess cognitive function and a short paediatric assessment to determine physical development including incidence of cerebral palsy, eyesight or hearing impairment etc.

6.2 Assessment of Tolerability

Serious adverse events which may be life threatening are common in very preterm infants, however, the proportion of **unexpected serious adverse events** (in the opinion of the investigator) is expected to be small.

AN UNEXPECTED SERIOUS ADVERSE EVENT (SAE) is any untoward medical occurrence that is not expected and:

- results in death, or
- is life-threatening (i.e. the subject is at risk of death at the time of the event), or
- requires prolongation of hospitalisation, or
- results in persistent or significant disability or incapacity

NOTE: The term “life-threatening” in the definition of “serious” refers to an event in which the baby was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

6.2.1 Reporting of Unexpected Serious Adverse Events and Outcome Events

The investigator is responsible for reporting all Unexpected Serious Adverse Events occurring during the study to the NHMRC Clinical Trials Centre within 1 working day.

Unexpected SAE reports should be faxed to +61 2 9562 5026.

The Trial Management Committee and all other Principal Investigators participating in the study will be informed. The investigator or delegate at each participating institution is responsible for reporting unexpected serious adverse events to their HREC. Serious adverse events will also be reported to the Safety Data Monitoring Committee.

7 STUDY STRUCTURE

7.1 Trial Management Committee

The NHMRC CTC, in conjunction with the Principal Investigators will appoint a Trial Management Committee (TMC). A Trial Executive Committee (TEC) may be selected from the TMC in order to expedite decision-making and will be led by the Study Chair.

The TMC responsibilities include protocol development, study planning, monitoring of progress and patient safety, review of information from related research and implementation of recommendations from other study committees and external bodies (e.g. HRECs), and publications. The TMC will be responsible for selection and support of local investigators,. The TMC will also monitor rate of recruitment and endpoint occurrence and will advise the SDMC of variations. The TMC will meet twice a year and/ or as required.

The TEC is a subset of the TMC which meets more regularly on key scientific and/or operational issues impacting on study conduct. The TEC will meet at least once every quarter (or as the stage of the trial dictates) as the study progresses.

7.2 Independent Safety and Data Monitoring Committee

An independent Data and Safety Monitoring Committee (IDSMC) will monitor the progress of all aspects of the study and will ensure that the study meets the highest standards of ethics and patient safety. It will review interim data and other emerging evidence, including relevant RCTs and overviews of RCTs. The IDSMC will advise the TMC if in their view there is proof beyond reasonable doubt of net clinical benefit or harm, for all infants or for a subset of infants, that might reasonably be expected to influence the management of many clinicians. Data on key study outcomes will be

monitored every 12 months, or more frequently if requested by the IDSMC, to ensure safety and that the event rates meet protocol projections. If the event rates are lower than expected, the IDSMC can recommend an increase in the study sample size to maintain study power.

The combined endpoint of death and/or major morbidity will be monitored using a modified Haybittle-Peto rule. The IDSMC supports the view that a difference of at least 3 standard deviations (SD) in a major endpoint (or in a combination of major endpoints) that suggested net clinical benefit or harm may be needed to justify recommending that the TMC consider either stopping the study prematurely, monitoring the trial more frequently or modifying the trial design.

7.2.1 Net Clinical Benefit: an important criterion for decision making

It should be noted that evidence of a significant difference in major morbidity is not necessarily an indication for early stopping of the trial since this might be balanced by a reduction in deaths or severe disability. For example one arm of the study may be associated with an increase in morbidity which is statistically significant, but may also be consistent with a reduction in deaths which is not statistically significant, and with net benefit (not statistically significant) for the other arm. Similarly, evidence of a significant increase in an intermediate measure of adverse outcome, such as intraventricular haemorrhage (IVH) or necrotising enterocolitis (NEC), in one arm of the study is not necessarily an indication to stop early, since this might be balanced by an overall reduction in deaths and/ or severe disability in the other arm of the study on follow up. Furthermore, major outcomes on follow up will not be determined until two years after trial entry. In both examples, it may be appropriate to continue the trial and indeed other related trials, until a clearer picture on net clinical benefit was obtained.

8 STATISTICS

8.1 Sample Size

Controls (early clamping) are expected to have a 30% rate of the primary combined outcome of mortality (10%) and major morbidity (20%) at 36 weeks post menstrual age. A sample size of 1600 would have 90% power to detect a reduction in absolute risk of 8% (27% relative risk reduction) in the primary outcome, allowing for 10% non-compliance at 2p = 0.05. If the non-compliance rate was 20%, this sample size would have more than 80% power to detect a reduction in absolute risk of 8%. About 534 babies are expected to be less than 27 weeks gestation.

The planned sample size of 1600 has 80% power at 2p=0.05 to detect an absolute risk reduction in mortality at 36 weeks post menstrual age of 4.2% from 10% to 5.8% and an absolute risk reduction of 8% (25% – 17%) in the outcome of mortality or severe disability to 24 months corrected gestation, assuming 10% non compliance. It gives 80% power to detect a reduction in absolute risk of major morbidity of 5.9% from 20% to 14.1%, assuming 10% non compliance. The expected sample of 534 babies < 27 weeks gestation gives 80% power to detect an absolute risk reduction of 13.4% from 60% to 46.6% in mortality and major morbidity, assuming 10% non compliance. The expected sample of 1066 babies of 27^{0} weeks gestation or more gives 80% power to detect an absolute risk reduction of 6.1% from 15% to 8.9% in mortality and major morbidity, assuming 10% non compliance.

8.2 Statistical Analysis

The primary analyses will be performed on assigned treatment (intention to treat). Continuous data will be summarised using means for normally distributed variables and medians otherwise. Binary outcomes will be summarised using percentages. Groups will be compared using chi-square tests or t-test where appropriate. Adjusted analyses will use logistic or linear regression where appropriate. Time to event outcomes will be analysed with proportional hazards regression. Safety outcomes may be analysed using a treatment received analysis. No adjustments will be made for multiple comparisons.

8.3 Prospective meta-analysis

Meta-analysis of multiple randomised controlled trials increases the overall sample size and power to demonstrate treatment effects. However, if meta-analysis is performed when results of trials are known, data dependent selection bias can occur. Trials with certain results can be deliberately included or excluded to manipulate the conclusions. A prospective meta-analysis (PMA) is a meta-analysis where randomised controlled trials are identified, evaluated and determined to be eligible before the results of any of them become known. PMA can therefore help to overcome some of the problems of retrospective meta-analyses. It enables: hypotheses to be specified a priori ignorant of the results of individual trials; prospective application of selection criteria; and a priori statements of intended analyses, including sub-group analyses, to be made before the results of individual trials are known. This avoids potentially biased, data dependent emphasis on particular subgroups. In addition, pooling of individual patient data allows more informative subgroup analyses to be conducted. The Australian Placental Transfusion Study will contribute to a proposed PMA of similar RCTs.

9 ADMINISTRATIVE ASPECTS

9.1 Ethics and regulatory compliance

This study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2007) and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008. The investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a mother or baby. In this circumstance the CTC, principal investigator and HREC will be advised. The trial will be registered with the Australian Clinical Trial Registry.

9.2 Study conduct

All study sites taking part in the trial will be required to participate in a start-up meeting on-site, to present the protocol and undergo training on study procedures and data collection methods. The Principal Investigator at each study site must apply for Human Research Ethics Committee (HREC) approval, submit all amendments and changes to the protocol and provide any necessary documentation for their site before they can enrol babies into the study.

9.3 Confidentiality

The study will be conducted in accordance with applicable Privacy Acts and Regulations. All data generated in this study will remain confidential. All information will be stored securely at the NHMRC Clinical Trials Centre, University of Sydney and will only be available to staff directly involved with the study.

9.4 Protocol amendments

Changes and amendments to the protocol can only be made by the Trial Management Committee. Approval of amendments by the Institutional HREC is required prior to their implementation. In some instances, an amendment may require a change to a consent form. The Investigator must receive approval/advice of the revised consent form prior to implementation of the change. In addition, changes to the Case Report Forms, if required, will be incorporated in the amendment.
The investigator should not implement any changes to, or deviations from, the protocol except where necessary to eliminate immediate hazard(s) to mothers or babies in the trial.

9.5 Data Handling and Record Keeping

Trial data will be recorded on the CRFs provided. All required data entry fields will be completed. Data corrections will be done according to the instructions provided. The investigator will be asked to confirm the accuracy of completed CRFs by signing key CRFs as indicated.

Source documents pertaining to the trial must be maintained by investigational sites. Source documents may include a mother or baby's medical records, hospital charts, clinic charts, the investigator's participant study files, as well as the results of diagnostic tests such as X-rays, laboratory tests, and electrocardiograms. The investigator's copy of the case report forms serves as part of the investigator's record of a baby's study-related data.

The following information should be entered into the baby's medical record:

a. Baby's name, contact information and protocol identification.
b. The date that the baby entered the study, and study number.
c. A statement that informed consent was obtained (including the date).
d. Relevant medical history
e. Results of key trial parameters.
f. Occurrence and status of any unexpected serious adverse events.
g. The date the baby exited the study, and a notation as to whether the baby completed the study or reason for discontinuation.

All study-related documentation will be maintained for 23 years following completion of the study.

9.6 Study Monitoring

Data from this study will be monitored by Clinical Trials Program staff from the NHMRC Clinical Trials Centre (CTC). Monitoring will include centralized review of CRFs and other study documents for protocol compliance, data accuracy and completeness. Monitoring may include monitoring visits to investigational sites for source data verification, review of the investigator's site file and drug handling records. The CTC will be given direct access to source documents, CRFs and other study-related documents. By signing the informed consent form, the parents give authorized CTC staff direct access to the mother and baby's medical records and the study data.

9.7 Audit and Inspection

This study may be subject to audit or inspection by representatives of the CTC or representatives of regulatory bodies.

9.8 Clinical Study Report

Data will be entered and statistical analysis will be conducted by the NHMRC CTC. A Clinical Study Report will be issued which may form the basis of a manuscript(s) intended for publication.

9.9 Publication Policy

The Trial Management Committee will appoint a Writing Committee to draft manuscripts based on the trial data. Manuscripts will be submitted to peer-reviewed journal(s). The initial publication will be the report of the full trial results based on the main protocol using the study group name, with subsequent publications of data subsets. The Writing Committee will develop a publication plan, including authorship, target journals and expected dates of publication.

10 REFERENCES

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Australian Placental Transfusion Study

Should very preterm babies receive a placental transfusion at birth? A randomised controlled trial

Protocol number
Version 4
1 July 2016

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SYNOPSIS

Primary clinical hypothesis:
An autologous transfusion of placental blood at birth will improve health outcomes in preterm babies. Placental transfusion by deferred cord clamping is associated with an 8% absolute risk difference, from 30% to 22% in mortality and/or major morbidity, at 36 weeks post menstrual age.

Study objective:
To establish if placental transfusion in preterm babies less than 30 weeks gestation will improve health outcomes compared with the standard practice of early cord clamping.

Study design:
This is a multicentre 2-arm parallel open label randomised controlled trial

Study interventions:
The two arms of the trial comprise:
(a) early cord clamping (which is the control arm)
(b) deferred cord clamping (for 60 seconds or more with the baby held below or at the level of the placenta)

Duration: 48 months for recruitment with a further 24 months for follow-up i.e. total study duration of 72 months.

Study population: babies of 29 ^{6} weeks gestation or less

Primary outcome
Death and/or major morbidity at 36 completed weeks post menstrual age

Secondary outcomes include
Incidence of the following outcomes up to 36 completed weeks post menstrual age unless specified otherwise:

1. Death
2. Major morbidity
3. Death and major morbidity in infants of 27 ^{0} to 29^{6} weeks gestation
4. Death and major morbidity in infants of 26 ^{6} weeks gestation or less
5. Death or major disability up to 3 years corrected age

Tertiary (hypothesis generating) outcomes include
Birth weight, number of exchange transfusions, number of partial exchange transfusions

Pre-defined subgroups:

i. Gender
ii. Age: Infants of 26 ^{6} weeks gestation or less or 27 ^{0} weeks gestation or more
iii. Caesarean Section vs Vaginal birth

Power and Sample Size
At least 1500 infants and a primary outcome rate in the immediate clamping group of 41.6% will have 80% power to detect a 24% RRR, allowing for 30% non-compliance at 2p = 0.05.

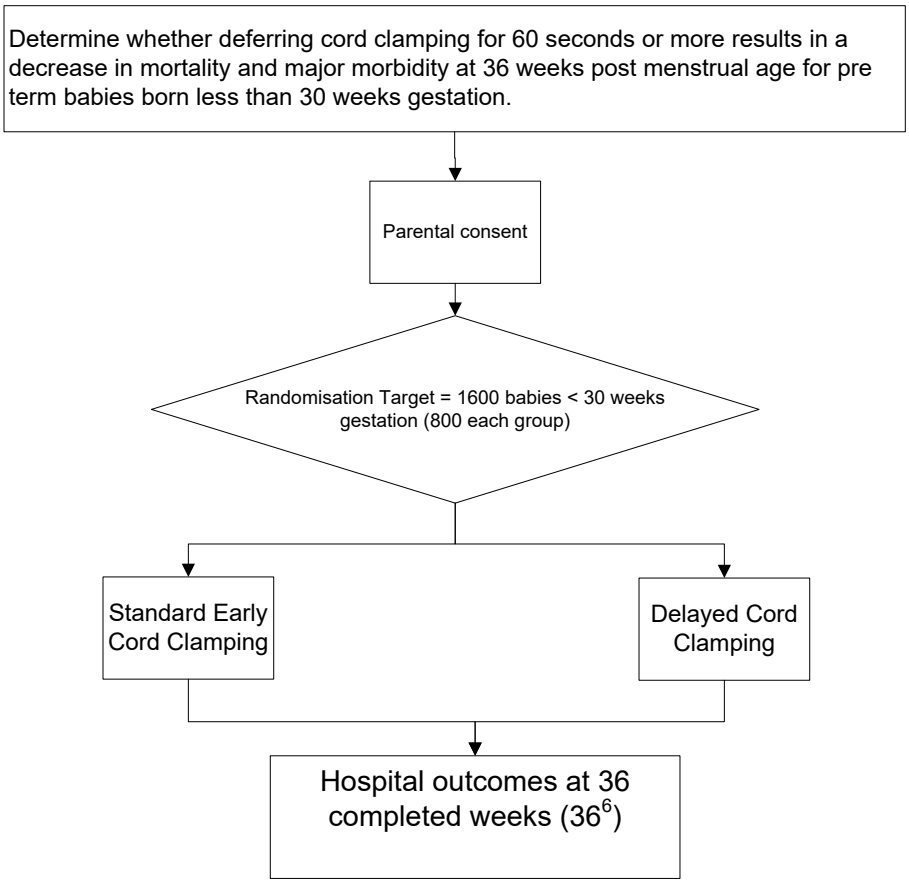


Figure 1. Australian Placental Transfusion Study schema.

1 BACKGROUND INFORMATION

Babies born less than 30 weeks of gestation have increased incidence of mortality, brain damage and other morbidities.[1] Death and disability often results from inflammatory damage mediated by hypoxia, hyperoxia, cardiovascular compromise, ischaemia, reperfusion injury, infection or a combination of these factors. Placental transfusion is a low cost, simple haemodynamic intervention that may reduce these morbidities and mortality in very preterm babies.

Summary of the clinical scenario

Placental transfusion at birth is achieved by deferring clamping of the umbilical cord or by the less frequently reported method of 'milking' residual cord blood into the baby after clamping. Table 1 summarises an unpublished updated meta-analysis of 11 RCTs of placental transfusion in 494 babies (Reynolds unpublished data). The majority of these data are derived from studies undertaking delayed cord clamping with only one study involving the 'milking' strategy.

Table 1: Effects of placental transfusion versus early clamping in 11 RCTs, 494 babies < 37 weeks gestation

Outcome	*No. of RCTs*	*No. of babies*	*Effect type*	*Estimate (95% confidence interval)*
Potential benefits				
• Haematocrit* 1 hr after birth	6	281	WMD[T]	3.00 [1.49, 4.51]
• Haematocrit* 4 hr after birth	4	134	WMD	5.40 [3.52, 7.28]
• Number of blood transfusions	5	212	WMD	-1.32 [-1.93, -0.70]
• Transfused for low blood pressure	2	58	RR ^	0.38 [0.18, 0.84]
• Intraventricular haemorrhage (IVH)	10	436	RR	0.54 [0.37, 0.78]
• Necrotising enterocolitis (NEC)	6	281	RR	0.61 [0.43, 0.88]
• Late onset sepsis (LOS)	1	72	RR	0.1 [0.01, 0.85]
• Mortality	11	480	RR	0.84 [0.43, 1.63]
• Temperature on admission (°C)[#]	3	143	WMD	0.14 [-0.03, 0.31]
Potential adverse effects				
• Peak serum bilirubin (micromol/L)	7	320	WMD	11.13 [1.75, 20.51]

* packed cell volume; [T] WMD weighted mean difference; ^ Relative risk;
[#] Rabe et al., 2008[2]

The evidence suggests that placental transfusion in babies <37 weeks gestation is safe, well tolerated and reduces the risks of intraventricular haemorrhage, necrotising enterocolitis and late onset sepsis by 40% or more. However, power was inadequate to exclude a moderate difference in mortality. Furthermore the results, especially for sepsis, are based on small numbers and must be viewed with caution. IVH reported above is for all grades of IVH and there is very little data on severe IVH. We were also unable to exclude from the analyses babies of 31-36 weeks gestation, who outnumber very preterm babies by more than 10:1 and who are at much less risk of adverse outcome. Nevertheless, the reductions in intraventricular haemorrhage, necrotising enterocolitis and late onset sepsis are important, and each is associated with mortality or later disability. If even a moderate decrease in these morbidities were confirmed in babies <30 weeks gestation, placental transfusion would have major implications for their survival and healthy development.

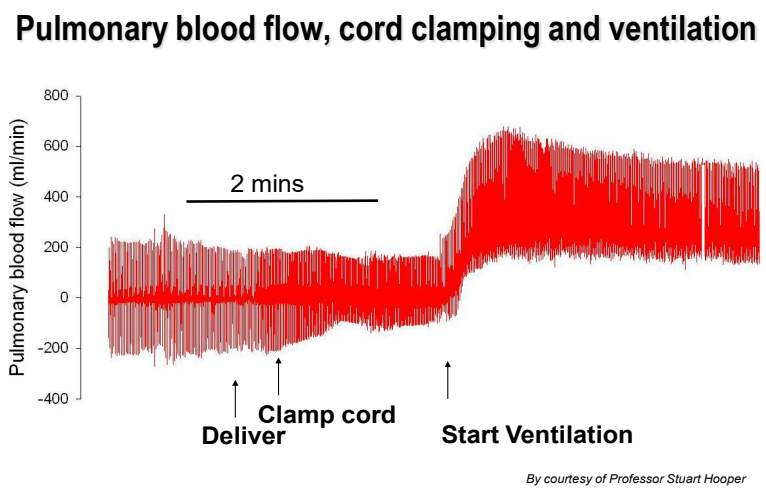
The main determinants of placental transfusion are time of cord clamping, uterine contraction and the position of the baby. Placental transfusion increases the blood volume by 35-60% in term babies if cord clamping is delayed for three minutes. If the infant is kept at or below the level of the placenta, about 25-30% of this transfusion occurs in the first 10-15 seconds. Umbilical arterial constriction is present by 45 seconds. Placental transfusion is completed by 1 minute if ergometrine is given early

in the third stage of labour, and by 30 seconds if the baby is lowered to 40 cm below the introitus, illustrating the importance of hydrostatic pressure.[3,4] Saigal *et al.* found that a complete placental transfusion produced a 47% and 50% increase in blood volume in preterm and term babies, respectively. Over half the full transfusion occurred by 1 minute in the preterm babies. Babies held 15 cm above the introitus for 1 minute received 60% of the transfusion received by babies held below it.[5]

The increase in blood volume provided by placental transfusion may be physiologically appropriate, and necessary, because the central haemodynamic event at birth is the rapid opening up of new vascular beds in the lungs.[4] Looked at another way, early clamping may deprive the baby of a substantial volume of blood. This causes volume depletion and systemic and peripheral hypoperfusion as pulmonary vascular beds must be filled with blood from the systemic circulation instead. This is well compensated for in normally delivered term babies, but may not be after Caesarean section (when the uterus is often not contracting) or in preterm babies. The preterm fetus responds to volume expansion by intrauterine blood transfusion with a rapid increase in plasma concentrations of natriuretic peptide.[6] Transudation or diuresis after placental transfusion could help explain why the mean haematocrit rises 1 and 4 hours after birth [Table 1].

Lung aeration and cardio-respiratory adaptation

Clamping the cord increases systemic vascular resistance by excluding the low resistance placenta from the neonatal circulation. Cord clamping also abolishes placental venous return to the ventricles and halves ventricular filling. [7] Both these effects substantially decrease output from the right ventricle. However, cord clamping does not necessarily decrease left ventricular output if the lung is aerated, because this reduces pulmonary vascular resistance, causing the shunt across the ductus arteriosus to reverse (left-to-right). This left to right shunting increases pulmonary blood flow (Figure), helping maintain venous return from the lungs and left ventricular output. [7]



Clamping the cord without first aerating the lungs leads to persistently high pulmonary vascular resistance, which prevents reversal of blood flow and left to right shunting through the ductus arteriosus. As a result, pulmonary venous return, left ventricular output and cerebral blood flow may all initially be low, but then rapidly increase with lung aeration.

Aeration of the lungs before clamping the cord, either through spontaneous breathing or by positive pressure support, may stabilize cerebral blood flow in a smoother transition to the postnatal circulatory state. In apnoeic infants, lung aeration can be achieved with the cord intact before cord clamping by applying mask IPPV, using air or oxygen.

Haematocrit correlates closely with red cell volume,[8] which is inversely associated with risk of respiratory distress syndrome.[9] In RCTs, placental transfusion increased initial haematocrit, blood pressure and oxygenation, and reduced use of surfactant and duration of ventilation or added oxygen. [10,11, 12] De Halleux *et al.* reported that decreased oxygen affinity after transfusion with adult blood increases the oxygen available to the tissues of pre-term babies.[12] This may increase oxygen toxicity to brain, eye[14] and lung. By reducing exposure to adult donor blood transfusions, [Table 1] placental transfusion may reduce this oxygen toxicity. Placental transfusion may also increase total transfer of immunoglobulin to the infant. Lastly, preterm cord blood is rich in haemopoietic precursor and stem cells. As well as enhancing bone marrow function and immunocompetence against infection, these may have anti-inflammatory, neurotrophic and neuro-protective effects.[15]

Although our meta analysis [Table 1] showed no statistically significant negative impact on safety from placental transfusion in babies < 37 weeks gestation overall,[2, 16] some preterm babies < 30 or <27 weeks gestation may be at higher risk of hypothermia, low Apgar scores, IVH , NEC or death if resuscitation is delayed until the cord is clamped after 60 seconds or more, especially the most immature or compromised, in whom myocardial dysfunction on echocardiography is common. Placental transfusion may increase serum bilirubin (a breakdown product of haemoglobin) and volume expansion with placental blood may be poorly tolerated in shock, asphyxia, or placental vascular disease.[17, 18, 19] *On the other hand, continuing perfusion with warm, oxygenated placental blood is well tolerated during the EXIT procedure, in which fetuses with complex airway problems are partially delivered by Caesarean section and supported by the placental circulation for 20 minutes or more while the airway is stabilized.* Continuing placental transfusion might improve hypothermia, hypoglycaemia, gas exchange, hypovolaemia and shock.

Results from the APTS pilot study comparing (i) delayed cord clamping, (ii) cord milking and (iii) both delayed cord clamping and milking combined with (iv) immediate cord clamping (unpublished) indicated that compliance with the 4 intervention arms was acceptable. There was no difference in haemoglobin at 6 hours after birth between the 4 treatment arms in the first 40 babies studied and no safety issues with any of the arms. It was thus recommended by the TMC to commence the main study comparing immediate cord clamping with deferred cord clamping, for which there is most data currently available.

This two arm study is proposed to establish if placental transfusion in preterm babies less than 30 weeks gestation will improve health outcomes compared with the standard practice of early cord clamping.

2 TRIAL OBJECTIVES

To establish if placental transfusion, using deferred cord clamping for 60 seconds or more while holding the baby at or below the level of the placenta, will improve health outcomes compared with standard early cord clamping in pre term babies less than 30 weeks of gestation.

3 TRIAL DESIGN

3.1 Design

This is a multi centre, two arm parallel, open label randomised controlled clinical trial

3.2 Consent

All mothers considered by the obstetric team to have a reasonable chance of delivering before 30 weeks gestation are eligible. Consent will be sought by members of the delivery team or appropriate research staff at any time during pregnancy. For example, if it is anticipated some weeks in advance that delivery will be necessary before 30 weeks gestation, consent may be obtained well in advance of randomisation.

3.3 Randomisation

Timing of randomisation
While consent can be attained at any time, randomisation should only take place when delivery is considered inevitable, for example when labour is considered irreversible or when the operating theatre is booked for Caesarean Section and the baby's estimated gestation is less than 30 weeks gestation. Randomisation should be performed allowing sufficient time to use the Interactive Voice Response System i.e. at least ten minutes before the expected birth, or longer than this for multiple births.

Method of randomisation
Randomisation can be done by any member of the obstetric, midwifery, or neonatal team using a phone to call a computerised interactive voice response system.
Randomisation will be performed by using an interactive voice response system built by an independent statistician at the NHMRC Clinical Trials Centre, University of Sydney. All data will be stored securely by the statistical group at the centre.
Randomisation will be by the method of minimisation with two strata: Institution and Gestational age (<27 weeks; >=27 weeks). Multiple births will be randomised separately.

3.4 Endpoints

3.4.1 Primary outcome

Death and/or major morbidity at 36 completed weeks post menstrual age.

Morbidity is defined by one or more of the following obtained from the medical record:

- Brain injury on ultrasound
- Severe retinopathy
- Necrotising enterocolitis
- Late onset sepsis

3.4.2 Definitions of primary outcome for study

Morbidity Measures

Based on definitions used by the Australian and New Zealand Neonatal Network.[20]

- Brain injury on ultrasound

Grade of 3 and 4 IVH (major intraventricular haemorrhage) seen on ultrasound according to the system of grading defined below:

1. Subependymal germinal matrix bleed
2. IVH without ventricular distension
3. IVH with ventricular distension with blood
4. Intraparenchymal haemorrhage

Or echodense intraparenchymal lesions, periventricular leukomalacia, porencephalic cysts or ventriculomegaly (97 percentile plus 4mm), or

- Severe retinopathy warranting treatment with laser surgery, cryotherapy or monoclonal antibody therapy or Stage 4 retinopathy (irrespective of whether treatment was warranted), or
- Necrotising enterocolitis diagnosis of proven Necrotising Enterocolitis with the following signs:
  1. at least one systemic sign: temperature instability, apnoea, bradycardia or lethargy and at least one intestinal sign: residual of 25% of the previous feed on 2 consecutive occasions, or abdominal distension, or vomiting or faecal blood
  2. profile consistent with definite NEC including at least one of the following: abdominal wall cellulitis and palpable abdominal mass, or pneumatosis intestinalis , or portal vein gas, or a persistent dilated loop on serial X rays, or a surgical or post mortem diagnosis.
  3. warranted treatment for NEC, which included nil by mouth and antibiotics
- Late onset Sepsis

  Systemic sepsis is defined as a clinical picture consistent with sepsis, and either a positive bacterial or fungal culture of blood and/or cerebrospinal fluid, or a positive urine culture by sterile collection only, and at least 5 days of antibiotic treatment. At least one episode of systemic sepsis with date of culture from at least 48 hours after birth.

  General guidelines for identifying positive cultures include:
  Isolation of organisms from one blood culture and, after considering clinical/laboratory evidence, decision made to give antibiotics with therapeutic intent against this organism. Infections with coagulase − negative staphylococci, and other potential contaminants, or group β streptococcal antigen detected in urine should be included only if the baby is considered clinically septic and there is supporting evidence such as raised white cell count or thrombocytopenia. Viral infections must be proven by culture and/ or haematological results consistent with infection. The following must not apply: mixed CNS or other skin flora contaminant; same blood organisms isolated from blood during the previous 14 days – repeat isolate.

3.4.3 Secondary outcomes

Incidence of the following outcomes up to 36 completed weeks post menstrual age unless specified otherwise:

1. Death
2. Death or brain injury
3. Major morbidity (see above for components of combined morbidity)
4. Death or major disability up to 3 years corrected age
5. Death up to 3 years corrected age
6. Major disability up to 3 years corrected age
7. Brain injury on ultrasound
8. IVH
   I. IVH all grades

II. grades 3 and 4
III. grade 4

9. Severe retinopathy defined as treatment for ROP or stage 4 ROP
10. Necrotizing enterocolitis
11. Late onset sepsis
12. Patent Ductus Arteriosis requiring treatment
13. Chronic lung disease (also known as bronchopulmonary dysplasia) defined as receiving supplemental oxygen or any form of assisted ventilation at 36 completed weeks post menstrual age for 4 consecutive hours in a 24 hour period

3.4.4 Descriptions of secondary outcomes – follow up

Assessment of death or major disability at follow up
Evaluation of major disability up to 3 years will be assessed by a short paediatric questionnaire that can be filled out from routine hospital assessments and the parent completed Ages and Stages questionnaire.[21]

Major disability is defined by a positive result on either:
(i) a Short Health Status Questionnaire completed by a qualified health professional documenting either: -

(a) Cerebral palsy with an inability to walk unassisted at or after 2 years corrected age, or
(b) Severe visual loss (legally blind i.e. corrected acuity <6/60 in both eyes), or
(c) Deafness, requiring a hearing aid or cochlear implants
(d) Major problems with language or speech at or after 2 years corrected for gestation, as defined by the inability to use more than 10 words (including signed words)[22, 23]

(ii) parent report of a score indicative of delay on the Ages and Stages Questionnaire (ASQ). The ASQ assessment will be calibrated against the BSID III using data from a sample of up to 180 study participants. The results of the calibration study will be used to refine the ASQ's contribution to the major disability endpoint, i.e. it will inform the choice of ASQ score cut-point that will be used to define the relevant degree of developmental delay. This work will be conducted blinded to treatment and finalised prior to database release for final analysis.

3.4.5 Tertiary outcomes

1. birth weight
2. blood transfusions (number of transfusions up to 36 weeks corrected for gestation)
3. admission temperature
4. peak bilirubin in the first 7 days after birth
5. peak haematocrit in the first 7 days after birth
6. length of hospital admission (to discharge)
7. maternal postpartum haemorrhage requiring blood transfusion
8. use of uterotonics
9. number of exchange transfusions and partial exchange transfusions up to 36 weeks gestation

3.4.6 Predefined subgroups

1. Gender
2. Age: Infants of 26 ^{6} weeks gestation or less or 27 ^{0} weeks gestation or more
3. Caesarean Section vs Vaginal birth

4 PARTICIPANT POPULATION

4.1 Participant Population

Male or female pre term babies less than 30 weeks gestational age.

4.2 Inclusion criteria

- mother imminently delivering < 30 weeks of gestation

- informed consent has been received from a parent

4.3 Exclusion criteria

Indication or contraindication to placental transfusion, in view of parent or doctor. Contraindications may include, but are not limited to

- fetal haemolytic disease
- fetal hydrops
- monochorionic twin pregnancies in which there is confirmed twin-twin transfusion syndrome
- major malformations considered incompatible with survival

4.4 Withdrawal criteria

Any family that wishes to withdraw from the trial may do so, without giving a reason and without any change in any other aspect of treatment. Parents of any baby who is withdrawn from the study after randomisation and before or after the intervention is administered will be asked to allow collection of outcome data. Information on hospital outcomes for any baby who is withdrawn after the baby is discharged from hospital will be used in the study.

5 TREATMENT OF PARTICIPANTS

Deferred cord clamping is performed against the background of usual care[24, 25]. For example, the timing of parenteral administration of uterotonic or the use of plastic wrap to warm the baby would be according to existing local protocols with no changes required for the study.

(a) Immediate cord clamping (< 10 seconds after birth)

- The cord is clamped 6 cm from the umbilicus within ten seconds of delivery of the baby.

(b) Deferred cord clamping

- A birth attendant holds the baby as low as possible below the level of the introitus or placenta for 60 seconds or more, then clamps the cord about 6 cm from the umbilicus.

Variation from the recommended procedures should be undertaken whenever this is judged necessary by the responsible clinician(s) acting in the best interests of the mother or baby. If the baby is *in extremis* or non-vigorous, the attending clinicians may use their discretion in administering the intervention.[24, 25]

There is concern that palpating the cord may restrict blood flow from placenta to infant. Therefore auscultation is encouraged rather than palpating the cord, subject to the clinician's discretion.

5.1 Compliance

As compliance with this intervention can vary, the following parameters will be recorded and monitored centrally:

- Duration in seconds of deferred cord clamping
- Estimated height in centimetres at which the baby was held in relation to placenta

5.2 Follow-up

To facilitate good follow up, regular contact by phone and/or post will be maintained with the families when the child is 6 months and 12 months to confirm the family's contact details. Sites will send participants birthday cards for each year until follow up has been completed.

6 EFFICACY AND TOLERABILITY

6.1 Assessment of Efficacy

Efficacy will be assessed through routinely collected hospital measures including demographic data, and outcomes such as necrotising enterocolitis and/ or brain injury which are all contained within the medical record.

Children will also have at least 2 follow up assessments: an Ages and Stages questionnaire to assess delay and a short paediatric assessment to determine physical development including incidence of cerebral palsy, eyesight or hearing impairment etc. A random sub-cohort of up to 180 of surviving children will also have a Bayley III Scales of Infant Development assessment, which will be used to calibrate the ASQ results.

6.2 Assessment of Tolerability

Serious adverse events which may be life threatening are common in very preterm infants, however, the proportion of suspected **unexpected serious related adverse events** (in the opinion of the investigator) is expected to be small.

AN UNEXPECTED SERIOUS ADVERSE EVENT (SAE) is any untoward medical occurrence that is not expected and:

- results in death, or
- is life-threatening (i.e. the subject is at risk of death at the time of the event), or
- requires prolongation of hospitalisation, or
- results in persistent or significant disability or incapacity

or

- other important medical events which, in the opinion of the investigator, are likely to become serious if untreated

NOTE: The term “life-threatening” in the definition of “serious” refers to an event in which the baby was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

An event is considered to be CAUSALLY RELATED if there is a reasonable possibility that the intervention caused the AE i.e. there is evidence to suggest a causal relationship between the intervention and the event.

6.2.1 Reporting of Unexpected Serious Related Adverse Events and Outcome Events

The investigator is responsible for reporting all Unexpected Serious Related Adverse Events occurring during the study to the NHMRC Clinical Trials Centre within 1 working day.

Unexpected related SAE reports should be faxed to +61 2 9562 5026.

The Trial Management Committee and all other Principal Investigators participating in the study will be informed. The investigator or delegate at each participating institution is responsible for reporting unexpected serious adverse events to their HREC. Serious adverse events will also be reported to the Independent Data and Safety Monitoring Committee.

7 STUDY STRUCTURE

7.1 Trial Management Committee

The NHMRC CTC, in conjunction with the Principal Investigators will appoint a Trial Management Committee (TMC). A Trial Executive Committee (TEC) may be selected from the TMC in order to expedite decision-making and will be led by the Study Chair.

The TMC responsibilities include protocol development, study planning, monitoring of progress and patient safety, review of information from related research and implementation of recommendations

from other study committees and external bodies (e.g. HRECs), and publications. The TMC will be responsible for selection and support of local investigators. The TMC will also monitor rate of recruitment and endpoint occurrence and will advise the SDMC of variations. The TMC will meet twice a year and/ or as required.

The TEC is a subset of the TMC which meets more regularly on key scientific and/or operational issues impacting on study conduct. The TEC will meet at least once every quarter (or as the stage of the trial dictates) as the study progresses.

7.2 Independent Data and Safety Monitoring Committee

An Independent Data and Safety Monitoring Committee (IDSMC) will monitor the progress of all aspects of the study and will ensure that the study meets the highest standards of ethics and patient safety. It will review interim data and other emerging evidence, including relevant RCTs and overviews of RCTs. The IDSMC will advise the TMC if in their view there is proof beyond reasonable doubt of net clinical benefit or harm, for all infants or for a subset of infants, that might reasonably be expected to influence the management of many clinicians. Data on key study outcomes will be monitored every 12 months, or more frequently if requested by the IDSMC, to ensure safety and that the event rates meet protocol projections. If the event rates are lower than expected, the IDSMC can recommend an increase in the study sample size to maintain power.

An interim analysis will be performed after the first 500 patients are recruited and then annually thereafter or as requested by the IDSMC.

The combined endpoint of death and/or major morbidity will be monitored using a modified Haybittle-Peto rule. The IDSMC supports the view that a difference of at least 3 standard deviations (SD) in a major endpoint (or in a combination of major endpoints) that suggested net clinical benefit or harm may be needed to justify recommending that the TMC consider either stopping the study prematurely, monitoring the trial more frequently or modifying the trial design.

7.2.1 Net Clinical Benefit: an important criterion for decision making

Evidence of a significant difference in mortality or in the primary outcome of mortality and morbidity or in mortality and brain injury between the study arms of more than 3 standard deviations would be of major concern and likely to lead to a recommendation to consider stopping recruitment. However, evidence of a significant increase in major morbidity in one arm is not necessarily an indication for early stopping of the trial since this might be balanced by a reduction in deaths or major disability. For example one arm of the study may be associated with an increase in morbidity which is statistically significant, but may also be consistent with a reduction in deaths which is not statistically significant, and with net benefit (not statistically significant) for the other arm. Similarly, evidence of a significant increase in an intermediate measure of adverse outcome, such as intraventricular haemorrhage (IVH) or necrotising enterocolitis (NEC), in one arm of the study is not necessarily an indication to stop early, since this might be balanced by an overall reduction in deaths and/ or major disability in the other arm of the study on follow up. Furthermore, major outcomes on follow up will not be determined until two years after trial entry. In both examples, it may be appropriate to continue the trial and indeed other related trials, until a clearer picture on net clinical benefit was obtained.

8 STATISTICS

8.1 Sample Size

At least 1500 infants and a primary outcome rate in the early clamping group of 41.6% will have 80% power to detect a 24% RRR, allowing for 30% non-compliance at 2p = 0.05. The table below shows other scenarios for larger sample sizes.

Table 2: Sample size scenarios using overall study estimate of event rate.

Sample Size	Immediate Cord Clamping %	Delayed Cord Clamping %	Overall rate %	Absolute Reduction%	RRR %	Power
1500	41.6	31.7	36.65	9.9	24	80

1550	41.6	31.8	36.7	9.8	24	80
1600	41.6	32	36.8	9.6	23	80

Infants recruited in some regions will not be included in the analysis for the extended follow-up. Assuming that this will exclude at most 150 infants, the table below gives an estimate for the absolute difference between the interventions that could be detected for a range of sample sizes and event rates for the primary endpoint at that time allowing for 30% non-compliance. Power is set at 80% and the absolute reduction that could be detected is calculated.

Table 3: Long term follow-up scenarios for absolute difference that could be detected for possible sample sizes

Secondary endpoint	Sample Size	Absolute difference	Power
Mortality and major disability to 36 months	1350	10% (35% to 25%)	80
	1400	9.8% (35% to 25.2%)	80
	1450	9.6% (35% to 25.4%)	80
	1350	10.3% (40% to 29.7%)	80
	1400	10.2% (40% to 29.8%)	80
	1450	10% (40% to 30%)	80

8.2 Statistical Analysis

The analysis population will not include babies that were randomised but stillborn. Additionally, babies that were randomised in anticipation of imminent birth, but were subsequently born after 30 weeks will not be included in the intention to treat population.

The primary analyses will be performed on assigned treatment (intention to treat). Continuous data will be summarised using means for normally distributed variables and medians otherwise. Binary outcomes will be summarised using percentages. Groups will be compared using chi-square tests or t-test where appropriate. Adjusted analyses will use logistic or linear regression where appropriate. Time to event outcomes will be analysed with proportional hazards regression. Safety outcomes may be analysed using a “treatment received” analysis. No adjustments will be made for multiple comparisons.

8.3 Prospective meta-analysis

Meta-analysis of multiple randomised controlled trials increases the overall sample size and power to demonstrate treatment effects. However, if meta-analysis is performed when results of trials are known, data dependent selection bias can occur. Trials with certain results can be deliberately included or excluded to manipulate the conclusions. A prospective meta-analysis (PMA) is a meta-analysis where randomised controlled trials are identified, evaluated and determined to be eligible before the results of any of them become known. PMA can therefore help to overcome some of the problems of retrospective meta-analyses. It enables: hypotheses to be specified a priori ignorant of the results of individual trials; prospective application of selection criteria; and a priori statements of intended analyses, including sub-group analyses, to be made before the results of individual trials are known. This avoids potentially biased, data dependent emphasis on particular subgroups. Also, pooling of individual patient data allows more informative subgroup analyses to be conducted. The Australian Placental Transfusion Study will contribute to a proposed PMA of similar RCTs.

9 ADMINISTRATIVE ASPECTS

9.1 Ethics and regulatory compliance

This study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2007) and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008. The investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a mother or baby. In this

circumstance the CTC, Chief Investigator and HREC will be advised. The trial will be registered with the Australian Clinical Trial Registry.

9.2 Study conduct

All study sites taking part in the trial will be required to participate in a start-up meeting on-site, to present the protocol and undergo training on study procedures and data collection methods. The Principal Investigator at each study site must apply for Human Research Ethics Committee (HREC) approval, submit all amendments and changes to the protocol and provide any necessary documentation for their site before they can enrol babies into the study.

9.3 Confidentiality

The study will be conducted in accordance with applicable Privacy Acts and Regulations. All data generated in this study will remain confidential. All information will be stored securely at the NHMRC Clinical Trials Centre, University of Sydney and will only be available to staff directly involved with the study.

9.4 Protocol amendments

Changes and amendments to the protocol can only be made by the Trial Management Committee. Approval of amendments by the Institutional HREC is required prior to their implementation. In some instances, an amendment may require a change to a consent form. The Investigator must receive approval/advice of the revised consent form prior to implementation of the change. In addition, changes to the Case Report Forms, if required, will be incorporated in the amendment.
The investigator should not implement any changes to, or deviations from, the protocol except where necessary to eliminate immediate hazard(s) to mothers or babies in the trial.

9.5 Data Handling and Record Keeping

Trial data will be recorded on the CRFs provided. All required data entry fields will be completed. Data corrections will be done according to the instructions provided. The investigator will be asked to confirm the accuracy of completed CRFs by signing key CRFs as indicated.

Source documents pertaining to the trial must be maintained by investigational sites. Source documents may include a mother or baby's medical records, hospital charts, clinic charts, the investigator's participant study files, as well as the results of diagnostic tests such as X-rays, laboratory tests, and electrocardiograms. The investigator's copy of the case report forms serves as part of the investigator's record of a baby's study-related data.

The following information should be entered into the baby's medical record:

a. Baby's name, contact information and protocol identification.
b. The date that the baby entered the study, and study number.
c. A statement that informed consent was obtained (including the date).
d. Relevant medical history
e. Results of key trial parameters.
f. Occurrence and status of any unexpected serious adverse events.
g. The date the baby exited the study, and a notation as to whether the baby completed the study or reason for discontinuation.

All study-related documentation will be maintained for 23 years following completion of the study.

9.6 Study Monitoring

Data from this study will be monitored by Clinical Trials Program staff from the NHMRC Clinical Trials Centre (CTC). Monitoring will include centralized review of CRFs and other study documents for protocol compliance, data accuracy and completeness. Monitoring may include monitoring visits to investigational sites for source data verification, review of the investigator's site file and drug handling records. The CTC will be given direct access to source documents, CRFs and other study-related documents. By signing the informed consent form, the parents give authorized CTC staff direct access to the mother and baby's medical records and the study data.

9.7 Audit and Inspection

This study may be subject to audit or inspection by representatives of the CTC or representatives of regulatory bodies.

9.8 Clinical Study Report

Data will be entered and statistical analysis will be conducted by the NHMRC CTC. A Clinical Study Report will be issued which may form the basis of a manuscript(s) intended for publication.

9.9 Publication Policy

The Trial Management Committee will appoint a Writing Committee to draft manuscripts based on the trial data. Manuscripts will be submitted to peer-reviewed journal(s). The initial publication will be the report of the full trial results based on the main protocol using the study group name, with subsequent publications of data subsets. The Writing Committee will develop a publication plan, including authorship, target journals and expected dates of publication.

10 REFERENCES

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11 Appendix 1: Sub-Study Protocol

Australian Placental Transfusion Study

The effect of placental transfusion on systemic blood flow (SBF) in very preterm babies less than 30 weeks gestation: An APTS echocardiographic sub-study.

APTS Sub-Study Protocol version 1.0
1 July 2011

Principal Investigator: Associate Professor David Osborn

Co-investigators:
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Signature Date

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CONFIDENTIAL

Table of Contents

1 SYNOPSIS

Aim:

The primary question is: does placental transfusion decrease or increase the risk of low systemic flow, determined by echocardiography in the first 24 hours after birth?

Clinical hypotheses:

Placental transfusion is associated with higher systemic blood flow in the first 24 hours.

Study design:

Sub-study of multi-centre, open label, randomised controlled trial

Study population:

Preterm babies less than 30 weeks gestational age enrolled in the APTS study.

Study assessments

Echocardiography and cardiorespiratory measurements performed at 3, 9 and 24 hours after birth.

Blood collection for Hb/HCT at birth (cord blood) and 6 hours.

Primary Outcome

Lowest SVC flow in 1st 24 hours – this is the lowest SVC recorded from echo assessments at 3 hours (range 3-5), 9 hours (range 6-12 hours) and 24 hours (20 – 28 hours).

Summary statistical methods

242 babies yield 90 % power, assuming 2-sided significance (5%) and 20% noncompliance, to detect a relative difference of 27% - from 55 ml/kg/min in the early cord clamping infants to 70 ml/kg/min in the delayed cord clamping infants [2]. Additional power will be obtained by further enrolments according to echo substudy resources at individual centers until completion of the main APTS study.

2 BACKGROUND INFORMATION

Infants born very premature account for approximately 1.5% of live births in Australia,[3] but account for a disproportionate burden of disability. Preterm delivery is the primary ascribed cause of death in over 15% of cases of neonatal mortality,[3] and very preterm infants develop cerebral palsy around 50 times the rate of infants born at term.[4] Of infants delivered before 30 weeks gestation, many will develop complications that may be associated with low systemic and organ blood flows during the first 24 hours after delivery, including necrotising enterocolitis (NEC - 7%) and severe (grade 3-4) intraventricular haemorrhage (IVH - 9%).[5] Mortality rates average from 70% for infants born at 23 weeks to 5% for those born at 29 weeks.[5] Around 15% of surviving infants born <30 weeks will have a moderate to severe developmental disability resulting in substantial costs to family and society.[5] Interestingly, delayed cord clamping which has been associated with increased haematocrit in newborn infants, has also been associated with reduced rates of intraventricular haemorrhage and necrotising enterocolitis, both of which are associated with reduced SBF.[6, 7] In addition, use of volume expansion in preterm infants with low SBF was found to increase SBF by a mean of 43%.[8] It is possible that infants exposed to early cord clamping practices may have a period of unrecognised hypovolemia increasing the risk of low SBF and organ injury. This study aims to evaluate the effect of delayed cord clamping on SBF and organ injuries related to low SBF.

Evidence for hypovolaemia

Hypovolaemia has been reported in newborn infants in the context of fetomaternal haemorrhage, twin-twin transfusion, vasopraevia and antepartum haemorrhage, caesarean section through an anterior placenta and with fetoplacental transfusion resulting from a tight nuchal cord (with the cord cut prematurely to facilitate delivery). However, early hypovolemia has not been thought to contribute to cardiovascular maladaptation in the majority of preterm infants for 2 reasons:

1. Studies using labeled albumin have reported that hypotensive preterm infants are not hypovolaemic compared to normotensive infants[9, 10], and
2. Trials of early volume expansion using various volume expanders (normal saline, albumin, fresh frozen plasma and gelofusine) have failed to show any benefit.[11, 12] Barr reported that the mean blood volume (derived from plasma volume [albumin space] and haematocrit value) of 26 hypotensive preterm infants (89.1 +/- 17.3 ml/kg) was not significantly different from that of 35 normotensive infants (91.4 +/- 14.6 ml/kg).[9] Bauer reported, using radiolabeled albumin, that blood volume in infants with a systolic BP >60 mmHg averaged 110 ml/kg and was significantly higher than in infants with a systolic BP 40-60 mmHg (78 ml/kg) and infants <40 mm Hg (75 ml/kg) was not predictive of hypovolemia.[10]

Systematic review[12] of trials of early volume expansion in preterm infants found 5 studies that compared volume to no treatment with most studies enrolling very preterm infants on the basis of gestation or birth weight, and 2 studies that compared different types of volume expansion in hypotensive preterm infants. Use of volume expansion was not associated with any improvement in any outcome including neonatal mortality, any morbidity or neurodevelopment up to 2 years. In hypotensive infants, volume expansion was effective in approximately 60% of infants in the trials in correcting hypotension but was not as effective as dopamine.[11] There was no difference in any clinical outcome.

However, of considerable interest are the following observations:

1. Several studies are consistent with the hypothesis that early volume expansion increases SBF in preterm infants. Pladys[13] reported 12 preterm neonates aged <7days presenting without cardiac dysfunction and with a low cardiac output who received 10% albumin solution 20 ml/kg, with all infants significantly increasing cardiac output from a median of 177 to 283 ml/kg/min. Six infants had a reduced index of systemic vascular resistance, 4 hypotensive infants responded by increasing mean BP, and capillary refill time decreased from mean 6.7 to 3.8 seconds. Lundstrom reported that volume expansion in normotensive very preterm infants

resulted in a mean (95% CI) increase in LVO +19.5% (-11.5 to +67.4) and an increase in CBF +15.4 (-3.1 to +37.3), with the increase in LVO significantly greater than seen in controls. Osborn[8] reported that volume expansion (10 ml/kg normal saline), in very preterm infants with low SBF, resulted in a mean 43% increase in SVC flow.

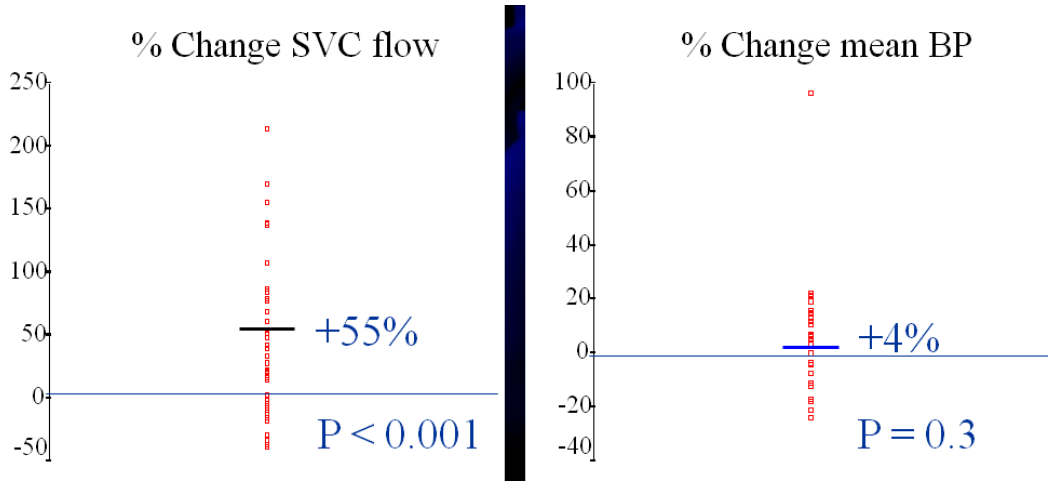

Figure 1: Percentage change in SVC flow after 10 ml/kg normal saline in infants born <30 weeks gestation with low SVC flow (<40 ml/kg/min):[8]

2. Studies have reported that central venous pressure in the first hours after birth are low and inversely related to the mean airway pressure. This raises the possibility that infants may have an early period of unrecognised hypovolaemia with subsequent intravascular redistribution;[14]

3. Systematic review of trials of delayed cord clamping[6, 7] have demonstrated a significant reduction in the incidence of transfusion for low blood pressure (2 studies; 58 infants; RR 0.38, 95% CI 0.18, 0.84) and several neonatal morbidities related to low systemic blood flow including IVH (10 studies; 436 infants; RR 0.54, 95% CI 0.37, 0.78) and NEC (6 studies, 281 infants; RR 0.61, 95% CI 0.43, 0.88).[6, 7, 15]

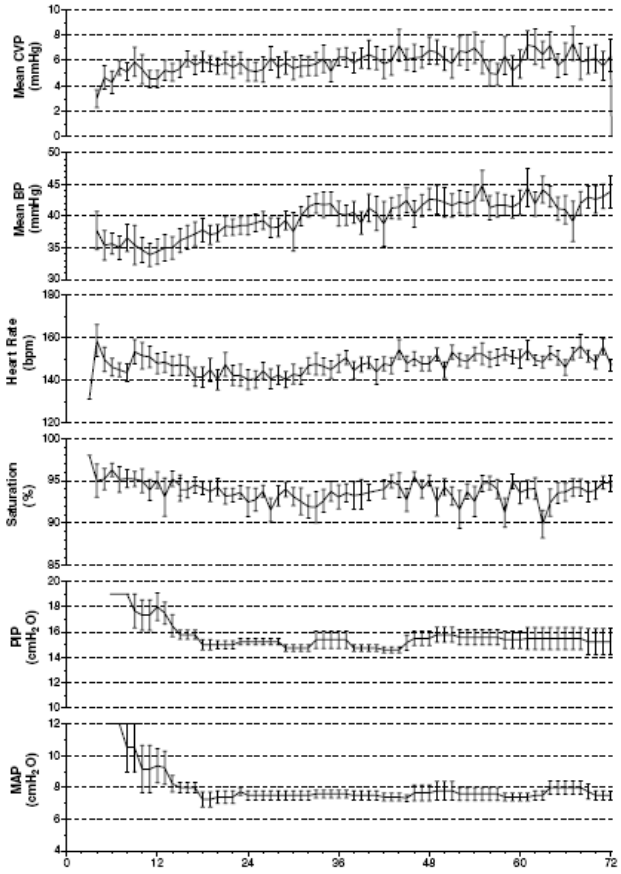

Figure 1. Plot of hourly physiological and ventilatory parameters. Data for nine infants on conventional mechanical ventilation were available for peak inspiratory pressure (PIP) and mean airway pressure (MAP). The error bars show the standard error of the mean.

Figure 2: Umbilical venous CVP and cardiorespiratory variables in preterm infants in the 1st day after birth:[14]

Measuring systemic blood flow in the preterm infant

Low ventricular outputs, the usual measure of systemic blood flow in most patients, are common in preterm infants with moderate and severe respiratory distress and are predictive of mortality.[16] However, many infants have substantial shunts across the adapting heart (ductus arteriosus or foramen ovale) in the first day, which means that measuring ventricular outputs potentially overestimates SBF by up to 100% at this time.[16] To overcome this issue, a novel approach of measuring cardiac inputs, which are not affected by cardiac shunts, has been developed and validated. Superior vena cava (SVC) flow is correlated to left ventricular output (LVO) in infants without a ductal shunt.[17, 18] Measurement of SVC flow is reproducible within the reported accuracies of echocardiographic measurements with median intra-observer variability for SVC flow measurement 8.1% and interobserver median variability 14%. Importantly, low SVC flow is associated with Doppler estimated cerebral blood flow (CBF),[19] neonatal morbidity,[17, 20] mortality[20, 21] and long term developmental outcomes.[20, 21]

Low systemic blood flow and neonatal outcome

Low SVC flow is common in very preterm infants, occurring in over 30% of infants born <30 weeks gestation and >60% of infants <27 weeks gestation.[17, 20] Low SVC flow is highly predictive of neonatal outcomes. Infants who developed low SBF were at substantially higher risk of mortality, IVH and NEC.[17, 20, 22] At 3 years of age the infants with low SBF had significantly lower

developmental scores (average 16 DQ points lower) and were at high risk of disability and motor impairments.[21, 23] Of importance, measures of SBF were better than blood pressure at predicting IVH,[17] mortality and subsequent motor impairments.[24] Low SVC flow was also predictive of hyperkalaemia and pulmonary haemorrhage.[25, 26] Hyperkalaemia has been associated with reduced Na^+K^+ATPase activity[27, 28] (presumably due to tissue ischaemia with cellular energy failure) and impaired renal function. Pulmonary haemorrhage almost always occurs in the reperfusion phase in infants with preceding low SVC flow and large diameter PDA.[25]

The importance of low systemic and organ blood flows have been confirmed by other groups. Several studies have now demonstrated low systemic and cerebral blood flows are an important risk factor for cerebral injury in preterm infants.[29-31]

Risk factors for low systemic blood flow

Risk factors for low SVC (≤40 ml/kg/min) have been consistent between two cohorts of infants studied by our group, and provide a basis for future prevention and treatment trials.[17, 20] In multivariate analysis, low SVC flow is predicted by lower gestation, an early large diameter DA and almost always occurs in ventilated infants with higher mean airway pressures. Infants with low SVC flow have higher calculated upper body systemic vascular resistance (UBSVR) and worse myocardial contractility as indicated by the relationship between mean velocity of circumferential fractional shortening (mVcfs) and left ventricular (LV) stress.[32]

To date, low SBF has not been related to hypovolaemia in preterm infants. Two early studies using albumin labeling techniques[9, 10] reported that clinical measures such as systemic hypotension were poorly correlated to blood volume in premature infants, suggesting that hypovolaemia is not a substantial factor underlying the cardiovascular maladaptation in the newborn infant.

Cardiovascular support of preterm infants

Most studies of cardiovascular support in preterm infants have focused on treating hypotension with volume expansion (colloid or crystalloid),[11, 12] inotropes (commonly dopamine and dobutamine)[33] and corticosteroids[34, 35] with increases in blood pressure reported for all interventions, but particularly so for inotropes with an α-adrenergic effect (dopamine and adrenaline).[34-36] Measures of systemic and organ blood flows have been reported by relatively few studies examining the effects of inotropes. Roze[37] reported that in hypotensive preterm infants, although dopamine produced greater increases in mean BP, dobutamine resulted in a significantly greater increase in LVO, with dopamine producing a trend to decreased LVO. Pellicer[35] reported, also in hypotensive preterm infants, that both dopamine and adrenaline produced similar increases in mean BP and NIRS measured cerebral blood flow. However, in trials of cardiovascular interventions in hypotensive preterm infants, no clinical improvements in neonatal outcomes have been reported and trials to date have failed to report long term developmental outcomes.

Only one study[8] has examined the effect of cardiovascular interventions in preterm infants with low SBF. We reported that in infants with low SVC flow <40 ml/kg/min in the 1st day, dobutamine produced a mean 35% increase in SVC flow at the highest dose reached compared to a mean 1% decrease for infants randomised to dopamine. In contrast, dopamine produced a significantly greater increase in mean BP and UBSVR. A significant proportion of infants failed to maintain SVC flows >40 ml/kg/min (dobutamine 27% versus dopamine 55%). Neither inotrope resulted in a significant improvement in neonatal or long term outcomes with over 70% of infants with low SVC flow in both groups dying or developing a significant disability.[23]

Observational studies have found increases in cardiac output after albumin infusion in sick preterm infants[13] and a small increase in systemic BP[9, 38]. However, we found that volume expansion (10 ml/kg normal saline), given over 20 minutes in very preterm infants with low SVC flow in the 1st hours after birth, resulted in a mean 43% increase in SVC flow.[8] As all infants were then given inotropes, there are no data to determine if this increase in SBF is maintained. However, 40% of infants failed to increase or maintain SVC flows above 40 ml/kg/min (the cut off for low SBF in this study).

There is also evidence of potential harm from excessive use of volume expansion in preterm infants with observational studies reporting an association with PIVH [36] and chronic lung disease[39] in preterm infants receiving volume expansion. A systematic review of albumin infusion in critically ill patients of all ages including those with hypovolaemia, burns and hypoalbuminemia found significantly increased mortality in patients receiving albumin compared to control.[40] Systematic reviews of randomised trials of early volume expansion in preterm infants[12] and early volume expansion versus inotropes in preterm infants[11] found that there is no evidence to support the routine use of early volume expansion in preterm infants in the first day, and that volume expansion is less effective than inotropes (dopamine) at increasing BP in hypotensive very preterm infants.

Evidence for placental transfusion

Systematic review of trials of delayed cord clamping[6, 7, 15] have demonstrated a significant reduction in the incidence of transfusion for low blood pressure (2 studies; 58 infants; RR 0.38, 95% CI 0.18, 0.84) and several neonatal morbidities related to low systemic blood flow including IVH (10 studies; 436 infants; RR 0.54, 95% CI 0.37, 0.78) and NEC (6 studies, 281 infants; RR 0.61, 95% CI 0.43, 0.88).[6, 7, 15]

In an observational study[2] of infants born <30 weeks gestation with early and delayed cord clamping (at discretion of neonatologist), median flow in the SVC in the first 24 hours was significantly higher in the group with delayed clamping (median 91 ml/kg/min; IQR 81–101) compared with 52 ml/kg/min (IQR 42–100) in the immediate clamping group (p=0.028). Fewer infants in the delayed group had low flow <55ml/kg/min (1 compared with 9; p=0.017). All three infants with IVH had low flow.

Table 1: Effects of placental transfusion versus early clamping in 11 RCTs, 494 babies < 37 wks gestation:

Outcome	*No. of RCTs*	*No. of babies*	*Effect type*	*Estimate (95% confidence interval)*
Potential benefits				
• Haematocrit* 1 hr after birth	6	281	WMD[T]	3.00 [1.49, 4.51]
• Haematocrit* 4 hr after birth	4	134	WMD	5.40 [3.52, 7.28]
• Number of blood transfusions	5	212	WMD	-1.32 [-1.93, -0.70]
• Transfused for low blood pressure	2	58	RR ^	0.38 [0.18, 0.84]
• Intraventricular haemorrhage (IVH)	10	436	RR	0.54 [0.37, 0.78]
• Necrotising enterocolitis (NEC)	6	281	RR	0.61 [0.43, 0.88]
• Late onset sepsis (LOS)	1	72	RR	0.1 [0.01, 0.85]
• Mortality	11	480	RR	0.84 [0.43, 1.63]
• Temperature on admission (°C)[#]	3	143	WMD	0.14 [-0.03, 0.31]
Potential adverse effects				
• Peak serum bilirubin (micromol/L)	7	320	WMD	11.13 [1.75, 20.51]

* packed cell volume; [T] WMD weighted mean difference; ^ Relative risk;
[#] Rabe et al., 2008

3 SUB-STUDY OBJECTIVES

In a sub-study of at least 242 babies, the primary question is 'Does placental transfusion decrease or increase the risk of low systemic flow, determined by echocardiography on the first day, in babies < 30 weeks gestation.

HYPOTHESES
Placental transfusion is associated with a reduction in SVC flow [1, 2] on echocardiography between 3 and 24 hours after birth.

4 SUB-STUDY DESIGN

4.1 Design

This is a sub-study of a multi centre, two arm parallel, open label randomised controlled clinical trial, the Australian Placental Transfusion Study. Echocardiography will take place on all babies recruited to APTS over approximately a 12 month period at selected sites.

4.2 Subject Population

Preterm babies less than 30 weeks gestational age enrolled in the APTS study.

4.3 Enrolment to sub-study

All infants randomised to the main APTS study at a participating centre will be included in the sub-study. If an echocardiographic fellow is temporarily not available, enrolment to the sub-study will be postponed for a pre-defined period while randomisation to the main study will continue.

4.4 Endpoints

Primary outcome:
Lowest systemic blood flow (defined as lowest recorded SVC flow of 3 measurements at 3, 9 and 24 hours after birth).

Secondary outcomes:
1. SVC flow recorded from echo assessments at 3 hours (range 3-5), 9 hours (range 6-12 hours) and 24 hours (range 20-28 hours) in babies less than 30 weeks gestation.
2. RVO recorded from echo assessments at 3 hours (range 3-5), 9 hours (range 6-12 hours) and 24 hours (range 20-28 hours) in babies less than 30 weeks gestation.
3. Ductal size and shunt direction in first 24 hours
4. Absolute change in haemoglobin / haematocrit concentration between baseline (from placenta at birth or, if unavailable, admission Hb/Hct) and at 6 hours after birth from baby
5. Hypotension: incidence of persistent hypotension (>15 minutes) receiving treatment with volume expansion or inotropes or corticosteroids. Hypotension is defined as mean BP below the gestation in weeks (mmHg) [approximately <10th percentile for gestation and postnatal age].
6. Treatment with volume expansion in 1st 24 hours
    - Type and volume of volume expansion
7. Treatment with inotropes in 1st 24 hours
    - Type and dose of inotrope
8. Nitric oxide dose
9. Indomethacin
    - Timing of administration
10. Symptomatic patent ductus arteriosus
11. Late intraventricular haemorrhage defined as intraventricular haemorrhage on <14 day HUS not present on initial (3 hour) HUS

5 EFFICACY AND SAFETY

5.1 Schedule of Study Assessments

Outcomes will be assessed according the schedule below.

Assessment / Time Point	Birth	3 hours (3 -5 hours)	6 hours	9 hours (6 - 12 hours)	24 hours (20 – 28 hours)	Before 14 days	36 weeks
Consent#	x						
ECHOCARDIOGRAPHY AND RELATED MEASURES							
Bloods/Haemoglobin	x		x				
ECHO		x		x	x		
Cardio-respiratory variables		x		x	x		
Total Fluids			x		x		
Study Specific Fluids		x	x	x	x		
CLINICAL OUTCOMES							
Head Ultrasound		x				x	
Nitric Oxide and Hypotension		x		x	x		
Symptomatic Patent Ductus Arteriosus							x

before birth

5.2 Assessments:

5.2.1 Consent

Consent forms must be signed prior to birth and prior to any assessments for this study being conducted.

5.2.2 Blood Collection

Haemoglobin and haematocrit values should be collected at:

- Baseline from cord bloods (where this is not possible, haemoglobin measures should be collected at admission)
- At 6 hours

5.2.3 Echocardiographic Assessment

All assessments will be de-identified by using a study code and echo number. Echocardiographic assessments will be recorded and stored in a standardized format with a copy stored centrally. The following parameters will be recorded at each echo:

Measures of Systemic Blood Flow:

- SVC flow (ml/kg/min) will be the median of five measures at each timepoint
- RVO (ml/kg/min) will be the median of five measures at each timepoint

Effect on the ductus arteriosus:

- Ductal diameter
- Ductal shunt direction - %right to left

5.2.4 Cardio-respiratory Variables

The following measures will be recorded at time of the ECHO assessments:
Cardiovascular variables

1. Blood pressure:
    - Invasive BP measurement where available will be recorded.
    - Where invasive BP is not available, a validated non-invasive oscillometric device will record BP (mean, systolic, diastolic).
2. Heart rate (prior to ECHO measures from monitor)

Respiratory variables

1. Respiratory support:
2. Mean airway pressure
3. FiO_2
4. Infant respiratory rate
5. Arterial blood gases (ABG) – pH, $PaCO_2$ PaO_2, Bicarbonate, base deficit, lactate. Blood gases may be collected by any means available

5.2.5 Total IV fluids in the first 6 hours and in the first 24 hours

Total IV fluids could be any of the following:

- Maintenance Fluids (ml)
- Normal saline (ml) used for volume expansion
- Fresh Frozen Plasma (ml)
- Albumin (ml)
- Packed Cells (ml)
- Platelets (ml)
- Other volume (ml)
- Intravenous Infusions (ml)
- Intra-arterial infusions (ml)
- IV Medications (ml)

5.2.6 Study-Specific Fluids

Volume expansion and Inotropes (type and volume)

5.2.7 Clinical Outcomes:

- Head ultrasound to assess brain injury (at 3 – 5 hours)
- Head ultrasound before 14 days (intraventricular haemorrhage) – main APTS data
- Incidence of persistent hypotension (>15 minutes) receiving treatment with volume expansion or inotropes or corticosteroids at each ECHO time point.
- Patent ductus arteriosus:
    - Large PDA on first echo
    - DETECT trial drug or indomethacin or ibuprofen in 1st 3 days
    - Treatment for symptomatic PDA (after 3 days age)
        - Indomethacin or ibuprofen
        - PDA ligation
- Nitric oxide

5.2.8 Inter-observer reliability:

All echocardiographs will be recorded and collected centrally. Echocardiography sets for the first 10 babies from each centre will be evaluated by a blinded independent reviewer for accuracy and standardization. After assessment of the first 10 babies, a decision whether to continue with central measurements will be made. In the event that inter-observer reliability is adequate (Intra-class correlation coefficient of absolute agreement >0.70), the echocardiographic measurements will be made at site.

5.2.9 Blinding of assessments

Echocardiographic assessments will be recorded and labelled by study number. Details of treatment allocation will be recorded separately as part of the main APTS trial. Echocardiographic measurements will be made at each study centre by the study fellow in batches blind to infant identity and treatment except for APTS study number and echo timing.

At the time of echocardiography details of ductal diameter, SVC flow and RVO will be given to the clinical team upon the clinical team's request due to clinical concern. However, if infants are detected as having a significant clinically unsuspected cardiovascular problem (SVC flow <41 ml/kg/min or RVO <100 ml/kg/min) this will be communicated by the echocardiography fellow to the clinical team.

5.3 Assessment of Safety

As per APTS protocol. It is not anticipated that clinical echocardiography or blood collection (0.2-0.4ml which may be incorporated into routine clinical measurement) will have an effect on safety requiring additional monitoring beyond that of infants in the main APTS study.

6 STATISTICS

6.1 Sample Size

242 babies yield 90 % power, assuming 2-sided significance (5%) and 20% noncompliance, to detect a relative increase of 27%, from a predicted 55 ml/kg/min in the early cord clamping infants to 70 ml/kg/min in the delayed cord clamping infants [2]. The sample size is calculated using log-transformed data, with a SD on the log scale of 0.21. All analyses will be performed using intention-to-treat principles.

6.2 Statistical Analysis

The primary analysis will compare the mean SVC flow between the treatment arms using an independent sample t-test on log transformed data. Linear regression may be used as a secondary analysis in order to adjust for any important prognostic factors.
Effect sizes and 95% confidence intervals will be presented for primary and secondary outcomes. For continuous variables, ratios (retransformed mean differences) and 95% confidence intervals for log-transformed variables, differences in means (and standard deviation) or medians (interquartile range) and for dichotomous and categorical factors, relative risks, odds ratios and risk differences will be presented.

7 ADMINISTRATIVE ASPECTS

7.1 Trial Management Sub-Committee

An echocardiography Sub-Committee has been appointed to oversee the conduct of the Echocardiography Sub-study. The Chair of the Sub-Committee (A/Prof David Osborn) will report on the progress and conduct of the study to the main Trial Management Committee (TMC).

The Sub-Committee's responsibilities include protocol development, study planning, monitoring of progress and patient safety. The Sub-Committee will meet as required.

7.2 Independent Data and Safety Monitoring Committee

An Independent Data and Safety Monitoring Committee (IDSMC) will monitor the progress of all aspects of the main APTS study. Echocardiograph assessments will not be submitted for routine assessment by the IDSMC.

7.3 Ethics and regulatory compliance

As for main study

7.4 Confidentiality

As for main study

7.5 Protocol amendments

As for main study

7.6 As for main study Data Handling and Record Keeping

As for main study

7.7 Study Monitoring

As for main study

7.8 Audit and Inspection

As for main study

7.9 Clinical Study Report

As for main study

7.10 Publication Policy

The APTS echo sub-study Committee will draft manuscripts based on the echo substudy data. Authors will have participated in the echo substudy. Manuscript(s) will be submitted to peer-reviewed journal(s).

8 LIST OF SUB-STUDY APPENDICES

Appendix 1.

Measurements:

Output (ml/kg/min) = heart rate x (π x diameter^2 / 4) x velocity time integral / weight (kg)

Superior vena cava flow measurement. A parasternal long axis view using M-mode or continuous 2-D recording of the superior vena cava entering the right atrium for measurement of average superior vena cava diameter (average of minimum and maximum diameters for 5 cardiac cycles). A transverse subcostal view of the superior vena cava entering the right atrium for measurement of superior vena cava flow velocities for calculation of the velocity time integral using pulsed Doppler (for 5 cardiac cycles).

Right ventricular output measurement: The right ventricular outflow tract diameter and Doppler flows are measured from a parasternal long axis view. The diameter is measured in 2-D at the level of the pulmonary valve leaflets in peak systole. Systolic flow velocities are measured by placing the pulse Doppler gate just below the level of the pulmonary valve and the velocity time integral measured in 5 consecutive cardiac cycles. Diastolic flow representing DA shunt flow or pulmonary reflection waves are not measured.

Measurement of ductal diameter and shunt direction: The colour Doppler minimum DA diameter is measured using a high parasternal view. Efforts should be made to ensure that colour Doppler is optimised to avoid ‘spill’ of colour in surrounding tissues resulting in overestimation of DA diameter. The ductus is measured at point of maximal constriction when there is optimal colour fill. At least 3 cardiac cycles are averaged. The pulsed Doppler DA shunt flow velocity is taken at the pulmonary end to the ductus when a left to right shunt is present and within the DA when bidirectional or right to left shunts are present. The percent time right to left shunt can be calculated by measuring the time interval the shunt is left to right divided by the cardiac cycle.

9 REFERENCES

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Summary of Changes

Changes to the APTS Protocol from **V1.0** (21 Jul 2010) to **V2.0** (24 Sep 2010) include:

- Administrative clarifications relating to background, trial objectives, randomisation, outcomes, 18-24 month assessment of death/severe disability, treatment, follow-up (throughout)
- Removal of measurements including haematocrit on admission, peak bilirubin, admission blood glucose concentration. Addition of measurements including uterotonic use. (Section 3.4.5)
- Update and clarification of sample size calculations to include minimum difference detected at 80% power and 10% non-compliance, as well as power for secondary follow-up outcome. (Section 8.1)

Note: Version 1.0 was submitted as part of original ethics application, however was amended to Version 2.0 prior to initial approval being granted. Version 2.0 was first protocol used by sites.

Changes to the APTS Protocol from **V2.0** (24 Sep 2010) to **V3.0** (12 Jul 2011) include:

- Addition of updated background information and references (Section 1)
- Addition of "Patent ductus arteriosus requiring treatment" as secondary outcome (Section 3.4.3).
- Addition of "Peak bilirubin in first 7 days after birth" and "Peak haematocrit in the first 7 days after birth" as tertiary outcomes (Section 3.4.3).
- Clarification of intervention administration where baby is in extremis or non-vigorous (i.e. that clinician's should act in best interest of baby), and to discourage cord palpation. (Section 5)
- Addition of "other important medical event" criteria for SAE definition (Section 6.2)
- Addition of sub-study protocol to Appendix (Section 11)

Changes to the APTS Protocol from **V3.0** (12 Jul 2011) to **V4.0** (1 Jul 2016) include:

- Chronic lung disease (CLD) removed from primary outcome definition as per IDSMC letter of recommendation. Long term respiratory support is now routine care and not a marker of CLD. CLD remains a secondary outcome. (Section 3.4.2)
- Addition of predefined subgroup: Caesarean section vs. vaginal birth. (Sections 3.4.6)
- Additional example of contraindication to placental transfusion (monochorionic twin pregnancies in which there is confirmed twin-twin transfusion) provided in exclusion criteria, in response to site queries. (Section 4.3)
- Sample size information updated to reflect observed compliance rates. Clarification of ITT population to exclude stillborn babies and babies born >30 weeks (Sections 8.1 and 8.2)
- Clarification that only suspected unexpected and related adverse events require expedited reporting, as unexpected SAEs are common in the preterm population. (Section 6.2)
- Clarification regarding Net Clinical Benefit and early stopping rules. (Section 7.2.1)
- Addition of interim analysis after the first 500 patients and then annually thereafter. Updates to reflect IDSMC request and current practice. (Section 7.2)
- Clarification of primary outcome definitions, including:
    - Severe retinopathy definition updated to include Stage 4 retinopathy regardless of whether it was treated. Stage 4 ROP is a severe condition, sufficient to meet the outcome definition regardless of treatment status. (Section 3.4.2)
    - Additional detail provided that "clinical picture consistent with sepsis" requires at least 5 days of antibiotic treatment to meet outcome. (Section 3.4.2)

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- Clarification of secondary follow-up outcome, including up to 3 years' corrected age and use of "major disability" terminology. (Section 3.4.2)
- Addition of "Death or brain injury" as a secondary outcome. Brain injury or death due to brain injury is considered an important secondary outcome for this cohort. (Section 3.4.2)
- Administrative clarification of "36 weeks post menstrual age" to mean 36 completed weeks (throughout)
- No formal amendments to the APTS Trial Protocol were made after **V4.0** (1 Jul 2016).

Changes to the APTS Primary Outcome in The APTS Childhood Follow Up Study:

- Death and disability in early childhood was a secondary outcome in the original APTS trial protocol of July 2010 and subsequent versions. In 2014, an application for the APTS Childhood Follow Up Study, APP1086865 was submitted to NHMRC, whose full title was *"Does placental transfusion prevent death and disability in very preterm infants? Childhood follow-up in the NHMRC Australian Placental Transfusion Study"* (see page 54). This application was funded and began on 1 January 2015. The Primary Outcome of the APTS Childhood Follow Up Study was defined in the section on the power calculation (see page 61) as "*death or major disability in the 3rd year after birth"*, which is the inverse of, and mutually interchangeable with, *"survival without major disability at 2-3 years"*. Thus, the power calculation stated that a sample of 1,320 infants "*gives 80% power to show a decrease of 8.5% in the primary outcome, from 30% to 21.5%, with two tailed significance of p<0.05, based on a non-adherence rate of 20% (5% in the early clamping group and 15% in the deferred cord clamping group)".* Using the inverse definition of the Primary Outcome, a sample of 1,320 infants also gives 80% power to show an increase of 8.5% in survival without major disability at 2-3 years from 70% to 78.5% with similar significance and non-adherence.

- Cerebral palsy, severe visual loss and deafness were determined from hospital records, physical examination or by parent report.

- Major disability was defined by a positive result on;
- (i) parent report on the Ages and Stages Questionnaire (ASQ),* or, if ASQ is unavailable,
- (ii) a modified Short Health Status Questionnaire completed by a medically qualified
- practitioner documenting either:-
- (a) major developmental delay, including language or speech problems, or
- (b) cerebral palsy with inability to walk unassisted at or after 2 yrs corrected age, or
- (c) severe visual loss (cannot fixate/ legally blind, or corrected acuity <6/60 in both eyes), or
- (d) deafness, requiring a hearing aid or cochlear implants.

Changes to the Primary Outcome in the Statistical Analysis Plan for the NHMRC APTS Childhood Follow Up Study. The Statistical Analysis Plan was finalised before any unblinding of investigators or any analyses of data, which were performed after 27 August 2020. In it, the Primary Outcome was changed from death or major disability in the 3rd year after birth (or survival without major disability at 2-3 years) to the following (Section 2.5, p 7): -

- The primary outcome is death and/or major disability at 24 months corrected age. Assessments of major disability must be performed between 18 months to 27 months. Any data outside of this window will require a central review.

- Definition of death

The survival data will be used up to 24 months corrected age. Only babies who are known to be alive at 21 months corrected or later will be deemed as 'alive' at 24 months. Deaths after 24 months are not included.

- Definition of major disability

Follow-up assessments should be performed on all APTS babies eligible for follow-up using the short health status questionnaire (SHSQ) and the Ages and Stages Questionnaire (ASQ-3). Additionally, some sites routinely use the Bayley scales of Infant and Toddler Development – Third edition (Bayley-III) which has been collected where available.

Major disability is defined by one or more of the following:
• Cerebral palsy with an inability to walk unassisted at or after 2 years corrected age,
• Severe visual loss (legally blind i.e. corrected acuity <6/60 in both eyes),
• Deafness, requiring a hearing aid or cochlear implants,
• Major problems with language or speech at or after 2 years corrected for gestation defined by the inability to use more than 10 words (including signed words)
• Cognitive delay as defined below.

A tiered approach (Table 2) will be used in order to obtain a disability status for as many babies as possible. If the data is not available for Tier 1, the disability status will be obtained from Tier 2, otherwise Tier 3. A central review blinded to treatment will be performed as appropriate and all of the information available at any time (i.e. Bayley-III) will be reviewed. If the outcome for an infant is not available from tier 1 or 2 results and can then not be ascertained with a central review, the primary outcome for this infant will be missing.

The ANZ Clinical Trials Register ACTRN12610000633088 was updated on 2 May 2021 to reflect these changes.

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Does placental transfusion prevent death and disability in very preterm infants? Childhood follow up in the NHMRC Australian Placental Transfusion Study.

OVERVIEW AND SYNOPSIS

1. Preventing complications from preterm birth is an urgent priority in Australia and globally.[1] About a million babies are born before 30 weeks gestation worldwide each year, with a disproportionate burden in indigenous or disadvantaged populations in Australia and overseas. Many die or face a lifetime of disability, with greatly increased risks of neuro-motor delay, low IQ, visual, hearing, learning, behavioural or psychiatric problems, diabetes and hypertension.[2-8] Enhancing placental transfusion in these infants by deferred clamping of the umbilical cord (DCC) is a simple procedure that may reduce mortality and major disability in childhood.[9]

2. The NHMRC Australian Placental Transfusion Study (APTS) is evaluating the effects of deferred (≥60 s) versus early (<10 s) clamping on death and major childhood disability in infants born before 30 weeks gestation. APTS is already the largest ever RCT of deferred clamping in very preterm infants ever conducted. It will complete recruitment of its target of 1320 infants <30 weeks gestation in 2015, with already over twice as many (630) as in all previous trials combined.[9,10]

3. Childhood follow up is essential in perinatal trials such as APTS. Many interventions in pregnancy and the newborn have shown short term benefit, but long term evidence of harm. Despite this, no previous RCTs of DCC have focused on childhood outcome in infants of <30 weeks gestation, even though their risks of death and disability are greatest. Only 96 survivors <30 weeks gestation in earlier trials have been assessed in childhood.[10] This is a major gap in the evidence. Without reliable data from RCTs with childhood follow up, like APTS, we will not know whether DCC may do more long-term harm than good in these vulnerable infants.

4. APTS was funded by NHMRC [Grant ID 571309] with a rating in Category 6 and will require no additional funding to fulfil its initial objectives. These were (a) to recruit the trial cohort and (b) compare in-hospital outcomes in each study arm.However, in that initial proposal, we clearly indicated that further funds would be sought to assess the project's primary outcome of death and disability in the 3rd year after birth, for which many assessments will fall due during 2015. ***It is thus critically important that support for this follow up is made available from the current funding round***. This will secure optimal return on NHMRC's investment by ensuring timely evaluation of the primary hypothesis of the overall project: ***that deferred versus early clamping reduces death and childhood disability.***

5. The need to complete the project is urgent and compelling. If the primary hypothesis is confirmed, deferred cord clamping will improve disability-free survival in very preterm infants in Australia and in about a million in total in high, middle and low-income countries annually, at minimal cost. Whatever the result, as in our previous international RCTs, *[INIS (3,493 infants), N Engl J Med 2011;[11] BOOST II (2,448 infants), N Engl J Med 2013[12]],* the scientific excellence and clinical significance of this project will profoundly influence local and global practice.

AIMS AND HYPOTHESES:

This pragmatic, randomised controlled trial (RCT) in children who were born before 30 weeks gestation aims to test one primary and three secondary hypotheses: that, in comparison with early (<10 s) clamping, deferred (≥60 s) clamping of the umbilical cord at birth improves:

I: Primary outcome ***(for which new funds are now sought, as stated in the original proposal)***

Survival without major disability at 2-3 years

Major disability is defined by a positive result on;

(i) parent report on the Ages and Stages Questionnaire (ASQ),* [13,14] or, if ASQ is unavailable,

(ii) a modified Short Health Status Questionnaire completed by a medically qualified practitioner [11,15] documenting either:-

(a) major developmental delay, including language or speech problems, or
(b) cerebral palsy with inability to walk unassisted at or after 2 yrs corrected age, or
(c) severe visual loss (cannot fixate/ legally blind, or corrected acuity <6/60 in both eyes), or
(d) deafness, requiring a hearing aid or cochlear implants.

* using a cut-off score on ASQ equivalent to 2 SDs below the trial norm for cognitive scores on the Bayley-III Scales of Infant and Toddler Development (Bayley-III),[16] derived from Bayley-III results within the study, performed by trained assessors in a random sub-cohort of 15% of surviving children stratified with equal numbers <27 or ≥ 27 weeks gestation.

II: Secondary outcomes ***(for which new funds are now sought, as stated in the original proposal)***

(1) Death at any time up to 3 years.
(2) Components of major disability at 3 years.
(3) ASQ overall and domain scores

III: Tertiary outcomes ***(already funded in Grant ID 571309: no new funding sought)***

Short term outcomes including in-hospital mortality and morbidity at 36 weeks corrected gestation.

Predefined subgroups

Male or female; Gestational age of infants less than 27 weeks or 27 weeks or more

BACKGROUND

1. Ensuring a healthy start to life for very preterm babies

One in ten babies, or 15 million per year, is born <37 weeks gestation and the number is rising.[1] A million deliver before 30 weeks. [1] Babies born <30 weeks account for <1% of all births but a disproportionately high burden of death, chronic lung disease, poor growth, hospital re-admissions, visual deficit, cerebral palsy and neuro-sensory, cognitive and behavioural impairment. Later in life, they face increased risks of attention deficit, school failure, asthma, hypertension, diabetes, obesity, autism and other psychiatric disorders.[1-5,7,8]

2. Placental transfusion may prevent mortality and disability

Enhancing placental transfusion by deferring clamping of the umbilical cord at birth is a simple, inexpensive intervention that may prevent death and disability in very preterm infants. This hypothesis is supported by four lines of evidence.

2.1 Cochrane Reviews of 13 RCTs of cardiovascular interventions and placental transfusion

Our Cochrane Review of placental transfusion by deferred cord clamping (12 RCTs) or cord milking (one RCT) in 738 infants <37 weeks gestation,[9] showed that placental transfusion reduced use of inotropes, intra-ventricular haemorrhage (IVH), anaemia and necrotizing enterocolitis. (Table 1) However, there was inadequate power for reliable conclusions about the primary outcomes of death,

severe IVH and peri-ventricular leukomalacia (PVL). Also, several studies were at risk of bias, with missing data for several outcomes.[9]

Table 1: Cochrane Review of 13 RCTs of enhanced placental transfusion at preterm birth[9]

Short term outcome	No. RCTs	No. infants	Risk Ratio or *Mean Difference [95% CI]	P value
Intropes for low blood pressure	4	158	0.42 [0.23, 0.77]	0.0045
IVH (all grades)	10	539	0.59 [0.41, 0.85]	0.0048
Transfused for anaemia	7	392	0.61 [0.46, 0.81]	0.00053
Necrotising enterocolitis	5	241	0.62 [0.43, 0.90]	0.011
% Haematocrit (HCT) at 24 hr	3	199	3.28 [1.34, 5.22]	0.00093
Death	13	668	0.63 [0.31, 1.28]	NS
Severe IVH: Grades 3 or 4	6	305	0.68 [0.23, 1.96]	NS
Peri-ventricular Leukomalacia	2	71	1.02 [0.19, 5.56]	NS

Importantly, no trials in our Cochrane review reported data on disability beyond 7 months. A more recent systematic review of DCC in extremely low birth weight infants of less than 30 weeks gestation has identified only 96 children who have been evaluated later in childhood.[10] This is a major gap in the evidence. Clearly, a RCT addressing childhood outcomes is needed before endorsing a policy that may help, or harm, millions of preterm babies globally. [17-20]

Importantly, rigorous, standardised evaluations of outcome for very preterm infants in early childhood are not routinely available and must be specifically funded within each trial.

2.2 Low superior vena cava flow and ischaemic/ reperfusion injury

Low cerebral and systemic blood flow may cause brain and gut injury. We were first to report superior vena cava (SVC) flow as a measure of cerebral and systemic blood flow[21] and that many babies born <30 weeks were at risk of critically low SVC flow, leading to IVH after birth[22] and to death or disability at 3 years.[23] Others have confirmed that low SVC flow is a risk factor for death and IVH.[24,25] Using echocardiography, we also showed that volume expansion by saline or albumin increases systemic flow.[25-28] Volume expansion by placental transfusion may thus prevent low systemic flow, yielding short term benefits as in Table 1.

2.3 Deferring cord clamping until after ventilation begins: potential benefits and risks

Deferring cord clamping until after ventilation is established may smooth transition of the cerebral and cardio-pulmonary circulation[29] and allow more time for spontaneous breathing to begin before the umbilical cord is clamped. This may also reduce the risk of invasive, potentially injurious intubation and resuscitation. [30,31] On the other hand, if DCC delays urgently needed resuscitation, it may cause long-term harm. A reliable RCT with childhood outcomes is thus of critical importance.

2.4 Potential benefits of additional red cells and stem cells

Increased transfer of red cells from placental transfusion may increase iron stores, reducing childhood anaemia and cognitive deficit.[32] Preterm blood is rich in haemopoietic precursor and stem cells. As well as enhancing bone marrow function and immune-competence, these cells may have anti-inflammatory, neuro-trophic and neuro-protective effects.[33,34]

3. Why is childhood follow up in perinatal trials like APTS essential?

Childhood follow up is critical in perinatal trials. Many interventions in pregnancy and the newborn have shown short term benefit, but long term evidence of harm. The need for caution in interpreting short term outcomes in perinatal trials, such as those in Table 1, is illustrated by several examples.

3.1 High dose, early postnatal steroids; impact on chronic lung disease and cerebral palsy.

Toward the end of last century, many babies at risk of chronic lung disease were treated with high-dose steroids soon after birth. Early trials had suggested short term benefits, e.g. less oxygen dependence and chronic lung disease, so high dose steroids were increasingly adopted world-wide. Follow up studies showed no survival benefit, and some showed an increase in cerebral palsy.[35,36]

3.2 Maternal antibiotics: impact on short term benefit, long term impairment and cerebral palsy

In our ORACLE trials of antibiotics in women at risk of preterm birth, we showed, in 2,226 singletons born to women with preterm rupture of membranes, that antibiotics reduced major cerebral abnormality in infants before discharge.[17] Despite this, there was no improvement in functional impairment at seven years old.[19] Furthermore, two antibiotics given to women in pre-term labour with intact membranes increased the risk of cerebral palsy by between 60 and 90%.[20]

3.3 Antenatal TRH: impact on hospital death, chronic lung disease, and motor delay

Initial trials of antenatal thyrotrophin releasing hormone (TRH) in women at risk of preterm birth suggested that TRH reduced subsequent neonatal chronic lung disease[37] and hospital mortality[38], two important short-term outcomes. However, two further RCTs in over four times as many women found no benefit of antenatal TRH for neonatal chronic lung disease[39] or hospital death[39], but an increased risk of motor delay at 12 months[39] and of mental delay at 12 and 24 months.[40]

3.4 Supplemental oxygen, eye disease, spastic diplegia and survival

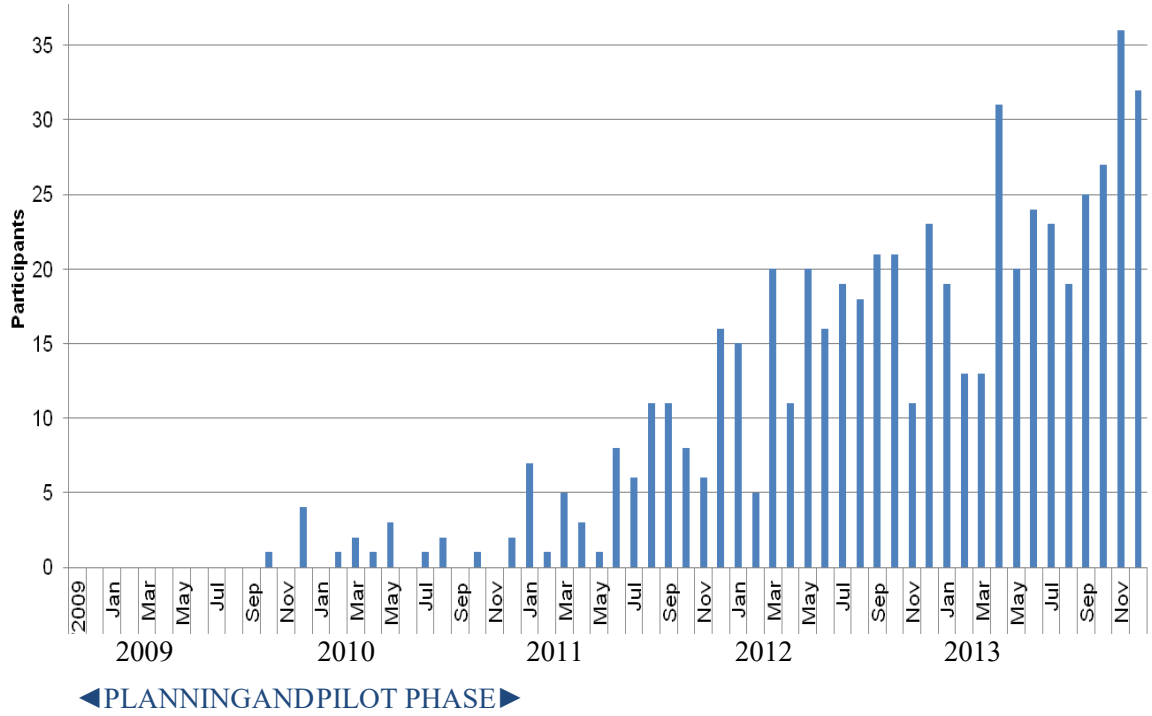
Perhaps the most dramatic example of the need for childhood follow up in trials is described by Silverman.[41] In 1955, a RCT of liberal versus restricted oxygen therapy in preterm infants suggested that oxygen curtailment did not increase mortality, but reduced the rate of cicatricial eye disease, a major cause of blindness, by two thirds.[41,42] Restriction of oxygen therapy to concentrations <40% became routine, regardless of the presence of hypoxia. Follow up of survivors in the RCT was never conducted.[41] Later, widespread excesses in the incidence of spastic diplegia and death were attributed to hypoxic respiratory failure due to arbitrary, inappropriate oxygen restriction. It was estimated that every sighted infant gained "may have cost some 16 deaths" and may have led to numerous avoidable cases of spastic diplegia, a form of cerebral palsy. [43]

These examples clearly show that, despite evidence of improved short term morbidity [9] (Table 1), DCC may not result in long term benefit and could cause net injury. The NHMRC APTS project is the first RCT of DCC to focus on infants <30 weeks gestation and to plan prospective childhood follow up between the second and third years after birth. Ensuring timely evaluation of its primary outcome of death or major disability is now essential. Without these data, we cannot rule out the possibility that DCC may do more harm than good.

4. Preliminary data: Recruitment in the Australian Placental Transfusion Study

APTS has already enrolled 630 infants between January 2011 and February, 2014 and is now recruiting ~32 per month in 20 sites in Australia, New Zealand and the United Kingdom (Figure 1). The initial grant funding for APTS (ID 571309) will enable full recruitment to be completed and no supplementary funding is required or sought to achieve its planned objective, to compare short term in-hospital outcomes between the study arms.

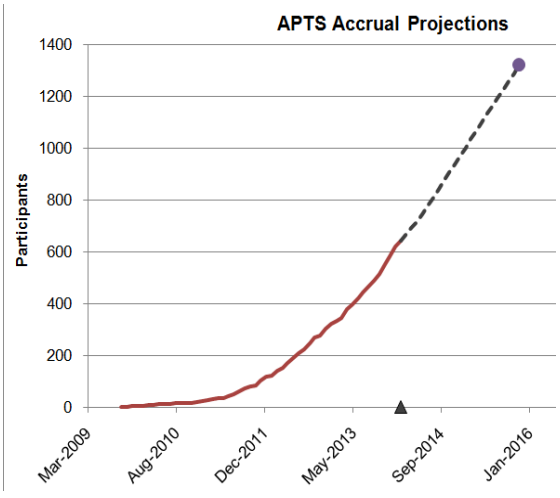
Figure 1: Monthly recruitment in the Australian Placental Transfusion Study



4.1 Projected recruitment and power

Seven new sites will join in 2014. At least 704 more infants from Australia, New Zealand and the United Kingdom are expected in the 22 months to December 2015, yielding >1,320 in which to assess the primary outcome of death or major disability at 3 years. (Figure 2)

Figure 2: Projected recruitment of 1,320 infants for evaluation of the primary outcome



The projected total of 1,320 infants gives 80% power to show a decrease of 8.5% in the primary outcome, from 30% to 21.5%, with two tailed significance of $p < 0.05$, based on the current non-adherence rate of 20% (5% in the early clamping group and 15% in the deferred cord clamping group).

An additional 280 infants will be enrolled in sites in Pakistan, India and the United States, yielding a total of 1600 for comparison of in-hospital outcomes. Childhood follow up, the primary outcome for the project, will not be undertaken in these infants because of the increased logistic difficulties in maintaining prolonged contact with families in these countries.

4.2 Adherence to protocol: first Independent Safety and Data Monitoring Committee report

As pre-specified in the trial protocol and the Independent Safety and Data Monitoring Committee (IDSMC) charter, the Trial Management Committee receives periodic reports from the IDSMC to monitor differences in times to clamping and peak haematocrit between study arms. (Table 2)

Table 2: Differences in time to clamping and peak haematocrit (488 infants: November 2012)

	Early clamping	**Deferred clamping**	P value
No. infants	246	242	
Time to cord clamping (s) median (range) *analysed by Wilcoxon rank sum Test*	5 (0-58)	60 (0-80)	<0.001
% Haematocrit in first week* [peak HCT before transfusion of adult blood] mean (SD) *analysed by student t test*	46.9 (7.3)	50.0 (8.2)	<0.001
Difference in peak % HCT in first week	3.1 (95% CI: 1.7-4.5)		

There were highly significant differences between study arms of 55 seconds in median time to clamping and of 3.1% in mean HCT, similar to the difference in HCT in the Cochrane Review.[9] (Table 1) Thus APTS is strongly placed to assess differences in outcome at 3 years. After a confidential review in December 2012, the IDSMC advised the Study Chair (CIA) that it had no concerns and that recruitment should continue.

5.0 Assessing major childhood disability in large perinatal RCTs by parent report

Although evaluation by trained assessors with tools like the Bayley Scales is a de facto 'gold-standard' for assessing disability, the predictive value and equivalence of BSID II and Bayley-III are the subject of ongoing research. We and others have defined cut-offs for major disability on BSID II and Bayley-III suitable for RCTs in cohorts that include Australian children.[11,16,44,45]

However, assessor-administered scales have become prohibitively expensive in investigator-initiated, public-good perinatal trials that are large enough to change practice.[11,46] Reliable, cost-effective measures of disability are essential to contain costs and maintain scientific validity in perinatal RCTs.[44,45,47] Also, parent-administered scales may more accurately reflect infant behaviours, because they reflect observations over longer periods, and in more natural settings, than one-off evaluations by trained assessors in unfamiliar environments.

"Parent-completed assessments of children's abilities represent a more practical and cost-effective method for collecting longer-term outcome data in neonatal clinical trials ... than formal neuro-developmental appraisals performed by trained assessors." [44]

We have therefore tested several measures of major disability using parent report. [11,13,14,44,45,48-50]

5.1 Parent report in 3,493 children in the International Neonatal Immunotherapy Study (INIS)

In the INIS RCT, published in *New England Journal of Medicine*, [11] we assessed cognitive delay at 2 years with the Parent Report of Children's Abilities–Revised (PARCA-R).[48,49] Its parent report composite score (PRC) can range from 0-158, higher scores indicating higher function.[45] Before unblinded analysis, we derived a cut-off value for severe disability on the PARCA-R PRC score by calibrating it against the BSID II Mental Development Index (MDI) performed by trained assessors in a sub-cohort of Australian and New Zealand children, representing 17% of all survivors.[11,45] The parent assessment successfully identified major cognitive delay. The area under the ROC curve (AUC) was >0.90 (a value of 1 indicating perfect prediction). A cut-point corresponding to a BSID II MDI score less than 3 SD below the norm for this sub-cohort was used to identify severe disability in the whole trial. Using a PARCA-R PRC cut-off of <2 SD below the sub-cohort norm to define major (i.e. moderate to severe) disability did not alter the conclusions. (Table S2, Supplementary Appendix, NEJM.org).[11] Importantly, different cut-offs do not affect the internal validity of comparisons in RCTs.

5.2 Detecting major disability by parent report using the Ages and Stages Questionnaire [50-52]

The Ages and Stages Questionnaire (ASQ) [53] is a widely validated tool completed by parents to assess development from one month to 5.5 years.[13,14,52,53] Previous versions have been validated against BSID I and II to detect severe developmental delay in Australian children.[54,55] We validated ASQ against concurrent evaluations for disability by trained assessors in about 1,000 children followed in the Magpie Trial of magnesium sulphate in pre-eclampsia.[13,14,50,52] The correlation of ASQ and Bayley-III is higher after 18 months,[56] supporting our plan to assess children later.

We selected ASQ to assess disability in APTS because ASQ can be completed between 2-4 years (and between 2 and 5.5 years if necessary), unlike the PARCA-R,[11] which should be completed close to a child's 2nd birthday. It might be argued that using a parent report to detect disability could introduce bias, because parents know which intervention their child received and may have a strong preference. This is unlikely, however, because only parents with no strong preference for either study arm consent to join APTS. Also, using ASQ in a RCT like APTS (where within-centre randomisation tends to balance confounding variables evenly between study arms) is considerably less open to confounding than in non-randomised comparisons across centres or cohorts.

6.0 International collaboration: the UK CORD Trial and a planned prospective meta-analysis

Professor Lelia Duley, who is a CI both on APTS Grant ID 571309 and on the current application, is also the principal investigator of the UK CORD Trial Collaboration. This is funded by the UK National Institutes of Health Research to undertake (i) a parallel multicentre trial of deferred cord clamping in very preterm infants in the UK[57] and (ii) a collaborative, individual patient meta-analysis [58-60] of the UK CORD Trial, APTS and similar trials of enhanced placental transfusion. The Trial Management Committees of APTS, CORD and other trials will share their data after publication. These trials will produce a meta-analysis of a few thousand infants, similar to our NeOProM Collaboration,[59] substantially enhancing the precision of future comparisons of childhood outcome and in-hospital mortality.

RESEARCH PLAN – METHODS AND TECHNIQUES TO BE USED

AIM: To determine whether, compared with deferred cord clamping (DCC), early clamping improves survival without major disability in children who were born < 30 weeks gestation.

Primary outcome: ***(for which new funds are now sought, as stated in the initial proposal)***
Survival without major disability at 2-3 years

Major disability is defined by a positive result on;

(iii) parent report on the Ages and Stages Questionnaire (ASQ),* [13,14] or, if ASQ is unavailable,
(iv) a modified Short Health Status Questionnaire completed by a medically qualified practitioner [11,15] documenting either:-
  (e) major developmental delay, including language or speech problems, or
  (f) cerebral palsy with inability to walk unassisted at or after 2 yrs corrected age, or
  (g) severe visual loss (cannot fixate/ legally blind, or corrected acuity <6/60 in both eyes), or
  (h) deafness, requiring a hearing aid or cochlear implants.

* using a cut-off score on ASQ equivalent to 2 SDs below the trial norm for cognitive scores on the Bayley-III Scales of Infant and Toddler Development (Bayley-III),[16] derived from Bayley-III results within the study, performed by trained assessors in a random sub-cohort of 15% of surviving children stratified with equal numbers <27 or ≥ 27 weeks gestation.

II: Secondary outcomes ***(for which new funds are now sought, as stated in the initial proposal)***

(1) Death at any time up to 3 years.
(2) Components of major disability at 3 years.
(3) ASQ overall and domain scores

III: Tertiary outcomes ***(already funded in Grant ID 571309: no new funding requested)***

Short term outcomes including in-hospital mortality and morbidity at 36 weeks corrected gestation.

Eligibility:

The sole eligibility criterion is enrolment in the APTS trial, which included parental consent for childhood follow up to 3 years. (Specific eligibility criteria for recruitment to APTS were: mothers imminently delivering babies of 29 ^{6} weeks gestation or less; informed consent received from a parent; no indication or contraindication to placental transfusion, in view of parent or doctor).

Intervention:

(a) immediate cord clamping (< 10 seconds after birth)
(b) deferred cord clamping (≥ 60 seconds after birth)

Sample size and power:

1,320 infants gives 80% power to show a decrease of 8.5% in the primary outcome, from 30% to 21.5%, with two tailed significance of $p < 0.05$, based on a non-adherence rate of 20% (5% in the early clamping group and 15% in the deferred cord clamping group).

Predefined subgroups:

Gender: male or female
Gestational age: infants of 26 ^{6} weeks gestation or less or 27 ^{0} weeks gestation or more

Statistical analysis:

AI Martin, NHMRC Clinical Trials Centre, is statistical lead. Using similar methodology as in our *New England Journal of Medicine* study,[11] before unblinding, ASQ scores will be calibrated against Bayley-III cognitive scores obtained concurrently by trained assessors in a random sample (stratified by gestation at birth) of 15% of survivors aged 2 to 3 years (n = ~180). Assuming a 20% event rate and a true area under the ROC curve (AUC) of 0.89 (similar to the result of our previous analysis with the PARCA-R,[11,45]) a sample of N=180 would yield an estimate of AUC with a 95% confidence interval ranging from 0.83 to 0.93. A cut-off score on ASQ corresponding to 2SD below the Bayley-III norm for the sample (i.e. moderate to severe delay) will be applied to the whole trial.

Comparisons between treatment arms on pre-specified endpoints will be undertaken at the two-sided 5% level of significance according to the intention-to-treat analysis principle. Binary endpoints will be analysed by two-sided chi-squared tests. Continuous variables (e.g. ASQ score) will be compared by t-tests or non-parametric equivalent as necessary. Treatment effects adjusted for baseline characteristics will be estimated in a series of sensitivity analyses using a general linear modelling approach. These methods will also be used to identify clinically important prognostic factors and heterogeneity in subgroup analyses. No adjustments will be made for multiple comparisons, but secondary analyses will be interpreted in proper context.

Independent Data and Safety Monitoring Committee:

An Independent Data and Safety Monitoring Committee (IDSMC) is monitoring the progress of all aspects of APTS recruitment and subsequent childhood follow up to ensure that they meet the highest standards of ethics and patient safety. It is also reviewing interim data and other emerging evidence, including relevant RCTs and overviews of RCTs. During the recruitment phase, the IDSMC will advise the TMC if in their view there is proof beyond reasonable doubt of net clinical benefit or harm for all infants, or for a subset of infants, that might be expected to influence the management of many clinicians. Data on study outcomes are being monitored annually, or more often if requested by the IDSMC, to ensure safety and that event rates meet protocol projections.

Feasibility, teamwork and collaboration:

We anticipate no problem in recruiting and following up the planned subjects. Ethics approval and parental consent have been obtained. The CIs and AIs have a strong record of teamwork and collaboration in trials, cohort and follow up studies in Australia, New Zealand and overseas, funded

by NHMRC, NZ HRC, UK MRC, UK DFID, WHO and others, with publications in *New England Journal of Medicine, Lancet* [4,11,12, 17-20,50,61,62] and other peer-reviewed journals.[3,6,13,14,23,44,45,48,49,63]

Expected results:
Our hypothesis is that DCC will improve the primary outcome of death or major disability on follow up, with no increase in major disability or in any of its components, including cerebral palsy. DCC will improve the secondary outcomes of (i) mortality and (ii) disability and (iii) ASQ score.

Limitations:

Although we have already validated the PARCA-R parent report against BSID II [11,45] and Bayley-III[44] in high risk newborn Australian and New Zealand infants across a wide range of gestational ages in our *NEJM* report,[11] the PARCA-R must be completed close to the child's second birthday, corrected for gestation, and some children will have passed this age during follow up in this project.

However, we have also validated the Ages and Stages Questionnaire (ASQ) against specialist evaluations by trained assessors in a large cohort of survivors in the Magpie Trial. [13,14,50,52] The ASQ can be used between one month and 5.5 years, and is thus appropriate for calibration against Bayley-III in this study, using similar methods, [11] (see section 5.2 and Statistical Analysis, p 8).

MILESTONES & PLANNING

A four year time span is needed to achieve the aims of the project.

	2015	**2016**	**2017**	**2018**
Finalise Manual of Operations and study materials	X			
Recruit coordinating centre staff	X			
Appoint local follow up staff, begin follow up	XXXX	XXXX	XXXX	XX
Expected numbers of infants followed up each year	156	214	390	440
Analysis, writing up and publication				XX

OUTCOMES, INNOVATION & SIGNIFICANCE

- In acknowledgement of the scientific importance of the NHMRC APTS project and its significance for global practice, CIA has been commissioned by the Editor of the American Journal of Obstetrics and Gynecology, a global opinion-leading journal with a circulation of 45,000, to submit an open access Clinical Opinion and commentary, co authored with other CIs, AIs and collaborators, describing the background and rationale of the NHMRC APTS project, entitled "Timing of clamping of the umbilical cord in very preterm infants."

- The NHMRC APTS project will provide insight into the development of reliable, cost-effective methods of follow up in perinatal trials, by calibrating the Ages and Stages Questionnaire, based on parent report, against the Bayley-III Scales of Infant Development, performed by trained assessors, in a sub-cohort of the trial population, as we did with the PARCA-R parent report in the *New England Journal of Medicine*.[11] As event rates fall and trial sample sizes rise, this will be increasingly important in ensuring scientifically valid and financially and logistically viable methods of childhood follow up in large populations in investigator-initiated, public-good trials.

- If the primary hypothesis is confirmed, DCC will improve disability-free survival in 2,000 very preterm infants every year in Australia and New Zealand and in about a million annually worldwide, at no extra cost to health services or families. For a very modest outlay per child enrolled, confirmation of this hypothesis would be a major advance in preventing complications of preterm birth in mainstream, indigenous and disadvantaged populations in Australia and overseas. That is an urgent global priority,[1] and accords with the Australian Government National Research Priority – a *Healthy Start to Life*. Completion of childhood follow up in this project (as proposed in the initial application) is therefore an eminently worthwhile investment.

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Australian Placental Transfusion Study

Should very preterm babies receive a placental transfusion at birth?

Childhood follow-up to the Australian Placental Transfusion Study

STATISTICAL ANALYSIS PLAN

Prepared by: Kristy Robledo

Signed by:

William Tarnow Mordi (Principal Investigator)

Val Gebski Digitally signed by Val Gebski Date: 2020.08.27 22:23:06 +10'00'

Val Gebski (Head of Statistics, NHMRC CTC)

Date: 27 August 2020

Contents

Version number	Changes	Author
1 (May 2019)	Initial draft prepared by Kristy and reviewed by Adrienne	Kristy Robledo
2 (July 2019)	Updates to incorporate inputs from Val Gebski	Kristy Robledo
2.1 (June 2020)	Updates to include treatment adherence weighted analysis	Kristy Robledo
2.2 (August 2020)	Updates to include additional treatment adherence weighted analysis and multiple imputation analysis	Kristy Robledo/William Tarnow-Mordi

1 SYNOPSIS

1.1 Primary clinical hypothesis:

An autologous transfusion of placental blood at birth will improve health outcomes in preterm babies born less than 30 weeks.

1.2 Study design:

This is a multicentre 2-arm parallel open label randomised controlled trial

1.3 Study interventions:

The two arms of the trial comprise:

- early cord clamping (which is the control arm) is clamping the cord within 10 secs of birth
- delayed cord clamping (for 60 seconds or more with the baby held below or at the level of the placenta)

1.4 Outcomes for the follow-up study:

Primary outcome Death and/or major disability at 24 months

Secondary outcomes Incidence of the following outcomes at 24 months:

1. Death
2. Major disability
3. Components of major disability
4. ASQ domain scores

1.5 Power and Sample Size

Infants recruited into the main APTS study are expected to complete extended follow-up. Assuming that, at most, 150 infants will be lost or not followed to two years, the table below gives an estimate for the absolute difference between the interventions that could be detected for a range of sample sizes and event rates for the primary endpoint allowing for 30% non-compliance. The absolute reduction that could be detected maintaining 80% power is shown.

Table 1: Long term follow-up scenarios for absolute difference that could be detected for possible sample sizes

Secondary endpoint	Sample Size	Absolute difference	Power
Mortality and major disability up to 36 months	1350	10% (35% to 25%)	80
	1400	9.8% (35% to 25.2%)	80
	1450	9.6% (35% to 25.4%)	80
	1350	10.3% (40% to 29.7%)	80
	1400	10.2% (40% to 29.8%)	80
	1450	10% (40% to 30%)	80

1.6 Subgroups

The following subgroups will be investigated for evidence of a difference in the treatment effect on death or major disability at 24 months: gender and gestational age (<27 weeks vs ≥27 weeks).

2 STATISTICAL ANALYSIS

The primary analyses will be performed on assigned treatment. As randomisation occurs when birth is imminent, occasionally babies are not born immediately following randomisation. Babies that are stillborn or born after 30 weeks gestation are not to be included in the intention to treat population.

As consent for the babies recruited into the pilot APTS study did not include collection of follow-up data, these pilot babies are not included in the APTS FU study (n=35).

P-values less than 0.05 will be considered statistically significant and there will be no adjustments for multiple comparisons.

2.1 Flow chart

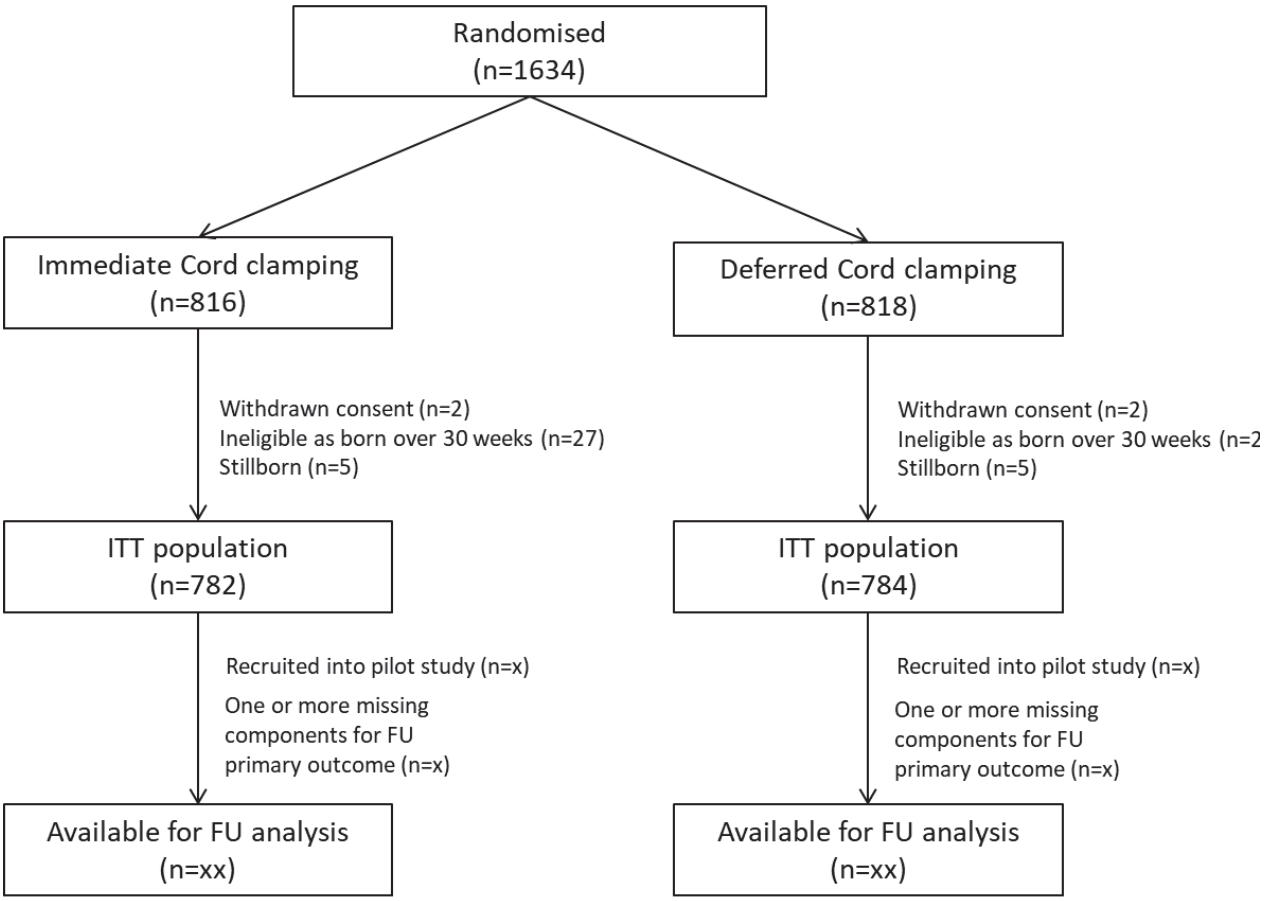

Figure 1: Patient flow chart

2.2 Follow-up cohort

Table 2: Children with follow-up data available

	Immediate cord clamping	Delayed cord clamping
Total babies randomised	816	818
Babies not included in ITT		
• Ineligible babies (born >30 weeks)	27	27
• Withdrawn consent (prior to delivery)	2	2
• Stillborn babies	5	5
Babies included in main study ITT	**782**	**784**
Babies removed because included in the pilot study		
Babies available for the APTS Followup study		
No follow-up obtained		
Partial follow-up obtained, but incomplete		
Data available for primary outcome at 24 months		

2.3 Treatment adherence in FU cohort

Table 3: Summary of overall treatment adherence by treatment

Variable	Immediate cord clamping	Delayed cord clamping
Babies in intention to treat population		
Not as randomised, intervention received will never be known‡		
Not as randomised (>10sec for ICC , <60sec for DCC, milking in either arm)		
As randomised (≤10sec for ICC, ≥60sec for DCC, no milking in either arm)		

‡These babies include babies that were that withdrew consent, transferred prior to delivery or information has not been recorded.

2.4 Delivery and admission characteristics in FU cohort

We will also compare the baseline characteristics of those that are in the FU cohort compared to those that are not in the FU cohort.

Table 4: Patient characteristics at admission and delivery by treatment

		Immediate Cord Clamping	Delayed Cord Clamping
N			
Males			
Gestational Age (weeks)*			
Babies <27 weeks gestation*			
Birth details			
Mode of delivery	Vaginal with instruments		
	Vaginal without instruments		
	Caesarean in labour		
	Caesarean not in labour		
Presentation at birth	Cephalic		
	Breech		
	Other (including transverse)		
	Unknown		
Infants of multiple births (only counting births in the ITT population)	Singletons		
	Twins		
	Triplets		
	Quadruplets		
Maternal assessment			
Ethnicity	Caucasian		
	Aboriginal/TSI		
	Asian		
	Pacific Islander & Maori		
	African‡		
Variables collected pre and post intervention			
Uterotonic agents administered			
If yes, what agent?	Syntocin		
	Ergometrine		
	Carbetocin		
	Syntometrin		
	Other (including multiple agents)		
If yes, what route?	IV		
	IM		
If yes, timing?	Pre-clamping		
	Post-clamping		

All characteristics measured prior to intervention were p>0.xx.
*Gestational age is gestational age at birth, as originally specified by the site. Note that reassessed GA has not been used.
‡ includes African American. Statistics are given as N (%), mean (SD) or median (Q1-Q3).

2.5 Outcome definitions

The primary outcome is death and/or major disability at 24 months corrected age. Assessments of major disability must be performed between 18 months to 27 months. Any data outside of this window will require a central review.

2.5.1 Definition of death

The survival data will be used up to 24 months corrected age. Only babies who are known to be alive at 21 months corrected or later will be deemed as 'alive' at 24 months. Deaths after 24 months are not included.

2.5.2 Definition of major disability

Follow-up assessments should be performed on all APTS babies eligible for follow-up using the short health status questionnaire (SHSQ) and the Ages and Stages Questionnaire (ASQ-3). Additionally, some sites routinely use the Bayley scales of Infant and Toddler Development – Third edition (Bayley-III) which has been collected where available.

Major disability is defined by one or more of the following:

- Cerebral palsy with an inability to walk unassisted at or after 2 years corrected age,
- Severe visual loss (legally blind i.e. corrected acuity <6/60 in both eyes),
- Deafness, requiring a hearing aid or cochlear implants,
- Major problems with language or speech at or after 2 years corrected for gestation defined by the inability to use more than 10 words (including signed words)
- Cognitive delay as defined below.

A tiered approach (Table 2) will be used in order to obtain a disability status for as many babies as possible. If the data is not available for Tier 1, the disability status will be obtained from Tier 2, otherwise Tier 3. A central review blinded to treatment will be performed as appropriate and all of the information available at any time (i.e. Bayley-III) will be reviewed. If the outcome for an infant is not available from tier 1 or 2 results and can then not be ascertained with a central review, the primary outcome for this infant will be missing.

Table 5: Identification of major disability†

Disability type	Tiers to move through in order to determine disability status	Definition of Disability present within each tier	Definition of Disability absent within each tier
Cerebral palsy	Tier 1: SHSQ competed after 22 months.	'CP = Yes' and the inability to walk unassisted	'CP = No' or 'CP=Yes' but only an unsteady walk
	Tier 2: ASQ-3 completed after 18 months	Score of 0 for Gross Motor domain	Score >0 for gross motor domain
	Tier 3: Central review required		
Severe visual loss	Tier 1: SHSQ completed at any age	Legally blind = Yes	Legally blind = No
	Tier 2: Central review required		
Deafness	Tier 1: SHSQ completed at any age	Hearing loss = Yes with requirement for hearing aids	Hearing loss = No or Hearing loss = Yes with no requirement for hearing aids
	Tier 2: Central review required		
Major problems with language or speech	Tier 1: SHSQ completed between 22-27 months (inclusive)	Unable to speak 10 words	Able to speak >10 words
	Tier 2: ASQ-3 completed after 18 months	Communication score indicative of delay (based on published ASQ cut-off)	Communication score not indicative of delay (based on published ASQ cut-off)
	Tier 3: Central review		
Cognitive delay	Tier 1: ASQ-3 completed after 18 months	Problem solving score indicative of delay (based on published ASQ cut-off)	Problem solving score not indicative of delay (based on published ASQ cut-off)
	Tier 2: Central review		

† the windows given above for the determination of disability using the various sources were determined by clinicians involved in the APTS Follow-up study. All ages are corrected ages.

If the baby is alive and does not have one of the above major disabilities, and has been assessed for all of the above components, then they will be classified as alive and disability free. If the infant was alive and has no disability for CP, severe visual loss, deafness and major problems with language or speech, but missing information for cognitive delay, then the infant will be assumed to be alive and disability free at 2 years. Otherwise the infant will be considered as missing for the primary analysis.

Table 6: Summary of completeness of the primary outcome and its components

Endpoint	Complete data	Missing data
Death or disability at 24 months		
Death status		
Cerebral palsy†		
Severe visual loss†		
Deafness†		
Major problems with language or speech†		
Cognitive delay†		

†These analyses are restricted to those that were assessed for these outcomes

Additionally, the baseline characteristics will be compared between those that have outcome data at 24 months versus those that have missing data at 24 months.

2.6 Analysis of outcomes

2.6.1 Primary analyses

The analysis of these outcomes will be a generalised linear model analysis using generalised estimating equations (GEE) with a log link function, and adjusting for multiple births. The estimate of effect will be a relative risk, with a 95% CI.

Analyses of the disability components will be restricted to those that survived to 24 months. A formal analysis will only be performed if there are adequate event numbers (greater than 10 events) at 24 months in each treatment arm.

Table 7: Outcomes at 24 months corrected age, by treatment

Outcomes at 24 months	ICC n/N (%)	DCC n/N (%)	Relative risk (95% CI)	p-value
Primary outcome: Death or major disability*				
Secondary outcomes:				
• Death				
• Major disability*				
• Cerebral palsy†				
• Severe visual loss†				
• Deafness†				
• Major problems with language/speech†				
• Cognitive delay†				

*major disability is one or more of the following: Cerebral Palsy, severe visual loss, deafness, major problems with language or speech or cognitive delay.
†These analyses are restricted to those that were assessed for these outcomes

2.6.2 Adjusted analyses

An additional analysis will use GEE as described above and will additionally adjust for appropriate baseline characteristics including gestational age (<27 weeks vs ≥ 27 weeks), gender, birthweight (into quartiles), multiple birth (singleton vs multiple birth) and mode of delivery (vaginal versus caesarean). This will only be performed for the primary outcome and death by 24 months.

Table 8: Adjusted analyses for outcomes at 24 months corrected age*

Outcomes at 24 months	ICC n/N (%)	DCC n/N (%)	Relative risk (95% CI)	p-value
Primary outcome: Death or major disability*				
Death				

*Models are adjusted for gestational age (<27 weeks vs ≥ 27 weeks), gender, birthweight (into quartiles), multiple birth (singleton vs multiple birth) and mode of delivery (vaginal versus caesarean).

2.6.3 Treatment adherence weighted analysis

The primary outcome will also have the following treatment adherence weighted analyses performed.

2.6.3.1 Simple weighted approach

Firstly, an overall treatment adherence adjusted estimate will be calculated. This will be calculated using a simple weighting of the overall estimate of relative risk reduction (RRR):

$$RRR_{\text{adjusted}} = \frac{RRR}{100 - (\text{non compliance}^{(\text{ICC})} + \text{non compliance}^{(\text{DCC})})}$$

Where

RRR is the relative risk reduction (%) for the primary outcome (Table 7)
$\text{non compliance}^{(\text{ICC})}$ is the treatment non-adherence percentage for ICC in Table 3
$\text{non compliance}^{(\text{DCC})}$ is the treatment non-adherence percentage for DCC in Table 3

2.6.3.2 Weighted subgroups by treatment adherence approach

Additionally, a weighted average approach, weighting by measures of treatment received will be undertaken, following the same principles used in the APTS main study analyses, while preserving the effect of randomisation. In these planned analyses we propose the following two approaches:

(a) sites which enrolled 50 or more babies will be considered a cluster and sites with less than 50 babies will be amalgamated to make a cluster of over 50 with other sites in the same geographic area;

(b) sites which enrolled 10 or more babies will be considered a cluster and amalgamated into a cluster with sites with less than 10 babies will be amalgamated with others in the same geographic area to form another 'cluster' with 10 or more babies (as shown in Table 9).

These clusters are slightly different from those proposed in the main paper as we have fewer patients with the follow-up component of APTS (1531 vs 1566), and also fewer with complete data at 2 years. Analysis (b), in which sites of 10 or more babies will be considered a 'cluster' as per Table 9, will allow us to assess the effect of placing less emphasis on the arbitrary grouping of sites by geographical location in analysis (a).

The main measure to be used to estimate treatment adherence will be the mean difference in clamp time (in seconds) between the ICC and DCC arms. Additional measures to be investigated include:

- mean clamp time in seconds in the DCC arm only
- proportion of compliant babies, where compliant is <10 seconds and not milked versus >=60 seconds and not milked
- proportion of babies who are compliant in the DCC arm only (>=60seconds and not milked)

For each measure of treatment adherence, the average for each treatment in each cluster will be calculated. For some measures, the difference between the two treatments will be calculated while for others, the measure of treatment received in the delayed clamping arm alone will be used. This value will then be used to rank the (a) 15 and (b) 22 clusters (as below in Table 9) for increasing adherence to protocol. These clusters in (a) and (b) will be grouped into 3 sets according to adherence to protocol, i.e. (a) 5 'low', 5 'medium' and 5 'good' adherence to protocol treatment (total 15 clusters) or (b) 7 'low', 8 'medium' and 7 'good' adherence to protocol treatment (total 22 clusters).

The main measure to be used to estimate treatment adherence will be the mean difference in clamp time (in seconds) between the ICC and DCC arms. For example, if all babies in the control group complied with the protocol specifications we might expect an average clamp time to be 5 seconds, whereas in the delayed arm, the average time would be 60 seconds, so the achieved difference would be 55 seconds. The value of the treatment adherence to be used for that cluster would be 55.

For each measure of treatment adherence, a weighted analysis will be carried out to obtain an estimate of the effect of the intervention, weighted by the degree of treatment adherence. An estimate of the achieved effect of delayed cord clamping, for each of the 3 groups of clusters, will be obtained from a generalised linear model. A weighted average of the treatment adherence for each group will be calculated using a weighted average of the values for each cluster. Finally, an estimate of the effect of the intervention weighted for non-adherence will be derived by a weighted average of the 3 group estimates.

The details of the method are in Appendix One.

Table 9: Listing of sites within each cluster

25 APTS sites	15 clusters, obtained from individual sites with ≥50 patients or by amalgamating sites with <50 patients with others by geographic area	22 clusters, obtained from individual sites with ≥10 patients or by amalgamating 3 sites with <10 patients with others by geographic area
Aga Khan University	Pakistan	Pakistan
Hopital Antoine Beclere	France/N America	France
Fletcher Allen Health Care Medical Center		N America
Texas Children's Hospital		
IWK Health Centre		
Royal Jubilee Maternity Hospital	Northern Ireland	Northern Ireland
Craigavon Area Hospital		
Royal Hospital for Women	NSW and ACT	Royal Hospital for Women
Canberra Hospital		Canberra Hospital
Liverpool Hospital		Liverpool Hospital
Royal North Shore Hospital	RNS hospital	RNS hospital
Royal Prince Alfred Hospital	RPA hospital	RPA hospital
John Hunter Hospital	John Hunter	John Hunter
Mater Mothers Hospital	Queensland	Mater Mothers Hospital
Royal Brisbane and Women's Hospital		RBWH
Townsville Hospital		Townsville Hospital
Mercy Hospital For Women	Melbourne	Mercy Hospital For Women
Monash Medical Centre		Monash Medical Centre
Flinders Medical Centre	Flinders	Flinders Medical Centre
King Edward Memorial Hospital	KEMH	King Edward Memorial Hospital
Waikato Hospital	NZ	Waikato Hospital
Dunedin Hospital		Dunedin Hospital
Auckland Hospital	Auckland	Auckland Hospital
Christchurch Hospital	Christchurch	Christchurch Hospital
Wellington Hospital	Wellington	Wellington Hospital

2.6.4 Multiple imputation analysis

A multiple imputation analysis[1,2,3] using chained equations[4] will be conducted for the primary outcome as a sensitivity analysis. Covariates to be included in both the imputation stage and the modelling stage include: treatment, gestational age (<27 weeks vs ≥ 27 weeks), gender, birthweight (into quartiles), multiple birth (singleton vs multiple birth) and mode of delivery (vaginal versus caesarean).

2.6.5 Survival analysis

A Kaplan-Meier curve will summarise time to death by treatment group. Time to death is defined as randomisation to date of death, or last known alive. All APTS follow-up babies will be included in this analysis and censored at date of last follow-up. A cox regression will be used to obtain a hazard ratio for treatment, adjusted for gestational age.

2.6.6 Causes of death

Causes of death will be summarised by treatment.

Table 10: Primary causes of death for deaths by 24 months corrected age by treatment

Primary cause of death	ICC N (%)	DCC N (%)
Total deaths		
Congenital abnormality		
Pulmonary hypoplasia		
Severe respiratory distress syndrome (RDS)		
Chronic lung disease		
Pneumonia		
Grade 3 / grade 4 IVH		
Meningitis		
Septicaemia		
Necrotising enterocolitis (NEC)		
Other		

2.7 Subgroups

The treatment effect for death or major disability at 24 months will be investigated in the following defined subgroups:

- Gender (Males vs. Females)
- Age: Infants of <27 weeks vs ≥27 weeks

This will be performed by fitting an interaction term between the subgroup of interest and treatment in a GEE model adjusted for multiple births. The two sided level of significance is 0.05. The relative risk for treatment will be determined from a model for each of the levels of the subgroup, e.g. a model for only females.

Robledo KP, *et al. Arch Dis Child Fetal Neonatal Ed* 2025;0:1–6. doi: 10.1136/archdischild-2024-327762

Table 11: Subgroup analyses for the primary outcome of death or major disability at 24 months

Subgroup		ICC n/N (%)	DCC n/N (%)	Relative risk (95% CI)	Interaction p-value
Gender	Males				
	Females				
Gestational Age	<27 weeks				
	≥27 weeks				

2.8 Bayley-III and ASQ-3 calibration sub-study

The aim in this sub-study is to validate the cut-offs provided by ASQ-3 developers in the APTS population. The APTS follow-up study was able to obtain funding for a sample of children to be assessed using the Bayley-III. In this sample of children, the Ages and Stages Questionnaire (ASQ-3) published cut-offs will be calibrated against the Bayley-III definitions of developmental delay.

A stratified sampling approach was taken in order to obtain a high risk sample of developmentally delay in order to calibrate these two instruments. Additionally, some sites routinely perform Bayley-III, and therefore we were also able to obtain an opportunistic sample of babies with a Bayley-III and matching ASQ.

Table 11 below maps the ASQ domains to the Bayley-III scores. Scatterplots, spearman correlations and ROC analyses may be used to compare these scores.

Table 12: Mapping of ASQ-3 domains to Bayley-III scores.

ASQ-3 domains	Bayley-III scores
Problem solving	Cognitive
Communication	Communication
Gross motor	Motor scale – gross motor
Fine motor	Motor scale – fine motor
Personal-social	Social emotional

Appendix One

Method to provide an estimate of the effect of the delayed cord clamping that might be achieved if the intervention was delivered according to the protocol.

Initially, the study sites will be grouped into **clusters** of (a) 50 or more infants based on geography and size of the neonatal unit or (b) of 10 or more infants. These clusters are shown in Table 9.

The primary measure of treatment adherence is the time in seconds to cord clamping ($\mu(\text{diff in sec})$). For this measure, and for each cluster, the average difference between the two interventions in time to clamping (ΔTrAdh_i) is calculated:

$$\Delta\text{TrAdh}_i = \mu(\text{diff in sec})_i^{(DCC)} - \mu(\text{diff in sec})_i^{(ICC)} \qquad \text{for } i = 1,..,15$$

The clusters will be ranked in order of increasing magnitude of this measure (ΔTrAdh_i) and then grouped into 3 sets, low, medium and high adherence to treatment. For the primary measure, this will be by the difference in the average time to clamping between the interventions.

Within each of these three groups, using the same methods as for the analysis of the primary endpoint, a GEE model (logistic regression with a log-link) will be used to calculate the observed effect of delayed cord clamping on death and disability at 2 years to obtain RR_j, (*j*= 1 to 3) where RR_j is the relative risk for the *j*th group and the associated variance for the log of the estimate being $\text{Var}(\ln(RR_j))$.

The weights for each of the low, medium and high groups (w_j) will be a weighted average of the ΔTrAdh_ivalues of the clusters within a group:

$$w_j = \begin{cases} \dfrac{\sum_{i=1}^{5} n_i \times \Delta\text{TrAdh}_i}{\sum_{i=1}^{5} n_i}, & \text{for } j = 1 \text{ (low)} \\ \dfrac{\sum_{i=6}^{11} n_i \times \Delta\text{TrAdh}_i}{\sum_{i=6}^{11} n_i}, & \text{for } j = 2 \text{ (medium)} \\ \dfrac{\sum_{i=12}^{16} n_i \times \Delta\text{TrAdh}_i}{\sum_{i=12}^{16} n_i}, & \text{for } j = 3 \text{ (high)} \end{cases}$$

where n_i is the number of babies within the i^{th} cluster.

A weighted analysis will be performed by multiplying the effect for each group by its weight and dividing this quantity by the sum of the weights.

Thus, a weighted estimate (G) is given by

$$G = \ln(RR) = \frac{\sum_{j=1}^{3} w_j \times \ln(RR_j)}{\sum_{j=1}^{3} w_j}.$$

The variance (V) of this weighted quantity will be calculated as,

$$V = \mathrm{Var}(G) = \frac{\sum_{j=1}^{3} {w_j}^2 \times \mathrm{Var}(\ln(RR_j))}{(\sum_{j=1}^{3} w_j)^2}.$$

G is the log of the weighted estimate of the effect of delayed clamping on death and morbidity at 36 weeks, and $\exp(G)$ provides an estimate of the effect of the intervention, weighted by difference in the time to cord clamping (i.e. treatment adherence).

The 95% CI for this estimate can be calculated by using

$$\exp(G \pm 1.96 \times \sqrt{V}).$$

References:

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3. Donald B. Rubin (1996) Multiple Imputation after 18+ Years, Journal of the American Statistical Association, 91:434, 473-489, DOI: 10.1080/01621459.1996.10476908

4. van Buuren, S., & Groothuis-Oudshoorn, K. (2011). mice: Multivariate Imputation by Chained Equations in R. *Journal of Statistical Software, 45*(3), 1 - 67. doi:http://dx.doi.org/10.18637/jss.v045.i03