Study protocol of the WashT Trial: transfusion with washed versus unwashed red blood cells to reduce morbidity and mortality in infants born less than 28 weeks’ gestation - a multicentre, blinded, parallel group, randomised controlled trial

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ABSTRACT

Introduction Many extremely preterm newborns develop anaemia requiring a transfusion, with most receiving three to five transfusions during their admission. While transfusions save lives, the potential for transfusion-related adverse outcomes is an area of growing concern. Transfusion is an independent predictor of death and is associated with increased morbidity, length of hospital stay, risk of infection and immune modulation. The underlying mechanisms include adverse pro-inflammatory and immunosuppressive responses. Evidence supports an association between transfusion of washed red cells and fewer post-transfusion complications potentially through removal of chemokines, lipids, microaggregates and other biological response modifiers. However, the clinical and cost-effectiveness of washed cells have not been determined.

Methods and analysis This is a multicentre, randomised, double-blinded trial of washed versus unwashed red cells. Infants <28 weeks’ gestation requiring a transfusion will be enrolled. Transfusion approaches will be standardised within each study centre and will occur as soon as possible with a recommended fixed transfusion volume of 15 mL/kg whenever the haemoglobin is equal to or fails below a predefined restrictive threshold, or when clinically indicated. The primary outcome is a composite of mortality and/or major morbidity to first discharge home, defined as one or more of the following: physiologically defined bronchopulmonary dysplasia; unilateral or bilateral retinopathy of prematurity grade ≥2, and; necrotising enterocolitis stage ≥2. To detect a 10% absolute reduction in the composite outcome from 69% with unwashed red blood cells (RBCs) to 59% with washed RBCs with 90% power, requires a sample size of 1124 infants (562 per group). Analyses will be performed on an intention-to-treat basis with a prespecified statistical analysis plan. A cost-effectiveness analysis will also be undertaken.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This will be the first neonatal trial that is adequately powered to demonstrate clinical benefit of transfusion with washed red cells.
⇒ The use of a waiver of consent ensures the sickest infants, who often require very early blood transfusion as part of initial neonatal stabilisation, are still eligible for randomisation.
⇒ As washed red cells have a reduced shelf life and increased cost relative to standard red cells, the within-trial short-term and modelled long-term economic analysis of the intervention will accurately evaluate cost-effectiveness.
⇒ The blood product development and infrastructure support established for the trial is specific for the Australian setting and may need modification to ensure rapid research translation and adoption into routine neonatal clinical practice in other countries.

INTRODUCTION

Very preterm newborns rapidly become anaemic from a combination of frequent phlebotomy loss and an immature haematopoietic system. As a result, 90% of babies born <29 weeks’ gestation and/or 1250 g will receive at least one red blood...
cell (RBC) transfusion,² with most receiving three to five transfusions during their primary admission.³ ⁴ While transfusions save lives in the neonatal intensive care unit (NICU) setting,⁵ the potential for RBC transfusion-related adverse outcomes is an area of growing interest and concern.⁶

For all ages, transfusion is an independent predictor of death and is associated with longer hospital stay and an increased incidence of multiple-organ system failure, infection and long-term immune modulation.⁷–⁹ The very preterm newborn is at particular risk of developing mostly inflammatory problems, specifically related to prematurity, following RBC transfusion. For example, RBC transfusion exposure is associated with the development of bronchopulmonary dysplasia (BPD), necrotising enterocolitis (NEC) and retinopathy of prematurity (ROP), with both incidence and severity related to the number and volume of transfusions received.¹⁰⁻¹⁴ Importantly, the occurrence of these morbidities is predictive of late death or survival with disability at 5 years of age.¹⁶

The underlying processes linking transfusion exposure to adverse outcomes are multifactorial. They include reduced RBC quality and quantity, accumulation of bio-active substances in RBC components over time (termed the red cell storage lesion)¹⁷ and exposure to immune-active cells such as leucocytes.¹⁸ This has led to a number of approaches to minimise risk, including the use of ‘fresh’ versus ‘older’ blood;¹⁹ ²⁰ modified RBC processing, such as prestorage leucodepletion;²¹ and the use of restrictive versus liberal transfusion thresholds.³ ⁴ ²² Only leucodepletion is associated with reduced mortality and transfusion-related morbidity²³ but clinical studies, including our own, have shown that leucodepleted RBC transfusion still leads to immune activation in the transfusion recipient.²⁴ ²⁵

The immune activation following transfusion exposure includes both adverse pro-inflammatory and immunosuppressive responses. Termed transfusion-related immunomodulation (TRIM),²⁶ these may play an important role in the pathogenesis of all the morbidities seen in relation to transfusion exposure. TRIM is likely a ‘two-insult’ process.²⁷ The first insult is initial sensitisation to inflammatory processes resulting in host neutrophil priming (first insult). Subsequent exposure to biological response modifiers, transfused along with the RBCs (second insult), then results in a pro-inflammatory immune response.²⁶ In support of this, a supernatant derived from RBC packs alters immune and endothelial cell function in vitro and in vivo.²⁸ Biological substances thought to mediate this effect include donor antibodies, bioactive lipids, free haemoglobin (Hb), red cell membrane fragments and cytokines that accumulate during blood storage.²⁹ For example, free Hb is implicated in vascular pathology, lung injury and thrombosis²⁹ altering endothelial and vascular function, while microparticles promote inflammation and thrombosis³⁰ likely contributing to TRIM.³¹

Epidemiological and observational evidence supports an association between transfusion of washed RBCs and fewer post-transfusion cardiopulmonary complications.³² Further, in patients with acute leukaemia use of washed RBCs reduced transfusion-related adverse immunological effects and improved survival.³³ However, a significant knowledge gap with respect to potential beneficial effects remains. A review of the literature (Cochrane Controlled Trials Register (CENTRAL) and MEDLINE (last searched in January 2019)) identified two randomised trials comparing washed versus standard RBC transfusions.³³ ³⁴ Transfusion of washed RBCs was associated with improved survival of adult patients diagnosed with acute myeloid or lymphoid leukaemia³³ and reduced transfusion-related pro-inflammatory cytokine production in paediatric cardiac surgical patients.³⁴ Our Cochrane review of the neonatal literature³⁵ identified a single prospective randomised study of 21 preterm infants, providing insufficient evidence to support or refute the clinical and economic value of transfusion of washed RBCs in preterm babies.³⁶ While the knowledge gap in the adult population has been recognised and is currently being addressed by the Washing of Allogeneic Red blood cells for the Prevention of Respiratory Complications (WAR-PRC) study (ClinicalTrials.gov Identifier: NCT02094118),³⁷ our proposed trial will definitively answer this question in the very preterm newborn.

Washing RBCs removes soluble mediators in the RBC supernatant including chemokines, biologically active lipids, cellular debris, microaggregates and other biological response modifiers.³⁸ In fact, washing RBC eliminates more microparticles than leucodepletion.³³ ³⁹ Further, washing RBCs reduces pro-inflammatory cytokine concentrations in stored RBCs,⁴₀ while supernatant from washed RBCs results in decreased endothelial permeability and pro-inflammatory cytokine and chemokine release compared with standard RBCs.³¹ While these studies have focused on adult washed RBC packs, we have demonstrated that the process of washing has beneficial effects on transfusion packs specifically produced for neonatal transfusion.⁴² These effects include reduced cytokines, complement proteins, RBC microparticles, supernatant potassium and IgA. Further, incubation with supernatant from washed RBCs led to decreased endothelial cell activation and cell surface expression of CD62E (E-selectin) and CD106 (VCAM).⁴² With respect to clinical studies, transfusion of washed compared with standard RBCs is associated with reduced ratio of pro-inflammatory interleukin (IL)-6 to anti-inflammatory (IL-10) cytokines in paediatric cardiac surgery patients.³⁴ While IL-6 concentrations were undetectable preoperatively, the post-transfusion increase in IL-6, peaking at 6 hours, was lower in patients transfused with washed packed RBCs. Therefore, the aim of the current study is to determine both the clinical and cost-effectiveness of transfusion with washed cells in the extremely preterm newborn.
METHODS AND ANALYSIS

Study design
The WashT trial is a randomised controlled, blinded (clinician, researcher and participant), multicentre clinical trial, investigating the effectiveness of washed leucodepleted RBCs versus unwashed leucodepleted RBCs (current standard practice) in infants born less than 28 weeks’ gestation. As many very preterm newborns require emergency transfusion in the first hours of life as part of their initial resuscitation and stabilisation, a waiver of consent approach will be employed. Infants requiring a transfusion will be randomised to receive either unwashed leucodepleted RBCs (control) or washed leucodepleted RBCs (intervention). Infants will remain in their allocated arm for every subsequent transfusion required until neonatal nursery discharge. The trial will be reported according to the Consolidated Standards of Reporting Trials checklist. A within-trial and modelled cost-effectiveness analysis will be reported according to the Consolidated Health Economic Evaluation Reporting Standards checklist.

Patient and public involvement
While patients were not involved in the design of the trial, a key stakeholder Australian Red Cross Lifeblood was directly involved in the design and the ongoing conduct of the study. Trial reporting and dissemination will involve both Australian Red Cross Lifeblood and the Miracle Babies Foundation.

Sample size
Using data from a previous neonatal trial conducted by the trial investigators in our unit, the expected incidence of this composite outcome from 69% to 59% with 90% power and overall two-sided alpha 0.05 (0.049 for clustering due to multiple births) is required. No adjustment for 10% missing data is required in these calculations, since infants from a multiple birth will be randomised individually. Randomisation will be performed using Research Electronic Data Capture (REDCap) by neonatal nursery staff when the infant requires their first RBC transfusion.

Randomisation and blinding
A computer-generated randomisation schedule using randomly permuted blocks will be generated by an independent statistician not involved with trial participants or data analysis. Infants will be randomised into washed or unwashed groups with an allocation ratio of 1:1, with stratification for study centre and gestational age <25 weeks’ and 25+0 to 27+6 weeks’ gestation. Infants from a multiple birth will be randomised individually. Randomisation will be performed using Research Electronic Data Capture (REDCap) by neonatal nursery staff when the infant requires their first RBC transfusion.

Transfusion practice
The predefined restrictive Hb threshold from the Transfusion of Prematures study (table 1) is recommended for use in each study centre. The Hb threshold will be

<table>
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<th>Table 1</th>
<th>Transfusion algorithm based on the Transfusion of Prematures study</th>
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<tr>
<td>Age (days)</td>
<td>Blood sampling</td>
</tr>
<tr>
<td>≤7</td>
<td>Complete blood examination</td>
</tr>
<tr>
<td>8–14</td>
<td>Complete blood examination</td>
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<td>≥15</td>
<td>Complete blood examination</td>
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Haemoglobin values are displayed as g/L. Respiratory support refers to any O₂, conventional mechanical ventilation, high frequency oscillatory ventilation, continuous positive airway pressure, high flow.
BPD is defined as a continued need for any form of respiratory support (assisted ventilation, continuous positive airway pressure (CPAP) or supplemental oxygen) and by postnatal age. The protocol does not dictate how or how often [Hb] will be determined, but all values will be recorded to determine compliance with the transfusion threshold algorithm. Transfusion will be indicated whenever the [Hb] equals or falls below the threshold value or when clinically indicated. RBC transfusions will be given as soon as possible after the attainment of a threshold value and at least within 6 hours of [Hb] determination. While a transfusion volume of 15 mL/kg is recommended, the volume used can be study centre specific to comply with local clinical practice guidelines. Exposure to other transfusion products (platelets, fresh frozen plasma, cryoprecipitate) will be recorded.

RBC manufacture and supply

Standard (leucodepleted) RBCs will be supplied from Australian Red Cross Lifeblood, Melbourne, to the transfusion departments at the study sites, as per current standard practice. Two-day-old group O Rh—crossmatch compatible RBC packs will be washed at Lifeblood as per current clinical protocols and subsequently divided into four equivalent paediatric quad-packs using closed techniques. The prepared washed RBCs will be supplied to the participating centres on a weekly basis. A 14-day maximum shelf life for both the washed and unwashed packs will be employed controlling for red cell storage lesion. The principal advantage of this system is the ease of transition of this process to any Blood Service nationally or internationally with minimal resource requirement.

Outcomes

Primary outcome

The primary outcome is a composite of mortality and/or major neonatal morbidities associated with organ dysfunction or failure diagnosed prior to first discharge home. Major neonatal morbidity is defined as one or more of the following:

- Death defined as death from any cause.
- BPD is defined as a continued need for any form of respiratory support (supplemental oxygen and/or assisted ventilation) at 36 weeks post-menstrual age, or discharge home (whichever occurs first). A standardised diagnostic approach for BPD will be used.46 On or shortly after 36 weeks +0 days postmenstrual age, infants not requiring respiratory support (intubation/CPAP/high flow nasal cannula oxygen ≥2 L/min) but receiving oxygen with a fractional inspired oxygen (FiO2) of less than 0.3 will have a trial of room air. For infants on nasal cannula oxygen the effective FiO2 will be determined using the Benaron-Benitz formula.45 Those infants with an FiO2 < 0.3 will have stepwise FiO2 reductions 5 min apart until either room air is being administered or oxygen saturation (SpO2) is no longer in the target range (91–95%). A successful air trial will be defined as SpO2 readings in the target range for 30 min in room air with nasal prongs removed. Infants requiring respiratory support (intubation/CPAP/high flow nasal cannula oxygen ≥2 L/min), and those failing the room air trial, will be deemed to have physiological BPD.

- ROP defined as grade 3–5 (unilateral or bilateral).48
- NEC is defined as stage 2 or greater using the grading criteria of Bell et al.49 A composite measure is reasonable as the individual morbidities are all frequent; have inflammation implicated in their pathogenesis; and an association with RBC transfusion exposure has been described in the neonatal literature. With a primary event rate of 69% in the study centres44 a 10% reduction in this outcome would provide persuasive evidence of significant net clinical benefit.

Key secondary outcomes

1. The individual components of the composite outcome, described in the primary outcome:
   - Death.
   - BPD.
   - ROP grade >2 (unilateral or bilateral).
   - NEC stage ≥2.

2. Intraventricular haemorrhage (IVH) grade ≥2 (unilateral or bilateral).

Other secondary outcomes

- Respiratory:
  - Severity of BPD (grade 1, 2 or 3).50
  - Postnatal steroids for BPD.
  - Total hours of invasive ventilatory support (via endotracheal tube, nasal continuous positive airway pressure, nasal/non-invasive ventilation, nasal high flow, any respiratory support.
  - Total hours of high frequency oscillatory ventilation.
  - Home oxygen therapy.
- Cardiac: Patent ductus arteriosus requiring treatment (pharmacological and/or surgical).
- Infection:
  - Bacterial, fungal or viral at >48 hours of age (blood or cerebrospinal fluid culture or PCR positive and treatment with antibiotics with therapeutic intent).
  - Spontaneous intestinal perforation (not NEC associated).
- Neurological: Porencephalic cysts, periventricular leucomalacia and any intracranial haemorrhage.
- Ophthalmic:
  - Maximal grade ROP to 3 months’ corrected age.
  - Retinal ablation or medical treatment (anti-vascular endothelial growth factor) to 3 months’ corrected age.
- Nutrition and growth:
  - Total hours of parenteral nutrition.
  - Weight, length and head circumference z-score at first discharge home.
Length of hospital stay from birth to first discharge home.

Economic evaluation
The economic evaluation will consist of within-trial cost-effectiveness analysis. Costs will be measured in terms of direct costs to the healthcare system up to hospital discharge using trial case report forms and individual participant medical records. The total volume of major categories of healthcare use (eg, RBCs, diagnostic tests, specialist doctor visits, procedures and length of NICU/ward hospitalisation stay) will be collected. Volumes of healthcare use and SD, and costs with 95% CIs for differences by allocated group will be tabulated. The outcomes for the economic analysis will be major neonatal morbidity/death avoided (ie, primary endpoint) at hospital discharge. Both costs and outcomes will be reported separately for each group (following best practice methods) and an incremental cost-effectiveness ratio or incremental net benefit statistic will be reported for the cost per neonatal morbidity/death avoided of the washed RBC group compared with the unwashed RBC group.

Given the morbidity from ROP, NEC, BPD, IVH has a longer-term impact on visual impairment, gastrointestinal dysfunction and asthma; a longer-term modelled cost-effectiveness analysis is necessary. Healthcare use beyond the first hospital discharge will be obtained from a number of administrative data sets including the 2–3 years follow-up of the Australian and New Zealand Neonatal Network, National Perinatal Data Collection, the Medicare Benefits Schedule and the Pharmaceutical Benefits Scheme. The data linkage will enable the per participant calculation of health system resource use and costs up to 2 years after the primary hospital discharge. Healthcare use will be valued using the most relevant unit pricing. Further details will be documented in the health economics analysis plan. The evidence of cost-effectiveness will support reimbursement decision-making at the local level (ie, hospitals and newborn services) and national level (ie, Red Cross blood bank).

Data collection
Outcome variables will be collected by study research nurses and entered into the REDCap data management system. The REDCap database will be password protected with defined user-level access for study personnel at each site, investigators and data analysts. Access to the study data will be restricted with study personnel in each centre only able to access their own data. There will be an audit trail of any changes made to the database. All study personnel will be trained in the study requirements, data collection and data query systems. Training will also include the ICH Good Clinical Practice (GCP) program and general information regarding obtaining research quality data. In order to ensure the accuracy of data collected, representatives from the coordinating centre and regulatory authorities will have access to source documents (ie, mother’s or infant’s medical records). Permission will be sought from parent(s) of participants who withdraw from study participation to (1) retain data already collected and (2) complete data collection.

Data analysis
Analyses will be performed according to a prespecified statistical analysis plan, written to conform with recent recommendations on the content of clinical trial analysis plans (online supplemental material). Broadly, analyses will follow the intention-to-treat principle, with all participants followed-up and analysed as randomised irrespective of protocol compliance. Further detail on study estimands and handling of intercurrent events are provided in the statistical analysis plan (see online supplemental file 1).

The incidence of mortality and/or major morbidities prior to first discharge from NICU will be compared between groups using log binomial regression. Generalised estimating equations with an independence working correlation structure will be used to account for clustering due to multiple births. Adjustment will be made for variables used to stratify the randomisation strata (study centre, sex and gestational age), with the difference between groups expressed as a relative risk with a CI and two-sided p value. Statistical significance will account for a single prespecified interim analysis using the O’Brien-Fleming approach.

Secondary outcomes will be analysed using linear, log-binomial and negative binomial regression models for continuous, binary and count outcomes, respectively, with generalised estimating equations used to account for dependence due to multiple births. In a planned secondary analysis of the primary outcome, effect modification by gestational age will be investigated by including a treatment-by-gestational age interaction term in the model. Relative risks and CIs will be reported for a range of gestational ages, along with a p value for the interaction between gestational age and randomised group.

Study oversight
An independent Data Safety and Monitoring Committee (DSMC) will be established to safeguard the interests of trial participants. The DSMC will consist of a neonatologist, haematologist and a biostatistician, collectively experienced in the conduct and monitoring of randomised trials. The DSMC will meet annually and review general study progress (recruitment, compliance and missing data), key safety outcomes and protocol modifications suggested by the investigators. Additionally, the DSMC will review results of a single interim analysis of the primary outcome once 50% of participants have outcome data available. Using O’Brien-Fleming stopping criteria a two-sided p value<0.0031 at the interim analysis will be taken to provide statistical evidence in support of early stopping. The DSMC will act according to a formal charter which will be agreed on at the first meeting. In addition to monitoring safety and trial quality data, the DSMC will review results of a single unblinded interim analysis of the
primary outcome and review all serious adverse events (SAEs).

**Serious adverse events**

There are no SAEs that are considered to be attributable to the intervention. The following are SAEs that could be reasonably expected to occur in this population of infants during the course of the trial, or form part of the data collection. They do not require reporting by the trial centres as SAEs:

- NEC or focal intestinal perforation.
- BPD.
- Intraventricular haemorrhage.
- Pulmonary haemorrhage.
- Patent ductus arteriosus
- ROP.

All deaths, irrespective of cause will be required to be reported by trial sites as an SAE. Any adverse event not listed and considered to have been related to transfusion exposure must be reported as an SAE. All SAEs must be reported within 24 hours of the event to the coordinating centre. Any significant safety issue which adversely affects the safety of participants will be reported to the Therapeutic Goods Administration, Human Research Ethics Committee and all investigators within 72 hours. Any SAE which does not involve a significant safety issue but requires an amendment to the protocol will be reported within 15 days.

**Ethics and dissemination**

National Mutual Acceptance Ethics approval has been sought and approved by the Women’s and Children’s Health Network (WCHN) Human Research Ethics Committee (HREC/12/WCHN/55). Site-specific approval for all sites will be obtained prior to first patient enrolment. There is no specific additional provision for ancillary and/or post-trial care. Trial insurance is provided through SAHMRI (South Australian Health and Medical Research Institute) as the trial sponsor. South Australia, Victoria, Western Australia, Tasmania and Queensland will provide indemnity in respect to the clinical treatment that will be provided. The principal investigator and trial management committee will have access to the final trial data set. Findings from this study will be disseminated to the scientific community in both abstract and oral presentation format at international and national conferences. Authorship for the primary results paper will take the form of the investigators, statisticians and trial management team. All contributors will be listed at the end of the report with their contribution to the trial specified. All published manuscripts will be published in open access journals.

**Trial status**

Currently eight tertiary neonatal units are enrolling patients. The first patient was randomised on 8 April 2021 and as of the 16 June 2023 196 patients had been randomised. The final inclusion of recruiting sites is expected in 2023. The current article is based on protocol V.5 (15 November 2022).

**Clinical significance**

Extremely low gestational age newborns are at a greater risk of poor outcome than any other population thereby incurring substantial societal and economic cost. As such any reduction in mortality in combination with improvement in survival free of significant neonatal morbidity would be of substantial short-term and long-term benefit. Emerging evidence in other critically ill populations supports significant benefit from modifications in blood product processing including RBC washing, though this has never been studied in the preterm newborn.

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**Competing interests** None declared.

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