Title: The effects of freshly-irradiated versus standard red cell transfusion on cerebral oxygenation in preterm infants: A randomized controlled trial

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KEY POINTS

Question: Are freshly irradiated red blood cells (RBCs) more efficacious in oxygen delivery capacity than irradiated and stored RBCs?

Findings: In a proof-of-concept randomized trial transfusion of freshly irradiated RBCs in preterm infants resulted in favorable cerebral oxygenation kinetics compared with transfusion of irradiated and stored RBCs as per the Australia and New Zealand Society of Blood Transfusion Guideline.

Meaning: 'On demand' irradiation of RBCs may be considered at institutions where this is practicable to optimize oxygen delivery in the recipient.

ABSTRACT

Importance: Gamma-irradiation of leukoreduced red blood cells (RBCs) prevents transfusion-associated graft-versus-host disease, but it also exacerbates storage lesion formation in RBCs. It is currently unknown whether freshly irradiated RBCs are more efficacious than irradiated and stored RBCs in preterm infants with high transfusion requirement.

Objective: To determine *in-vivo* efficacy freshly irradiated RBCs (intervention) versus irradiated and stored RBCs (control) in anemic preterm infants.

Design: In this single-center randomized controlled trial, 64 non-urgent transfusion episodes in 42 preterm infants were studied. Transfusion episodes were randomized to the intervention (RBC irradiated on the day of transfusion, n=32) or control arm (RBCs irradiated and stored as per the ANZSBT guidelines, n=32). Cerebral regional oxygenation ($crSO_2$) and fractional tissue oxygen extraction (FTOE) were measured by blinded clinicians using Near Infrared Spectroscopy (Sensmart X-100, Nonin) for 3hrs immediately before, immediately after, as well as 1 and 5 days after transfusion. Data were analyzed with a covariate-adjusted linear mixed model, which accounted for multiple transfusions in some infants.

Setting: Wellington Neonatal Intensive Care Unit, New Zealand

Participants: Forty-two preterm infants who are <34 weeks gestation at birth and ≥14 days of age

Intervention: Transfusion of freshly irradiated RBCs

Main Outcomes: Changes in $crSO_2$ from immediately before to immediately after the trial transfusion was selected as the pre-specified primary outcome measure.

Results: Compared to the control group, there was a covariate-adjusted mean increase of 2.0% (95%CI: 1.2-2.8%) in $crSO_2$ and a mean decrease of 0.025 (95%CI: 0.011-0.039) in FTOE immediately after transfusion in infants who received freshly irradiated RBCs. These

differences were sustained up to 5 days after transfusion. There were negligible mean changes in $crSO_2$ or FTOE in infants in the control group at any of the time points.

Conclusion and Relevance: Transfusion of freshly irradiated RBCs confers a small advantage in cerebral oxygenation that persists for at least 5 days post-transfusion compared to transfusion of irradiated and stored RBC components. 'On demand' irradiation of RBC components may be considered at institutions where this is practicable to optimize oxygen delivery in the recipient. Further research is needed to ascertain the clinical significance of this physiological finding.

Trial Registration: Australia and New Zealand Clinical Trial Registry (ACTRN12617001581358)

KEY WORDS

Transfusion Practices (Neonatal, Pediatrics), Blood Component Preparations, RBC Transfusion

INTRODUCTION

Preterm infants in Neonatal Intensive Care Units (NICUs), particularly those with extremely low birth weight (<1000g), almost invariably require multiple transfusions of red blood cells before their predicted term 'due dates'.[1-3] The vast majority of blood transfusions are for medically stable infants with chronic anemia ('anemia of prematurity; AOP'), with the aim of increasing oxygen delivery to the metabolically active organs during the critical phase of growth and neurodevelopment. Some infants in NICUs may receive up to 200% cumulative replacement of their total circulating volume at birth by means of transfusion.[4] Therefore, ensuring both the safety and efficacy of this common clinical intervention is of utmost importance.

In modern transfusion practice, donors and their donated blood products undergo stringent screening and processing to ensure a high standard of safety for recipients. One processing method commonly utilized is gamma-irradiation of leuko-reduced RBCs, which effectively prevents proliferation of viable donor leukocytes thus eliminating the risk of transfusion associated graft-versus-host disease (TA-GvHD) in the recipient.[5] TA-GvHD is a rare but life-threatening complication of RBC transfusion affecting those with established immunodeficiency. A series of case reports suggests preterm infants with immature immunity may be at risk of TA-GvHD.[6] In these infants, whether modern pre-storage leukoreduction alone is sufficient in preventing TA-GvHD remains uncertain. A number of institutions worldwide have adopted a universal irradiation policy due to the potential risk of TA-GvHD in those with previously undiagnosed immune dysfunction.[7] Similarly, irradiation of RBCs given to neonates is routine practice in many NICU settings.

Recommended dosimetry and shelf life of irradiated RBCs differ between countries and continents. In Europe and Australasia, it is safe to store irradiated RBCs for up to 14 days (up to 28 days in the United States).[8-10] While these recommendations are primarily based on the acceptable levels of hemolysis and extracellular potassium concentrations in stored units, there is a paucity of literature on the efficacy of irradiated and stored RBCs with regards to oxygen delivery capacity. Limited *in-vivo* evidence to date has highlighted a potentially detrimental effect of storage after irradiation on the ability of RBCs to increase vital organ oxygenation.[11] This, in conjunction with *in-vitro* evidence of accelerated storage lesion formation in irradiated and stored RBCs[12-18], raises a clinically relevant question: does storage following irradiation compromise the primary function of transfused RBCs to improve oxygen delivery in the recipient?

The aim of the current study is to examine, in a randomized controlled trial (RCT), whether transfusion of freshly irradiated RBC components, compared with transfusion of RBC components irradiated and stored as per the Australia and New Zealand Society of Blood Transfusion (ANZSBT) guidelines[10], resulted in an improved cerebral oxygen delivery in anemic preterm infants. We hypothesized that infants receiving freshly irradiated RBC components would have increased cerebral regional oxygenation ($crSO_2$) and cerebral fractional tissue oxygen extraction (cFTOE), compared with infants receiving irradiated and stored RBC components.

MATERIALS AND METHODS

Study Design

The Near Infrared Spectroscopy for Monitoring Brain Oxygenation: Randomized Controlled Trial of Freshly Irradiation versus Standard Red Cell Transfusion for Anemia of Prematurity

(NIMO-Rad) trial was a single-center, randomized, double-blinded, proof-of-concept study comparing transfusion of RBCs irradiated on the day of transfusion ('freshly irradiated') with standard issue RBCs (irradiated and stored for up to 14 days as per the ANZSBT guidelines). The decision to give RBC transfusion to enrolled infants was made solely by the attending clinical team using the high transfusion thresholds adopted from the Premature Infants in Need of Transfusion (PINT) trial.[19] Written informed consent was obtained prospectively from a legal guardian in all cases. The trial protocol was registered on the Australia and New Zealand Clinical Trial Registry (ACTRN12617001581358) prior to enrollment.

Study Population

Preterm infants (<34 weeks gestation at birth) who were ≥14 days of age in Wellington NICU, NZ were considered for inclusion in the trial between December 2017 and November 2018. Eligible infants were enrolled if written informed consent was given by a legal guardian, and the attending clinician made a decision to give non-urgent RBC transfusion for anemia of prematurity. Infants on invasive respiratory support, undergoing treatment for systemic infections, or those who had hemodynamically significant ductus arteriosus or oedema (due to potential interference with signal acquisition) were excluded. If enrolled infants received multiple RBC transfusions during the trial period, each transfusion episode was randomized independently provided that infants continued to meet the inclusion criteria, no exclusion criteria were identified, and the full 5-day follow up data collection from the previous study transfusion was complete. No participant received an additional transfusion during the 5-day follow up period.

Randomisation

A randomisation sequence was generated with no restriction using a computerized random sequence generator 'Sealed Envelope' (www.sealedenvelope.com) by the Biostatistician (GA) based at the Teesside University, UK. It was concealed in a brown envelope and given to the New Zealand Blood Service Hospital Blood Bank prior to enrollment. Once RBC transfusion is prescribed for an enrolled infant by the attending clinician, the study transfusion notification was sent to the Blood Bank service and the trial RBC component was issued according to the randomisation sequence. There was a 3-hr interval between the study transfusion notification and issuing of the study RBC component to allow sufficient time for 'on-demand' gamma-irradiation to be performed. Control RBC components were issued with the same time lag to maintain blinding of the clinical team. As RBC irradiation 'on-demand' was not a standard practice at Wellington Regional Hospital, trial transfusion was only performed Mondays to Saturdays, excluding NZ Public Holidays. If enrolled infants were eligible for multiple study transfusions, each study transfusion episode was randomized chronologically and independently to the intervention or control arms in accordance with the pre-generated randomisation sequence. For the participants who received more than 1 study transfusion, this was taken into account in the linear mixed model in order to avoid pseudoreplication – see statistical analysis section and the supplementary file.

Red Cell Components provided for transfusion

Red cells used for transfusion were produced from whole blood, from known cytomegalovirus antibody negative donors, collected in Citrate-Phosphate Dextrose (CPD) anticoagulant. Plasma was removed, either with or without removal of the buffy coat, and the red cells were re-suspended in an additive solution containing saline, adenine, glucose and mannitol (SAG-M) followed by removal of the leucocytes to a maximum residual white cell content of 5×10^6 per unit. The unit was subsequently divided into 4 satellite packs ('Pedi-

Packs') using a closed system and components stored between 2 and 6 degrees Celsius. Irradiation was performed in accordance with ANZSBT Guidelines.[10] Briefly, red cell components less than 14 days old were subjected to irradiation with a minimum dose achieved in the irradiation field of 25 Gy with no part receiving greater than 50 Gy.

Blinding

Trial RBC components were issued by unblinded Blood Bank personnel who were not part of the clinical or trial team. The expiratory date and date of irradiation of the issued RBC components were checked by the NICU Acting Charge Nurse Managers as part of routine transfusion safety protocols, and then masked to maintain blinding of the attending clinicians, cot-side nurses, researchers and parents.

Intervention and comparator arms

All enrolled infants received transfusion of 15ml/kg of the neonatal red cell component over 3 hours. Infants in the intervention arm received RBC components irradiated on the day of study transfusion, whilst those in the control arm received RBC components which were batch irradiated and subsequently stored for up to 14 days (as per the ANZSBT guidelines[10]). To account for the potentially confounding effects of time since donation and donor characteristics, 4 Pedi-Packs from each adult donor were equally divided between the study arms (2 Pedi-Packs for the intervention and control arms respectively).

Outcomes

As a proof-of-concept study, changes in $crSO_2$ from immediately before to immediately after trial transfusion was selected as the pre-specified primary outcome measure. Pre-specified

205 secondary outcome measures were cFTOE immediately after transfusion, and $crSO_2$ and
206 cFTOE at 24hrs and 5 days after transfusion.
207
208 To obtain the physiological outcome measures, spatially-resolved Near Infrared Spectroscopy
209 (Sensmart Model X-100, Nonin, USA) was applied to measure $crSO_2$ at a sampling rate of
210 0.25Hz for 3hr at the following time points in relation to trial transfusion: immediately
211 before, immediately after, 24hrs and 5 days after. In all cases, a neonatal sensor with light
212 penetration depth of 25mm (EQUANOX 8004CB-NA Advanced, Nonin, USA) was placed
213 on the left forehead avoiding hair and the midline. Peripheral arterial saturation (SpO_2) was
214 recorded concurrently for calculation of cFTOE [$(SpO_2 – crSO_2)/SpO_2$].
215
216 Sample size estimation
217 Based on data from a previously published observational study[11], we estimated *a priori* that
218 a total of 60 transfusion episodes were required to detect a 5% difference in $crSO_2$ response
219 between the intervention and control groups with a 2-tailed unpaired statistical test, 96%
220 power and *p*-value of .05 (G power 3.1). We based this estimation on an unpaired test
221 between study arms in the absence of information about how many infants would ultimately
222 receive more than one transfusion. We predicted that the presence of paired (within-subjects)
223 cases would ultimately increase, rather than decrease, statistical power when modelled
224 appropriately (see below).
225
226 Statistical analysis
227 Data were analyzed with the SPSS v24 software (IBM, USA). A linear mixed model[20] was
228 formulated to quantify mean treatment effects, with associated 95% confidence intervals
229 (95%CI) – Also refer to Data analysis plans in the Supplementary File. Mean treatment

effects were *a priori* defined as the covariate-adjusted difference between study groups in terms of the change from baseline at each follow-up timepoint (immediately after, 24 h after and 5 days after transfusion). In order to avoid "pseudoreplication" in the analysis results,[21] this model took into account that a small number of cases in treatment (n= 5) and control (n=3) had more than one transfusion in the trial period. i.e. the design was unbalanced.[20] Mean treatment effects were covariate-adjusted for baseline (pre) values of the measured outcome as well as gestational age.[22] Sensitivity analyses were also undertaken using unadjusted models and models with added covariates of birth weight and pre-transfusion Hb+/-Hct. These covariates were selected on the basis of hypothesized influence on the study outcome variables. Selection of covariance structure for the model was based on the smallest Akaike's Information Criteria (see supplementary data analysis file). Normal distribution of model residuals was confirmed using a histogram.

Ethics

Prospective approval for the trial was granted by the Human Disability and Ethics Committee of New Zealand (HDEC Ref: 17/CEN/202).

RESULTS

A total of 61 infants met inclusion criteria and were considered for non-urgent RBC transfusion by the attending clinical team between 1st December 2017 and 30th November 2018. Of these, 42 infants (69%) were enrolled in the trial. Reasons for exclusion of eligible infants are listed in Figure 1. In the enrolled infants, a total of 64 transfusion episodes were randomized as per the trial protocol. No infant received more than 3 trial transfusions. Four transfusion episodes (6%) were lost to follow up (Figure 1) and were excluded from the final analysis. The commonest reason for loss to follow up was development of signs of sepsis

needing broad spectrum antibiotics during the 5-day follow up (n=3). No infants had sepsis confirmed by a positive blood culture. These episodes of presumed sepsis were not felt by clinicians to be related to the transfusion of RBC components.

Characteristics of infants receiving trial transfusions are shown in Table 1. Mean baseline values of gestational age, postnatal age, birth weight, weight at the time of trial transfusion, hemoglobin count, hematocrit ratio, baseline oxygenation kinetics, or age of RBC components since donation were similar between the infants randomized to treatment and control groups (Table 1). Mean (range) age of RBC components since irradiation in the control group was 9 (3 – 14) days.

The main effect for treatment across all follow-up time-points was 2.1% (95%CI: 1.6-2.7%, P<0.0005). The treatment x follow-up time interaction was not statistically significant, indicating relatively consistent mean treatment effects at each follow-up time-point (P=0.61). Compared with the control group, infants receiving freshly irradiated RBCs showed a higher covariate-adjusted mean $crSO_2$ immediately after transfusion of 2.0% (95%CI: 1.2 to 2.8%) (Figure 2). Compared with control, in infants receiving freshly irradiated RBCs the post transfusion increase in covariate-adjusted mean $crSO_2$ was sustained at 24hrs (2.4%, 95%CI: 1.8 to 3.1%) and 5 days (2.0%, 95%CI: 0.8 to 3.2%). However, there remained negligible changes in $crSO_2$ in infants receiving standard RBCs over the post-treatment follow-up time-points (Figure 2). Mean treatment effects at each follow-up timepoint were similar in magnitude in the unadjusted models, and mean treatment effects at each follow-up time-point were, again, statistically significant (Supplementary file).

For cFTOE, the main effect for treatment across all follow-up time-points was statistically significant (P<0.0005) and amounted to a reduction of 0.027 (95%CI: 0.017-0.037). The treatment x follow-up time interaction was not statistically significant, indicating relatively consistent mean treatment effects at each follow-up time-point (P=0.93). Compared with the control group, transfusion of freshly irradiated RBCs was associated with a statistically significant covariate-adjusted mean reduction in cFTOE immediately after transfusion of 0.025 (95%CI: 0.011 to 0.039) and at 5 days of 0.028, 95%CI: 0.013 to 0.043). In infants receiving standard RBCs there were negligible differences in cFTOE at these timepoints (Figure 2).

DISCUSSION

This is the first RCT to quantify the mean treatment effects of storage on oxygen delivery capacity of gamma-irradiated transfused RBCs. Our study findings indicate that storage of irradiated RBCs within the ANZSBT recommended timeframe (<14 days), significantly reduces oxygen delivery capacity of transfused RBCs. Furthermore, the observed difference in oxygen kinetics was maintained up to 5 days after transfusion, indicating that, contrary to previous suggestions, function of transfused RBCs does not recover *in-vivo*.[23, 24]

To date, clinical trials addressing the efficacy and safety of storage of RBCs have focused almost exclusively on *time since donation*.[25, 26] However, it is plausible that this timeframe is not the best indicator of structural, biochemical and functional degradation of stored RBCs.[27] Gamma irradiation is associated with an exponential acceleration in red cell hemolysis with associated increases in extracellular potassium and free-iron, reduced bioactivity of nitric oxide, rheiological changes altering the ability of RBCs to pass through the microvasculature, decreased 2,3-DPG, ATP concentrations, reduced pH, and microparticle and microvesicle

formation (jointly referred to as ‘storage lesion’ formation).[16-18, 28-30] Preclinical studies have demonstrated that, unlike other medical interventions, transfused RBCs are not a functionally homogeneous entity, yet there is a paucity of published data on the effect of processing and subsequent storage of RBC components on their *in-vivo* function which must be urgently addressed.

Preterm infants in NICU represent a unique cohort of medically stable patients with chronic anemia. The causes for anemia of prematurity are multifactorial in nature and include breakdown of fetal hemoglobin following exposure to *ex-utero* ‘oxygen rich’ environment, immature haemopoietic system in the context of rapid postnatal growth, iatrogenic blood loss, nutritional deficiencies and chronic inflammation.[31, 32] While optimal transfusion thresholds for anemia of prematurity are currently under review, it is generally accepted that these infants require so-called ’top-up’ transfusions for stable oxygen delivery to vital organs during crucial phases of growth and neurodevelopment.[33, 34] Whilst commonly adopted transfusion-related trial outcome measures capture important variables such as all-cause mortality and multi-organ dysfunction[35, 36], they are less informative on the physiological efficacy of RBC transfusion. We suggest that a more direct measure of *in-vivo* oxygen kinetics, using non-invasive cerebral regional oximetry, provides a valuable insight into function and efficacy of transfused RBCs.

The current study was conducted at a center practicing high transfusion thresholds adopted from the PINT trial. Previous studies using NIRS have demonstrated that pre-transfusion hemoglobin and hematocrit counts are correlated inversely with the magnitude of changes in cerebral oxygenation following RBC transfusion.[37] At high transfusion thresholds in preterm infants small or no changes in cerebral oxygenation were observed following transfusion,

indicating that changes in oxygen delivery capacity may be compensated by cardiovascular adaptation at these thresholds.[38] Our findings of small increases in cerebral oxygenation, both in the freshly irradiated and the irradiated and stored arms (2.0% and 0.2% respectively), are consistent with previously published data.

We observed no substantial difference in demographic variables and hematological parameters between the freshly irradiated and the irradiated and stored arms. Additionally, we accounted for the potential confounder of donor characteristics by equally allocating single donor Pedi-Packs between the two study arms. We also analyzed our data with a covariate-adjusted statistical model. Of note, the age of red cell components since donation was shorter for the freshly irradiated group although this difference did not reach statistical significance (10 days vs. 13 days, $p = 0.08$). It is plausible that unblinded Blood Bank staff may have had a natural bias towards selecting red cell components with shorter shelf life for the intervention arm. However, given that the age of red cell components since donation has been shown to have a negligible impact on oxygen kinetics or clinical outcomes, we believe this potential difference would not have altered our trial outcomes. We therefore attribute the difference in oxygen kinetics following trial transfusions in our randomized trial to the practice of storage following gamma-irradiation.

We acknowledge that our study has a number of limitations. As a physiological study, clinical significance of the small changes in cerebral oxygenation kinetics are not fully understood. Currently, clinical trials are ongoing to determine whether reduction in cerebral hypoxia and hyperoxia burdens could improve long-term outcomes in preterm infants.[39-41] Mean increase in cerebral oxygenation over the 5 days post-transfusion by about 2.0% was smaller than our anticipated change of 5.0%. It is possible that these changes may

preferentially benefit critically ill infants than those with chronic anemia. However, due to logistical challenges 'on-demand' irradiation may not be suitable for those who require urgent RBC transfusion. The current study excluded those who required mechanical ventilation or had significant neonatal co-morbidities such as sepsis. In light of TOP and ETTNO trials favoring restrictive transfusion practice in preterm infants[42, 43], a larger clinical trial is required to re-examine the effect of irradiation practice on cerebral oxygenation kinetics and clinical outcomes in this vulnerable patient group.

Nevertheless, our proof-of-concept study highlights new evidence that irradiated and stored RBCs function differently to freshly irradiated RBCs *in-vivo*. Based on the current study findings, we postulate that those who are transfusion dependent for chronic anemia, including preterm infants, may benefit from freshly irradiated RBC components as this may confer superior oxygen delivery to vital organs. 'On demand' irradiation of RBC components prior to transfusion is still within the safety framework of current international guidelines and could be considered at institutions where this is practicable. However, long-term clinical implications of our findings warrant further investigation.

Conclusion

In the current study transfusion of freshly irradiated RBCs conferred a small advantage in cerebral oxygenation that persist for at least 5 days post-transfusion compared to transfusion of irradiated and stored RBC components as per the ANZSBT guidelines. 'On demand' irradiation of RBC components may be considered at institutions where this is practicable, for this practice remains within the safety framework of current international guidelines.

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Authorship contributions

All authors have approved the final manuscript as submitted.

Access to data statement

MSB and GA had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Figure legends

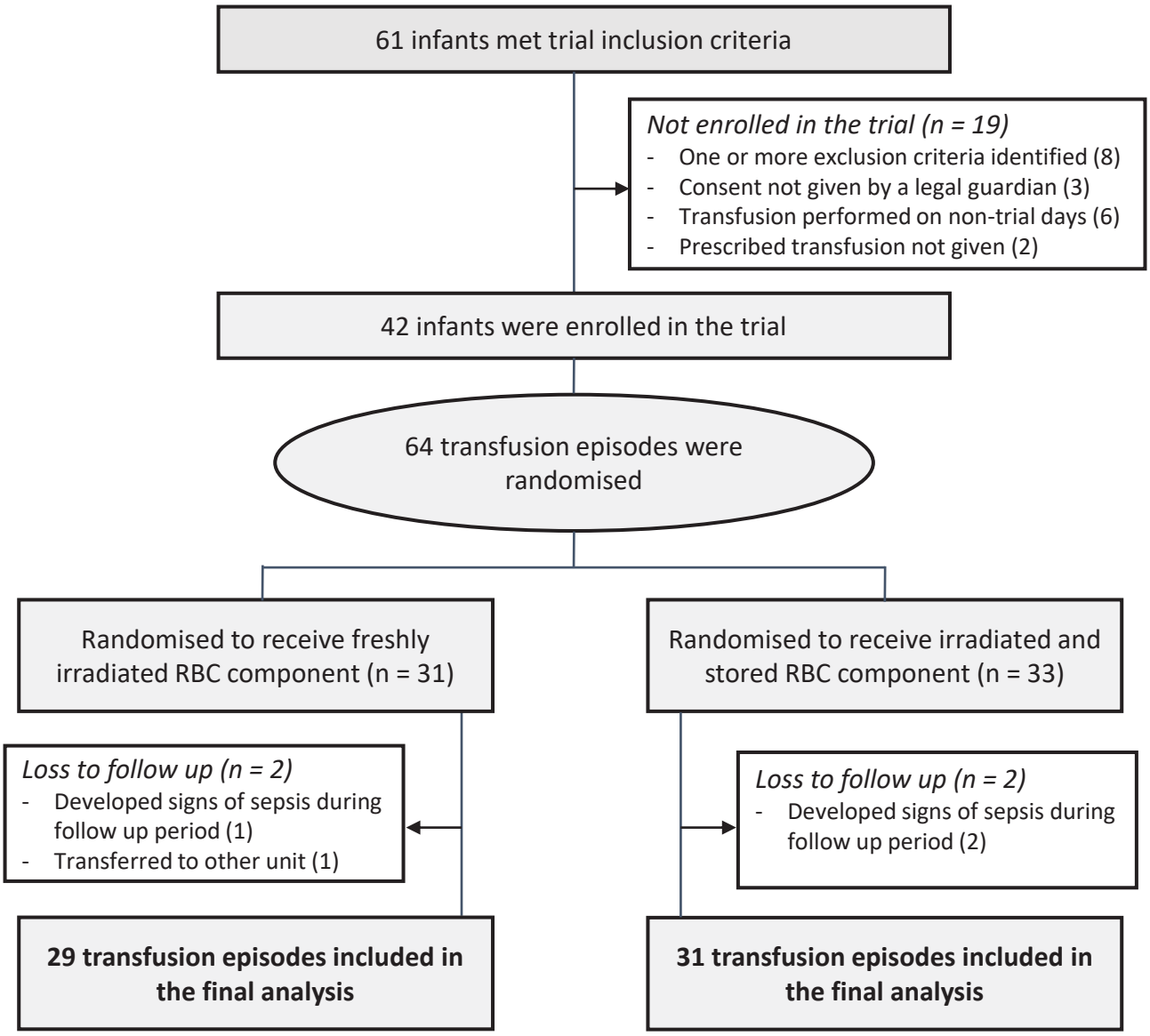
Figure 1: Flow diagram of infants who were considered for inclusion between December 2017 and November 2018 in Wellington NICU, NZ and reasons for exclusion.

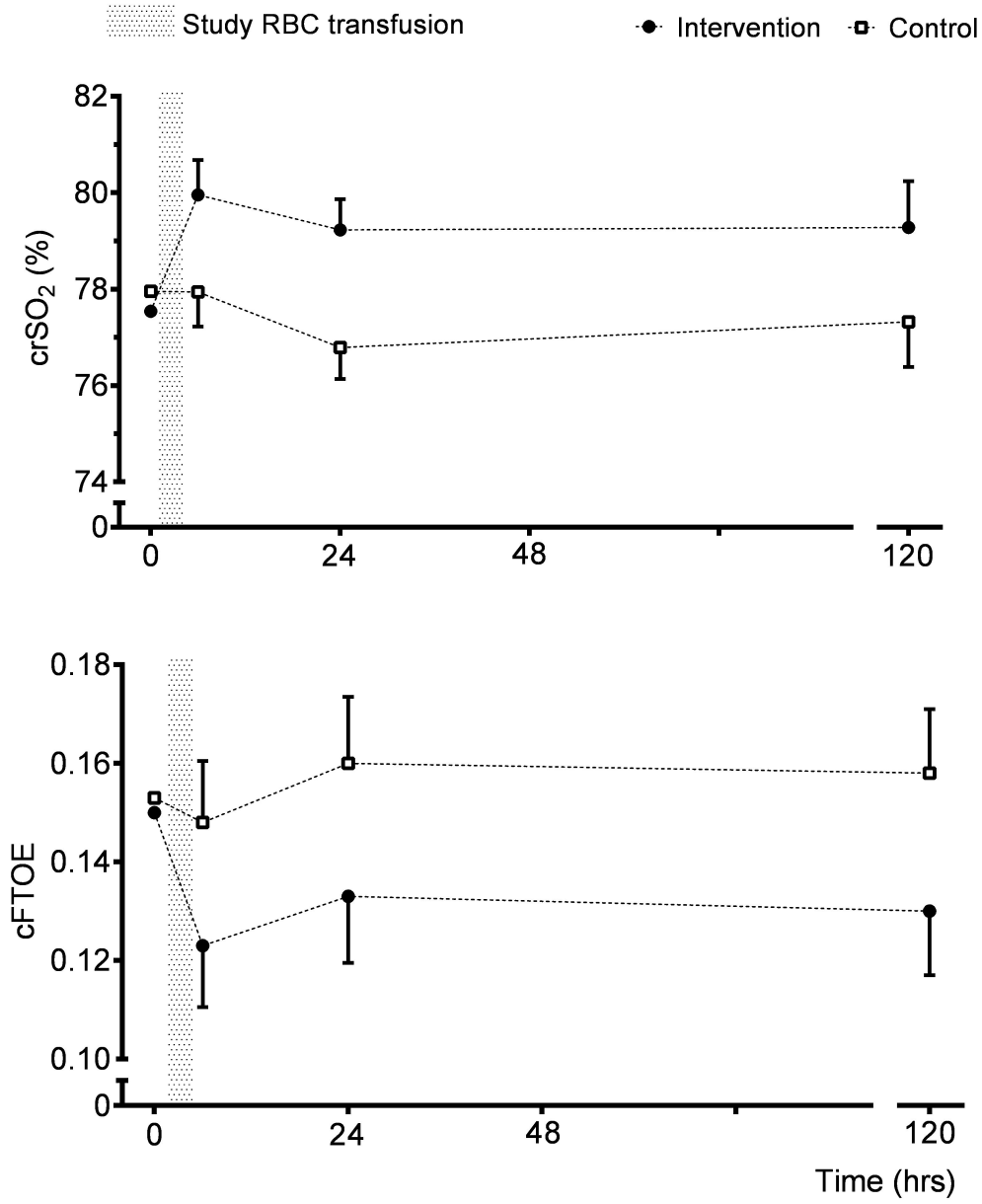
Figure 2: Comparison of freshly irradiated RBCs and control (irradiated and stored) RBCs on cerebral regional oxygenation ($crSO_2$) and cerebral fractional tissue oxygen extraction (cFTOE). A sustained increase in $crSO_2$ and reduction in cFTOE up to 5 days after transfusion were observed in infants who received freshly irradiated RBCs. Negligible changes in $crSO_2$ or cFTOE was observed at any of the time points in infants who received control RBCs. Data are presented as unadjusted means ± 95% confidence interval, apart from the baseline (zero timepoint) values which were used as a covariate in the statistical model.

Table 1: Participants' characteristics prior to trial transfusion

	Red blood cell groups	
Participants' characteristics prior to trial transfusion	Freshly irradiated (n=29)	Irradiated & stored (n=31)
Gestational age (weeks + days)	26+3 (24+0 – 31+5)	26+3 (24+0 – 31+5)
Corrected postnatal age (weeks + days)	32+3 (27+3 – 38+2)	32+4 (28+0 – 38+2)
Birth weight (g)	875 (± 237)	923 (± 269)
Current weight (g)	1572 (± 319)	1602 (± 351)
Haematology		
Haemoglobin (g/L)	86 (± 9)	84 (± 8)
Haematocrit (ratio)	0.26 (± 0.03)	0.26 (± 0.03)
Oxygenation kinetics		
Cerebral regional oxygenation (%)	77 (± 3)	78 (± 3)
Peripheral arterial saturation (%)	91 (± 3)	92 (± 3)
Fractional tissue oxygen extraction (ratio)	0.15 (± 0.04)	0.15 (± 0.03)
Age of RBC components		
Since donation (days)	10 (± 4)	13 (± 5)
Since irradiation (days)	0	9 (± 3)

Data are presented as mean (± SD) for continuous variables, except for gestational and postnatal age which are presented as mean (range).


61 infants met trial inclusion criteria
Not enrolled in the trial (n = 19)
- One or more exclusion criteria identified (8)
- Consent not given by a legal guardian (3)
- Transfusion performed on non-trial days (6)
- Prescribed transfusion not given (2)
42 infants were enrolled in the trial
64 transfusion episodes were randomised
Randomised to receive freshly irradiated RBC component (n = 31)
Randomised to receive irradiated and stored RBC component (n = 33)
Loss to follow up (n = 2)
- Developed signs of sepsis during follow up period (1)
- Transferred to other unit (1)
Loss to follow up (n = 2)
- Developed signs of sepsis during follow up period (2)
29 transfusion episodes included in the final analysis
31 transfusion episodes included in the final analysis


Study RBC transfusion
Intervention
Control
82
80
78
76
74
0
crSO2 (%)
0
24
48
120
0.18
0.16
0.14
0.12
0.10
0
cFTOE
0
24
48
120
Time (hrs)