INFORM Study Statistical Analysis Plan

1. Introduction

This document outlines the analysis plans for the INFORM Study, an international pragmatic trial in which patients were randomized in a 1:2 fashion to receive either the freshest available red blood cells or standard care red blood cells. The primary outcome is in-hospital death and the comparison is to be based on a logistic regression model adjusting for center and blood group.

2. Datasets to be analysed

The primary comparison between treatment arms will be based on patients with group A and O blood.

The primary dataset for analysis will be based on a modified intention-to-treat principle in which outcomes will be attributed to the randomized intervention but with the restriction that patients must have received at least one RBC transfusion and must have been admitted to hospital (NOTE: in-hospital death could not occur if the patient was not admitted to hospital).

Secondary datasets will include patients of all blood groups.

3. Analysis Objectives

The primary goal of the analysis is to compare in-hospital mortality between the randomized groups among hospitalized patients who received at least one transfusion.

4. Endpoints and Covariates

In-hospital death is the primary binary outcome. The time to in-hospital death will also be analysed as a secondary outcome. Stratification variables include centre and recipient A/O status.

The analyses will be restricted to data from randomized patients who were admitted to hospital.

6. Statistical Analyses

6.1 Descriptive Analyses

Baseline data will be summarized using descriptive statistics such as means and standard deviations and as numbers and proportions, as appropriate.

6.2 Primary Analyses

A stratified logistic regression model (McCullagh and Nelder, 1989) will be fitted with in-hospital death as the outcome: (patients not observed to die who are still in hospital on Nov 20, 2015 (30 days after last recruitment date - Oct 21, 2015), will be classified as alive), stratified by centre and blood group, with a single binary covariate indicating assignment to the freshest blood available. For such a logistic regression model, this amounts to specifying a linear predictor comprised of main effects for centre, recipient blood group (A/O) and intervention level (freshest blood or standard issue). The odds ratio and associated 95% confidence interval will be reported and the p-value will be calculated to test the null hypothesis of no difference in the in-hospital mortality between the two arms.

6.3 Secondary Analyses

6.3.1 Competing Risk and Cox Regression Modeling of Time to In-hospital Death

Nonparametric estimates of the cumulative probability of in-hospital death will be computed for each randomized arm treating discharge as a competing risk (Crowder, 2012). The relationship between arm and time of in-hospital death will also be assessed using stratified Cox regression with time-dependent covariates reflecting exposure to different storage durations (Kalbfleisch and Prentice, 2002). Center and recipient blood group will be the stratification factors. The time of in-hospital death will be censored at the time of discharge for the hospitalization in which patients were randomized, or at the time they were last known to be in hospital for those not observed to die or become discharged. Model diagnostics will be carried out for all proportional hazards assumptions using tests based on Schoenfeld residuals (Therneau and Grambsch, 2000). When there is evidence of non-proportionality, models will be generalized to allow the age of blood effects to vary with time by fitting interactions between the respective covariates and a covariate which is a defined function of time (Kalbfleisch and Prentice, 2002).

6.3.2 Assessment of Heterogeneity of Treatment Effects (group A and O patients)

To determine if there is statistically significant variation in the treatment effect between countries and between blood types, two more general logistic regression models will be fitted based on the full analysis dataset with main effects for centre, patient blood group treatment arm and interaction terms: in one model interaction terms will involve country

and treatment arm; in the second model an interaction term will be added involving blood group (A/O) and treatment arm.

Country* treatment arm interaction: We hypothesize that the beneficial effect of receiving fresh blood in Canada will be significantly smaller than other countries due to the whole blood processing method used in Canada as supported by recent evidence from an RCT (Lacroix et al) and an observational study (Heddle et al).

Blood group*treatment arm interaction: An analysis of recipient blood group by treatment arm will also be carried out with no *a priori* hypothesis.

A significance level of 0.05 will be used for each test involving interaction terms. Separate logistic regression models will be fitted to the data to explore effects if interaction terms if they are significant. The main effect of patient blood group will also be examined to assess whether the mortality rates differ by blood group based on literature indicating that group O patients have lower levels of von Willibrands factor. We hypothesize that mortality will not be significantly different between group A and O individuals.

Further analyses will be directed at assessing the homogeneity of the treatment effect according to the primary diagnosis: cardiovascular disease, cancer, trauma, digestive diseases and other. A 4-degree-of freedom likelihood ratio test will be used to determine the significance of interaction terms involving treatment and most responsible diagnosis.

Three sub-group analyses will also be carried out by restricting attention to patients who had cardiovascular surgery, patients who had non-cardiovascular surgery, and ICU patients. These sub-groups are not mutually exclusive and so the primary analysis will be repeated for each of them using a logistic regression model with stratification variables for centre and patient blood group along with the main effect of treatment. A Bonferroni adjustment will be made to address the multiple comparisons and a significance level of 0.05/3 will be used to determine significance.

6.3.3 Using all Patients with Any Blood Group

The above analyses will be repeated for patients of blood groups A, O and B, and for all blood groups.

6.4 Additional Exploratory Analyses

6.4.1 Analyses Directed at More Detailed Summaries of Exposure History

A tertiary exploratory analysis will be directed at modeling the relationship between exposure to blood of different storage durations and risk of in-hospital death but when in-hospital death is:

a. treated as a binary outcome and analyses are based on stratified logistic regression, and

b. when the time of in-hospital death is used and analyses are carried out using a stratified Cox regression model.

The first analysis is similar to how the primary comparison is to be made between arms. The latter analysis is most suitable for the setting where exposure variables are dynamic. In this case time-dependent summaries of the exposure to blood of different storage durations will be examined including the maximum cumulative storage duration, the average cumulative storage duration, and minimum cumulative storage duration.

Categorical versions of these continuous time-dependent exposure variables (i.e. using quartiles of storage duration of transfused units), will also be created and used as predictors in Cox regression models to facilitate the examination of a dose-response relationship between age-exposure and in-hospital mortality. These models will permit estimation of relative risks by the exposure status for different durations of storage. Relative risks will then be computed to examine whether there are particular durations of storage above which risks become unacceptably higher than chosen reference levels. Note that while this modeling exercise is similar to an observational analysis since it is not directed at simple comparisons across the two randomized treatment arms, randomization ensures that there will be a larger than normal amount of variation in the storage duration for the transfused blood and that more patients will be consistently exposed to fresher or older blood over multiple transfusions than would occur in standard practice; this should enhance the power of these analyses.

6.4.2 Exploratory Analyses Pertaining to Policy Implications

If the INFORM trial results demonstrate superiority of freshest available vs. standard-issue blood, we will carry out a modeling exercise combining clinical outcome data with administrative information obtained from blood banks and blood suppliers to evaluate the impact of potential changes to blood shelf-life on inventory, the availability of supply, and cost. Using a stratified approach, we will create models to evaluate the utility of various static issuing policies vs. dynamic policies that might consider current inventory state, expected demand, and anticipated supply over a short to medium (e.g., 3-5 days) time horizon. We will, in addition, evaluate the potential impact in terms of inventory outdates and product shortages for a range of potential age of blood cut points as well as estimating the increased volume of donors or additional funds required to operate a blood system in which blood shelf-life was reduced from the maximum (35-42 days).

Additional analyses will also be carried out by considering interaction terms between the sex of the donor and treatment arm. The interaction term will be time-dependent since the sex of the donor on successive transfusions may vary. Specifically we will create a binary time-dependent variable which indicates whether the patient has been exposed to blood from a female donor and this indicator will be used to create an interaction term

with the treatment arm. This will then be tested to examine whether the effect of storage duration is different according to whether the donor was female or male.

6.4.3 Additional Descriptive Analyses of Out-dating Rates

The number of red blood cells discarded by centers will be summarized and compared to historical rates in descriptive analyses.

Plan for Figures

Figure 1: Consort Diagram (to discuss)

Figure 2: Distribution of the age of blood transfused by allocated arm (Panel A), distribution of minimum (Panel B), average (Panel C) and maximum (Panel D) storage duration of RBC units each patient received over the course of their admission.

Figure 3: Nonparametric estimates of the cumulative incidence functions for in-hospital death for each study arm (freshest or standard issue RBCs)

Figure 4: Forest plots of hazard ratios from Cox regression models with estimates for overall analysis and estimates by country (Panel A) and estimates according to the underlying condition (specific list of conditions to be examined is to be defined; Section 6.3.2 of SAP lists cardiovascular disease, cancer, critical care, trauma, non-cardiac surgery, cardiac surgery, or other, but we can revise); patients of A/O blood group

[Plot will contain estimates of hazard ratios, 95% confidence intervals and p-values for each line, along with the p-value for the test of the null hypothesis that the effects are homogeneous across sub-groups.]

Figure 5: Similar to Figure 4 but not restricting to patients of A/0 blood group.

References

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