Near **I**nfrared spectroscopy for **M**onitoring brain **O**xygenation: a single-centre single-blind randomised controlled trial of freshly ir**rad**iated versus standard red cell transfusion for treatment of anaemia of prematurity (**NIMO-Rad**)

– Study Protocol –

Investigators:

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Trial site: Neonatal Intensive Care Unit and Blood Bank, CCDHB

Study type: Prospective, single-centre, randomised controlled trial (RCT)

Background:

Premature infants require frequent blood sampling but their immature bone marrow produces red blood cell at a rate slower than that of their term-born peers. Consequently, anaemia is prevalent in this patient cohort ('anaemia of prematurity') and this is routinely treated with non-urgent transfusion of packed red blood cells.

Premature infants are intrinsically immunocompromised and ensuring the safety of blood products given to them is essential. Currently the internationally accepted method of preventing transfusion-associated graft-versus-host disease, a rare but potentially life-threatening complication of transfusion in immunocompromised patients, is to irradiate leuko-reduced packed red blood cells prior to transfusion. Gamma wave irradiation effectively inhibits proliferation of any residual lymphocytes. However, it also alters the intra- and extracellular biochemical composition of packed red blood cells and accelerates haemolysis and cell death, limiting their viable lifespan(1-4).

The Australian and New Zealand Society of Blood Transfusion guidelines recommend that irradiated red blood cells can be stored for up to 14 days(5). This

recommendation is based on previously published *in-vitro* studies demonstrating acceptable levels of haemolysis in irradiated packed red blood cells stored for the recommended duration(6). However, currently there is no published *in-vivo* study supporting the safety of this practice or the efficacy of transfusion of stored irradiated red blood cells.

Post-hoc analysis from our recent observational study (HDEC ref: 16/CEN/18) showed a negative correlation between the storage duration following irradiation and the magnitude of improvement in cerebral tissue oxygenation ($crSO_2$) after transfusion. Furthermore, a more pronounced improvement in the frequency and duration of peripheral arterial desaturation was seen in those who received 'fresher' irradiated packed red blood cells (unpublished data). These findings suggest that storage of irradiated red blood cells may have a detrimental effect on the *in-vivo* oxygen carrying capacity of transfused red blood cells, and that transfusion of stored irradiated red blood cells may be less efficacious than transfusion of freshly irradiated red blood cells in premature infants.

The post-hoc analysis was limited by the small sample size (n=24), and by the fact that no participant received packed red blood cells that were stored for less than 6 days following irradiation. Therefore, this potentially clinically significant finding warrants further investigation, and we propose to conduct a prospective, single-centre, randomised controlled trial of freshly irradiated versus standard red cell transfusion for treatment of anaemia of prematurity.

Objectives:

To determine whether premature infants with anaemia of prematurity (AoP), when transfused with red cell components irradiated on the day of transfusion, have a different response in the cerebral and peripheral oxygenation levels, compared with those infants receiving red cell components irradiated in accordance with standard NZBS protocols based on the current Australian and New Zealand Society of Blood Transfusion (ANZSBT) guidelines for the prevention of transfusion associated graft versus host disease (TA-GvHD) published in 2011.

Study methods:

Informed parental consent will be obtained prospectively in all cases.

Inclusion and exclusion criteria:

Preterm infants (<34 weeks) who are at least 14 days old will be considered for recruitment into the study if the clinical team in Wellington Neonatal Intensive Care Unit (NICU) makes a decision to give non-urgent transfusion for treatment of anaemia of prematurity (AoP).

Patients will be excluded from the study if they satisfy the following criteria:

- Urgent blood transfusion is required
- Mechanically ventilated at the time of transfusion
- Undergoing treatment for systemic infection with broad spectrum antibiotics
- Receiving medical treatment, or are awaiting surgery for a haemodynamically significant patent ductus arteriosus (PDA)

- Undergoing any other planned surgical intervention within 5 days of the blood transfusion episode to be studied
- Significantly oedematous

Intervention

Participating infants will be randomised to the intervention or control groups as follows:

> *Intervention*: Infants will receive 15ml/kg of appropriately cross-matched red blood cells **irradiated on the day of blood transfusion**. All other aspects of preparation and storage of red blood cells including the irradiation dose will be as per the departmental guidelines of the CCDHB Blood Bank.
>
> *Control*: Infants will receive 15ml/kg of appropriately cross-matched red blood cells, prepared and stored as per the current departmental guidelines of the CCDHB Blood Bank.

Randomisation

Randomisation sequence is generated in advance and is kept in the Blood Bank.

On the day of study transfusion, NICU staff will allocate a study number to the participating infant and will send the NIMO-Rad Study Notification Form (kept in the NIMO-Rad study folder in NICU) to the Blood Banks **before 10:30am**. On receipt of the Study Notification Form, the Blood Bank will send back the Confirmation of Receipt of NIMO-Rad Notification Form to NICU.

Study Notification Forms will be filed in the 'NIMO-Rad folder: Blood Bank copy' kept in the Blood Bank until completion of the study. Confirmation of Receipt forms will be filed in the 'NIMO-Rad folder: NICU copy' kept in NICU.

Re-randomising infants

It is anticipated that some participating infants will receive more than one blood transfusion for AoP during the study period. If the transfusion episodes are more than 7 days apart and parents have agreed for their infants to be re-randomised, each transfusion episode will be treated as a new study.

Outcome measures

The primary outcome measure is the change in cerebral regional tissue oxygenation ($\Delta crSO_2$) and this will be measured non-invasively using the **Near Infrared Spectroscopy** (Nonin SenSmart™ Model X-100) for 3hrs at the following time points in relation to blood transfusion:

- Immediately before
- Immediately after
- 1 day after
- 5 days after

Peripheral arterial saturation (SaO_2) will be monitored concurrently using the Nonin SenSmart™ Model X-100 in order to calculate the fractional tissue oxygen extraction (FTOE), which will account for fluctuations in SaO_2 affecting $crSO_2$.

Cerebral tissue oximetry sensors:
An adhesive or non-adhesive regional oximetry sensor will be applied to the fronto-parietal area of the infant's head (EQUANOX Advanced 9004CB Paediatric/Neonatal). Non-adhesive sensors will be preferentially used if the infants' skin is considered fragile, or if they can be held in place easily by CPAP hats already in use as part of routine clinical care of the participating infants.

Additional investigations
Blood test prior to blood transfusion (<24hrs). It is a standard practice for infants receiving blood transfusion for AoP to have venous full blood count (FBC) taken prior to transfusion as part of the clinical decision-making process. However, if venous FBC is not performed as part of routine clinical care (e.g. decision to give blood transfusion is based on capillary blood test results) then a venous blood sample will be taken by a skilled investigator/clinician using appropriate analgesia (e.g. sucrose) at the same time as the transfusion cannula is inserted, to avoid additional discomfort or bruising. A total of 0.35ml (less than $1/10^{th}$ of a teaspoon) is required for this test.

Statistical analysis:

Based on the post-hoc analysis from the study we recently completed in Wellington NICU (HDEC ref: 16/CEN/18), we calculated that a sample size of at least 60 infants is required to detect a difference of 5% in cerebral tissue oxygenation immediately after transfusion between the intervention and control groups, with power of 0.96 and p-value of 0.05 (t-test, G power 3.1).

References:

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