Research Protocol

1. Project Details:

Protocol/Research Project Title:	Associations of nadir haemoglobin level and red blood cell transfusion with mortality and length of stay
Protocol Number (Version and Date):	Version 2: Version Date: 08/11/2017
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2. Synopsis

Anaemia is a global public health problem[1,2] and is common in developing and developed countries, particularly among hospitalized patients. The World Health Organization defines anaemia as a haemoglobin concentration below 130 g/L in males and 120 g/L in females. A recent systematic review and meta-analysis on preoperative anaemia and outcomes after cardiac and non-cardiac surgery reported 39% of patients were admitted anaemic. These anaemic patients had three-fold higher odds of mortality, four-fold higher odds of acute kidney injury and twice the odds of infection. Not surprisingly, anaemia was also associated with increased transfusion, with anaemic patients five-times more likely to receive a red blood cell transfusion.[3]

As these results indicate, red blood cell transfusions are often administered to correct low haemoglobin levels. However, correcting anaemia with transfusion is problematic as red blood cell transfusion has a dose-dependent association with increased mortality, morbidity, hospital and ICU length of stay, readmissions, and cost.[4,5] Large risk-adjusted observational studies demonstrate that even transfusing a single unit of red blood cells is associated with increased adverse outcomes in surgical patients,[6,7] thus recommending caution before transfusing.

In an attempt to find the “optimal” transfusion threshold, many randomized controlled trials have investigated the difference between using restrictive pre-transfusion haemoglobin thresholds compared with liberal pre-transfusion haemoglobin thresholds. A restrictive strategy refers to a policy of administering red blood cell transfusions at comparatively lower pre-defined haemoglobin levels, with the goal of minimizing the use of blood.[8] Though not always the case, restrictive transfusion

thresholds are often defined as haemoglobin levels between 70 g/L and 80 g/L and liberal transfusion thresholds are commonly defined as haemoglobin levels between 90 g/L and 100 g/L.

A recent systematic review and meta-analysis published in the Cochrane Library concluded there is no difference in morbidity or mortality between restrictive and liberal transfusion strategies.[9] However, these trials are often confounded by transfusions administered pre-randomization,[10] a lack of comparable transfusion dosing regimens between studies, and at times small differences in actual mean pre-transfusion haemoglobin levels between control and intervention arms. In addition, these randomized controlled trials still leave many important questions unanswered. For example, are haemoglobin thresholds lower than 70 g/L just as effective as haemoglobin levels higher than 70 g/L? Some have suggested lower haemoglobin thresholds may be just as effective.[11] Interestingly, lower thresholds are already applied in clinical practice in challenging patient populations.[12-15]

Other studies have investigated mortality in patients with very low haemoglobin levels who do not receive red blood cell transfusions.[16,17] These studies found that each 10 g/L decrease in haemoglobin below 80 g/L was associated with a 1.8 to 2.5 times increased odds of mortality, with sharper increases in mortality observed when the haemoglobin fell below 50 g/L.[18] However, these studies do not address what effect red blood cell transfusion would have on outcomes at various nadir or pre-transfusion haemoglobin levels.

Additionally, while many studies have investigated the effect of anaemia on outcomes independent of red blood cell transfusion, and the effect of red blood cell transfusion on outcomes independent of anaemia, very few investigate if and how these two independent risk factors interact with each other.

The aim of this study is to determine what relationship red blood cell transfusion has with mortality and length of stay at various levels of nadir haemoglobin. To achieve this objective a retrospective observational study will be conducted, involving three hospitals from Western Australia. The study will include all elective and emergency surgical admissions over a 9 year period (July 2008 – June 2017).

3. Objectives

The specific objectives addressed by the study are:

1. Is there an association between in-hospital mortality, 30-day mortality, and hospital length of stay and red blood cell transfusion at various levels of nadir haemoglobin?

2. Is there an effect modification between levels of nadir haemoglobin and presence of a red blood cell transfusion on in-hospital mortality, 30-day mortality, and length of stay?

4. Benefits

This study proposal includes important strengths. First, it involves admissions over a 9 year period from three hospitals across the largest surgical specialties responsible for transfusion and therefore will provide a large sample size for analyses. Second, this study will be conducted in a health service with low mean pre-transfusion haemoglobin thresholds, indicating there are likely more transfused and non-transfused patients at comparatively lower nadir haemoglobin levels available for analyses. Given these two important characteristics this study will likely have more power to detect differences in outcomes between transfused and non-transfused patients at lower levels of haemoglobin than previously reported.

Third, while some studies have investigated the impact of nadir haemoglobin and transfusion on outcomes, they focused on specific patient groups.[19-22] This study will focus on the major surgical groups responsible for the majority of red blood cell transfusion and thus will increase the generalizability of results to other tertiary hospitals.

Thus the results of this proposed study will make an important contribution to the current body of literature and may guide future decisions on what transfusion thresholds researchers select for randomized controlled trials, or whether these randomized trials are even asking the correct research questions.

5. Literature Review

Rhode and colleagues[23] pooled the results of 18 trials investigating what effect restrictive pre-transfusion haemoglobin thresholds have on hospital acquired infections. They found patients assigned to restrictive transfusion thresholds had a significantly lower risk of infection when compared to patients assigned to liberal transfusion thresholds (risk ratio 0.82, 95% CI 0.72 to 0.95). This significant risk reduction remained unchanged in a sub group analysis of trials using leukocyte reduced red blood cells.

Curley and colleagues[24] conducted a systematic review and meta-analysis of randomized controlled trials comparing restrictive to liberal transfusion haemoglobin thresholds in patients undergoing cardiac or vascular surgery. In the pooled results from seven studies they report no statistically significant difference between the two groups in terms of mortality, length of stay or other outcomes. One limitation is the small difference in haemoglobin thresholds between the restrictive and liberal protocols studied, often a 10 g/L difference. The impact of this small difference is evident in the mean units of red cells transfused between groups, approximately half a unit, with patients in the liberal arm transfused a mean 1.9 red cell units and patients in the restrictive arm a mean of 1.4 units.

Docherty and colleagues also conducted a systematic review and meta-analysis[25] looked at the effect of restrictive versus liberal transfusion strategies in patients with cardiovascular disease undergoing non-cardiac surgery. The main outcome investigated was 30-day mortality and cardiovascular events. The authors found no statistically significant difference in 30-day mortality between groups (risk ratio 1.15, 95% CI 0.88 to 1.50). However, there was a significant increased risk of acute coronary syndrome in patients assigned to a restrictive threshold when compared with patients assigned to a liberal transfusion threshold (risk ratio 1.78, 95% CI 1.18

to 2.70). As is common with other the other meta-analyses reviewed, one limitation of this review is the overlapping transfusion thresholds studied. Of the studies pooled the cut-off values for the restrictive transfusion thresholds ranged from 70 to 97 g/L, the cut-offs for the liberal transfusion thresholds ranged from 90 to 113 g/L.

The largest of these systematic reviews and meta-analyses to date is the study by Carson and colleagues[9] published in the Cochrane Database of Systematic Reviews. The review's aim was to compare mortality and other outcomes in patients randomized to restrictive versus liberal red blood cell transfusion thresholds. The authors found no statistically significant difference in hospital mortality (risk ratio 0.86, 95% CI 0.73 to 1.01) or 30-day mortality (risk ratio 0.97, 95% CI 0.81 to 1.16), or any difference in the other outcomes measured. Hospital length of stay was recorded as part of the review, however the results were not included in the analysis. One of the limitations of this study is that the majority of randomized controlled trials pooled applied a restrictive transfusion threshold of 80 g/L or higher. In clinical practice many hospitals report lower mean pre-transfusion haemoglobin thresholds, and when applied with other strategies to appropriately manage a patient's own blood (referred to as Patient Blood Management) this is often associated with improved outcomes.[12-15,26]

At face value, the results of the three systematic reviews and meta-analyses investigating mortality tell a consistent story: no significant difference in mortality when restrictive transfusion thresholds are applied compared with liberal transfusion thresholds. These results suggest hospitals adopting restrictive transfusion threshold policies would likely reduce transfusions, reducing patient exposure to transfusion risk, with no evidence of increased mortality.

However, another systematic review and meta-analysis investigated the impact of applying more restrictive pre-transfusion haemoglobin thresholds. Salpeter and colleagues[27] pooled results from trials using a restrictive haemoglobin threshold of less than 70 g/L and compared the outcomes between the restrictive and liberal transfusion arms. They found that in trials applying a lower restrictive threshold (less than 70 g/L), patients assigned to the restrictive arm had significantly lower in-hospital mortality (risk ratio 0.74, 95% CI 0.60 to 0.92) and total mortality (risk ratio 0.80, 95% CI 0.65 to 0.98), when compared to liberal transfusion group. A separate analysis was conducted to study the outcomes in trials applying a higher "restrictive" transfusion threshold, between 75 g/L and 100 g/L. The authors found no significant difference in in-hospital mortality (risk ratio 0.65, 95% CI 0.37 to 1.15) or total mortality (risk ratio 1.03, 95% CI 0.81 to 1.31). This important difference may explain why some randomized controlled trials report reduced mortality with restrictive transfusion thresholds compared to liberal thresholds, while others report no significant difference.

Still these five systematic reviews and meta-analyses of randomized controlled trials leave important questions unanswered. While patients assigned to restrictive transfusion threshold arms are less likely to receive a transfusion the fact remains that among these trials nearly half of patients in the restrictive group still receive transfusions. In one meta-analysis 48% of the restrictive group were transfused compared with 84% in liberal group.[9] Some have used the results from these trials to draw conclusions about whether red blood cell transfusions are harmful.[10,24] However by design, results from these trials alone cannot be extrapolated to

describe the effect of transfusion versus no transfusion on mortality or other outcomes. Further limiting the interpretation of results is the fact that many large trials do not include all the units of blood transfused during hospital admission, as blood transfused prior to randomization is often overlooked.[10]

Some observational studies in the surgical setting have sought to address this gap in the literature by comparing outcomes between patients transfused and those not transfused across various levels of anaemia. For example, in patients undergoing cardiac surgery, Shaw and colleagues[22] used a propensity-matched analysis to compare the outcomes of patients transfused to those not transfused at four preoperative haematocrit levels (a measure of anaemia). They found that transfused patients had higher mortality rates than those not transfused, at all levels of haematocrit. Kougais and colleagues[21] studied the impact of postoperative nadir haemoglobin levels and transfusion on outcomes in patients presenting for elective operations for atherosclerotic vascular occlusive disease at one hospital. They found that neither red blood cell transfusion nor nadir haemoglobin level was associated with the composite end point of death and/or myocardial infarction. Interestingly, there was no significant interaction between transfusion and nadir haemoglobin, suggesting transfusion had the same effect on outcome regardless of the nadir haemoglobin level.

While these studies provide some insights into differences between outcomes in transfused and non-transfused anaemic patients they also leave many unanswered questions. For example, what are the outcomes in patients with haemoglobin thresholds lower than 70 g/L?

Two case series have investigated mortality in patients with very low haemoglobin levels who do not receive red blood cell transfusions.[16,17] These studies found that each 10 g/L decrease in haemoglobin below 80 g/L was associated with a 1.8 to 2.5 times increased odds of mortality, with sharper increases in mortality observed when the haemoglobin fell below 50 g/L.[18] However, these studies do not address what effect red blood cell transfusion would have on outcomes at various nadir haemoglobin levels.

6. Research Plan

6.1. Description of population and sample.

This project will include all adults admitted as elective or emergency cases for selected surgical specialties to three hospitals in Western Australia between July 2008 and June 2017. Local studies show that the following six specialties account for the majority of blood transfused in surgical patients.[26]

- Orthopaedics
- Gastrointestinal Surgery
- Vascular Surgery
- Urology
- Cardiothoracic surgery
- Neurosurgery

Any patients transfused fresh frozen plasma, platelets, and cryoprecipitate will be excluded from the study. This is to reduce the possibility of confounding as the purpose of this study is to focus on nadir haemoglobin levels, red blood cell transfusion and their effect of mortality and length of stay.

Also excluded are patients receiving a massive transfusion (defined as 5 units of red blood cells in 4 hours or 10 units in 24 hours), patients transfused any blood products 90 days prior to admission, patients with a length of stay of less than 2 days (same day and overnight admissions), patients under the age of 18 years at admission, and patients with no haemoglobin results.

6.2. Description of data gathering methods, including definitions of variables.

The majority of data needed for this project will be sourced from the *Western Australian Patient Blood Management System*. The advantages of sourcing the majority of data from one system include consistent data collection processes and classification of variables.

The exposure variables of interest are degree of anaemia and red blood cell transfusion. Anaemia will be defined using the nadir haemoglobin result during admission for those not transfused and the pre-transfusion haemoglobin result in patients transfused, as described previously.[20] The pre-transfusion haemoglobin will be the closest level measured prior to the first red blood cell transfusion administered. Fridges are available for storing blood in theatres and because of this the timing of red blood cell transfusion in the operating room is unknown. Therefore for patients transfused in the operating room the nadir haemoglobin result will be used as a surrogate for the pre-transfusion haemoglobin result. Haemoglobin levels will be grouped into the following categories: below 50 g/L, 50-59 g/L, 60-69 g/L, 70-79 g/L, 80-89 g/L, 90-99 g/L, 100 g/L or over. These cutoffs were selected after reviewing similar studies.[17,20,28]

The main outcome variables of interest are mortality and hospital length of stay of current admission. For the mortality analysis, both in-hospital and 30-day all-cause mortality will be assessed. In-hospital and 30-day mortality will be calculated from date of death recorded in hospital patient admission systems (TOPAS/WebPas). Potential confounders collected will include age at admission, sex, hospital, discharge year, admission type (emergency or elective), clinical specialty, and patient comorbidities.

Patient comorbidities will be assigned a score based on the Charlson Comorbidity Index derived from ICD-10 codes for the comorbidities identified.[29] To ensure the Charlson score represents patient comorbidities on admission, hospital-acquired complications will be excluded from the calculation.

Other data items to be collected include admission date, discharge date, discharge specialty, diagnosis related group, hours in ICU, presence of a red blood cell transfusion (yes/no), had a massive transfusion (yes/no), and number of red blood cell units transfused.

6.3. Validity and reliability of data

The research methodology for this project is based on methodologies used in similar studies.[17,20,21] To improve the validity of results, potential confounding variables will be statistically adjusted for in the analysis, and inpatients receiving transfusions other than red blood cells will be excluded.

This study will investigate consecutive adult surgical admissions to three public hospitals for specialties where blood is commonly transfused. Given the study design and the broad inclusion criteria applied, the results are expected to have external validity and are likely generalizable to other surgical inpatients admitted to large teaching hospitals internationally.

Exposure and outcome variables have been carefully selected and are known to be accurately measured. Results from laboratory testing will be used to measure nadir haemoglobin values to accurately define the degree of anaemia. The cut-offs for haemoglobin levels have been selected based on other studies.[17,19]

The in-hospital and 30-day mortality outcome measures selected for this study are well-defined objective measures and will be coded as binary variables (yes/no). Hospital length of stay, also a frequently studied objective outcome measure will be calculated in days from admission to discharge. All outcomes variables will be measured in the same way between the different subgroups.

6.4. Statistical methods.

Exposure and outcome variables will initially be described by univariate and bivariate statistics, the latter using the chi-squared test for categorical variables and independent samples t-test for continuous variables. Logistic regression models will be applied to test the relationship that both red blood cell transfusion and nadir haemoglobin levels have with in-hospital mortality and 30-day mortality. A zero-truncated negative binomial regression model will be applied to the length of stay outcome. All regression models will adjust for the same potential confounders. A robust variance adjustment will be applied to the regression models to account for any potential correlation between multiple admissions for the same patient.

For this analysis we are also interested in whether the presence of a red blood cell transfusion modifies the effect of nadir haemoglobin on in-hospital mortality, 30-day mortality and length of stay. To test any effect modification on outcome an interaction term between nadir haemoglobin level and red blood cell transfusion will be added to the multivariable logistic regression models. Only significant interactions (defined as p-values <0.05) will be reported, with calculated odds ratios for interaction terms presented.

In addition, Kaplan-Meier curves and Cox proportional hazards models will be plotted to show time to death in patients transfused red blood cells when compared to patients not transfused red blood cells across the various nadir haemoglobin levels.

6.5. Sample size estimation.

Given the inclusion and exclusion criteria, there are expected to be between 60,000-70,000 consecutive inpatient admissions in the study cohort.

6.6. Ethical Considerations

Ethical approval for this study will be sought from the Royal Perth Hospital Human Research Ethics Committee (HREC).

Because this study involves accessing a large amount of existing data stored in the Western Australia Patient Blood Management Data System and date of death from the TOPAS/WebPas system. A request for a waiver of consent will be made as part of the ethics application.

It is impractical to seek informed consent given the study is expected to include over 60,000 inpatient admissions over a 9 year retrospective period. Given the data is already collated and stored in a secure manner and will be provided to researchers by a data custodian in initially re-identifiable format (and later made non-identifiable), it is considered to be of negligible risk to patients or their communities.

6.7. Data extraction.

The data extraction process will apply a separation principle with a hospital-employed data manager with usual access to the data sources and not involved in the project or analysis of the data completing the following steps:

1. The data manager will extract and merge the data from the WA Patient Blood Management System and TOPAS/WebPAS.
2. Identifiers will be removed and replaced with a study number (made *re-identifiable*).
    a. The patient identifier (the Unit Medical Record Number) will be replaced with a study number during the extraction process in step 1. As this step is applied during the extraction process the data manager will not see the Unit Medical Record Numbers, however they have the ability to re-identify them.
3. The “re-identifiable” data will be held and used by the CPI and AIs for analysis but they will not have the ability to re-identify them.
4. At the completion of the analysis the data manager will destroy the file linking the patients to their study number rendering the study data *non-identifiable* (anonymisation).
    a. The analysis of data is expected to take between 3-6 months. The data will be made non-identifiable 6 months from extraction. The data

manager will receive instructions to delete the extraction code used 6 months from the date of extraction.

7. Budget

No funding is necessary for this project.

8. References

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