Supplementary Materials for
“Estimating the effect of donor sex on red blood cell transfused patient mortality: a retrospective cohort study using a targeted learning and emulated trials-based approach”

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For simplicity the DAG only includes the 2 first days of follow-up (day 0 and 1). The DAG can however be extended to cover the full follow-up period without introducing additional confounding than that presented in the simplified DAG. Day 0 and 1 can therefore be interpreted as day $k-1$ and $k$. The time axis is from left to right. The red arrows indicate unadjusted confounding. In the following, the included covariates will be explained:

**Disease severity:** The disease severity of the patient at day $k$

**Hemoglobin:** The hemoglobin level of the patient at day $k$

**Hospital:** Hospital of admission at day $k$
RBC units: The number of RBC units transfused on day $k$

Male/female donor ratio: The treatment. This is the ratio between RBC units from male and female donors of the RBC units transfused on day $k$. The coding of the treatment variable is elaborated in the methods section of the supplementary.

Total RBC units received: The cumulative number of RBC transfusions received prior to day $k$ not including the RBC units transfused on day $k$ (“RBC units”)

Month and year: Year and month at day $k$

Patient age: Patient age at baseline

Patient sex: Patient sex

Patient blood type: ABO/RhD blood group of the patient

Using DAGitty the minimal sufficient set of covariates that needed to be adjusted for to estimate the total effect was “RBC units_1”, “Hospital_1”, and “Month and year_1”. As shown in the DAG below, adjusting for these 3 covariates blocks all backdoor paths (no red arrows).
Minimal sufficient set adjustment: RBC units_1, Hospital_1, and Month and year_1 (calendar period).

To block for confounding from random variability and residual confounding we adjusted for additional covariates as shown in the DAG below.
Main analyses adjustment
Table 1: Distribution of treatment and patient characteristics by hospital (“center-effect”)

|                                | BISPEBORG  | BORNHOLMS  | HERLEV   | HUDOVRE  | NORDSJAELLAND | RIGSHOSPITALET | ALL  |
|--------------------------------|------------|------------|----------|----------|---------------|----------------|------|
|                                | (N=11772)  | (N=2209)   | (N=19671)| (N=18742)| (N=13669)     | (N=2754)       | (N=90917)     |
| Patient age (years)            |            |            |          |          |               |                |      |
| Mean (SD)                      | 74.5 (14.8) | 72.5 (15.3) | 70.8 (16.0)| 69.3 (18.3)| 71.7 (15.2)   | 62.5 (15.7)    | 68.7 (16.7)   |
| Median [1th, 99th]             | 77.0 [20.0, 98.0] | 75.0 [24.0, 96.0] | 73.0 [24.0, 96.0] | 73.0 [23.0, 97.0] | 74.0 [26.0, 96.0] | 66.0 [20.0, 89.0] | 71.0 [23.0, 96.0] |
| Patient sex                    |            |            |          |          |               |                |      |
| Male patients                  | 4606 (40.9%) | 1005 (45.5%) | 8803 (44.8%) | 6343 (62.7%) | 5983 (43.1%)   | 14060 (53.4%)  | 41256 (45.4%)  |
| Female patients                | 6666 (59.1%) | 1204 (54.5%) | 10868 (55.2%) | 10399 (57.3%) | 7776 (56.9%)   | 12748 (46.6%)  | 49861 (54.6%)  |
| Charlson score                 |            |            |          |          |               |                |      |
| Mean (SD)                      | 2.71 (2.28) | 2.43 (2.34) | 2.62 (2.07) | 2.26 (2.39) | 2.50 (2.42)    | 2.90 (2.56)    | 2.87 (2.46)    |
| Median [1th, 99th]             | 2.00 [0.10, 10.0] | 2.00 [0.10, 10.0] | 2.00 [0.10, 10.0] | 2.00 [0.10, 10.0] | 2.00 [0.10, 10.0] | 2.00 [0.10, 10.0] | 2.00 [0.10, 10.0] |
| Death during follow-up         |            |            |          |          |               |                |      |
| Yes                            | 1709 (15.2%) | 286 (13.9%) | 2962 (13.5%) | 2234 (13.3%) | 1930 (14.1%)   | 2915 (10.7%)   | 11736 (12.9%)  |
| No                             | 9563 (84.8%) | 1923 (87.1%) | 17009 (86.5%) | 14508 (86.7%) | 11739 (85.9%)  | 24439 (89.3%)  | 79181 (87.1%)  |
| Total number of RBCs received  |            |            |          |          |               |                |      |
| Mean (SD)                      | 3.38 (3.21) | 3.27 (3.17) | 3.34 (3.00) | 3.61 (3.88) | 3.41 (3.56)    | 5.23 (8.50)    | 3.97 (5.58)    |
| Median [1th, 99th]             | 2.00 [1.00, 17.0] | 2.00 [1.00, 16.0] | 2.00 [1.00, 16.0] | 2.00 [1.00, 20.0] | 2.00 [1.00, 17.0] | 3.00 [1.00, 40.0] | 2.00 [1.00, 25.0] |
| Percentage of RBCs from male donors |           |            |          |          |               |                |      |
| Mean (SD)                      | 51.6 (34.4) | 58.8 (34.3) | 51.3 (34.2) | 51.6 (33.5) | 52.2 (33.9)    | 51.7 (32.4)    | 51.8 (33.5)    |
| Median [1th, 99th]             | 50.0 [0.100] | 50.0 [0.100] | 50.0 [0.100] | 50.0 [0.100] | 50.0 [0.100]   | 50.0 [0.100]   | 50.0 [0.100]   |
| ABO blood group patient        |            |            |          |          |               |                |      |
| O                              | 4673 (41.5%) | 818 (37.0%) | 8183 (41.4%) | 6955 (41.5%) | 5892 (41.6%)   | 11166 (40.8%)  | 37487 (41.2%)  |
| A                              | 4780 (42.4%) | 1090 (49.3%) | 8559 (43.5%) | 7132 (42.5%) | 5994 (43.9%)   | 12057 (41.1%)  | 39592 (43.5%)  |
| B                              | 479 (4.2%)  | 97 (4.0%) | 882 (4.5%) | 719 (4.3%) | 579 (4.2%)     | 1172 (4.3%)    | 3528 (4.3%)    |
| AB                             | 1340 (11.9%) | 204 (9.2%) | 2041 (10.4%) | 1996 (11.7%) | 1805 (12.9%)   | 2095 (8.0%)    | 6910 (10.3%)   |
| RHD blood group patient        |            |            |          |          |               |                |      |
| Negative                       | 1762 (15.6%) | 357 (16.2%) | 3173 (16.1%) | 2614 (15.6%) | 2147 (15.7%)   | 4201 (15.4%)   | 14254 (15.7%)  |
| Positive                       | 9510 (84.4%) | 1852 (83.8%) | 14698 (83.9%) | 14128 (84.4%) | 11552 (84.3%)  | 23153 (84.6%)  | 76663 (84.3%)  |
Methods

Coding the treatment variable

The time-varying treatment variable, $A$, is defined as the proportion of male donor RBC units received on each separate person-day (each day $k$). Thus, the treatment variable is defined as:

$$A(k) = \begin{cases} \frac{T_{\text{Males},k}}{T_{\text{Total},k}}, & T_{\text{Total},k} > 0 \\ 0.5, & T_{\text{Total},k} = 0 \end{cases}$$

Where $T_{\text{Males},k}$ is the number of RBC units from male donors received on day $k$, and $T_{\text{Total},k}$ is the total number of transfusions received on each day $k$ (including both male and female donated RBCs). Thus, the treatment variable can take values between 0 and 1. Here, 0 if no RBCs from male donors are received and 1 if all RBCs received are from male donors. On days where no transfusions are received, we set this ratio to 0.5 because it corresponds to receiving the same number of male and female RBCs (one could say that 0 male RBCs and 0 female RBC where received). Thus, by using this coding, the days where no transfusions are received does not affect the ratio of RBC received from the male and female donor during the complete follow-up/transfusion history. Previous studies have used the cumulative exposure to male donor RBCs that varies with time which can be easily implemented in a Cox regression. This is not a suitable solution when using TMLE because TMLE uses inverse probability weighting and the g-formula to adjust for confounding. When estimating inverse probability weights for each day $k$, the model needs to know the exact treatment received on each day $k$ and the treatment received up to day $k$ to properly estimate the inverse probability weight. Here, using the cumulative exposure will not be a correct definition of the
treatment on day $k$, and the inverse probability weights will most likely not be correctly estimated. For further details on how one can estimate the inverse probability weights in this complicated blood transfusion setting where multiple RBC units are received over time, the reviewer is referred to our previously published paper.¹

Another way the treatment could be coded was to use the number of male RBC units received on each day $k$ instead of estimating the proportions. However, by doing this, the treatment variable would contain information on the number of transfusions received on each day $k$, which we want to adjust for. Thus, it would be problematic to code this information into the treatment variable. Further, days where no transfusions were received would be coded similar to days where no RBCs from male donors were received (treatment variable taking the value 0 in both cases), which may have unwanted consequences for the estimation. We believe that our approach of modeling the treatment variable is the better choice in a blood transfusion setting.
Targeted Maximum Likelihood Estimation

Targeted Maximum Likelihood Estimation (TMLE) is a well-established semi-parametric estimation method which can be used to estimate average treatment effect (ATEs) and other statistical quantities of interest (target parameters).\textsuperscript{2,3} Compared with traditional parametric regression methods (e.g. logistic regression and Cox regression), TMLE has double robustness properties. The double robustness is obtain because TMLE includes a “targeting step” that utilizes both G-computation and inverse probability weighting to optimize the bias-variance trade-off for the target parameter.\textsuperscript{3} Further, TMLE can be coupled with data-adaptive machine learning algorithms to place minimal assumptions on the distribution of the data and thus minimize the amount of potential model misspecifications bias. For further technical details of TMLE the reader is referred to literature by van der Laan, for example: “Laan MJ van der, Rose S. Targeted Learning: Causal Inference for Observational and Experimental Data. Vol 20.; 2011.”

Super learning

Instead of relying on the estimates obtain from a single machine learning algorithm, TMLE, can be coupled with an ensemble of machine learning algorithms known as Super learning.\textsuperscript{4} The ensemble can include a combination of both linear models (e.g. logistic regression) and non-linear models (e.g. Random Forest). Because one can not know which algorithm that will perform the best in any given setting, combining multiple machine learning algorithms with different capacities may be a better option. Using cross-validation the Superlearner estimates how to weight the prediction from each individual machine learning algorithm as to minimize the loss function (e.g. the mean squared error). Thus, the Superlearner is expected to
perform at least as well or better than any of the included individual base learners (algorithms). For further technical detail about Super learning the reader is referred to this paper by van der Laan et al.: “Van Der Laan MJ, Polley EC, Hubbard AE. Super learner. *Stat Appl Genet Mol Biol.* 2007;6(1). doi:10.2202/1544-6115.1309”.
Figure 2: Estimated average treatment effects

Estimated average treatment effects separately for male (A) and female (B) patients under treatment with RBC units from exclusively female donors vs. natural course (the current practice), male vs female donors, and male donors vs. natural course on day 28 after the baseline-transfusion with 95% confidence intervals. A positive ATE implies a higher survival for the treatment on the left-hand side of “vs.” compared with the right-hand side.

A  Male patients

B  Female patients

Treatment contrast  Female donors vs. natural course  Male donors vs. female donors  Male donors vs. natural course
S1: Sensitivity analyses of the estimates obtained from the trials on male patients

Because we utilized an objective approach using an ensemble of data-adaptive machine learning algorithms, testing for model misspecifications were not applicable. Instead, we simulated how the estimate would change under the assumption of causal bias up to three times larger (in both directions) than adjusted for in the main analyses.\(^5\)

Average treatments effects and 95% confidence intervals under assumed levels of causal bias for treatment with RBC units from female donors vs. natural course.
Average treatments effects and 95% confidence intervals under assumed levels of causal bias for treatment with RBC units from male donors vs. natural course.

Average treatments effects and 95% confidence intervals under assumed levels of causal bias for treatment with RBC units from male vs. female donors.
S2: Sensitivity analyses of the estimates obtained from the trials on female patients

Average treatments effects and 95% confidence intervals under assumed levels of causal bias for treatment with RBC units from female donors vs. natural course.

Average treatments effects and 95% confidence intervals under assumed levels of causal bias for treatment with RBC units from male donors vs. natural course.
Average treatments effects and 95% confidence intervals under assumed levels of causal bias for treatment with RBC units from male vs. female donors.

Unadjusted estimate
Adjusted estimate

Assumed causal bias relative to difference between adjusted and unadjusted estimates

Female patients
Table 2: Average weighting of individual learners

Table 1: Average weights across cross-fitting folds and all days of follow-up for each base learner included in the Super learner. The weights are presented for the estimation of the outcome model (g-computation) and the assignment model (inverse probability weighting) and for the models on male and female patients.

| Base learner | Outcome model | Assignment model |
|--------------|---------------|-----------------|
|              | Male patients | Female patients | Male patients | Female patients |
| glm          | 0.08          | 0.08            | 0.02          | 0.02            |
| LASSO        | 0.52          | 0.56            | 0.00          | 0.00            |
| MARS         | 0.05          | 0.06            | 0.38          | 0.40            |
| XGBoost1     | 0.06          | 0.04            | 0.07          | 0.09            |
| XGBoost2     | 0.02          | 0.01            | 0.02          | 0.02            |
| XGBoost3     | 0.19          | 0.18            | 0.46          | 0.42            |
| XGBoost4     | 0.07          | 0.06            | 0.06          | 0.05            |
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