Supplementary Online Content

Small Volume Tubes to Reduce Anemia and Transfusion (STRATUS) Trial

SUPPLEMENT 2: Trial Protocol and Statistical Analysis Plan

CLINICAL STUDY PROTOCOL

STRATUS Study

Small-Volume Tubes to Reduce Anemia and Transfusion:
A Pragmatic Stepped Wedge Cluster Randomized Trial

Version 2.0

2019-05-17

PROTOCOL APPROVAL

Signature:

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Date:

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LIST OF ABBREVIATIONS

ACS	Acute Coronary Syndromes
CI	Confidence Interval
EDTA	Ethylenediaminetetraacetic Acid
ICU	Intensive Care Unit
IRB	Independent Review Board
GCP	Good Clinical Practice
GRACE	Global Registry of Acute Coronary Events
Hb	Hemoglobin
ICH	International Conference on Harmonisation
MODS	Multi-Organ Dysfunction Score
NSTEACS	Non ST Elevation Acute Coronary Syndromes
OR	Odds Ratio
PHRI	Population Health Research Institute
RBC	Red Blood Cells
SD	Standard Deviation
STEMI	ST Elevation Myocardial Infarction
TCPS	Tri-Council Policy Statement
TIMI	Thrombolysis In Myocardial Infarction
WHO	World Health Organization

1. BACKGROUND

Laboratory Testing Results in Blood Loss

Blood sampling can result in significant blood loss, particularly in hospitalized patients who undergo frequent blood testing. Intensive care unit (ICU) patients, the sickest in the hospital, often undergo blood testing multiple times each day, with sample volumes up to 41 mL per day reported (equivalent to donating a unit of blood every 9 days)[1]. Importantly, only about 10% of the blood collected is actually used for testing procedures with the remainder discarded as waste[2]. This suggests that blood sample volumes could be decreased without compromising patient care.

Adverse Consequences of Blood Loss from Laboratory Testing

Exacerbation of Anemia

Anemia is defined by the World Health Organization [WHO] as hemoglobin less than 120 g/L in women and 130 g/L in men. Up to 50% of hospitalized patients and 75% of the hospitalized elderly experience anemia[3]. Patients in the intensive care unit (ICU) are at particularly high risk for anemia with 60% anemic upon ICU admission, 90% by day 3 and 97% by day 8[1,4,5]. In hospitalized patients, anemia is often considered multifactorial and generally attributed to acute or chronic hemorrhage and/or reduced erythropoiesis from infection, inflammation, and reduced erythropoietin production[6]. Limited evidence suggests that frequent laboratory testing is a potentially modifiable cause of blood loss and a contributor to anemia. As a proof of concept, phlebotomy of 314 mL in healthy volunteer subjects led to a small but significant reduction in average hematocrit from 44.2% to 39.9%[7]. Patients with acute medical illness are expected to be more susceptible to the impact of blood loss due to a reduced production of and response to erythropoietin. For example, in non-anemic patients with MI, every 50 mL of total blood drawn for testing has been shown to increase the risk of moderate to severe anemia (hemoglobin ≤ 110 g/L) by 15% in adjusted analysis[8]. In hospitalized patients, blood loss of 100 mL was associated with a reduction in hemoglobin of 7 g/L[9].

Anemia is Associated with Major Adverse Cardiovascular Outcomes and Death

Anemia is associated with major adverse cardiovascular outcomes and death in a wide spectrum of patients including those with myocardial infarction[[10-14], heart failure[15-17], diabetes[18] and those undergoing surgery[19,20]. ICU patients with lower hemoglobin levels during admission have been shown to have longer ICU and hospital length of stay [LOS] and higher mortality at 30 days[4]. In the prospective observational CRIT study, nadir (but not baseline) hemoglobin less than 100 g/L was associated with a longer ICU LOS compared to nadir hemoglobin 100 g/L or more (hemoglobin less than 80 g/L: LOS ratio 1.41, 95%CI 1.29-1.53; hemoglobin 80 to 89 g/L: LOS ratio 1.57, 95%CI 1.47-1.69; hemoglobin 90 to 99 g/L: LOS ratio 1.30, 95%CI 1.23-1.39, p<0.0001)[4]. Similarly, nadir (but not baseline) hemoglobin less than 100 g/L was associated with longer hospital LOS compared to hemoglobin 100 g/L or more (hemoglobin less than 80 g/L: LOS ratio 1.17, 95%CI 1.08-1.27; hemoglobin 80 to 89 g/L: LOS ratio 1.23, 95%CI 1.16-1.31; hemoglobin 90 to 99 g/L: LOS ratio 1.14, 95%CI 1.08-1.21, p<0.0001). Baseline hemoglobin levels were not statistically significantly associated with ICU or hospital length of stay. In multivariate logistic regression analysis, the odds ratio for 30-day mortality was higher in ICU patients with nadir hemoglobin less than 90 g/L compared to those with hemoglobin 100 g/L or more in that study (hemoglobin less than 80 g/L: odds ratio [OR] 1.54, 95%CI 112-2.12, p<0.009; hemoglobin 80 to 89 g/L OR 1.49, 95%CI 1.13-1.95, p<0.004).

In patients with MI, the development of moderate to severe hospital acquired anemia (defined as hemoglobin concentration ≤ 110 g/L) was associated with higher mortality (8.4% vs. 2.6%; HR 1.82, 95%CI 1.11-2.98) and poorer health status at 1 year compared to patients without anemia after adjustment for known confounding factors [21]. In patients with ST elevation MI, each 10 g/L decrement in hemoglobin is associated with increased cardiovascular mortality (adjusted odds ratio (OR) 1.21, 95% confidence interval [CI] 1.12-1.30) based on data from 16 Thrombolysis in Myocardial Infarction (TIMI) trials[10]. Similarly, each 10 g/L decrement in hemoglobin was associated with an increased risk of the composite outcome of cardiovascular death, recurrent MI or recurrent ischemia in patients with non-ST elevation acute coronary syndrome (ACS) (adjusted OR 1.45, 95%CI 1.33-1.58). Among ACS patients, baseline hemoglobin <110 g/L increased the risk of the composite outcome by about 2-fold (OR 2.26, 95%CI 1.83-2.79.

Red Blood Cell Transfusion

Indications for red blood cell (RBC) transfusions in critically ill patients include hemorrhagic shock, acute hemorrhage with hemodynamic instability or evidence of inadequate oxygen delivery, and hemoglobin concentration of less than 70 g/L or less than 80 g/L depending on the patient population[22,23]. Current guidelines from the AABB recommend a hemoglobin transfusion threshold of 70 g/L for hemodynamically stable hospitalized adult patients (including critically ill patients) and a threshold of 80 g/L for patients undergoing orthopedic or cardiac surgery, or those with underlying cardiovascular disease[24]. The Transfusion Requirements in Critical Care (TRICC) was a landmark trial that demonstrated reduced hospital mortality in patients randomized to a restrictive RBC transfusion strategy (< 70 g/L) compared to a conservative RBC transfusion strategy (<100 g/L) and, in conjunction with subsequent meta-analyses, established the recommended threshold for RBC transfusion in critical illness.

About 40% of ICU patients receive one or more red blood cell (RBC) transfusions to correct anemia, half of which are administered in the absence of clinically significant hemorrhage[1,4,25,26]. Up to 75% of patients with ICU stays of longer than one week receive RBC transfusion[1]. In a prospective observational study, 17.8% were admitted to ICU for more than 7 days[1]. Diagnostic blood loss is associated with an increased likelihood of RBC transfusion in non-bleeding ICU patients and accounts for 50% of the variation in the amount of RBC transfusion[25,27]. Among ICU patients admitted for 21 days or longer, increases in average diagnostic blood loss of 3.5 mL per day are associated with a 2-fold increase in the odds of transfusion[27].

Health Risks of Red Blood Cell Transfusion

Transfusions are a limited resource and are associated with health risks such as infection, transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), hemolytic transfusion reactions, immunosuppression, allosensitization and allergy[28,29]. RBC transfusion is associated with death, longer ICU and hospital admissions, infection, prolonged mechanical ventilation and organ dysfunction[1,4,30]. Two observational studies showed that receipt of a RBC transfusion in the ICU increased the odds of dying by 1.37 (95%CI 1.02-1.84) and 1.65 (95% CI 1.35-2.03) in adjusted analyses[1,4]. For example, in the CRIT study, RBC transfusion was significantly associated with an increased risk of death after propensity matching (1059 transfused and 1059 non-transfused patients) with an adjusted mortality ratio of 1.65 (95% confidence interval, 1.35–2.03; log-rank, p<0.001)[4]. The number of RBC units transfused was associated with increased ICU LOS (1-2 units: LOS ratio 1.47, 95%CI 1.35-1.59; 3-4 units: LOS ratio 1.84, 95%CI 1.66-2.03; more than 4 units: LOS ratio 3.20, 95%CI 2.88-3.55; p<0.0001) and hospital LOS (1-2 units: LOS ratio 1.32, 95%CI 1.22-1.42; 3-4 units : LOS ratio 1.61, 95%CI 1.47-1.75; more than 4 units: LOS ratio 2.51, 95%CI 2.28-2.76; p<0.001) compared with patients who did not receive transfusions. The median ICU and hospital LOS in non-transfused patients were 4.6 days and 11.0 days, respectively. Patients who received 1-2, 3-4, or more than 4 RBC units had increases in median ICU LOS of 2.1, 3.8, and 10.1 days, and increases in median hospital LOS of 3.5, 6.7, and 16.6 days, respectively.

Red Blood Cell Transfusion is Costly

One unit of RBCs costs between $400 and $500 (personal communication Dr. K. Webert, Canadian Blood Services). However, this estimate does not account for the cost of preparing and administering transfusion and managing adverse events. Between 2012 and 2015, approximately 30,000 RBC units were transfused in ICUs and cardiac care units in Hamilton, Ontario at a direct cost of approximately $15,000,000 (unpublished data, D. Siegal). Interventions that reduce RBC transfusion have been shown to decrease cost[31,32]. For example, one initiative which reduced RBC transfusion by 25% produced estimated savings of $5.9 million over 4 years by reducing RBC unit acquisition costs[32]. This does not account for additional potential sources of savings to hospitals including reduced nursing workload (administration of transfusions, monitoring and management of adverse reactions), reduced laboratory workload (testing for transfusion and adverse reactions), reduced length of stay, and 30-day readmissions.

Diagnostic Blood Loss, RBC Transfusion and Anemia in a Cohort of Canadian ICU Patients

We assessed blood loss for diagnostic testing, RBC transfusion and anemia in a consecutive cohort of patients admitted to medical-surgical ICUs in Hamilton, Ontario from 2012 to 2015 (unpublished data, D. Siegal). Among patients admitted for ≥48 hours (n=7,273), the median estimated blood loss was 195 mL (IQR 123-348). RBC transfusions were administered to 47.5% of patients among whom the median number of RBC units transfused was 3 (IQR 2-7). The mean RBC transfusion rate for the entire cohort (including both transfused and non-transfused patients) was 2.7 units per patient. The proportion of patients with

severe anemia (hemoglobin ≤90 g/L) increased from 39.6% on ICU admission to 61.9% during ICU admission. In patients admitted to ICU for ≥24 hours, cumulative blood loss of 200 mL was associated with an increased risk of RBC transfusion (hazard ratio [HR] 2.34, 95% CI 2.07-2.65) in analysis adjusted for known covariates.

Small-Volume Blood Collection Tubes Decrease Blood Loss from Laboratory Testing

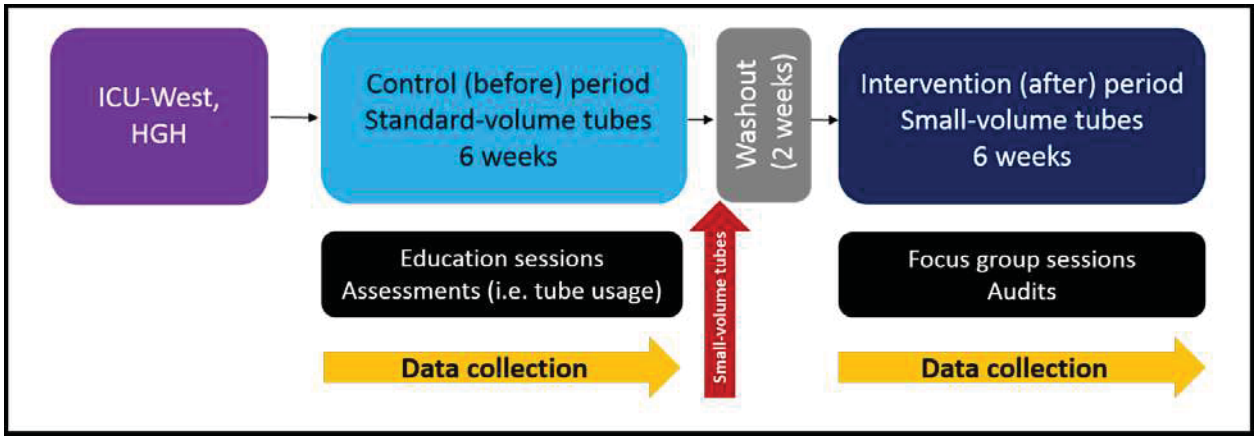
Small-volume (soft-draw) Vacutainer® tubes for blood collection have the same cost and physical dimensions as standard-draw Vacutainer® tubes, but draw less blood (2 to 3 mL vs. 4 to 6 mL) due to lower vacuum inside the tube which fills to a smaller pre-determined volume. When maximally filled, soft-draw tubes have the correct concentration of anticoagulant required to ensure validity and reliability of test results. The soft-draw tubes are routinely used in children and are compatible with current laboratory equipment. Their routine use could decrease blood loss from laboratory testing in ICU patients.

There is a paucity of studies evaluating the benefits and harms of small-volume blood collection tubes as a strategy to mitigate diagnostic blood loss in adults. We conducted a systematic review (published as abstract) which identified only 2 small observational studies (judged to have serious risk of bias) that evaluated reduced-volume blood collection tubes to reduce diagnostic blood loss in ICU[33,34]. In those studies, the volume of blood collected for laboratory testing was reduced by 47% and 74%; clinical outcomes (e.g. transfusion, length of stay, mortality) and potential harms were not evaluated. An additional small observational study (n=248) published since our review showed a 42% reduction of diagnostic blood loss using small-volume tubes in a medical-surgical ICU population[35]. Incident severe anemia (hemoglobin < 70 g/L) was less frequent in the reduced-volume group (10% vs. 22%, p=0.01). There was a 27% reduction in RBC transfusion in the reduced-volume tube group, but this was not statistically significant. Although this study was limited by small sample size and the likely presence of confounding factors that could influence results, the study suggested that reduced-volume tubes may be a feasible and effective strategy for reducing diagnostic blood loss and RBC transfusion.

STRATUS Pilot Study

We conducted a 14-week mixed-methods pilot study in the ICU-West at Hamilton General Hospital coordinated by the Population Health Research Institute (PHRI) to determine whether a larger study will be feasible (Figure 1). This consisted of a control period (current practice of standard-volume tubes) and an intervention period (small-volume tubes), each lasting 6 weeks and separated by a 2-week washout period. The main objective was feasibility with focus on evaluating the study protocol, assessing potential harms, assessing implementation issues, and evaluating data collection procedures. The primary outcome was feasibility defined as follows: (i) successful switch from standard-volume to small-volume tubes by the end of the 2-week washout period (≥95% correct tubes); (ii) adherence to correct tube size during the intervention period (≥95% correct tubes); (iii) sufficient volume for testing with small-volume tubes (<3% of samples); (iv) acceptability of intervention by end-users (nurses and laboratory staff); (v) identification of barriers and facilitators of implementation; and (vi) complete primary data collection from hospital administrative databases and electronic medical records (≥95% complete data collected). We conducted simple, targeted (5 minute) education sessions for nursing and laboratory staff prior to the intervention period. The cohort consisted of 412 consecutive patients, 204 during the control period and 208 during the intervention period (unpublished data, D. Siegal). Preliminary results show that a successful switch and adherence to tube size were achieved. There were 4197 blood specimens received in the laboratory during the control period and 3641 during the intervention period. The proportion of blood specimens which were cancelled/not done due to insufficient volume of blood was similar between the control and intervention periods (0.19% and 0.27%, p=0.13). Preliminary results from focus group discussions suggest that the educational resources provided were effective and that the intervention was acceptable to end-users.

Figure 1. Pilot Study Schematic



2. RATIONALE

Although it may seem obvious that soft-draw tubes should be used, this has not occurred likely due to a lack of high-quality evidence of benefit with minimal harm. There are barriers to implementation including concerns about workload, compatibility with laboratory equipment, validity of test procedures, reduced number of tests that can be performed and need for repeat testing which could lead to additional blood collection and delays in obtaining critical results. Thus, there is equipoise as to whether soft-draw tubes provide overall net benefit. A randomized trial is needed for an unbiased assessment of whether the use of soft-draw tubes can reduce the need for RBC transfusion while avoiding harms to patients or hospitals.

An individual patient randomized trial design would have significant feasibility challenges and would not address important implementation issues. A stepped wedge cluster randomized trial is an innovative methodological approach which can assess both effectiveness and implementation by incorporating the intervention of interest into routine practice; it is well suited to determine whether interventions work and are implementable in a “real world” setting. Using this design, ICUs (clusters) start with a strategy of blood collection using standard-draw tubes (control) and then at a pre-defined time point switch to soft-draw tubes (intervention). The timing at which individual ICUs switch to the intervention is randomly assigned prior to the start of the trial (Figure 2). Therefore, each ICU provides both control and intervention observations, in effect acting as their own controls.

If it can be shown that soft-draw tubes can be implemented for blood collection in adult ICU patients without significant adverse consequences, and that their use is associated with improved patient outcomes, this could lead to widespread practice change regarding blood collection for laboratory testing. Further, this study has the potential to influence policy to reduce waste and encourage stewardship of valuable blood products.

3. PRIMARY OBJECTIVE

To determine if an ICU-wide policy of using only soft-draw blood collection tubes (smaller volume tubes that collect 2 – 3 mL of whole blood) as compared to standard-draw blood collection tubes (4 – 6 mL capacity) improves patient outcomes without compromising blood testing procedures.

4. STUDY OUTCOMES

Primary Outcome

The primary outcome is the average number of RBC units transfused per patient during ICU admission among patients admitted to ICU for 48 hours or longer.

Secondary Outcomes

1. Number of RBC units transfused per patient for the whole cohort
2. Change in hemoglobin concentration from ICU admission to ICU discharge adjusted for number of RBC transfusions received
3. Proportion of patients who get at least one RBC transfusion
4. Number of blood specimens (tubes) received in the laboratory with insufficient volume for testing
5. Number of blood specimens (tubes) received in the laboratory during ICU admission
6. Number of blood tests ordered and resulted during ICU admission
7. ICU and hospital length of stay
8. ICU and hospital mortality

5. ELIGIBILITY

ICUs will be eligible if they meet the following criteria:

1. Large size ICU (at least 14 level 2-3 ICU beds) with capacity for invasive mechanical ventilation
2. Use BD Vacutainer® blood collection tubes
3. Use standard-draw tubes for blood sampling in adults
4. Electronic information is available for extraction of administrative and health record data

6. METHODS

STUDY DESIGN

The study design is a stepped wedge cluster randomized trial in which 25 ICUs (clusters) switch from a strategy of blood collection using standard-draw tubes (control) to soft-draw tubes (intervention). In this design, the timing at which individual ICUs switch to the intervention is randomly assigned (Figure 2). Therefore, each ICU provides before and after observations and switches from control to intervention, but at different times. There will be an initial period during which no ICUs are exposed to the intervention. Then, at each of 13 intervals (steps) of approximately 6 weeks' duration, 2 ICUs will switch to the intervention and last crossover will have 3 ICUs based on the randomly allocated schedule stratified by the cluster size. All patients at each study site will have blood drawn for routine and emergent laboratory testing (hematology, chemistry, coagulation) using the tube size to which the site is allocated at that time (e.g. soft-draw or standard-draw blood collection tubes) as the standard procedure.

A 1-week washout period will occur after switching to soft-draw tubes during which the tubes are used, but no patients admitted to ICU at washout period will be included to reduce the likelihood of carryover effect from previous blood collection that could affect results. There will be patients who span the washout period; such patients will have blood drawn into both size tubes. However, they will be analyzed with their initial allocated strategy. For the purposes of the secondary clinical outcomes, all analyses will assign patients to their initial tube volume even if some of their data are derived from the other group. Data will be collected for 30 days after ICU admission, or until hospital discharge or death.

The stepped wedge design allows the introduction of a new "policy" of using soft-draw tubes and evaluation of both effectiveness and implementation. The advantages of this pragmatic design are: (i) use of existing hospital electronic data to assess outcomes; (ii) focus on clinical effectiveness; (iii) low cost allowing the inclusion of a sufficient number of patients to answer the research question reliably; and (iv) implementation of soft-draw tubes into clinical practice at all sites by the end of the study.

Figure 2. Schematic diagram of stepped wedge cluster randomized trial design

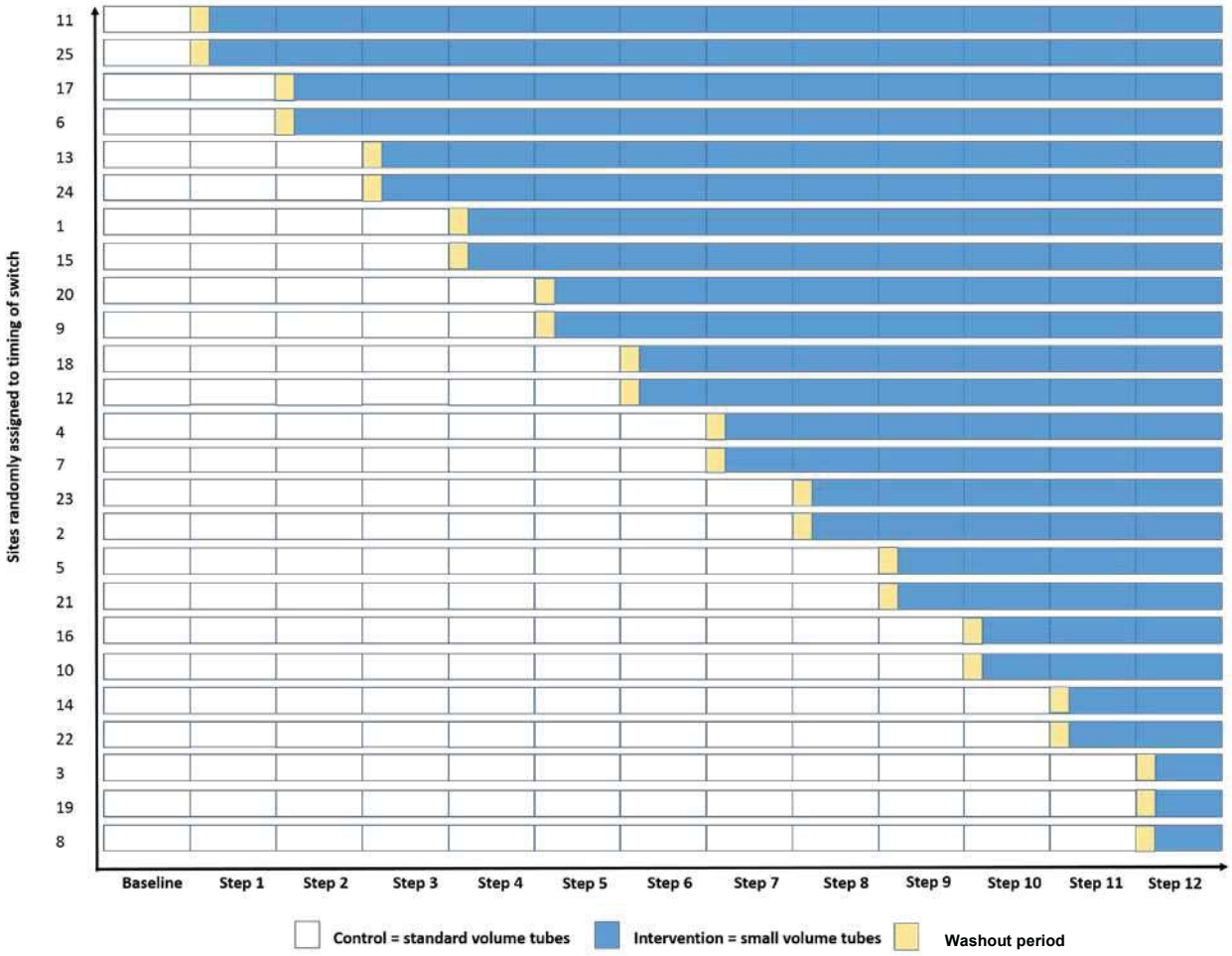


Figure 2. Individual ICUs (clusters) are shown on the y-axis and time points (steps) are shown on the x-axis. At the start of the study, patients are managed as per routine clinical practice (standard-draw tubes). At each step (approximately 2 months), 2 sites are randomly assigned to introduction of soft-draw tubes such that they have been introduced at all sites by the end of the study.

By incorporating the research protocol and interventions into routine clinical care and evaluating outcomes that use the electronic hospital information systems, the trial will be pragmatic, cost-effective and readily implementable in the “real world” setting. Our group has experience conducting both large multi-centre pragmatic randomized trials, cluster randomized trials and those done in the ICU involving blood sampling. Overall, this is the best way to test if a policy of using soft-draw blood tubes improves clinically relevant outcomes when this intervention is applied at the level of the hospital. The results of this study have the potential to influence blood sampling widely across Canada and internationally.

CONSENT

The cluster design challenges conventional approaches to clinical research because patients cannot choose to avoid the intervention, nor consent for the study because the intervention is applied at the level of the health care environment and not the patient. Because both interventions (soft-draw and standard-draw blood collection tubes) fall within current standard of care and are already in use in our hospital system, we will seek waiver of individual patient consent according to criteria proposed by the Tri-Council Policy Statement (TCPS 2): Ethical Conduct for Research Involving Humans[36]. This study fulfills TCPS criteria in that: (i) the study poses minimal risk to patients; (ii) waiver of consent will not adversely affect patient rights and welfare; (iii) it would be impracticable to carry out the research if prior consent is required; and (iv) patients and/or families will be provided with information about the study using an information

brochure and/or poster in ICU waiting areas. Members of our team have successfully implemented this consent process for previous low-risk studies.

STUDY INTERVENTIONS

Both soft-draw (2 – 3 mL) and standard-draw (4 – 6 mL) ethylenediaminetetraacetic acid (EDTA), lithium-heparin, citrate, fluoride and silica tubes (Vacutainer®, Becton, Dickinson and Company) are commercially available (Table 1). The standard-draw tubes are routinely used in hospitals and ICUs as standard practice, with some hospital areas (i.e. pediatrics) using the soft-draw tubes. The tubes are evacuated to create a vacuum inside the tube allowing filling with a predetermined volume of blood. The soft-draw tubes have similar physical dimensions as the standard-draw tubes, but have reduced vacuum and draw less blood during collection. Therefore, both tubes can be used on the same standard laboratory instruments without process modification. In addition, the cost is the same for both tubes.

Although very small volume capillary blood collection tubes (e.g. Microtainer®) are commercially available, they are more expensive and require manual collection and aliquoting at the bedside and in the laboratory, thereby introducing safety concerns and substantially increasing workload to unacceptable levels which would make them infeasible for widespread large-scale use outside a clinical trial setting. During the study period, only the allocated tubes will be stocked and used unless specially requested. To ensure reliable stocking of the correct sized tubes, the research coordinator at each site will conduct audits of tubes in storage areas.

Table 1. BD Vacutainer® Blood Collection Tube Volumes

Description	Standard-Draw (mL)	Soft-Draw or Reduced Volume (mL)
EDTA (lavender) BD Hemogard™ Conventional	 10.0 6.0 4.0 3.0 4.0	 2.0
Fluoride (grey) BD Hemogard™ Conventional	 6.0 4.0 10.0	 2.0
Lithium/Sodium Heparin (green) BD Hemogard™ Conventional	 6.0 4.0 10.0	 2.0
Plasma separation (light green) BD Hemogard™ Conventional	 4.5 3.5 10.0	 3.0
Citrate (light blue)	4.5 2.7	1.8
Serum (red)		

BD Hemogard™ Conventional	6.0 5.0 4.0 10.0	4.0 2.0
Serum separator (gold) BD Hemogard™ Conventional	 5.0 4.0 10.0 8.5 7.5	 3.5

Source: BD Life Sciences – Preanalytical Systems Product Catalogue 2016

STUDY CONDUCT

Following enrolment, sites will be randomized to a schedule of switching from standard-draw to soft-draw tubes. This will ensure sufficient lead-in time to plan for transition between tubes. A clinical research assistant (CRA) from the PHRI study coordination team will coordinate with site leads, purchasing and laboratory medicine to stock the new tubes at each site at the beginning of the washout period. During the treatment period, the CRA will conduct an audit of tubes in the ICU storage areas to evaluate adherence with regards to stocking the correct tube size. Data will be collected using data collection infrastructure that is already in place and downloaded into a secure electronic database stored on a password-protected server. Minimal personal health information and no direct identifiers will be entered into the system in order to protect patient privacy. The study will be conducted in accordance with the Declaration of Helsinki.

Coordinating Centre Responsibilities
- Assistance with Research Ethics Board Submission
- Assisting sites with procurement and stocking of blood collection tubes
- Education of nurses and laboratory staff at individual sites
- Assisting individual sites to identify data collection processes
- Central collection and management of electronic data
- Random audits of blood collection tube storage areas at sites
- Collection of feedback and troubleshooting in real-time

Site Responsibilities
- Research Ethics Board submission
- Identification of an individual to communicate with the study team
- Ensure appropriate tubes are available in accordance with the randomization schedule
- Identification of data collection processes and/or individuals responsible for data download at individual sites
- Ensure data transfers are cumulative, comprehensive and completed in accordance with study protocol and other operational instructions for the study

7. DATA COLLECTION AND MANAGEMENT

All data will be collected by PHRI from hospital administrative databases and electronic medical records. Datato be collected includes patient demographics, number of blood specimens received in the laboratory, number of laboratory tests ordered and resulted, specific laboratory tests, RBC transfusions administered, ICU mortality, in-hospital mortality, duration of ICU stay, duration of hospital stay, and primary and secondary diagnoses (ICD10 codes). Additional data will include use of mechanical ventilation and renal replacement therapy, and illness severity (as measured by multi-organ dysfunction score [MODS]) as these are additional covariates that may affect hemoglobin level and influence the likelihood of RBC transfusion.

Encryption of patient identifiers will be used to ensure confidentiality. Data will be validated, managed, and stored in a de-identified database on a secure server at PHRI.

8. STATISTICAL CONSIDERATIONS

SAMPLE SIZE

Because the rationale and hypothesis of our study are based on the cumulative effects of diagnostic blood loss during ICU admission, our primary analysis will be limited to patients admitted to the ICU for 48 hours or longer. We chose this patient population for our primary analysis as ICU patients represent the highest risk population; they have longer lengths of stay, undergo frequent blood testing and RBC transfusion making it more likely to detect an effect of the intervention. To ensure sufficient statistical power, we used the following conservative estimates based on local (n=7273 patients admitted to 3 ICUs for ≥48 hours) and published data:

(i) Average length of stay of 5 days based on the median length of stay in ICU of 5.1 days (IQR 3.0-10.5) in our cohort of ICU patients admitted for ≥48 hours. The mean (SD) ICU length of stay reported in a large prospective cohort study of similar ICU patients admitted to medical/surgical ICU with anticipated stay >48 hours was 7.4 (7.3) days[4]. A recent study in Ottawa, Ontario showed the median ICU length of stay was 4 days (IQR 2-10) for all admitted ICU patients[37].

(ii) Baseline RBC transfusion rate of 2 units per patient during ICU admission. In our cohort, the RBC transfusion rate for patients admitted for ≥48 hours was 2.7 RBC units per patient. Published data show the average number of RBC units transfused during ICU admission (among transfused patients) is approximately 4 units[1,4,38].

(iii) Admission rate of 40 patients per month. In our cohort, the mean admission rate was 67 patients per ICU per month.

Assuming a power of 90% with a Type I error of 5% (2-sided) and intra-cluster correlation coefficient (ICC) of 0.01, 25 sites with 729 patients per site (total 18,240 ICU patients) are required to detect a minimum difference of 5 units per 100 patients (or 2.5% reduction). Because the interventions are applied in hospital, we expect minimal loss to follow-up.

STATISTICAL ANALYSES

The primary analysis will evaluate the effect of soft-draw compared to standard-draw blood collection tubes on the number of RBC units transfused per patient during ICU admissions 48 hours or longer as they are most likely to be affected by the cumulative effects of diagnostic blood loss. Secondary analyses will evaluate the effect of the intervention on RBC transfusion in all ICU patients. A generalized poisson mixed model (hierarchical model) will be used to analyze the effect of tube size (intervention) on the number of RBC units transfused per patient at ICU stay. Intervention and periods will be modelled as fixed effects and the centers will be modelled as a random effect. Analyses will also be conducted with adjustment for known covariates that may affect RBC transfusion (age, sex, use of renal replacement therapy, illness severity as assessed by MODS score, duration of ICU admission). For all analyses, a p-value of ≤0.05 will be considered significant, and all statistical comparisons will be two-tailed. There may be patients who span the washout period such that they will have blood drawn into both size tubes; however, all analyses will assign patients to their initial tube volume even if some of their data are derived from the other group. All patients admitted to ICU during the washout period will not be include. Planned subgroup analyses will include sex, age, most responsible diagnosis category (surgical vs. medical), most responsible or post-admit diagnosis of bleeding, and hemoglobin concentration at ICU admission.

9. STUDY ORGANIZATION

STEERING COMMITTEE

The study will have a Steering Committee consisting of the Principal Investigator (who will chair the committee) and several key investigators and regional leaders. The functions of the Steering Committee include advice on the scientific and clinical aspects of the study protocol and related documents, overall responsibility for the execution and scientific reporting of the trial, responsibility for the conduct of the trial, resolution of policy issues that might be encountered during the trial.

COORDINATING CENTER

The trial will be managed by the Population Health Research Institute (PHRI), a joint institute of McMaster University and Hamilton Health Sciences (Hamilton, Ontario, Canada). The Coordinating Center will consist of a study team consisting of the Principal Investigator, Program Manager and/or Research Coordinator, Research Assistant(s) and Data Management Assistant(s). The study team will facilitate and oversee execution of the study in collaboration with the Steering Committee. Coordinating Center responsibilities encompass study design and set-up, data management, statistical analysis, study initiation, maintenance and close-out, publications and dissemination of study results.

10. INVESTIGATOR AND ADMINISTRATIVE REQUIREMENTS

INSTITUTIONAL REVIEW BOARD

The protocol and request for waiver of informed consent for this study must be reviewed and approved by an appropriate IRB before the study commences. It is the responsibility of the investigator to ensure that the study is conducted in accordance with current country and Local Regulations, International Conference on Harmonisation, Good Clinical Practice, and the Declaration of Helsinki. A letter, documenting the approval that specifically identifies the protocol by number and title as well as the Investigator, must be received by PHRI before initiation of the study. Amendments to the protocol will be subject to the same requirements as the original protocol. After the completion or termination of the study, the Investigator will submit a report to the IRB as per local requirements.

RETENTION OF DATA

The investigators must retain trial records for the amount of time specified by applicable laws and regulations or by ICH E6 Good Clinical Practice guidelines, whichever is longer. All trial documents shall be made available upon request from relevant health authorities. Any investigative center will consult the PHRI Coordinating Centre before discarding trial and/or subject files.

DEVIATION FROM THE PROTOCOL

The investigators should not deviate from the protocol except where necessary to eliminate an immediate hazard(s) to clinical trial patients. Deviations will be reported to the PHRI Coordinating Centre.

STUDY MONITORING

The Investigator will allow representatives of PHRI to periodically audit relevant portions of the investigative site. The representative will conduct audits of tubes in storage areas during regular inspections to ensure reliable stocking of the correct tubes. The monitoring visits provide PHRI with the opportunity to evaluate the progress of the study; ensure that all protocol requirements, applicable regulations, and Investigator's obligations are being fulfilled; and resolve any issues that may arise.

11. DISCLOSURE OF DATA

Individual patient medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. Patient confidentiality will be further assured by utilizing patient identification code numbers to correspond to treatment data in the computer files. The study personnel, employees of the regulatory agencies, including Health Canada, and the study sponsor, PHRI, and its agents may need to review patient medical records in order to accurately record information for this study. If results of this study are reported in medical journals or at meetings, the patient's identity will remain confidential.

12. OWNERSHIP OF DATA AND USE OF STUDY RESULTS

The STRATUS Steering Committee has the ownership of all data and results collected during this study.

13. PUBLICATION POLICY

All study presentations and/or publication of the results will be based on clean, checked and validated data in order to ensure the accuracy of the results. All analyses for publication will be provided by the PHRI Coordinating Centre. The responsibility for presentations and/or publications belongs to the Steering Committee. The final content of the manuscript is the responsibility of the Steering Committee. Publication of the main findings of this study will be made jointly in the name of all wholehearted collaborators. Other papers will be authored based on the contributions of the individuals to the overall study. All the trial participants (investigators and committee members) make a prior delegation of responsibility for primary presentation and/or primary publication of the results to the Steering Committee. No other publication is allowed before the primary publication. Any presentation or publication of trial material must mention the trial and has to be approved by the Steering Committee. Moreover, it is mandatory to make reference to the primary publication.

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Small Volume Tubes to Reduce Anemia and Transfusion:

A Pragmatic Stepped Wedge Cluster Randomized Trial

STATISTICAL ANALYSIS PLAN

Version: Final 1.0

Issue Date: 2021-10-15

Authors: Deborah Siegal MD MSc
Principal Investigator
McMaster University
Population Health Research Institute (PHRI)

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Statistician
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APPROVAL

Version #	**Final 1.0**
Version Date	2021-10-15

By signing the below, I designate my approval of the above-named version of the STRATUS Statistical Analysis Plan on behalf of all named authors.

Name	**Deborah Siegal**
Role	**Principal Investigator**
Signature	DocuSigned by: Deborah Siegal 9D517875EF2449E...
Date (yyyy/mm/dd)	2021-Nov-04 | 09:54 EDT

By signing the below, I designate my approval of the above-named version of the STRATUS Statistical Analysis Plan on behalf of PHRI Statistics.

Name	**Shun Fu Lee**
Role	**Study Statistician**
Signature	DocuSigned by: Shun-Fu Lee F16F0B626DEC4F2...
Date (yyyy/mm/dd)	2021-Oct-18 | 15:00 EDT

1 TABLE OF CONTENTS

2 LIST OF ABBREVIATIONS

ACS	Acute Coronary Syndrome
CI	Confidence Interval
EDTA	Ethylenediaminetetraacetic Acid
ICU	Intensive Care Unit
GRACE	Global Registry of Acute Coronary Events
Hb	Hemoglobin
MODS	Multi-Organ Dysfunction Score
MRN	Medical Record Number
NSTEACS	Non ST Elevation Acute Coronary Syndrome
NSQ	Inadequate Volume for Testing
OR	Odds Ratio
PHRI	Population Health Research Institute
RBC	Red Blood Cells
SD	Standard Deviation
STEMI	ST Elevation Myocardial Infarction
TIMI	Thrombolysis In Myocardial Infarction

3 INTRODUCTION

Background:

Blood sampling can cause significant unnecessary blood loss particularly in the intensive care unit (ICU) where the volume of blood drawn is similar to donating a unit of red blood cells (RBC) every 8 days. Only 10% of patient blood collected is used for testing which indicates that volumes drawn can be drastically reduced without compromising patient care or laboratory processes. Blood loss contributes to anemia which is highly prevalent in the ICU (>90% of patients after 3 days) and is associated with major adverse cardiovascular outcomes and death. Diagnostic blood loss increases the likelihood of RBC transfusion which is given to correct anemia in about 40% of ICU patients (half of transfusions are given in absence of hemorrhage) and transfusions have significant health risks. Small-volume blood collection tubes, which collect about 50% less blood, are available, but rarely used in adults. They are compatible with the same laboratory equipment and have the same cost as standard-volume tubes. The rationale for the continued use of standard-volume tubes is a theoretical concern about inadequate volume for testing and the absence of data showing the benefit of small-volume tube use on an important clinical outcome.

Study Objective:
The specific aim of the STRATUS study is to evaluate whether the routine use of small-volume blood collection tubes (1.8 – 3 mL) reduces RBC transfusion compared to standard-volume blood collection tubes (4 – 6 mL) in adult ICU patients.

Methods:
Using a stepped wedge cluster randomized trial design, ICUs (clusters) will switch from current standard-volume tubes (control) to small-volume tubes (intervention), but at different times which are randomly allocated. The primary analysis will focus on adult ICU patients who undergo frequent blood testing and RBC transfusion where the effect of the intervention will be largest and will be seen most quickly. Administrative and electronic health data will be used to assess outcomes. The study will be conducted by a multi-disciplinary team and coordinated by the Population Health Research Institute at McMaster University which has extensive experience coordinating multi-centre studies including cluster trials.

The statistical analysis plan (SAP) specifies the details of the statistical analysis of the STRATUS study described in the Clinical Study Protocol (version 1.0, dated 2018-05-01). The SAP is a working document that will be amended as new information becomes available. Approval is provided for the content of the appendices at the time of approval. Appendices may be updated as required during the course of the study without obtaining approval for the changes.

4 STUDY HYPOTHESES/OBJECTIVES

4.1 Primary Objectives

To determine if an ICU-wide policy of using only soft-draw blood collection tubes (smaller volume tubes that collect 1.8 – 3 mL of whole blood) as compared to standard-draw blood collection tubes (4 – 6 mL capacity) improves patient outcomes without compromising blood testing procedures.

4.2 Primary Outcome

The primary outcome is the number of RBC units transfused per patient during ICU admission among patients admitted to ICU for 48 hours or longer.

4.3 Secondary Outcomes

1. Number of RBC units transfused per patient during ICU admission for the whole cohort
2. Change in hemoglobin concentration from ICU admission to ICU discharge adjusted for the number of RBC transfusions received
3. Proportion of patients admitted to ICU for 48 hours or longer who get at least one RBC transfusion
4. Proportion of patients within the whole cohort who get at least one RBC transfusion
5. Number of blood specimens (tubes) received in the laboratory with insufficient volume for testing
6. Number of blood specimens (tubes) received in the laboratory during ICU admission
7. Number of blood tests ordered and resulted during ICU admission
8. ICU and hospital length of stay
9. ICU and hospital mortality

5 SAMPLE SIZE

Assuming a power of 90% with a Type I error of 5% (2-sided) and intra-cluster correlation coefficient (ICC) of 0.01, 25 sites with 729 patients per site (total 18,240 ICU patients) are required to detect a minimum difference of 5 units per 100 patients (or 2.5% reduction). Because the interventions are applied in hospital, we expect minimal loss to follow-up. Assuming an average of 40 patients per month, two clusters (3 in the last period) will cross-over for each period of a total of 13 periods each with a duration of 6 weeks.

6 POPULATIONS TO BE ANALYZED

Data was collected from administrative data sources for all patients admitted to the participating ICUs between February 05, 2019, and December 22, 2020, with the last date to be January 21, 2021 for a 30 days follow-up (Refer to diagram of the stepped wedge design in APPENDIX A). Only data for patients with admission times falling within the study period and with age ≥ 18 years will be included in the analysis. The primary analysis will be conducted on patients who were admitted to the ICU for 48 hours or longer. Patients will be followed until hospital discharge, 30 days, or death, whichever is earliest.

A 1-week washout period occurred after switching to soft-draw tubes during which the tubes are used, but no patients admitted to ICU during the washout period will be included.

There were patients admitted during the control period and remaining in ICU during the washout period (i.e. span the washout period). These patients will be analyzed according to the initial allocated strategy. For the purposes of the secondary clinical outcomes, all analyses will assign patients to their initial tube volume even if some of their data are derived from the other group.

6.1 Multiple hospitalizations

If a patient is hospitalized more than once during the study period, the first eligible hospitalization i.e. ICU stay >=48 hours will be utilized and the patient will be assigned to the treatment allocation of the eligible hospitalization. All other hospitalizations will be excluded.

If a patient has multiple hospitalizations across multiple sites and we are able to identify this, the first eligible ICU admission (i.e. ICU stay >=48 hours) will be utilized and the patient will be assigned to the treatment allocation and ICU of the eligible ICU admission. All other hospitalizations will be excluded.

6.2 Population affected by COVID-19

The planned transitions to the intervention were delayed at 7 sites due to the COVID-19 pandemic. We considered the period between March 2 and August 17, 2020 to have been critically impacted by the pandemic, interfering with reliable implementation of the intervention. This interference was due to enhanced clinical workload of staff, clinical and research staffing shortages, pandemic preparedness activities, and infection control precautions. In addition, the types of patients admitted to ICU (and their prognoses) may have changed substantially after the start of the COVID-19 pandemic, with variable proportions of patients admitted for COVID-19 and elective surgery across sites. The

prevalence of other underlying conditions leading to ICU admissions also changed in unanticipated ways. Therefore, all patients admitted during this period will not be included in the primary analysis but will be considered for the sensitivity and/or subgroup analysis. Transitions to the intervention at the remaining sites were resumed according to the randomization schedule on August 18, 2020, since the impact of COVID-19 was considered minimum after the power assessment under different scenarios.

6.3 Population without COVID-19

A sensitivity analysis will include patients admitted to ICU for 48 hours or longer during the pre-pandemic period only (February 5, 2019 and March 1, 2020). Data will be collected until March 31, 2020 (30 day follow-up of the last patient admitted on March 1, 2020).

6.4 Entire cohort

The secondary population proposed in the protocol will be all patients admitted to ICU between February 5, 2019 and December 22, 2020. Data will be collected until January 21, 2020 (30 day follow up of the last patient admitted on December 22, 2020). Approach to determine the population for the primary analysis is as follows.

We propose to investigate the effects of the COVID-19 by dividing the study population into three phases.

Phase I: study period prior to the COVID-19 pandemic (February 5, 2019 to March 2, 2020),

Phase II: study period considered to be critically impacted by the COVID-19 pandemic and during which time implementation of the intervention was paused (March 1, 2020 to August 17, 2020),

Phase III: study period considered to be less impacted by the COVID-19 pandemic and implementation of the intervention was resumed (August 18, 2020 to December 22, 2020).

We will tabulate the baseline characteristics of the patients based on the patient's admission date by three phases described above (Table A1).

The patients enrolled in phase I and III will be the population to determine the primary outcome. We will also run sensitivity analyses by repeating similar analyses for patients enrolled in all three phases.

7 TREATMENT ALLOCATION

After study initiation, there will be an initial period during which no ICUs are exposed to the intervention. Then, at each of the 13 intervals (steps) of approximately 6 weeks' duration, 2 ICUs will switch to the intervention (3 ICUs in the last interval), based on the randomly allocated schedule stratified by the cluster size. The allocation can be referred to in the design diagram in APPENDIX A.

8 ANALYSIS

The primary analysis will evaluate the effect of soft-draw (intervention) compared to standard-draw (control) blood collection tubes on the number of RBC units transfused per patient during ICU admissions 48 hours or longer as these admissions are most likely to be affected by the cumulative effects of diagnostic blood loss. A negative binomial mixed model (hierarchical model) will be used to analyze the effect of tube size (intervention) on the number of RBC units transfused per patient per ICU stay with periods modelled as a fixed effect and centers as a random effect. We will adjust the model for known covariates that may affect RBC transfusion such as age and sex. This model will be referred to as the primary model.

We will also evaluate for temporal trends and the impact of COVID-19 (Phase I vs Phase III) using an interaction term between the periods and treatment effects and the phase and treatment effect, respectively.

Since we will not have data for all covariates from all the sites (except age and sex), the covariates will first be assessed between the intervention groups using the standardized mean. We will then conduct a sensitivity analysis for the primary model including the intervention effect and any imbalance covariates with a standardized mean >0.10

The secondary outcomes will be analysed using the linear or logistic mixed models accounting for the stepped wedge design for the outcomes according to whether they are continuous or binary, respectively. The mean differences with 95% CIs will be reported for the continuous outcomes. The relative risks with 95% CIs will be reported for the count or binary outcomes.

All the analyses will be conducted in SAS 9.4. A p-value of ≤0.05 will be considered significant, and all statistical comparisons will be two-sided.

Summary Tables of Baseline characteristics and Outcomes are shown in Appendix B. Description of the data variables are provided in Appendix C.

8.1 Handling of Missing Values

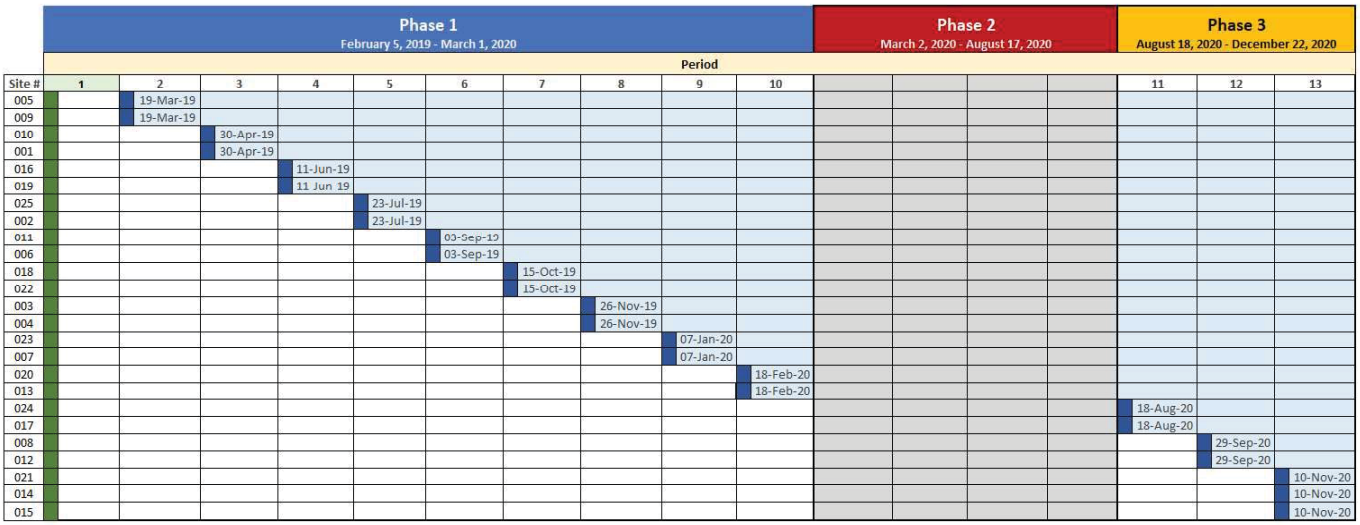
We will impute the ICU admission date plus the median ICU stay days or the death date, whichever is earliest for the patients missing the ICU discharge date. Similarly, we will impute the hospital admission date plus the median hospital stay days or the death date, whichever is earliest for the patients missing the hospital discharge date. For patients with missing admission or discharge times, the time will be replaced with 23:59.

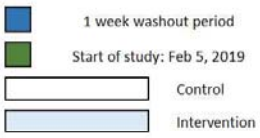
In general, missing values will be treated as ‘missing’. No attempt will be made to impute missing values and only observed values will be used for analysis.

8.2 Planned subgroup analyses

Planned subgroup analyses will include COVID phases, sex, age, most responsible diagnosis category (surgical vs. medical), and hemoglobin concentration at ICU admission. It is noted that except for sex and age, subgroup analyses will be conducted for those sites that have the variables available.

APPENDIX A. DIAGRAM OF STEPPED WEDGE DESIGN





Period #	Period Start Date	Period End Date	
Period 1	2019-Feb-05	2019-Mar-19	
Period 2	2019-Mar-19	2019-Apr-30	
Period 3	2019-Apr-30	2019-Jun-11	
Period 4	2019-Jun-11	2019-Jul-23	
Period 5	2019-Jul-23	2019-Sep-03	
Period 6	2019-Sep-03	2019-Oct-15	
Period 7	2019-Oct-15	2019-Nov-26	
Period 8	2019-Nov-26	2020-Jan-07	
Period 9	2020-Jan-07	2020-Feb-18	
Period 10	2020-Feb-18	2020-Mar-01	Original end date March 24 cut short due to COVID
COVID HOLD	2020-Mar-02	2020-Aug-17	
Period 11	2020-Aug-18	2020-Sep-29	
Period 12	2020-Sep-29	2020-Nov-10	
Period 13	2020-Nov-10	2020-Dec-22	
Follow-up start	2020-Dec-23		
End date	2021-Jan-21		

APPENDIX B. SUMMARY TABLES

Table A1. Baseline Characteristics stratified by phase

	PHASE I	PHASE II	PHASE III
Total admitted, n			
Age, yrs mean(SD) median (IQR)			
Sex, n(%) Female Male			
Most responsible diagnosis*			
Pre-admission comorbidities			
Hemoglobin concentration at ICU admission (female) mean(SD) median(IQR)			
Hemoglobin concentration at ICU admission (male) mean(SD) median(IQR)			
Anemia at ICU admission, n(%)** None Mild (111-120) Moderate (90-110) Severe (≤90 g/L)			
Creatinine at ICU admission mean(SD) median(IQR)			
MODS score available, n(%) mean(SD) median(IQR)			

*ICD codes

**For women: None >/= 120 g/L, Mild (110-119 g/L), Moderate (90-99 g/L), Severe <90 g/L For men: None >/= 130 g/L, Mild (120-129 g/L), Moderate (100-119 g/L), Severe <100 g/L.

Table 1. Baseline Characteristics

	Overall	Control	Intervention
Total admitted, n			
Age, yrs mean(SD) median (IQR)			
Sex, n(%) Female Male			
Most responsible diagnosis*			
Comorbidities in hospital*			
Duration of ICU admission, days mean(SD) median(IQR)			
Hemoglobin concentration at ICU admission (female) mean(SD) median(IQR)			
Hemoglobin concentration at ICU admission (male) mean(SD) median(IQR)			
Anemia at ICU admission, n(%)** None Mild (111-120) Moderate (90-110) Severe (≤90 g/L)			
Creatinine at ICU admission mean(SD) median(IQR)			
MODS score available, n(%) mean(SD) median(IQR)			

*ICD codes

**For women: None >/= 120 g/L, Mild (110-119 g/L), Moderate (90-99 g/L), Severe <90 g/L For men: None >/= 130 g/L, Mild (120-129 g/L), Moderate (100-119 g/L), Severe <100 g/L.

Table 2. Outcomes

	Overall	Control	Intervention
Duration of ICU admission, days mean(SD) median(IQR)			
Duration of hospital admission, days mean(SD) median(IQR)			
Creatinine, umol/L Highest during ICU admission mean(SD) median(IQR)			
Dialysis exposure during ICU admission, n(%)			
Mechanical Ventilation, n(%)			
Mortality, n(%) In ICU In Hospital			
Lab Testing Blood specimens sent for laboratory testing Specimens not analyzed due to insufficient volumes, n(%)			
RBC Transfusion Patients receiving RBC transfusion in ICU, n(%) 1 unit RBC 2 units RBC 3 units RBC 4 units RBC 5 or more units RBC RBC units transfused in ICU mean(SD) median(IQR) Time from admission to first RBC transfusion, hrs mean(SD) median(IQR)			
Hemoglobin Last hemoglobin within 48hrs prior to ICU discharge mean(SD) median(IQR) Adjusted† last hemoglobin within 48hrs prior to ICU discharge mean(SD) median(IQR) Change in hemoglobin from ICU admission to ICU discharge mean(SD) median(IQR)			

Adjusted change in hemoglobin from ICU admission to ICU discharge mean(SD) median(IQR)			
Anemia‡ Incidence of anemia during ICU stay Severe Moderate Mild No anemia			

† Hemoglobin adjusted for RBC transfusion 1 transfusion = Hb – 10 g/L

‡ For women: None >/= 120 g/L, Mild (110-119 g/L), Moderate (90-99 g/L), Severe <90 g/L For men: None >/= 130 g/L, Mild (120-129 g/L), Moderate (100-119 g/L), Severe <100 g/L.

APPENDIX C. LISTING OF VARIABLES

Category	Suggested Variable Name
Administrative	MRN
	Gender
	PatientAgeYears
	AdmitDate
	AdmitTime
	ICUadmit
	ICUadmitTime
	ICUdischarge
	ICUdischargeTime
	DischargeDate
	DischargeTime
	DateofDeath
	Dialysis
	MechanicalVent
	ICD10DiagnosisCode_BKey
	DiagnosisTypeDescription
	APACHEscore
	MODScore
Transfusion	MRN
	Product
	UnitNumber
	Specimen#
	TransfusionDate
	TransfusionTime
Laboratory	MRN
	Specimen#
	CollectedDate
	CollectedTime
	TestName
	Result
	ResultUnit
	Comment