Effects of donor-recipient sex-matched versus sex-mismatched red blood cell transfusion on outcomes in critically ill adult patients

Protocol for a randomized trial

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BACKGROUND

Red blood cell (RBC) transfusions are selected based on **donor and recipient blood group compatibility** without consideration of donor and recipient sex.

- There is new evidence of **biological mechanisms** potentially underlying observations.
- Studies investigating **sex-matched stem cell and solid organ transplants** reported improved outcomes for sex-matched patients.
- A **systematic review**, **meta-analysis,** and a 40,000-**patient exploratory analysis** conducted by our group and others, supported the hypothesis that **sex-matched RBCs improve survival in** recipients over sex-mismatched RBCs.
  - However, the studies' **retrospective** designs poses a limitation to findings.

The recent **iTADs** study, a large randomized controlled trial (RCT) comparing mortality in hospitalized patients receiving **male-only or female-only RBCs** (i.e. male donor to any sex patient, and female donor to any sex patient) concluded **no significant differences in survival.**

METHODOLOGY: a superiority RCT with pragmatic features

INTERVENTION GROUP: donor-recipient sex-matched RBCs.
COMPARATOR GROUP: donor-recepient sex-mismatched RBCs.

via randomization

STUDY OBJECTIVE

Develop a precision transfusion strategy based on **sex-matching of blood donors and recipients,** to minimize adverse effects and **improve patient outcomes** post-transfusion.

PATIENT POPULATION

Adult patients (age ≥18) hospitalized with admission to an eligible **ICU;** undergoing **RBC transfusions** while in the ICU, across any of the **participating sites in Ontario.**

SAMPLE SIZE

The target sample size is **11,082** calculated using:

- **3% absolute risk reduction** rate
- **two-sided** test with **90% power** and **5% type 1 error rate.**

STUDY OUTCOMES

PRIMARY OUTCOME: 30 day mortality.

SECONDARY OUTCOMES: 30-day in-hospital mortality; 90-day mortality; time to 30-day in-hospital mortality; 90-day survival analysis; 90-day in-ICU mortality; time to 90-day in-ICU mortality; hemoglobin increment (per RBC transfusion); need for continuous renal replacement therapy/hemodialysis; ICU/hospital lengths of stay; number/type/volume/dose of transfused product; transfusion reactions; and cost effectiveness measured using the incremental cost per life year saved between sex-matched and mismatched transfusions.

SUBGROUP ANALYSES: Analysis considering sex, age, and dosage effect from the number of sex-mismatched units received.

DATA SOURCES: Patient data will be collected through electronic medical record extraction, and the primary outcome will be obtained from the Institute of Clinical Evaluative Sciences (ICES) database.

RESEARCH QUESTION

In transfused **adult patients** admitted to the **Intensive Care Unit (ICU)**, do **donor/recipient sex-matched RBC transfusions** result in a **lower in-hospital mortality rate** compared to **sex-mismatched RBC transfusions?**

COLLABORATORS:

The **iTADS** study team informed the methods and objectives of the proposed trial in which we aim to conduct a **CIHR-funded** RCT comparing the effect of sex-matched and sex -mismatched RBC transfusions on patient outcomes. **Canadian Blood Services (CBS)** will be a collaborator as the major blood supplier in this study. Results will be disseminated with the help of the **Canadian Transfusion Trials Group (CTTG).**

Canadian Blood Services

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1. BACKGROUND

1.1 WHAT IS THE PROBLEM?

Blood transfusion is one of the most common procedures performed during hospitalization.[1] Approximately 85 million red blood cell (RBC) units are transfused globally each year.[2,3] RBC units are matched for blood groups, but matching for other donor characteristics such as sex is not considered. By the current standard of care, male or female patients can receive RBCs from male or female donors. However, there is concern with regards to donor sex and transfusion risk.[4–7] Plasma from female donors is associated with an increased risk of transfusion related acute lung injury and hypotension;[8,9] sex-mismatched heart transplantation is associated with increased transplant-associated mortality;[10–12] and stem cell transplants from female donors are associated with worse outcomes.[10,13–16]

Anemia is common during critical illness;[17] 20 – 40% of critically ill patients require a mean of 2 – 5 RBC units during admission to the intensive care unit (ICU). Given blood bank inventories, sex-mismatched transfusions are inevitable once a patient receives more than 6 RBC units (>97% chance) (**Figure 1**). The population of transfused adult ICU patients is already at high risk of death, with a 90-day all-cause mortality of 35-37%.[18] Locally, based on historic data, we have found ~30% in-hospital mortality for this population (**Table 1a**), and based on our CIHR-funded multisite pilot data, 29% 30-day and 34% 90-day in-hospital mortality (**Table 1b**). Thus, optimizing supportive care strategies is needed to improve outcomes of this highly vulnerable patient group and a strategy as simple as matching RBC transfusions for donor sex may have significant impact.

1.1.1 Background & Conflicting Data

To support this study, we completed an exploratory analysis,[19] meta-analysis[20] and a CIHR-funded multisite feasibility pilot randomized controlled trial (RCT) (see **Appendix B**).[21] The exploratory analysis included transfusion data linked to donor sex spanning a 6-year period. We analyzed 25,219 transfusion recipients and found significant association between male to female RBC transfusions and death [hazard ratio (HR) 1.31, 95% confidence interval (CI) 1.02-1.69].[19] A trend towards higher mortality was also noted with female to male RBCs (HR 1.13: 95% CI 0.92-1.39), and with sex-mismatched versus sex-matched RBCs overall (HR 1.23: 95% CI 1.04-1.45). These findings suggest that sex-mismatched RBC transfusions may contribute to a higher mortality among ICU patients.

Several observational studies have explored the association between donor sex on recipient mortality after RBC transfusions[4–6,19,22–27] We performed a systematic review and meta-analysis that showed an increased risk of death with sex-mismatched RBC transfusions compared with sex-matched transfusions (pooled HR 1.13: 95% CI 1.02-1.24) (**Figure 2**).[20] Data were derived from five retrospective observational studies (n= 86,737). The certainty of evidence was low due to confounding, selection and reporting

bias of the primary studies; no country has implemented a sex-matched RBC transfusion strategy as a result of these observational data.

Since the meta-analysis was published, additional observational studies have emerged. A large multi-database study explored associations of donor sex, prior pregnancy, and sex-mismatched transfusions on mortality.[28] The authors concluded that transfusion of donor-recipient sex-mismatched RBCs was not associated with increased risk of death; however, subsequent analysis showed increased risk of death with sex-mismatched transfusions.[28] Another study investigated associations between donor-recipient sex and post-transfusion mortality and morbidity in transfused critically ill patients from either male-only donors or from female-only donors.[29] Transfusion of female RBCs to male patients was associated with an increase in ICU mortality compared with transfusion of female RBCs to female patients (OR 2.43; 95% CI, 1.02-5.77).

Finally, a large RCT examined impact of donor sex on mortality after transfusion (iTADS*).* [30–32] The iTADS trial addressed whether transfusion of male-only RBCs (male donor to male or female recipients) was associated with improved survival compared with female-only RBCs (female donor to male or female recipients) in all hospitalized patients. Results showed no important difference in survival with either transfusion strategy. Subgroup analysis suggested a lower risk of death among male patients assigned to female-only RBCs than among those assigned to the male RBC group (hazard ratio [HR], 0.90; 95% CI, 0.81 to 0.99). In addition, among recipients from donors 20 to 30 years of age, there appeared to be a higher risk of death among patients assigned to the female donor group than those in the male donor group (HR 2.93; 95% CI, 1.30 - 6.64), and a post hoc analysis showed fewer deaths among recipients of sex-mismatched RBC transfusions compared with sex-matched (unadjusted HR, 0.89; 95% CI, 0.82 - 0.96). Further discussion on this trial is in **Section 1.2** below.

1.1.2 Biological Hypotheses:

Important biochemical and biophysical differences between male and female RBCs include differences in hematocrit, cell volume, hemolytic propensity, deformability and mean cell hemoglobin content.[33–36] In addition, donor sex, particularly during the reproductive years (16 – 35), result in significant differences in the age distribution and physical characteristics of the young and old RBCs in circulation in male and female blood donors.[37–39] The impact of blood donor demographics, including sex, on patient outcomes is an emerging field of study with important potential for broad impact. To support our working hypotheses that sex-mismatched RBC transfusions may be harmful:

1.1.2a Why male RBC transfusions might be harmful to female recipients:

Transfusion of biologically older RBCs from male donors with a high hemolytic propensity, lower deformability and higher densities may critically affect oxygen delivery, coagulation and vasculopathy when transfused to female recipients.[40] The mechanisms underlying the reduced sensitivity of female RBCs to storage-induced stress can be partially ascribed to the increased proportion of more robust, biologically “young” subpopulations of RBCs in female donors, and the differential effects of testosterone, progesterone, and estrogen

on the hemolytic propensity of RBCs.[41] Larger amounts of hemoglobin, particularly free hemoglobin, found in male RBC products may overwhelm the haptoglobin scavenging capacity of the reticuloendothelial system in female recipients.[42,43] This overwhelmed pathway for scavenging of the toxic free hemoglobin / iron in circulation results in a reduced nitric oxide (NO) bioavailability, which can in turn lead to endothelial dysfunction, platelet aggregation, and oxidative injury.[42,44–46] RBC units from male donors have been shown to have elevated levels of immature RBCs[47] which have been shown to be immunosuppressive[48–52] and potentially enhance the erythrophagocytosis of mature RBCs and further release of hemoglobin into circulation.[53] In addition, hemodynamic changes in blood flow due to the transfusion of male RBC (higher ratio of older, denser, less deformable cells) could affect the margination of red cells in circulation of a female recipient, thus affecting platelet and white blood cell endothelial interactions.[54] It has been recently shown[55] that single unit sex-mismatched compared to sex-matched RBC transfusion in ICU patients resulted in elevated iron levels, enhanced endothelial activation and increased mortality which supports this hypothesis.

1.1.2b Why female RBC transfusions might be harmful to male recipients:

Characteristics of the RBC sub-populations in male and female donors may have different immunological characteristics. Sub-populations of erythroid precursors in young females and pregnant women are enriched in immunosuppressive cells which have been shown to impair the defense against pathogens in neonates[48–50,56] and cancer patients.[51,52] In a rat model of transfusion, male animals who received female RBCs had elevated markers of vascular injury and more entrapment of RBCs in the lung, liver and spleen.[57] Observational data suggest that male recipients exposed to ever-pregnant female donors were at higher risk of mortality compared to female recipients.[6] Further research is required to determine whether male recipients of female RBCs are at increased risk of morbidity and mortality from immunosuppressive side effects or from differences in the deformability/density/hemoglobin content of female RBCs.

1.2 WHAT THIS STUDY WILL ADD TO THE CURRENT KNOWLEDGE

Findings from observational studies are inconsistent. The iTADS RCT results are intriguing and require confirmation especially in ICU patients who have a much higher baseline risk of death than hospitalized patients, and for whom a procedural blood bank change could have a significant impact. iTADS did not have the power to detect potential differences in patients admitted to the ICU. The subgroup analyses of iTADS were found to have low credibility based on an external independent evaluation using the Instrument to assess the Credibility of Effect Modification Analyses (ICEMAN); this external analysis was performed by 2 independent methodology experts (unpublished).[58] Our proposed trial is different from iTADS because we plan to examine ICU patients only with increased RBC exposures and higher event rate and we specifically address matched vs. mismatched (not male vs. female). We have worked closely with the iTADS study team who, given our different aims and study design, are supportive of the Sex Matters design and have contributed significantly to study design. Changing policy to a sex-matched

transfusion strategy would be a major change for hospitals and blood suppliers and requires strong evidence from RCTs to support such a change.

Results of this RCT will inform policy on blood transfusion practices. If mortality is shown to be reduced with sex-matched transfusions, then a policy change can be implemented at the blood bank level. This will require re-evaluation of how blood bank inventories are managed and assigned. Results will be shared with the Canadian blood suppliers (Canadian Blood services [CBS] and Héma Québec) and disseminated with the help of the Canadian Transfusion Trials Group (CTTG) Patient Engagement Working Group and Knowledge Translation Working Group.

2. OVERALL GOAL

The goal of this study is to address the following question: *In adults admitted to the ICU who require RBC transfusions, do donor-recipient sex-matched RBC transfusions result in improved 30-day mortality compared with sex-mismatched RBC transfusions?* The outcomes of this prospective multi-center blinded pragmatic randomized trial are further described in **Section 3.4** below.

3. RESEARCH APPROACH AND METHODS

3.1 STUDY DESIGN

This study is designed as a superiority RCT with pragmatic features. We have selected ICU patients given the high mortality rate to maximize the chance of detecting a possible effect of sex-matching – moreover, a relatively high proportion (20-40%) of these patients will require RBC transfusions. Mortality in transfused ICU patients is ~33.1% based on historic data (**Table 1a**); and 30-day in-hospital mortality was 29% in our pilot RCT (**Table 1b**)**.**

The pragmatic features of this RCT include enrolment of all ICU patients who require transfusion to maintain generalizability; waived consent (addressed in **Section 4.5** and **Table 3**); electronic data collection based on existing electronic medical records (EMR)/sources to increase efficiency and decrease cost **(Section 5.1.1**); use of an objective outcome measure to minimize bias and maximize data completeness. Our research group (Michael G. DeGroote Centre for Transfusion Research, MCTR) has experience implementing large scale pragmatic RCTs, including the CHIR-funded INFORM trial (n=31,000),[60] which used a similar pragmatic design. We have also collaborated with the iTADS team to leverage their infrastructure and experience.

3.2 SETTING

Eight sites in Ontario (see Table 5) will participate in this study. Management of the trial will be coordinated by MCTR, an experienced group in transfusion-related research with

an extensive track record in coordinating multi-centre RCTs. Infrastructure for trial coordination and data management are well established within this group. The study data will be captured electronically and transferred via a dedicated FTP server (or equivalent) located in the Computer Services Unit (CSU) at McMaster University, then stored on MCTR's secure server also located in the CSU at McMaster University.

3.3 STUDY INTERVENTION AND DURATION

The interventions are donor-recipient sex-matched RBC transfusions (male donor to male recipient; female donor to female recipient) or sex-mismatched RBC transfusions (male donor to female recipient; female donor to male recipient). An ICU patient will be enrolled the first time a request is made to the blood bank for an RBC unit for them. All patients will receive ABO and Rh compatible RBCs as per routine blood bank practices, in addition to selecting for donor sex. All transfused RBCs will be obtained from Canadian Blood Services (CBS) and will be the standard RBC products provided in Canada.

RBC units will be labelled with a unique sticker representing donor sex. Only CBS will know the colour assignment for units. Inventory flow and study logistics were developed with input from iTADS investigators and successfully implemented in our pilot study (see **Figure 4**). The blood bank staff will be blinded to the treatment allocation, and the use of random block sizes will ensure that they cannot predict treatment allocation. The clinical staff, patient/family and research staff will be blinded to the intervention. Validation of sticker-labeling will be conducted at each site according to the protocol successfully implemented in the pilot (see **Figure 5**).

Patients will receive RBCs according to their treatment allocation throughout the hospital stay until discharge from hospital or death (see **Section 3.4.4** below).

3.4 STUDY OUTCOMES

3.4.1 Primary outcome

The primary outcome is 30-day mortality, defined as death within 30 days of randomization. A review of mortality in clinical trials of critically ill patients showed that half of all deaths occurred in the first two weeks following randomization and three-quarters by 28 to 30 days; with few additional deaths accruing over 6 months.[61] Use of 28-30 day mortality is consistent with other critical care trials.[62–66].

3.4.2 Secondary outcomes

Secondary outcomes for this study will include 30-day in-hospital mortality, 90-day mortality, time to 30-day in-hospital mortality, 90-day survival analysis, 90-day in-ICU mortality, time to 90-day in-ICU mortality, hemoglobin increment (per RBC transfusion), need for CRRT/HD, ICU/hospital lengths of stay, number/type/volume/dose of transfused product, transfusion reactions, and cost effectiveness measured using the

incremental cost per life year saved (ICER) between sex-matched and mismatched transfusions. Secondary outcomes were selected based on the quality and accuracy of data available in the source registries and to cover a clinically representative range of adverse short and long-term events after transfusion (renal, cardiovascular, oncology, mortality, infections).

3.4.3 STUDY DURATION

With eight sites and sequential activations, it will take approximately 40 months following enrollment of the first randomized patient to achieve the required sample size of 11,082.

For each patient, the treatment period begins when the patient is randomized just before receiving the first unit of RBCs in the ICU. Patients will receive their assigned RBC group throughout their hospital admission, even if moved to a different ward, until hospital discharge or death. Moreover, patients will continue to receive the assigned type of unit until 90 days post-randomization, even if they receive units in different admissions at the same hospital. Note: patients re-admitted to a different hospital would not follow this procedure. Instead, they would be considered “lost to follow-up”.

3.4.4 RANDOMIZATION

Patients will be allocated to a treatment group in a balanced (1:1) fashion using a secure, concealed, computer-generated, web-based (created on REDCap) randomization sequence.[60] Treatment assignment will be established by way of an electronic marker placed in each patient’s blood bank electronic record within the Laboratory Information System (LIS) (further details in **Figure 3**). Randomization will be performed by blood bank technical staff immediately prior to RBC issue. Blocked randomization will be used with random variable block sizes (2, 4 or 6) stratified by sites to ensure allocation concealment.

3.4.5 STUDY BLINDING

RBC units will be labelled with a unique sticker representing donor sex. Only CBS will know the colour assignment for units. Inventory flow and study logistics were developed with input from iTADS investigators and successfully implemented in our pilot study (see **Figure 4**). The blood bank staff will be blinded to the treatment allocation, and the use of random block sizes will ensure that they cannot predict treatment allocation. The clinical staff, patient/family and research staff will be blinded to the intervention.

The web-based randomization system with appropriate stratification will ensure that the randomization sequence remains concealed to blood bank technical staff and study investigators. Since RBC units are not labeled with donor sex and the stickers used to label the units are agnostic, caregivers and patients will not know whether they are receiving RBCs from a male or female donor. Health care professionals in the ICU will

be made aware of the study but will not know what sticker indicates which sex. Only hospitals with sufficiently large inventories will participate to decrease the chance of unblinding at the blood bank level given that there are more male blood donors (56%) (https://professionaleducation.blood.ca/en/transfusion/publications/surveillance-report). Other methods for protecting against sources of bias include: (a) computerized generation and concealment of the treatment allocation schedule by site; (b) use of an objective primary outcome measure that is not subject to ascertainment bias; and (c) anticipated near complete follow-up using hospital records and external databases for death data.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 INCLUSION CRITERIA

Adults (age ≥18), admission to a participating ICU, and requiring RBC transfusion.

4.2 EXCLUSION CRITERIA

Requirement for a specialized RBC product or unit not readily available in inventory (e.g., rare blood type, washed RBCs, complex RBC antibodies, etc.), massively bleeding patient (i.e., ≥4 units of blood ordered at one time, or Massive Hemorrhage Protocol initiated, or an urgent blood request made), sex unknown or sex other than male or female (i.e. intersex), and do not have a valid Ontario Health Insurance Plan (OHIP) health card number.

The eligibility criteria are meant to be pragmatic with few exclusions. In selecting only medical-surgical ICUs, our population is enriched for patients who have not had a recent transfusion prior to their ICU admission. Patients who were previously transfused prior to their ICU admission during the same hospital admission will be included; historic data show that 27% of those transfused in the ICU received a transfusion within 90 days prior to the ICU admission. In our pilot study, pre-randomization transfusion rate was ~42% with ~one-third of those receiving their first RBC transfusion pre-randomization in the ICU. We chose not to exclude such patients to facilitate more pragmatic enrolment. Also, we believe the percentage of pre-transfused patient will decrease over time; the pilot trial was conducted during pandemic-related critical staffing shortages in the blood bank; improved staffing levels and familiarity with the trial should increase the number of patients randomized on first RBC unit transfused in the ICU. If a patient is allo-immunized and is receiving a specially requested phenotype-matched product, it might be difficult to provide sex-matched units, depending on the specific frequency of the antigen. This represents approximately 2% of patients (these patients are not excluded unless antigen is rare, as above). We have focused on adult patients, as pediatric ICU patients have a much lower mortality rate compared to adults (though this is an important population for future study). We excluded patients where blood was urgently needed to ensure no delay in provision of blood due to randomization. Patients not identified as male or female cannot be stratified during randomization to receive either intervention and can therefore not be included in the study.

4.3 RECRUITMENT SCHEDULE

During the pilot RCT, sites were activated in a step-wise manner between January 7th and March 4th, 2022. During the period where all sites were enrolling, the blood bank technologists randomized ~120 patients/month. With eight sites and sequential activations, it will take approximately 40 months to achieve the required sample size.

4.4 STUDY ENROLLMENT PROCEDURES

We request waived consent authorization from the Research Ethics Board. Approval for the pilot study was obtained through Clinical Trials Ontario (CTO) with the Hamilton Integrated REB as the REB of record (see **Appendix A**). To meet the Tri-Council requirement to inform patients we will provide study information to all ICU patients at the time of discharge from the ICU/hospital. The information will be embedded in the patient electronic discharge summary or provided in paper form (as per site capabilities) and will state that they may have been included in this study and provides opt-out instructions. Similar brochures were used in the Sex Matters pilot, INFORM, and iTADS.

4.5 REQUEST FOR WAIVED CONSENT

We believe that it is appropriate to obtain waived consent for a number of reasons. First, for feasibility, transfusion requests are received on a 24/7 basis and our study population is comprised of ICU patients making it extremely difficult logistically to obtain consent in a timely manner from all patients in advance of their transfusion. Second, RBC units are currently released in random order without consideration of donor sex (ie. it is impossible to know what product is ordered). In our study, at time of study inclusion, the first transfusion will be administered at random (determined by our computerized system rather than “picking the next bag”) and this is no different than routine care. The only modification to the standard of care is that all subsequent transfusion (if any) will be of the same type as the first transfusion. We therefore follow the Tri Council recommendations that consent can be waive given that:

1. “the research involves no more than minimal risk to the participants”;
2. “the alteration to consent requirements is unlikely to adversely affect the welfare of participants”;
3. “it is impossible or impracticable to carry out the research and to address the research question properly, given the research design, if the prior consent of participants is required”;
4. “in the case of a proposed alteration, the precise nature and extent of any proposed alteration is defined”; and
5. “the plan to provide a debriefing (if any) which may also offer participants the possibility of refusing consent and/or withdrawing data and/or human biological materials, shall be in accordance with Article 3.7B”.

Patients at the participating centres routinely receive discharge papers at the time of discharge from the ICU informing them that they received a transfusion. To respect item

5, additional information will be provided about the nature of the study, stating that they may have been included in the study and providing opt-out instructions, while minimizing the potential for fear and apprehension for patients.

Furthermore, it should be noted that deferred consent would not be feasible as this would potentially impact our ability to accurately obtain our primary outcome data; it may be more difficult to obtain consent from substitute decision makers in the event of a patient death during their ICU stay thus adding to potential bias.

4.6 THREATS TO RECRUITMENT

Recruitment compliance (number of recruitments that are compliant/total number of patient randomized ×100%) from the pilot study was 97% with protocol adherence (number of patients who receive all RBC transfusions as group assigned/total number of randomized and transfused patients x 100%) of 88%. At sites where compliance and/or adherence were lower than others, root cause analysis, corrective action and preventative action plans were developed during monthly Technical Resource Committee meetings (**Section 7.1**). In addition, based on our experience with one of the largest pragmatic RCTs ever performed in Transfusion Medicine (31,497 patients randomized)[60] and our pilot experience, we expect excellent compliance for providing blood according to the patients' group assignment. Blood bank staff at each centre will be responsible for issuing the blood and will receive formal training in randomization procedures. A marker will be placed in the patient's electronic blood bank record within the LIS indicating treatment assignment. These electronic markers appear when the file is accessed and are checked prior to transfusion. The study budget includes resources to oversee blood bank adherence to study procedures. The Data Management Sub-Committee **(Section 7.1)** will monitor protocol violations monthly and implement corrective action if needed.

4.7 LIKELY RATE OF LOSSES TO FOLLOW-UP

We anticipate near complete follow-up since all patients will have in-hospital medical records available and patients' discharge status is recorded with 100% accuracy. Mortality data from Ontario Registered Persons Database can identify the true number of deaths within 1%.[70]

5. DATA COLLECTION AND QUALITY CONTROL

5.1 DATA COLLECTION

Data will be captured electronically at pre-specified intervals from the EMR and LIS. All participating centres have proven track records for collecting the data required for this study using an electronic approach. **Figure 6** illustrates the strategy for confidential data collection. Queries will be run to identify issues; sites will be asked to respond in a timely manner. The same level of scrutiny and follow-up will be applied to this electronic approach as would be to case report forms received during any RCT.

5.1.1 Data Collection from EMR:

Each patient's demographic and clinical information (primary and secondary diagnoses and interventions) is coded following discharge by trained medical chart abstractors and stored in the hospital's EMR. Data variables will be collected as per pilot and influenced by iTADS. Data variables available to extract are summarized in **Table 6**.

5.1.2 Data Collection from the LIS:

RBC product and patient transfusion data will be extracted monthly from the LIS (**Table 6**). All sites have the capability of extracting the relevant LIS data. The LIS is the gold standard source for accurate and complete transfusion data, validated to meet regulatory standards.[69]

5.1.3 Data Linking and Confidentiality:

Data from all sources will contain a specific patient identifier needed for linking. To protect patient identity and maintain confidentiality and compliance with privacy requirements, this unique patient identifier will be either protected, recoded, or given a new study ID as per individual site REB requirements. Once the identifiers have been managed at the site level, the data files will be exported to the Coordinating Centre. At the Coordinating Centre, the individual site files from medical records and the LIS will be linked using re-coded patient identifiers; this approach for maintaining privacy and confidentiality is compliant with current Canadian legislation.

5.1.4 Data Collection from CBS:

CBS will provide a list with each inventory shipment of RBCs containing units coded according to colour which correspond to donor sex, though the blood bank staff will be blinded to this. We will also be collecting de-identified data on donor age, hemoglobin level at time of donation, volume of unit and manufacturing process from CBS. There are currently two different manufacturing process for RBCs through CBS and the type of process has been shown to impact cell quality.[73,74]

5.1.5 Data Collection from Ontario Registered Person Database/ICES:

30- and 90-day mortality data will be obtained following methodology from iTADS.[32]

5.2 TREATMENT DEVIATIONS

All protocol deviations will be documented and logged. All precautions will be taken to exclude potential deviations before randomization. The Data Management Sub-Committee (**Section 7.1**) will monitor protocol violations monthly and implement corrective action if needed.

In the event a patient is randomized to either treatment arm and experiences a massive bleeding event during the course of their hospitalization, the wellbeing and safety of the patient comes before compliance. If a randomized patient subsequently requires blood in quantities such that blood per allocation cannot be provided by the blood bank without jeopardizing patient safety, this would constitute a protocol deviation with the

reason recorded. This situation occurred 3 times during the pilot. Such cases are included in the intention to treat analysis but excluded from the per protocol analysis and should be balanced across treatment arms.

5.2.1 Validation Errors

For patients who are randomized, a list of all units transfused with a corresponding unique patient and allocation group identifier will be kept at the blood bank and sent back to CBS monthly to confirm that each patient received the appropriate unit. On a regular basis, a member of the research team will conduct spot checks of the inventory list with colour allocation on units in inventory to ensure they are correctly labeled. If an incorrectly labeled unit is identified, the study team personnel will approach the blood bank staff to review, change the sticker and identify root cause of error. If more than 1% protocol deviation occurs, additional training, audits and study monitoring will occur at the sites to ensure appropriate randomization and study treatment allocation. While patient withdrawal is unlikely, it is possible that patients randomized may not require the RBCs by the time they reach the patient for transfusion due to a change in treatment or diagnosis. These patients will remain included in the study and analyzed using the intention-to-treat strategy.

5.2.2. Patient Withdrawal from the Study

For patients who indicate their desire to opt-out of the study after receiving notification of their enrollment, a note will be made in the blood bank management system. For these patients, future hospitalizations requiring RBC transfusions will have standard blood bank procedures applied - i.e. discontinuation with their enrolled study arm, and delivery of the most suitable RBC unit, selected at the discretion of the medical team and blood bank technician. The study team from sites will obtain REB approval to record the name, date of birth and contact information of the patient so that the research team can determine if the patient was included in the study. If the patient was included in the study, their data will be removed at sites and/or the coordinator center and a flag in the LIS will be added to indicate that the patient is not eligible for the study in case of future randomization.

6. STATISTICAL METHODS

6.1 ESTIMATED SAMPLE SIZE

Thirty-day in-hospital mortality in the pilot trial was 29% and evidence from the literature suggests that 30-day all-cause mortality in transfused patients admitted to the ICU is ~33%. In consultation with knowledge users and decision makers from CBS, intensivists, transfusion medicine experts, transfusion providers, methodologists, and patients, we have decided to target an absolute decrease in mortality of 3% (with 90% power based on a two-sided test with a type 1 error rate of 0.05) (**Table 4**), which will

require 5,037 patients/arm. Other transfusion studies in the ICU have used a 4.2%[67] and 5%[18] reduction in in-hospital mortality, with baseline rates of 24-33%. We target a sample size 10% higher (n=5541/arm) to accommodate potential non-compliance to allocated intervention and the possibility of variable mortality rates in some hospital sites, as seen in the pilot trial. The total sample size is therefore 11,082.

6.2 OUTCOME MEASUREMENTS

Outcome data will be captured electronically from the hospital electronic health record (Discharge Abstract Data, laboratory and transfusion data). Linkage with Ontario Registered Person Database/ICES will yield mortality status (where unknown from hospital EMR) as was done in iTADs.[30] **Figure 6** shows data sources and linkages.

6.3 DATA ANALYSES

A statistical analysis plan will be written and posted publicly before the study database is locked.

6.3.1 Principal analysis of primary outcome measure

For the primary binary outcome (30-day mortality) and binary secondary outcomes (30-day in-hospital mortality, 90-day mortality, 90-day survival analysis, 90-day in-ICU mortality, time to 90-day in-ICU mortality) logistic regression models will be fitted, controlling for centre and sex of recipient. Based on this, odds ratios (OR) will be computed for the treatment effect from the fitted models and accompanied by a 95% confidence interval (CI). Wald-based tests of significance will be carried out. Sensitivity analyses will adjust for factors such as whether they are surgical patients, and whether they had a malignancy or sepsis.

For hemoglobin increment at each RBC transfusion, a linear mixed model will be used with a patient level random effect to accommodate the association between increments in successive transfusions in the same recipient, centre and sex main effects, and an indicator of the treatment arm. The length of stay in the ICU will be analysed as ICU length of stay for all patients, hospital length of stay for all patients, ICU-free days (to day 30), and hospital free days (to day 90). The analysis will again be based on a linear regression model with main effects for centre, sex and treatment arm and a robust standard error will be computed.

Total number of transfused RBC units per patient will be compared between the two arms based on a working Poisson regression model, stratifying on centre and sex, with a robust variance used for protection against extra-Poisson variation. Transfusion reaction numbers will be compared using a log-linear model controlling for centre and sex of donor and using a robust variance estimate.

The time to in-hospital death within 30 days of randomization will be modeled using a cause-specific Cox regression model treating discharge from hospital as a competing

risk. Models will be stratified by centre and sex of patient. Follow-up will be censored at 30 days. Tests of the adequacy of the proportional hazard assumption will be carried out based on Schoenfeld residuals as associated test statistics. Hazard ratios will be computed along with 95% CI and Wald-based tests of significance will be based on the regression coefficient. Sensitivity analyses will assess intervention effects adjusting for surgical status, and whether patients had malignancy or sepsis.

6.3.2 Secondary Analyses

In exploratory secondary analyses a time-dependent covariate will be derived which records the cumulative proportion of units received that were from a sex-mismatched donor as well as cumulative binary indicator of receiving sex mismatched RBCs. In a Cox regression model for in-hospital death a further stratification factor will be added as the cumulative number of RBC transfusions received. In this model then the effect of the cumulative number of mismatched RBC units will be assessed in individuals who have received the same total number of RBC units of any kind. This analysis will be done in two ways: the first is to count only the number of units received after randomization and counting only sex-mismatched RBC units after randomization. The second exploratory analysis will define the cumulative number of units received as including those that were received prior to randomization – the time-dependent cumulative number of sex-mismatched units will likewise count those that were mismatched and transfused before randomization. Underlying diagnosis will be considered in the analysis.

6.3.3 Subgroup Analyses

Subgroup analyses will be carried out by broadening the primary regression models to include interactions between the treatment arm and the covariate identifying subgroups of interest. A centre by treatment interaction will be fitted to test for homogeneity of the effect of sex-matched blood across sites. With eight centres this will result in an 7 degree of freedom test based on the logistic regression model for the primary outcome. Additional subgroups to be explored include sex of recipient in order to assess whether matching the sex of the donor matters more or less to female and male recipients, race/ethnicity when available, donor/recipient age, medical/surgical patients. Subgroup analyses will be directed at assessing the effect of the sex-matched RBC units in individuals who had or had not been transfused prior to randomization. Finally regression models incorporating interactions between treatment arm and patient characteristics will be fitted to assess variation in effects across patient groups with different underlying conditions including whether they are surgical or medical patients and whether they have a malignancy or sepsis.

6.4 COST-EFFECTIVENESS ANALYSES

An economic analysis alongside the trial will be conducted over a 90-day time horizon from the public payer's perspective. We will estimate the cost to blood suppliers (e.g., the cost of producing recipient-donor sex-matched RBC units), the cost of hospitalization, and other health care costs during the 90 days using the ICES datasets. Ninety-day mortality will be used as the outcome measure for the economic analysis.

95% CI of the incremental cost per life year saved (ICER) will be assessed using the non-parametric bootstrapping method and cost effectiveness acceptability curve over a range of willingness-to-pay thresholds will be presented.

7. STUDY MANAGEMENT

This study is endorsed by the Canadian Transfusion Trial Group. Management of the trial will be coordinated by MCTR, an experienced group in transfusion-related research with an extensive track record in coordinating multi-centre RCTs. Infrastructure for trial coordination and data management are well established within this group. The study data will be captured electronically and transferred via a dedicated FTP server (or equivalent) located in the Computer Services Unit (CSU) at McMaster University, then stored on MCTR's secure server also located in the CSU at McMaster University. These servers are regularly maintained, monitored daily for any problems, and all data are backed-up regularly. Only the study biostatistician and the study coordinator will have access to the study data. Blood bank inventory sticker labelling is described in **Section 3.3** and **Figure 4**.

Randomization: Described in **Section 3.4.4.**
Data Collection: Data will be captured electronically at pre-specified intervals from the EMR and LIS. All participating centres have proven track records for collecting the data required for this study using an electronic approach. **Figure 6** illustrates the strategy for confidential data collection. Queries will be run to identify issues; sites will be asked to respond in a timely manner. The same level of scrutiny and follow-up will be applied to this electronic approach as would be to case report forms received during any RCT.
Data Collection from EMR: Each patient's demographic and clinical information (primary and secondary diagnoses and interventions) is coded following discharge by trained medical chart abstractors and stored in the hospital's EMR. Data variables will be collected as per pilot and influenced by iTADS. Data variables available to extract are summarized in **Table 6**.
Data Collection from the LIS: RBC product and patient transfusion data will be extracted monthly from the LIS (**Table 6**). All sites have the capability of extracting the relevant LIS data. The LIS is the gold standard source for accurate and complete transfusion data, validated to meet regulatory standards.[69]
Data Linking and Confidentiality: Data from all sources will contain a specific patient identifier needed for linking. To protect patient identity and maintain confidentiality and compliance with privacy requirements, this unique patient identifier will be either protected, recoded, or given a new study ID as per individual site REB -requirements. Once the identifiers have been managed at the site level, the data files will be exported to the Coordinating Centre. At the Coordinating Centre, the individual site files from medical records and the LIS will be linked using re-coded patient identifiers; this approach for maintaining privacy and confidentiality is compliant with current Canadian legislation.
Data Collection from CBS: CBS will provide a list with each inventory shipment of RBCs containing units coded according to colour which correspond to donor sex, though the blood bank staff will be blinded to this. We will also be collecting de-identified data on

donor age, hemoglobin level at time of donation, volume of unit and manufacturing process from CBS. There are currently two different manufacturing process for RBCs through CBS and the type of process has been shown to impact cell quality.[73,74]
Data Collection from Ontario Registered Person Database/ICES: 30- and 90-day mortality data will be obtained following methodology from iTADS.[32]

7.1 COMMITTEES

Steering Committee (SC): The SC will be comprised of the Principal Investigators (PIs), Co-Investigators, Technical Specialist, patient representative, and the Study Coordinator. Responsibilities include study oversight and problem solving. The SC will meet by conference call at least every 3 months during the trial. The Data Management Sub-Committee (DMSC), will be responsible for reviewing monthly reports and bringing issues to the SC.
Technical Resource Committee (TRC): The TRC will be comprised of the PI, Site PI (ICU or TM MD) Study Coordinator, and a technical and medical representative from the Transfusion Services at all participating sites. This Committee will meet every two weeks during the first 3 months of the study to identify and resolve implementation issues and as needed throughout the trial.

Data Safety Monitoring Committee (DSMC): Recruitment for this study will be approximately 40 months; an IDSMC will be formed (comprised of 3 members with expertise in Transfusion Medicine, Critical Care and Biostatistics) and will be responsible for reviewing aggregate outcome data every 6 month. They will make recommendations to the SC. They will provide an independent report related to any safety concerns including review of severe adverse events.

7.2 ETHICS, CONSENT AND PRIVACY

Individual consent waiver for patients randomized to receive sex-matched vs. sex-mismatched RBCs is consistent with the five criteria specified in the Tri-Council Policy Statement.[69] Our proposed study meets all five of these criteria (see **Table 3**). Approval for the pilot study was obtained through Clinical Trials Ontario (CTO) with the Hamilton Integrated REB as the REB of record (see **Appendix A**). To meet the Tri-Council requirement to inform patients we will provide all transfused ICU patients with information regarding the study at the time of discharge from the ICU/hospital. The information will be embedded in the patient electronic discharge summary or provided in paper form (as per site capability) and will state that they may have been included in this study and provides opt- out instructions. Similar brochures were used in the Sex Matters pilot, INFORM and, iTADS.

We will ensure approval from Research Ethics board of all involved institutions, as well as from Canadian Blood Services (for donor sex information). All data collection and management will be performed in accordance with the *Personal Health Information Protection Act of Ontario, Regulation 329/04.* A unique de-identified number will identify

all patients and no patient identifiers will be kept with clinical data. The data will be encrypted and stored centrally at MCTR during the trial. No patients will be recruited before institutional approval is obtained.

7.3 KNOWLEDGE TRANSLATION PLAN

From the early development of this research project, we involved stakeholders and experts in a wide range of fields involved with the organization, research and the care of patients receiving transfusions (hematologists, intensivists, transfusion specialists, health-care researchers, epidemiologists, blood organization decision makers and senior scientists). This diversity of expertise will ensure that the research questions, objectives, methods and result analysis and interpretation answer pertinent questions for clinicians, but also for stakeholders, patients and the overall population. Our design will allow us to perform the required analyses to impact major blood users.
Results of this RCT will inform policy on blood transfusion practices. If mortality is shown to be reduced with sex-matched transfusions, then a policy change can be implemented at the blood bank level. This will require re-evaluation of how blood bank inventories are managed and assigned. Results will be shared with the Canadian blood suppliers (CBS and Héma Québec) and disseminated with the help of the Canadian Transfusion Trials Group (CTTG) Patient Engagement Working Group and Knowledge Translation Working Group.

8. STRENGTHS AND POTENTIAL CHALLENGES

Knowledge users and decision makers from CBS are co-investigators on this study, providing their input and support to ensure they are aware of potential implications to the blood supplier. To support this study, we have previously completed an exploratory analysis,[19] meta-analysis of observational data,[20] and a CIHR-funded multisite feasibility pilot randomized controlled trial (RCT) (see **Appendix B**). The pilot met all feasibility outcomes and our experience has further informed construct and design of the proposed trial allowing us to address this research question in an efficient and cost-effective manner (see **Table 2** for feasibility outcomes and pilot study results).

A main task is to ensure that every RBC unit stored in the participating sites blood banks are correctly classified as being of one of the two arms (see validation section above). This study will also require the training of blood bank personnel to perform an additional step (randomization of new patients or determining the study assigned group) prior to the release of an RBC unit. We have involved blood bank managers that will help with personnel training and study monitoring. Because of our track record of conducting large trials, all of which required extensive blood bank collaboration, we are confident in conducting this study in collaboration with our blood bank colleagues.

9. ANTICIPATED RESULTS AND OUTPUTS

Our study will provide robust evidence whether a sex-matched compared to a sex-

mismatched RBC transfusion strategy improves survival of critically ill patients who receive a blood transfusion. We will also obtain important information regarding the recipient subgroups that may be less affected (or not at all) by such a practice and thus provide important information to the blood providers to help tailor transfusion practices to the patient. Our study results have potential to inform policy on blood transfusion practices in critically ill patients. If mortality is shown to be reduced with sex-matched transfusions, then a policy change can be implemented at the blood bank level. This will require re-evaluation of how blood bank inventories are managed and assigned as well as consideration of inventory, supply and cost from a systems level.

9.1 ANTICIPATED CONTRIBUTIONS

Knowing that anemia is common during critical illness, mismatched transfusions are inevitable once a patient received more than 6 RBC units (>97% chance) (**Figure 1**). Thus, optimizing supportive care strategies is needed to improve outcomes of this highly vulnerable patient group and a strategy as simple as matching RBC transfusions for donor sex may have significant impact.

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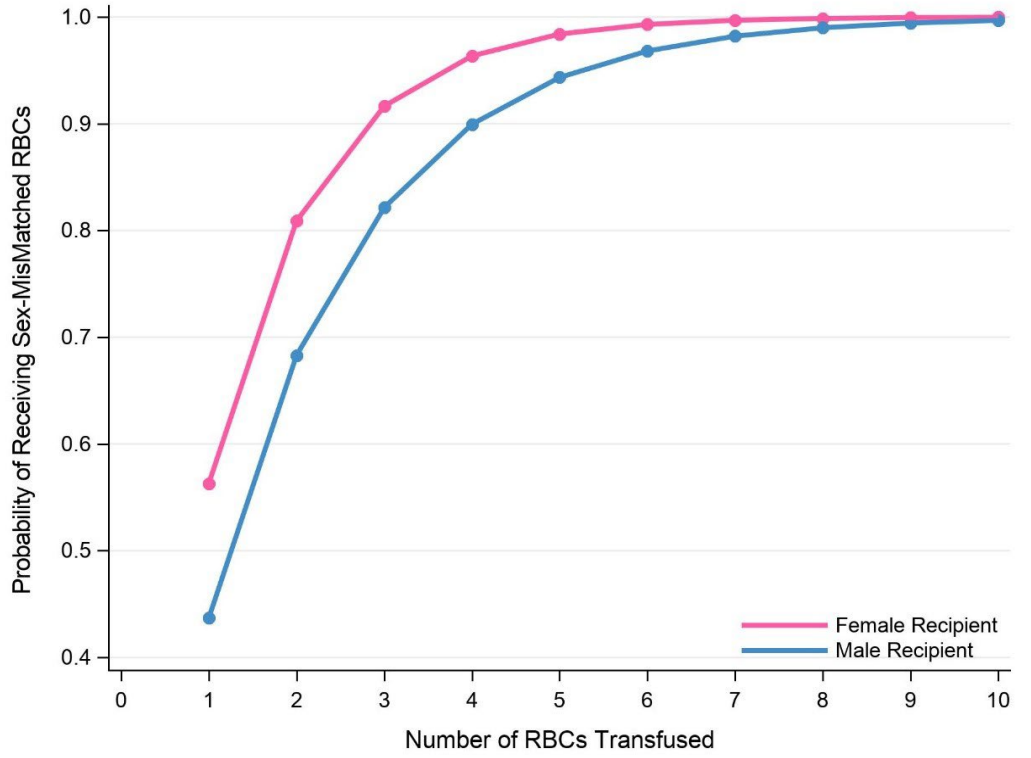
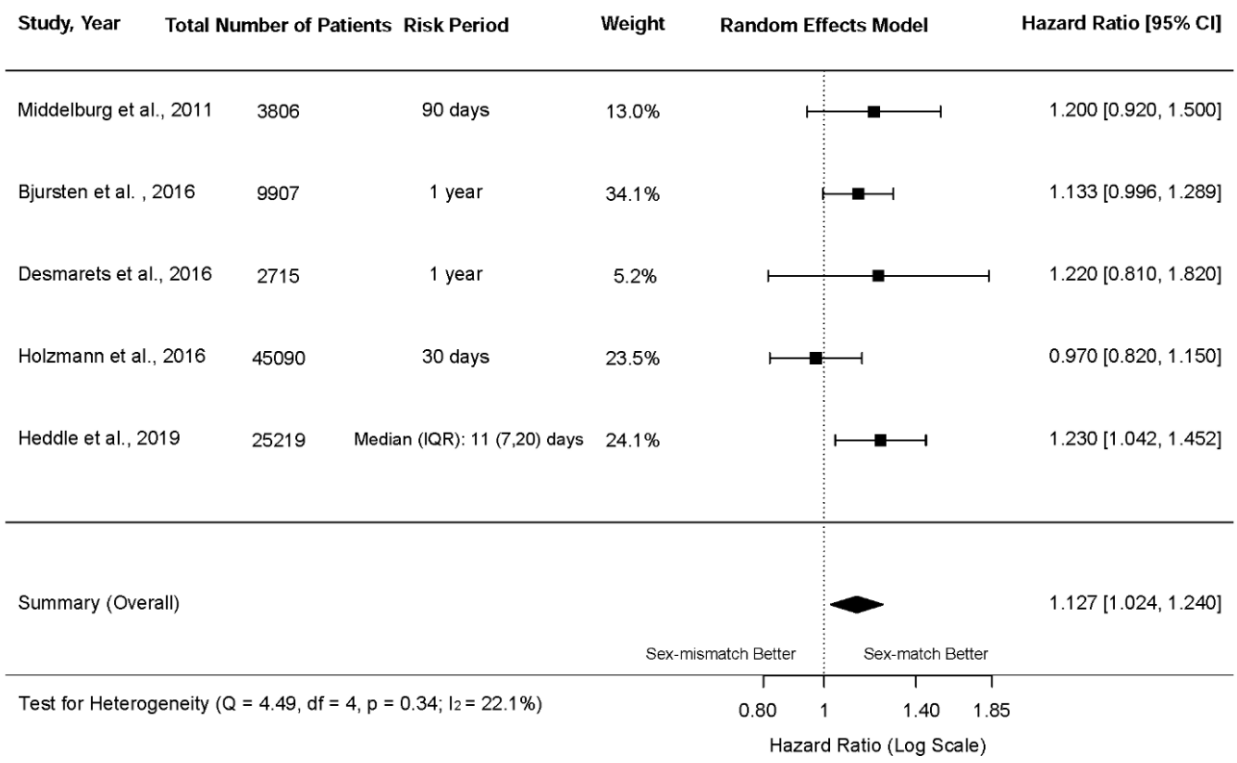
Figure 1. Plot of probability of receiving sex-mismatched RBCs by total number of RBCs transfused. The probability of receiving sex-mismatched RBCs was estimated assuming donor sex distribution of blood based on history data supplied from Transfusion Research Utilization Surveillance and Tracking (TRUST) (RBCs from female donors 43.7%; from male donors 56.3%). Probabilities of receiving sex-mismatched RBC unit when transfused >10 RBC units are extremely close to 1 and not shown. The actual probabilities for each point on the graph are summarized in the Table below.



	Proportions	
Number of RBCs	Female recipient	Male recipient
1	0.56300	0.43700
2	0.80903	0.68303
3	0.91655	0.82155
4	0.96353	0.89953
5	0.98406	0.94344
6	0.99304	0.96815
7	0.99696	0.98207
8	0.99867	0.98991

9	0.99942	0.99432
10	0.99975	0.99680

Figure 2. Random effects meta-analysis of observational data comparing recipient mortality outcomes in patients transfused sex-matched compared to sex-mismatched red blood cell transfusions.



A note on observational studies and their limitations:

Observational studies are always limited by confounding; hence, causation or lack of evidence for causation can only be demonstrated by randomized controlled trials. This concept has been clearly illustrated by research related to harm when stored red blood cells (RBCs) are transfused. Over 30 observational studies were conducted in this area of research with discrepant results. Subsequently, well conducted randomized controlled trials provided clarity demonstrating that fresh RBCs were not superior to stored RBC.[1,2] This area of research took over 25 years of laboratory, observational and experimental research to finally reach the conclusion that the storage lesion did not affect patient outcome.

1. Chai-Adisaksopha C, Alexander PE, Guyatt G, et al. Mortality outcomes in patients transfused with fresher versus older red blood cells: a meta-analysis. *Vox Sang*. 2017:1-11. doi:10.1111/vox.12495
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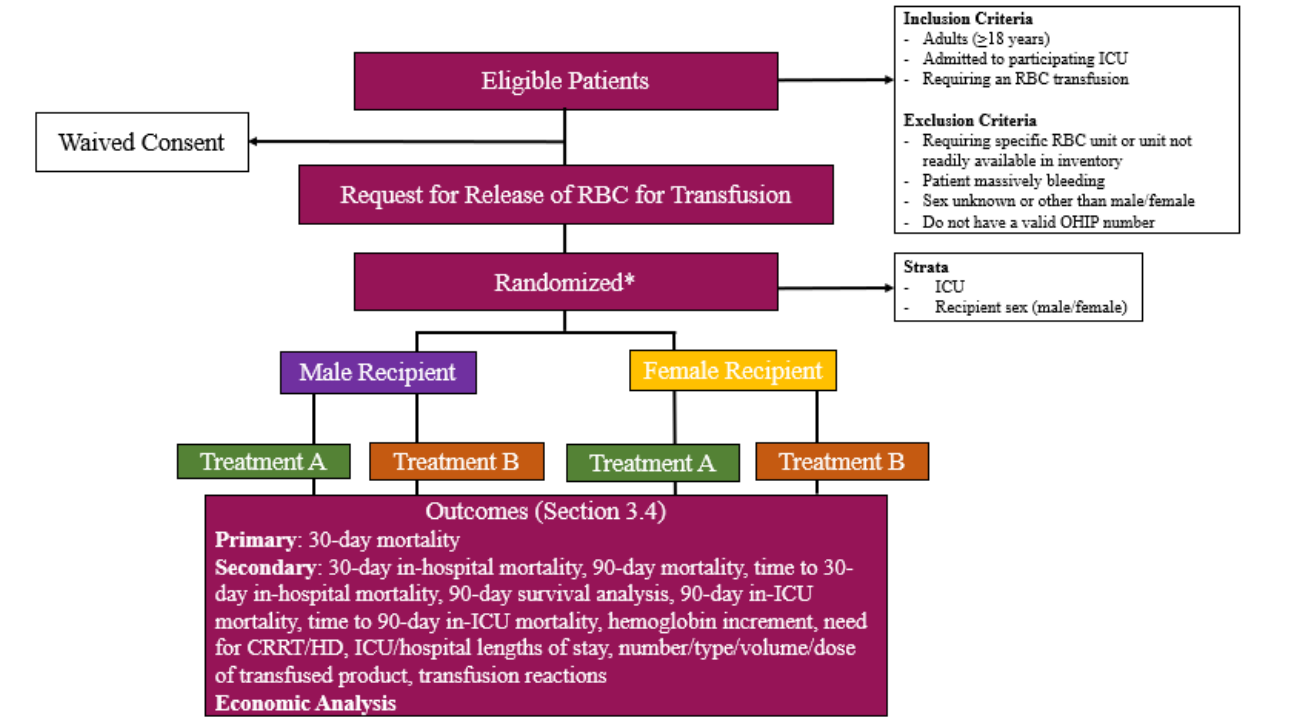
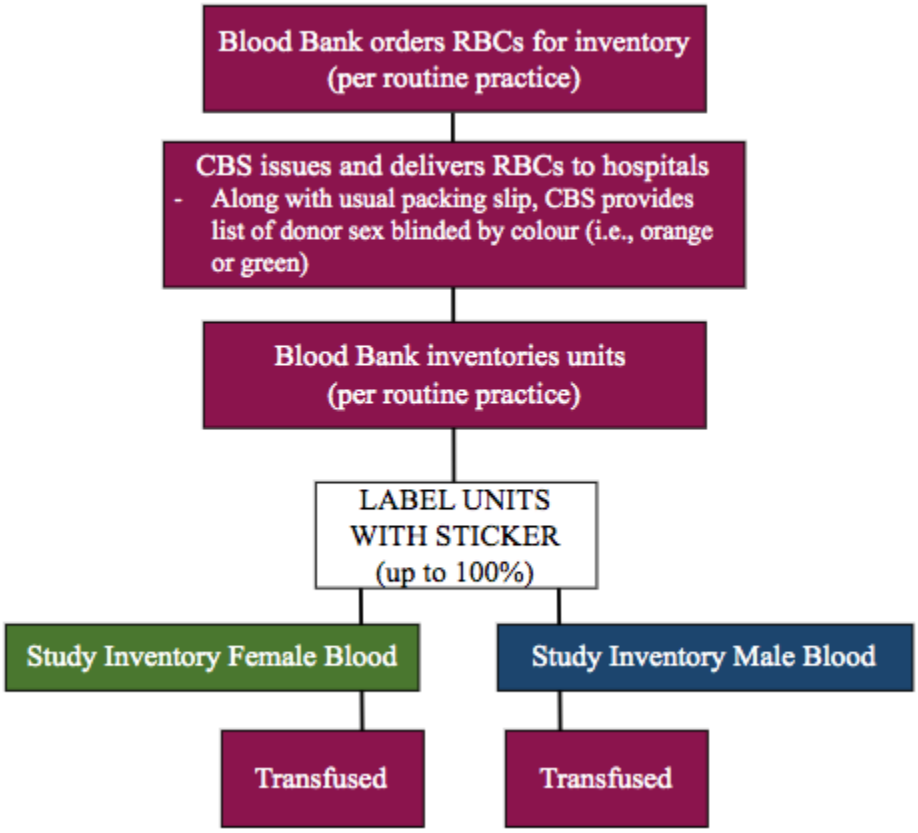
Figure 3. Flow diagram of effects of donor-recipient sex-matched blood transfusion on patient outcomes randomized controlled trial.



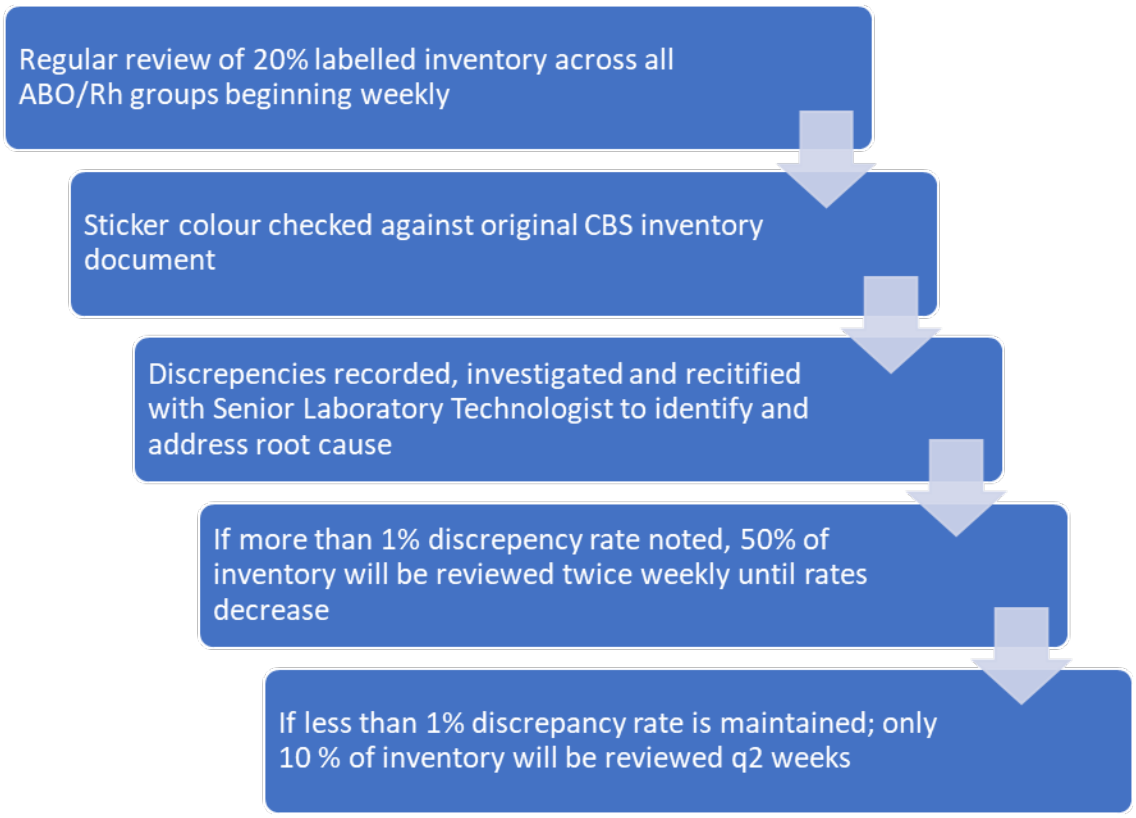
* Randomization: An electronic marker placed in each patient's blood bank electronic record within the LIS will indicate treatment allocation. This marker will be visible each time a patient's transfusion record is accessed (each time a RBC unit is issued). The patient will be transfused according to their assigned treatment allocation throughout their entire hospital stay. Once a study patient is discharged from hospital, the marker may be changed, indicating that they are not eligible to participate in the study if a hospital readmission occurs.

Figure 4. High level red blood cell inventory flow diagram.



Logistics: Study units of RBCs (labelled with a colour-coded sticker denoting donor sex) will be stored in the Blood Bank refrigerator. Blood Banks will aim to label 100% of inventory (as staffing allows). As the labelled inventory is depleted, it will be replenished. The Blood Bank staff be blinded to the treatment allocation schedule and the use of random block sizes in the allocation schedule will ensure that they are not aware of which treatment is coming next in the allocation sequence. The clinical staff will be blinded to the intervention, as CBS is now using coloured stickers for quality control measures on RBC units; for this study, sticker colours and/or shapes not already in circulation will be used.

Figure 5. Sticker validation quality control.



Note: During the pilot RCT, 6,576 units were labelled over the course of the study; 1,320 units were checked (20%), with a total of 6 units discrepant (0.5%) across all sites.

Figure 6. Data flow diagram illustrating the unique electronic data capture process to be used in the study, the integration of data from Canadian Blood Services (CBS) to identify the donor sex of each transfused RBC, the integration of data from TTISS-ON to identify transfusion reactions, and the strategy and the process to ensure privacy of the patient data.

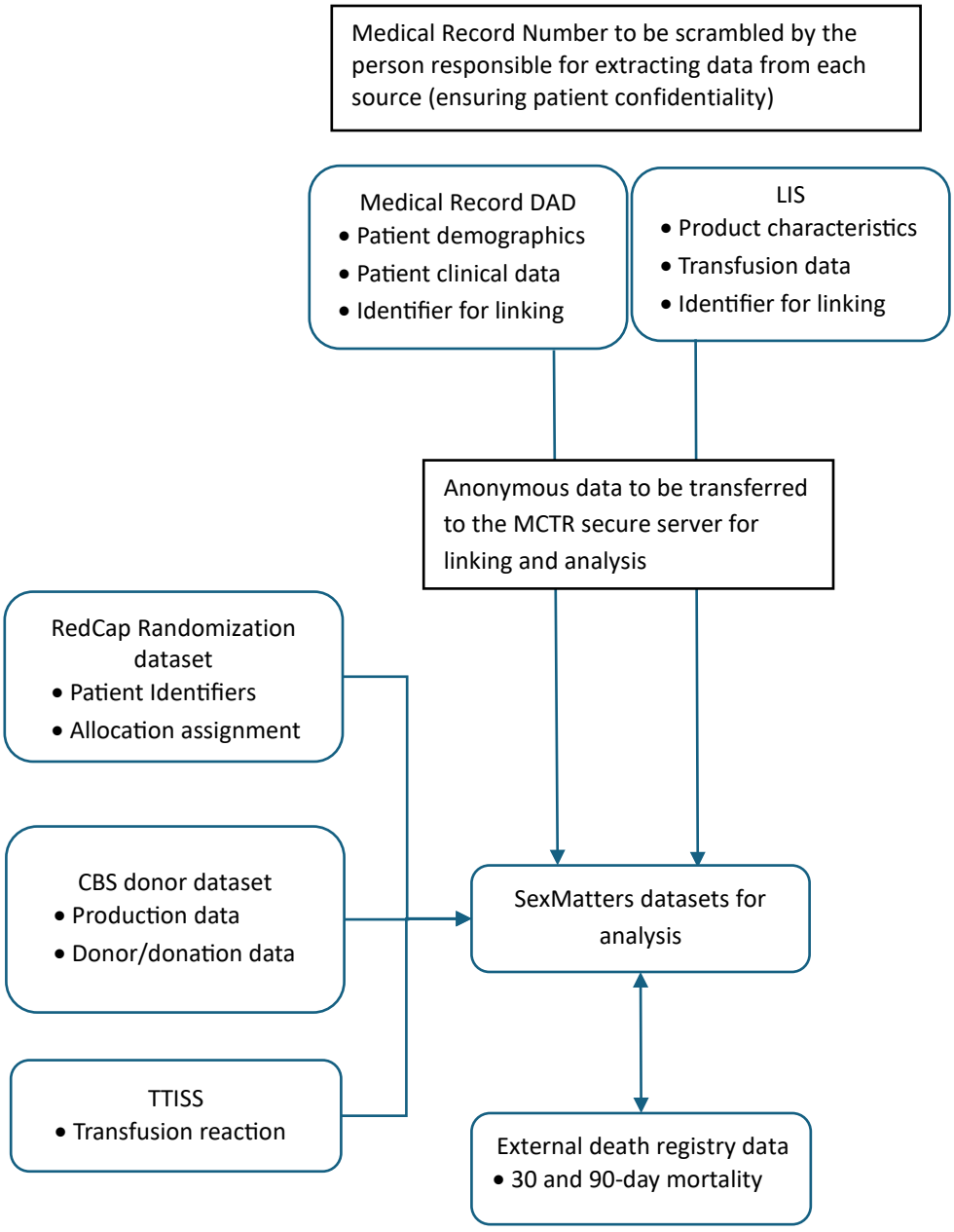


Table 1a. Mortality rates for transfused patients in select Hamilton Intensive Care Units with projected reductions in mortality rate per year estimated using three-year history data (2015-2017).

Yearly Data	**HHS – General ICU-EAST**	**HHS-General ICU-SOUTH**	**St Joes JI-ICU**	**Total**
# patients admitted to ICU	714	500	774	1988
# (%) patients transfused during ICU stay	184 (25.8)	123 (24.6)	195 (25.2)	502 (25.3)
# (%) deaths in ICU patients transfused	58 (31.8)	39 (31.4)	69(35.4)	166 (33.1)
Deaths reduced by 0.5% lower mortality	1	1	1	3
Deaths reduced by 1% lower mortality	2	1	2	5
Deaths reduced by 2% lower mortality	4	2	4	10

Table 1b. Overall 30- and 90-day in-hospital mortality rates in patients from the pilot RCT.

Pilot Outcomes	**Modified Intention-to-Treat Analysis**	**Per Protocol Analysis**
# patients	379	335
# (%) patients 30 day in-hospital mortality	110 (29.0)	98 (29.3)
# (%) patients 90 day in-hospital mortality	130 (34.3)	113 (33.7)

Table 2. Pilot feasibility outcome results.

	Proportion we expected to achieve during the pilot study	**Proportion that we set to define success for the feasibility outcomes**	**Actual proportion achieved during the pilot study[1]**
Randomizing eligible patients (patient level)	90%	80%	79%[2]
Recruitment compliance (patient level)	95%	90%	97%
Protocol adherence	97%	90%	88%[3]

(transfusion level)			

[1]Results from patients recruited during the feasibility period of recruitment only.

[2]We identified that staffing shortages in the Blood Bank was a significant barrier to randomizing eligible patients. Sites that were not experiencing staffing shortages during the pilot were able to recruit up to 100% of eligible patients. Moving forward, we will assess Blood Bank capacity and resources before initiated the study.

[3]We identified that inventory inadequacy and the inability to override automated blood choice mechanisms (ex. specialized fridges that automatically dispense RBCs based on ABO/Rh compatibility only) had a small impact on protocol adherence. Moving forward, we will ensure that sites are receiving and labeling a robust RBC inventory and have identified instances where choosing units based on sex compatibility stickers is not possible.

Table 3. Criteria specified in the Tri-Council Policy Statement for waiver of informed consent.

Criteria Specified in the Tri-Council Statement for Waiver of Informed Consent	**Justification for Waiving Informed Consent**
1) The research involves no more than minimal risk to the participants	The interventions in this study are two different strategies to distribute blood inventory for the provision of a RBC transfusion. Both interventions fall within the current practice for the provision of a RBC transfusion. The study arms include provision of sex-matched RBCs compared to sex-mismatched RBCs. Hence, the study presents no more than minimal risk to participants as standard of care does not take donor sex into consideration.
2) The alteration to consent requirements is unlikely to adversely affect the welfare of participants	There is no reason to suspect that the waiver will adversely affect the welfare of patients. The current standard of care does not consider nor match donor-recipient sex. In this study, some patients will receive blood from donors of the same sex and others will be sex-mismatched. The intent of the study is to see if there is any evidence that giving sex-matched blood is beneficial in reducing recipient mortality risk.
3) It is impossible or impracticable to carry out the research and to address the research question properly, given the research design, if the prior consent of participants is	Transfusion requests are received on a 24/7 basis and our study population is comprised of Intensive Care Unit (ICU) patients, making it extremely difficult logistically to obtain consent in a timely manner from all patients in advance of their transfusion. Attempting to obtain consent would ultimately compromise the

required	objective of the study to efficiently recruit a large representative population in a timely manner without delaying their blood transfusion.
4) In the case of a proposed alteration, the precise nature and extent of any proposed alteration is defined	The specific alteration that we are proposing for this study is waived consent to participate in the RCT. As mentioned, it would be impracticable to obtain consent from all patients in a timely manner and without delaying the transfusion. Furthermore, the study is evaluating the effect of sex-matched RBC transfusions on clinical outcomes. Outside of the study, consent for the provision of transfusion as a medical treatment has been obtained and the choice to transfuse a patient is at the discretion of the treating physician.
5) Participants will be provided a debriefing, which may also offer participants the possibility of refusing consent and/or withdrawing data and/or human biological materials	We will provide all transfused patients in participating ICUs with an information pamphlet about the study. This pamphlet will inform patients about the nature of the study while minimizing the potential for fear and apprehension for patients. We used this approach successfully in the INFORM study and the pilot trial for this RCT, and the approach and pamphlet was approved by the REB in both cases. See patient information entitled **"Blood Transfusion- Did YOU know?"**

Table 4. Thirty-day in-hospital mortality in the pilot trial was 29% and evidence from the literature suggests that 30-day all cause mortality in transfused patients admitted to the ICU is ~33%. In consultation with knowledge users and decision makers from CBS, intensivists, transfusion medicine experts, transfusion providers, methodologists, and patients, we will target an absolute decrease in mortality of 3% (90% power, Type 1 error 0.05), which will require 5,037 patients/arm. Other transfusion studies in the ICU have used 4.2%[64] and 5%[18] reduction in in-hospital mortality, with baseline rates of 24-33%. We target a sample size 10% higher (n=5541/arm) to accommodate potential non-compliance to allocation arm and possibility of variable mortality rates in some hospital sites, as observed in the pilot trial. The total sample size is therefore 11,082.

MisMatched Group (P2)	Matched Group (P1)																				
	0.20	0.21	0.22	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40
0.20	.	34247	8714	3939	2252	1464	1032	769	597	478	392	328	279	241	210	185	164	147	132	119	109
0.21	34247	.	35466	9014	4070	2325	1510	1063	792	614	491	403	337	286	246	215	189	167	150	135	122
0.22	8714	35466	.	36643	9303	4196	2394	1553	1093	813	630	504	413	345	293	252	219	193	171	153	137
0.23	3939	9014	36643	.	37778	9581	4318	2461	1595	1122	834	646	516	422	352	299	257	224	196	174	155
0.24	2252	4070	9303	37778	.	38871	9849	4434	2525	1636	1149	853	660	527	431	360	305	262	228	200	177
0.25	1464	2325	4196	9581	38871	.	39921	10107	4546	2587	1674	1175	872	675	538	440	367	311	267	232	203
0.26	1032	1510	2394	4318	9849	39921	.	40930	10353	4654	2646	1711	1200	890	688	548	448	373	316	271	235
0.27	769	1063	1553	2461	4434	10107	40930	.	41897	10590	4757	2703	1747	1224	908	701	558	456	379	321	275
0.28	597	792	1093	1595	2525	4546	10353	41897	.	42821	10816	4855	2757	1780	1247	924	713	568	463	385	326
0.29	478	614	813	1122	1636	2587	4654	10590	42821	.	43704	11031	4948	2808	1812	1269	939	724	576	470	391
0.30	392	491	630	834	1149	1674	2646	4757	10816	43704	.	44545	11236	5037	2856	1842	1289	954	735	585	477
0.31	328	403	504	646	853	1175	1711	2703	4855	11031	44545	.	45343	11430	5121	2902	1871	1308	968	745	592
0.32	279	337	413	516	660	872	1200	1747	2757	4948	11236	45343	.	46100	11614	5200	2946	1898	1326	980	755
0.33	241	286	345	422	527	675	890	1224	1780	2808	5037	11430	46100	.	46814	11788	5275	2986	1923	1343	992
0.34	210	246	293	352	431	538	688	908	1247	1812	2856	5121	11614	46814	.	47487	11951	5345	3025	1947	1359
0.35	185	215	252	299	360	440	548	701	924	1269	1842	2902	5200	11788	47487	.	48117	12103	5410	3060	1969
0.36	164	189	219	257	305	367	448	558	713	939	1289	1871	2946	5275	11951	48117	.	48706	12245	5471	3093
0.37	147	167	193	224	262	311	373	456	568	724	954	1308	1898	2986	5345	12103	48706	.	49252	12376	5527
0.38	132	150	171	196	228	267	316	379	463	576	735	968	1326	1923	3025	5410	12245	49252	.	49756	12497
0.39	119	135	153	174	200	232	271	321	385	470	585	745	980	1343	1947	3060	5471	12376	49756	.	50219
0.40	109	122	137	155	177	203	235	275	326	391	477	592	755	992	1359	1969	3093	5527	12497	50219	.

Type I error rate = 5% (Zα/2 = 1.96); Power = 90% (Z1−β = -1.28); Balanced design

*A 3% absolute risk reduction was selected as clinically important in consultation with knowledge users and decision makers from CBS, methodologists, intensivists, transfusion medicine experts, transfusion providers and patients. It is similar to the 4.2% and 5% absolute risk reduction that was used to calculate the sample size for other studies of ICU patients that assessed the impact of blood storage duration (Cooper NEJM 2017; Lacroix NEJM 2015).

Table 5. Participating sites

Hospital	**Site Co-investigators or leads**	**Participated in pilot RCT**
Hamilton General Hospital	Dr. Andrew Shih Dr. Paul Engels	Pilot participant
Sunnybrook Health Science	Dr. Yulia Lin Dr. Neill Adhikari	Pilot participant
London Health Sciences Centre	Dr. Ziad Solh Dr. Marat Slessarev	Pilot participant
Kingston Hospital Sciences Centre	Dr. Jeannie Callum Dr. Stephanie Sibley	Pilot participant
Lakeridge Hospital	Dr. Karim Soliman Dr. Nadia Gabarin	
Mount Sinai Hospital	Dr. Nadine Shehata	
Ottawa Hospital – General	Dr. Shane English	
University Health Network – General	Dr. Nadia Gabarin Dr. Jacob Pendergrast Dr. Eddy Fan	

Table 6. Relevant data that can be pulled from the Laboratory Information System (LIS) and/or medical records**,** and may be pulled for this study.

Data Extracted from LIS

Blood product inventory	**Transfused patients (inpatient and outpatient)**
Transfused blood product • Hospital site* • Product Unit Number* • Product names (Red cells, Plasma, Platelets, Cryoprecipitate)* • Product source (CBS collection center codes) • Product lot number* • CBS product code* • Product ABO group and RH type • Pooled product name and unit number • Product collection date and expiry	**Transfused patients** • Facility • Hospital ID (MRN)* • Encounter number • Patient age at transfusion* • Patient gender and/or sex* • Patient ABO group and RH type • Admission date/time and discharge date/time • Admission status (inpatient/outpatient/ER admission) • Patient antibody result and date • Autoantibody indicator • Patient marker (e.g. phenotype marker

date* • Product receipt date/time • Product final disposition (transfused or presumed transfused) • Product marker (CMVNEG, irradiated and etc.)* • Product antigen name and result • Issue date/time* • Volume issued • Ward at product issue **Product Orders** • Hospital ID (MRN) • Specimen number • Order date/time • Ward at order and service • Product ordered	or study SEMA markers etc.)* **Transfused patient laboratory testing** • Specimen collection date/time • Result verify date/time • Test name (Hemoglobin, Platelet CT, PT in INR, APTT, Clauss Fibrinogen, Creatinine) and result within 24 hours prior to randomization **Transfused patient status** • Hospital ID (MRN) • Encounter number • Admission date/time and discharge date/time • Admission status at time of data exaction (death indicator) • Death date/time (if different from discharge date/time) **Group and Screen Results** • Hospital ID (MRN) • Specimen number • Specimen collection date/time • Result verify date/time • ABO/Rh result • Screen Test (AG, AINTM, AIR) • Screen result (positive, negative, NP,ND)

Data Extracted from Medical Records

Inpatient admissions
Patient demographics • Institution* • Hospital ID (MRN)* • Patient age and gender and/or sex* **Admission information** • Encounter number* • Admission date/time and discharge date/time* • Length of stay • Acute length of stay • Admission category • Discharge disposition

Diagnosis

- Diagnosis type (primary, secondary and etc.)
- Diagnosis code (ICD 10)

Interventions

- Intervention Code (CCI codes)
- Intervention start date/time and end date/time
- Location (OR rooms, or departments, units)
- Intervention doctor service
- Unplanned return to OR

Doctors

- Attending physician service code

ICU

- ICU admission date/time and discharge date/time*
- ICU units *
- ICU LOS (days and hours)

Notes:

1. All variables listed in the tables above will be collected for all randomized patients. Only variables with * will be collected from all patients in participating intensive care unit(s) (regardless of randomization status) between date of last report and date of current report as part of **regular** denominator data pulls.
2. All variables listed in the tables above will be collected from all patients in participating intensive care unit(s) (regardless of randomization status) within the previous month as part of **start-up** data pulls. These data are required to confirm that sites are able to pull all required variables. This may be completed several times, as required, until confirmation of satisfactory data and subsequent site activation. These data will not be analyzed and will be destroyed upon confirmatory check.
3. In addition to the data above provided by sites, we will also collect data from the following sources:

a. Data on unit and donor characteristics from Canadian Blood Services
b. Data on transfusion reactions from the Ontario Transfusion Transmitted Injuries Surveillance System

Appendix A: Pilot Research Ethics Approval

HiREB

Hamilton Integrated Research Ethics Board

Date: 8 April 2021

To: Dr. Michelle Zeller, McMaster University

CTO Project ID: 3402

Study Title: Pilot Study of Sex-matched vs. Sex-mismatched Red Blood Cell Transfusion

Sponsor Study ID:

Study Sponsor: McMaster University/Hamilton Health Sciences Corporation

Application Type: Clinical Trial Provincial Initial/CHEER Initial Application

Review Type: Delegated

Meeting Date: 16/Feb/2021

Date Approval Issued: 08/Apr/2021

Study Approval Expiry Date: 08/Apr/2022

Dear Provincial Applicant,

Thank you for submitting the above referenced study on behalf of all Ontario centres through the Clinical Trials Ontario Streamlined Research Ethics Review System. The HiREB Panel A has reviewed the study and granted initial provincial approval as of the date noted above.

In light of the current COVID-19 pandemic, while this study has been reviewed by HiREB and given final approval status, the actual conduct of the research needs to be performed in accordance with institutional restrictions with respect to Coronavirus (which may mean new subjects cannot be actively enrolled and most research staff will be limited with respect to access to other data sources for the time being).

Provincial documents approved:

Document Name	Document Date	Document Version
Sex Matters TRUST only_Jan 22 2021	22/Jan/2021	V1
Sex Matters Appendices_Jan 22 2021	22/Jan/2021	V1
Sex Matters Protocol References FINAL	22/Jan/2021	V1
Sex Matters Protocol_March 16 2021_clean	16/Mar/2021	1.1 Clean
Sex Matters PatientBrochure_Mar 16 2021	16/Mar/2021	V1.1

Provincial documents acknowledged:

Document Name	Document Date	Document Version
SexMATTERS_IDMC_ToR_Jan22_2021	22/Jan/2021	V1
Sex Matters Budget Justifications 22Jan2021	22/Jan/2021	V1

Note: According to TCPS2 Article 11.10; All clinical trials shall be registered before recruitment of the first trial participant in a publicly accessible registry that is acceptable to the World Health Organization (WHO) or the International Committee of Medical Journal Editors (ICMJE). Please forward the clinical trial registration number for HiREB files when received.

Note: Provincial REB approval does not confer ethics approval for participating centres. Each participating centre, including that of the Provincial Applicant, must submit the "Centre Initial Application" and receive approval from this REB prior to the conduct of the study at that centre. All other required institutional approvals must also be obtained prior to the conduct of the study.

No deviations from, or changes to, the protocol should be initiated without prior written approval from HiREB Panel A, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial (such as a change in telephone number).

REB members involved in the research project do not participate in the review, discussion or decision.

Abstract

Sex-matched compared to sex-mismatched red blood cell transfusion: a pilot randomized controlled trial

Abstract Author Names :

Michelle Zeller [1 * ^], Richard Cook [2], Donald Arnold [3], Jeannie Callum [4], Bram Rochwerg [5], Jason Acker [6], Nour Alhomsi [7], Yulia Lin [8], Yang Liu [9], Kayla Lucier [10], Shuoyan Ning [11], Jungyoon Jane Park [12], John Principato [13], Ziad Solh [14], Theodore Warkentin [15], Kathryn Webert [16], Nancy Heddle [17]

Abstract Summary :

Introduction/Objective: Red blood cell (RBC) transfusions are selected based upon donor/recipient blood group compatibility. Whether RBCs should be selected based on donor sex is uncertain. This pilot randomized controlled trial (RCT) was designed to assess feasibility of a study examining impact of donor and recipient sex-matched RBC transfusions compared to sex-mismatched RBC transfusions on recipient mortality in critically ill adult patients.

Design/Methods: This was an allocation concealed, blinded, pilot RCT with pragmatic features conducted at five Ontario hospitals from January-May 2022. The study was conducted with staggered site activations to identify and overcome potential barriers in the first 130 of 400 patients randomized. We enrolled consecutive hospitalized adult patients (age ≥18) admitted to the intensive care unit (ICU) who were prescribed RBC transfusion for any indication. We excluded patients who required non-standard RBC units (e.g., phenotypically matched, rare blood, washed, complex RBC antibodies, etc.), ≥4 units of blood/massive hemorrhage protocol/urgent request for blood at the time of randomization, sex unknown or intersex. We employed a waived consent model.

Eligible patients were randomized to receive donor sex-matched or sex-mismatched RBC transfusions until hospital discharge or death. Feasibility targets were assessed for the final 270 of 400 patients and included: eligible patients randomized >80%; recruitment compliance >90%; and protocol adherence >90%. We also collected patient-important clinical outcomes such as 30 day in-hospital mortality and hospital length of stay. Post hoc, we conducted quality control assessment of masked (colour-coded) male and female labels for 20% of the RBC units.

Results: We found 79% of eligible patients were randomized, recruitment compliance was 97%, and protocol adherence was 88%. Feasibility metrics varied across study sites and generally improved over time following refinement of protocol exclusion criteria and enhanced training procedures. Forty-two percent of recruited patients were transfused prior to randomization; among those, approximately one-third received their first RBC transfusion in the ICU. Overall, in-hospital mortality rate was 29%. A total of 6,576 units of RBCs were labelled for the study; 1,320 units were checked (20%), with a total of 6 units discrepant (0.5%) across all sites (all discrepancies were corrected prior to RBC issue).

Conclusions: This pilot study demonstrated that a larger RCT examining the role of sex-matching versus sex-mismatched RBC transfusions in critically ill patients is feasible with ongoing efforts to ensure all eligible patients are captured. This pilot trial will inform design of a larger trial.

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