Out for blood: causal inference in clinical transfusion research

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Chapter 5

Transfusion of ever-pregnant donor red blood cells and mortality of male patients

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Abstract

Previous studies found exposure to red blood cell transfusions from female donors who have been pregnant reduces survival in male patients compared to exposure to male donor products, but evidence is not consistent. We postulate the previously observed association is modified by offspring sex, with an expected increased mortality risk for male patients receiving units from female donors with sons. Here, marginal structural models were used to assess the association between exposure to units from ever-pregnant donors, ever-pregnant donors with sons and ever-pregnant donors with daughters, and mortality. Clinical data were collected on first-ever transfusion recipients in the Netherlands and donor data were supplemented with information about offspring sex and date of birth. In this analysis, 56,825 patients were included, of whom 8,288 died during follow-up. Exposure to red blood cell units from ever-pregnant donors with sons was not associated with increased all-cause mortality risk among male transfusion recipients (hazard ratio [HR] 0.91, 95% confidence interval 0.83-1.01). Exposure to ever-pregnant donors, irrespective of offspring sex, was associated with mortality in male patients aged between 18 and 50 years (ever-pregnant donors: HR 1.81, 95% CI 1.31-2.51) compared to male donor units, but was protective in female patients. This study suggests that the observed increased mortality risk for exposure to red blood cell units from parous female donors does not depend on offspring sex. The increased risk of mortality seen in younger adult male patients is a finding consistent with previous observations, but the underlying biological mechanism could not be identified in this study.

Introduction

Red blood cell transfusions are given to improve tissue oxygenation in patients suffering from anemia and hemorrhage. There is substantial variation in clinical practice leading to possible over-transfusion[1], and furthermore transfusions are associated with harms, such as bloodborne infections and transfusion-associated circulatory overload.[2]

In 2011, an association was reported between transfusions of red blood cells from female donors and increased mortality in male patients under 50 years of age.[3] Later, this finding was replicated in an independent cohort.[4] This association was shown to be limited to female donors with a history of pregnancy, and it was estimated that this association could be responsible for one potentially preventable death per day in the Netherlands.[4,5] Although recent investigations from other countries have not found an effect of donor pregnancy on mortality after transfusion[6,7], differences between blood product production methods and used materials, differences between donor and patient populations, as well as differences in applied methodology could explain the discrepancies in results between studies. Evidently, transfusion practices should not be changed based on these contradictory findings, yet better understanding of the biological mechanisms that gave rise to these results might enable targeted changes to blood transfusion practice.

The observation that younger adult male patients exposed to ever-pregnant donors were at increased mortality risk compared to other patient subgroups suggests that these patients are somehow 'sensitive' to a component of the red blood cell product. This sensitivity could be due to the involvement of male-targeted minor histocompatibility antigens (HY-antigens) as well as the transfusion indication.[8] Pregnant women have been shown to immunize against male antigens (e.g. HY-antigens) during pregnancy or delivery. At the same time, young men often receive blood for the indication of trauma, which is known to cause a transient immune suppression.[9] Thus, younger male patients could be more sensitive to the effects of unintentionally transferred immune cells in red blood cell transfusions because of the indication for the transfusion. Furthermore, they could be more sensitive to immune cells primed against HY-antigens. Accordingly, we hypothesize blood products from female donors who have male offspring are harmful to young male patients. We hypothesize that the effect of exposure could become apparent early, but also later in life, as can be seen by the diverging Kaplan-Meier curves in a previous publication.[4]

To investigate this hypothesis, we aimed to first replicate the previously found association of increased mortality in male patients receiving red blood cells from female donors with a history of pregnancy. Second, we aimed to quantify the association between mortality and red blood cell transfusions from female donors who gave birth to a son or who gave birth to a daughter. Third, we aimed to investigate these associations in different age subgroups of male patients, as effect measure modification by patient age has been observed previously.[3,4]

Three comparisons were performed (outlined in *Figure 1*):

1. Male donors (reference) compared to ever-pregnant female donors (exposure group 1) and never-pregnant female donors (exposure group 2)
2. Male donors (reference) compared to ever-pregnant female donors with male offspring (exposure group 1) and female donors without male offspring (consisting of female never-pregnant donors and female ever-pregnant donors without male offspring, exposure group 2)
3. Male donors (reference) compared to ever-pregnant female donors with female offspring (exposure group 1), and female donors without female offspring (consisting of female never-pregnant donors and female ever-pregnant donors without female offspring, exposure group 2)

Methods

The 'Mortality After Transfusion of Ever-pregnant donor Red blood cells' (MATER) study is an observational cohort study, including data between the 1st of January 2005 and the 1st of January 2019 from two earlier cohort studies[3,4], supplemented with data from recent years (2015-2018) and additional exposure information pertaining to donor pregnancy history. Patient data were collected to the 'Risk Factors for Alloimmunization after red blood Cell Transfusions' (RFACT) study database (CCMO-NL29563.058.09; clinicaltrials.gov study number: NCT01616329).[3,10] During the study period, all blood products underwent a leukodepletion step as part of production, and an estimated 4% of units was irradiated prior to transfusion. The need for informed consent was waived by the Medical Ethics Review Board.

This large cohort of patients with transfusion data was supplemented with data from the national registration of Dutch inhabitants (Basisregistratie Personen, 'BRP') on registered offspring of donors. Mortality data were obtained from the hospital administration at the hospital's end of data collection or the administrative end of study.[3,4]

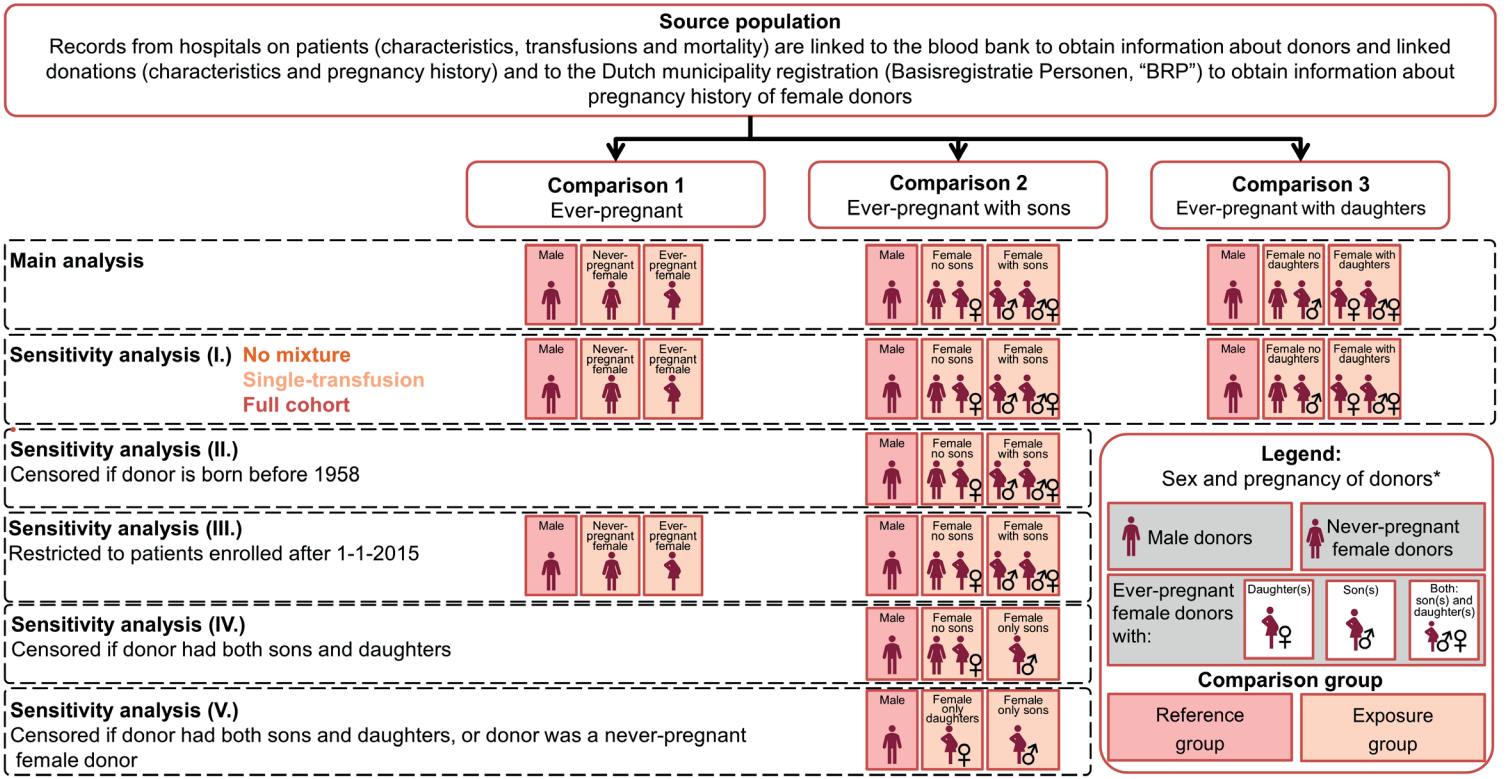

Figure 1. Schematic representation of exposure groups in main analysis and sensitivity analyses

The source population for the study and the different comparisons are visually represented. Comparisons were chosen with respect to donor pregnancy and sex of the offspring, and were adapted in the sensitivity analyses as shown, to correspond to the comparator of interest.

* Donors classified according to sex of the donor from blood bank records and the sex of the offspring registered in the BRP.

Although the MATER study is an observational study, we expected that the potential for confounding in this study was small. As the information about donor sex and pregnancy is not available to treating physicians, in practice red blood cell units are allocated independently of donor characteristics (notably, sex and parity of the donor). However, the logistics of the distribution of blood products depend on a number of factors that we consider to be potential confounders (*Figure S1*). In brief, confounders were included because they are predictive of both the distribution of blood products in the population, and the outcome. All information on potential confounders was obtained from the hospital administration and the R-FACT study at baseline.[3,10]

To be able to compare the effect of the different exposure categories, patients were censored at the time they received a transfusion from a different category than their previously received transfusions. This resulted in patients receiving more transfusions (and thus more likely to have a worse prognosis) being more likely to be censored, a phenomenon known as *informative censoring*.[11] Furthermore, the possibility exists that *treatment-confounder feedback* by hemoglobin present in the blood product further exacerbates the already existing bias in any analysis not adjusted for informative censoring.[6]

To correct for both confounding at baseline, and the informative censoring during follow-up and treatment-confounder feedback, inverse probability weighting (IPW) was applied.[12-14] Weights were trimmed at a fixed level of 10, to reduce instability of the IPW estimator. Weighted marginal structural Cox proportional hazards models were fitted using the R packages *ipw* and *survey*.[14]

Analyses were stratified by patient sex and age (0-17, 18-50, 51- 71 and ≥71 years), as prespecified in the statistical analysis plan and in line with previous studies.[4,7] Sensitivity analyses were performed to:

- evaluate alternative statistical analyses with methods similar to earlier research (I),
- test assumptions about data quality (II),
- form an independent study cohort not previously described (III),
- assess the effect of excluding donors with both sons and daughters (IV),
- assess the effect of excluding donors with both sons and daughters and in addition excluding never-pregnant female donors (V).

Analyses were performed in Stata, version 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC; data preparation and sensitivity analysis I), and R (version 3.6.3) and R Studio (version 2022.02.0+443)

software (sensitivity analyses II-V). An extended methods section can be found in the *Supplemental materials*.

Results

Population

Table 1 contains donor and patient characteristics of the complete study population and the population included in the main analysis. The complete dataset contained data on 546,102 transfusions, and the donations linked to these transfusions originated from 134,046 male donors and 135,992 female donors. In total, 98,676 patients were included, and 51% (n=50,138) of the patients were female. During a median follow-up of 278 days (counted from the date of the first transfusion to the date of death, censoring or end of follow-up) 33,487 patients died (34%).

Table 1. Patient and transfusion characteristics

Characteristics	Complete dataset		Main analysis*	
	Female patients	Male patients	Female patients	Male patients
Number of patients	N=48,538	N=50,138	N=28,115	N=28,710
Number of deaths, (%)	18,191 (37%)	15,296 (31%)	4,280 (15%)	4,008 (14%)
Follow-up, median (IQR), days†	1,081 (230-2,415)	1,372 (373-2,662)	151 (6-1597)	434 (11-2007)
Person-time, sum in years	191,573	223,156	69,558	85,898
Age of patients, median (IQR), years	65 (50-75)	65 (42-77)	64 (39-75)	65 (36-77)
0 to 17	6,681 (14%)	5,395 (11%)	5,931 (21%)	4,819 (17%)
18 to 50	5,626 (12%)	10,295 (21%)	2,644 (9%)	4,865 (17%)
51 to 70	18,412 (38%)	14,636 (29%)	9,687 (34%)	7,787 (27%)
≥71	17,819 (37%)	19,812 (40%)	9,853 (35%)	11,239 (39%)
Transfusions of red blood cell units per patient, median (IQR)	3 (2-6)	2 (2-5)	2 (1-2)	2 (1-2)
Units of red blood cells transfused, Number (%)‡	301,250	244,852	49,992	51,052
female donor, never-pregnant	49,607 (16%)	40,448 (17%)	4,467 (9%)	4,648 (9%)
female donor, ever-pregnant, male offspring	58,782 (20%)	47,378 (19%)	6,602 (13%)	6,721 (13%)
female donor, ever-pregnant, no male offspring	18,415 (6%)	15,089 (6%)	6,644 (13%)	6,749 (13%)
male donor	172,316 (57%)	140,126 (57%)	36,662 (73%)	37,447 (73%)

* Consists of all the follow-up time during which patients either received all their red blood cell transfusions exclusively from one exposure category: female donors without a history of pregnancy (never-pregnant donors), female donors with a history of pregnancy (ever-pregnant donors, with or without sons), or male donors.

† Median follow-up time is defined as the median of longest time any patient is in one of the comparisons. Exposure categories are: female donors without a history of pregnancy (never-pregnant donors), female donors with a history of pregnancy (ever-pregnant donors, with or without sons), male donors.

‡ Includes units from female donors with offspring of unknown sex.

From the complete study population, only 56,825 patients could be included in the cohort for the main analysis because they received only one exposure category on their first transfusion day, of whom 51% (n=28,710) were female. From this selected population, 8,288 deaths could be included in the main analysis (15%). The median age of the complete population was 65 (interquartile range (IQR) 46-76) and the median age in the main analysis was 64 (IQR 37-76). Compared to the complete study population, patients included in the main analysis were followed up for a shorter duration (median (IQR): 278 days (7-1,815) vs. 1,226 days (297-2,547)). Patients in the main analysis also received fewer transfusions (median (IQR): 2 (1-2) transfusions vs. 3 (2-6) transfusions) and were more likely to receive transfusions from male donors (73%) compared to the complete population (57%). Linkage of donor records resulted in complete exposure information (99.7% for comparison 1, 99.3% for comparison 2 and 3). Of note, male patients on average had a substantially shorter length of follow-up than female patients, which was more pronounced in the ever-pregnant and never-pregnant exposure arm (Table S1).

Donor and patient characteristics for the populations included in the sensitivity analyses can be found in Table S2. In Table S3, the study population restricted to patients aged 18 years and older is described. Absolute standardized mean differences (SMD) were calculated to assess balance after weighting for baseline factors for comparison 1 (*Figure S3*). Balance was sufficient after weighting for all baseline characteristics (SMD <0.1), for the population comparing ever-pregnant donor exposure to male donors.

No increased risk of mortality after exposure to ever-pregnant donor units

Results for the three comparisons in the main analysis are reported in *Figure 2*. Exposure to female donors who have previously been pregnant compared to male donors was not associated with mortality (hazard ratio (HR) 0.96 (95% confidence interval (CI) 0.88-1.04)) in male patients (*Figure 2*). Exposure to ever-pregnant donors with sons and ever-pregnant donors with daughters was not associated with mortality in this analysis (comparison 2: HR 0.91 (95% CI 0.83-1.01); comparison 3: HR 0.94 (95% CI 0.85-1.03)). Blood products from never-pregnant female donors were protective (HR 0.88 (95% CI 0.78-0.98)) in male patients, compared to exposure to male donors. No other significant associations were observed.

For female patients, exposure to blood products from ever-pregnant donors was associated with decreased mortality compared to exposure to male donor units (HR 0.91 (95% CI 0.83-0.99)). Exposure to units from female donors with sons was not associated with mortality (HR 0.93 (95% CI 0.84-1.03) and exposure to units from ever-pregnant donors with daughters was associated with decreased

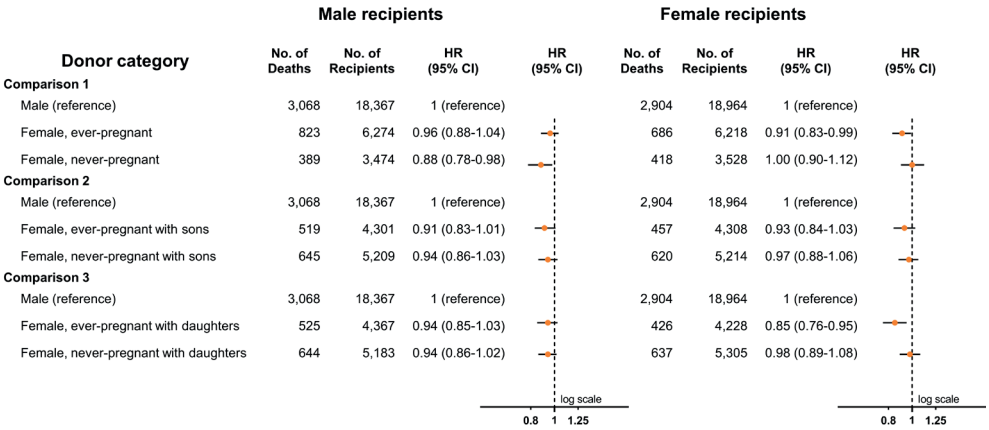

Figure 2. Mortality Hazard Ratio Of Male and Female Transfusion Recipients of Male, Ever-Pregnant (with Sons or Daughters) and Never-Pregnant Female Donor Red Blood Cell Products

Exposure to ever-pregnant donor red blood cell products compared to male donor exposure is not associated with mortality in the complete population of male patients, nor in the complete population of female transfusion recipients. Offspring sex is not predictive of patient mortality, with HRs similar in size and direction for both male and female offspring sex.

Abbreviation: HR, hazard ratio.

mortality, compared to male donor unit exposure (HR 0.85 (95% CI 0.76-0.95). No significant associations were observed for exposure to blood products from never-pregnant donors, female donors without sons and female donors without daughters.

For reasons of conciseness, the remainder of the Results section will focus on male patients only.

For the main analysis, restricted to patients aged 18 years and older, HRs were 0.99 (95% CI 0.92-1.09) for male patients exposed to ever-pregnant donors, 0.98 (95% CI 0.88-1.08) for exposure to ever-pregnant donors with sons and 0.99 (95% CI 0.89-1.10) for exposure to ever-pregnant donors with daughters (*Table S4*), all compared to exposure to male donors as reference. Exposure to never-pregnant female donors was significantly associated with decreased mortality (HR 0.87 (95% CI 0.78-0.98). No other significant associations were observed.

Association between exposure to ever-pregnant donors and mortality in younger adult male patients

Results for the analysis stratified by age for male patients are reported in *Table 2*. In male patients aged between 18 and 50 years, receiving units from ever-pregnant donors was associated with mortality (HR 1.81 (95% CI 1.31-2.51)). Receiving units from ever-pregnant female donors with sons was similarly associated with mortality in this subgroup, with a HR of 1.86 (95% CI 1.27-2.71), and

Table 2. Mortality Hazard Ratio of Male Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female (Never-Pregnant With Male Offspring or Ever-Pregnant With Male Offspring) vs Male Donors Stratified by Patient Age

Donor category	0-17 y			18-50 y			51-70 y			≥71 y			p value for interaction*
	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	
Comparison 1													
Male (reference)	187	3,702	1 (reference)	160	1,803	1 (reference)	1,058	6,408	1 (reference)	1,663	6,454	1 (reference)	
Female, ever-pregnant	79	1,578	0.99 (0.74-1.32)	61	518	1.81 (1.31-2.51)	269	2,047	0.91 (0.79-1.05)	414	2,131	0.95 (0.85-1.08)	0.0001
Female, never-pregnant	21	651	0.63 (0.38-1.03)	13	323	0.56 (0.30-1.02)	129	1,232	0.93 (0.72-1.20)	226	1,268	0.93 (0.80-1.08)	0.3140
Comparison 2													
Male (reference)	187	3,702	1 (reference)	160	1,803	1 (reference)	1,058	6,408	1 (reference)	1,663	6,454	1 (reference)	
Female, ever-pregnant with sons	51	1,166	0.90 (0.65-1.26)	40	343	1.86 (1.27-2.71)	168	1,353	0.91 (0.77-1.09)	260	1,439	0.92 (0.79-1.06)	0.0007
Female, never-pregnant with sons	41	1,036	0.77 (0.53-1.11)	34	459	1.19 (0.78-1.81)	215	1,815	1.01 (0.83-1.25)	355	1,899	0.96 (0.84-1.09)	0.3861
Comparison 3													
Male (reference)	187	3,702	1 (reference)	160	1,803	1 (reference)	1,058	6,408	1 (reference)	1,663	6,454	1 (reference)	
Female, ever-pregnant with daughters	60	1,176	0.98 (0.71-1.35)	34	354	1.58 (1.05-2.37)	172	1,396	0.92 (0.78-1.10)	259	1,441	0.93 (0.81-1.08)	0.0197
Female, never-pregnant with daughters	37	1,029	0.73 (0.49-1.06)	30	468	0.97 (0.64-1.47)	227	1,778	1.04 (0.86-1.24)	350	1,908	0.90 (0.79-1.02)	0.3144

*For the trend in interaction across the 4 presented categories of patient age.

exposure to units from ever-pregnant female donors with daughters was also associated with mortality (HR 1.58 (95% CI 1.05-2.37)). There was a significant interaction of exposure with age in the exposure groups of ever-pregnant donors, ever-pregnant donors with sons and ever-pregnant donors with daughters (p-value of 0.0001 (comparison 1); 0.001 (comparison 2); 0.020 (comparison 3)).

Results for female patients can be found in *Table S5*. No significant associations were observed. The fully independent cohort of patients included after 1st of September 2015 showed a similar magnitude and direction of the association between exposure to ever-pregnant donors and mortality for male (*Table S6*) and female patients (*Table S7*).

Sensitivity analyses

Sensitivity analyses were performed to verify the previously described assumptions about the data and the used methods, and the results were in agreement with the main result showing robustness of the methods to changes in these assumptions. Results for the sensitivity analyses can be found in the Supplemental materials (I, *Table S8-10*, and II-V, *Table S11*).

Discussion

In this study of donor characteristics and transfusion recipient mortality, the observed mortality of male patients after exposure to ever-pregnant donor units was not explained by donor offspring sex. In the subgroup of male patients aged between 18 and 50 years, exposure to red blood cell products from ever-pregnant donors, regardless of the donor's offspring sex, was significantly associated with worse outcomes after transfusion (HR 1.86 (95% CI 1.27-2.71)). This result is consistent with a previous publication from our research group, and constitutes an independent replication of those earlier findings.[3,4] The same association in female patients was actually in the direction of moderate protection; an unexpected finding which we cannot explain (HR 0.91 (95% CI 0.83-0.99)). Notably, a recent publication[15] on a large pragmatic randomized controlled trial investigating donor sex found an increased risk of mortality after female donor exposure in patients aged 20-29 years, although the population was small and not stratified by patient sex.

Analyses using traditional methods (*sensitivity analysis I*) were used to evaluate the magnitude and direction of bias due to informative censoring.[16] Indeed, in the single-transfusion cohort investigating exposure to ever-pregnant donors, potential bias in the direction of harm from 'rare' exposure was visible in these most selective, most censored analyses (HR 1.14 (95% CI 1.02-1.28)). This, as op-

posed to the main analysis, with a HR of 0.96 (95% CI 0.88-1.04). We postulate previous work could have suffered more from this bias, due to missing data in the pregnancy history of the donor necessitating more frequent censoring of patient follow-up. Treatment-confounder feedback, with more transfusions given to patients receiving blood from female donors through lower hemoglobin concentration in products donated by female donors as compared to male donors, is a potential cause of bias here.[6] If chosen as exposure, any variable which affects the hemoglobin dose of the product may lead to bias if not accounted for correctly, because the hemoglobin dose of the product affects (in part) the time to next transfusion, and the number of transfusions is associated with underlying disease severity. As women have a lower normal level of hemoglobin compared to men, treatment-confounder feedback should be accounted for in the analyses. It is recommended that future investigations of blood product characteristics that relate to hemoglobin-raising capacity, e.g. product storage and any traits related to red blood cell storage and stress hemolysis[17], incorporate measures to counteract this methodological artefact.

One of the strengths of this study is the large cohort of real-world data that was used and analyzed using appropriate methods. By pooling together into combined exposure groups the subgroups of ever-pregnant donors with both sons and daughters, and never-pregnant donors (depending on the comparison made), the main analysis had a large sample size. Expected challenges with regards to data quality and appropriateness of used methods were thoroughly investigated using sensitivity analyses, and these results were consistent with the main analysis. Thereby, these challenges were adequately addressed.

Limitations of the study include the granularity of the data, as the data were organized per day. This necessitated the exclusion of patients receiving transfusions from multiple categories on their first transfusion day, which could have led to bias and limited generalizability to patient populations requiring multiple transfusions early in the treatment course. Second, findings presented here are applicable to the study population of transfusion recipients between 2005 and 2019 in six hospitals in the Netherlands who received a median of two transfusions, and may not be generalizable to other settings, especially those with higher disease burden. Third, the use of inverse-probability weighted methods was only possible with larger intervals following the initial 4-week follow-up that was analyzed by transfusion day owing to sparse multivariable data, and this interval-censoring is a potential source of bias. Fourth, multiple comparisons were made but no adjustments for multiple testing were applied. However, all comparisons were pre-specified and no post-hoc analyses were included. Fifth,

pregnancies resulting in miscarriages and stillbirths are not reported to the BRP and could therefore not be included in this study. These limitations are mitigated by using multiple control conditions (e.g. never-pregnant donors and never-pregnant donors with daughters) and the inclusion of separate analyses for the fully independent cohort.

The aforementioned methodological limitations apply to the full population, and would not explain the repeated observation of increased mortality in younger adult male patients. The association between mortality and exposure to ever-pregnant donors in male patients aged between 18 and 50 years was also present in the population included after September 2015, which was not previously described in other publications, and thereby constitutes an independent replication of this previously observed finding (*Table S10* for male patients; *Table S11* for female patients). Methodological explanations were sought, and we hypothesized these male patients received multiple transfusions on their first day due to their transfusion indication, excluding them from the analysis and potentially introducing bias. However, after examining the frequency of exclusion due to mixture of exposures on the first day, this was not different between male and female patients for the different exposure categories (*Table S12*). More research, specifically on transfusion indications and causes of death, could further clarify the clinical relevance of this repeated observation. Transfusion policy changes which could be considered in the future (e.g. irradiation, matching for patient subgroups) must be based on a solid understanding of the underlying biological mechanism.

If some male patients are indeed sensitive to blood products from ever-pregnant female donors, there should be a biological rationale. Male recipients of allogeneic stem cell transplantations (aSCT) from female donors who previously gave birth to a son have a poor prognosis due to increased risk of Graft-vs-Host disease (GvHD), which could be due to micro-chimerism of the male offspring inducing an immune response directed at male cells.[18,19] However, transplants from female donors with only daughters are not associated with GvHD, with these women having a more tolerogenic immune status comparable to that of nulliparous donors.[19] Furthermore, male patients could be sensitive to external stimuli due to their transfusion indication, as they more often receive large volumes of blood products in a short timeframe, in a trauma setting. Micro-chimerism has been detected following transfusions for trauma indications, with reports of long-term engraftment of donor cells, but evidence is conflicting.[20] An alternative explanation of the observed mortality in young male patients, not related to sex of the offspring, is immunization of the female donor against inherited paternal

human leukocyte antigens (IPA) of the fetus. However, the exact mechanisms underlying the observed increase in mortality following transfusions from ever-pregnant female donors in young men are incompletely understood and may be multifactorial.

To conclude, in young adult male patients, blood products from ever-pregnant female donors are consistently associated with mortality, which continues to be a concern. With a large observational database containing complete exposure information on over 99% of red blood cell units, and an appropriate methodology that could account for possible treatment-confounder feedback, this research question was thoroughly investigated.

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Disclosure of Conflicts of Interest

JJZ is in the scientific advisory council of Novartis / Amgen / Sanofi and received a speakers fee. All other authors declare no competing financial interests for the research in this manuscript.

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Supplemental materials

Title: Transfusion of ever-pregnant donor red blood cells and mortality of male patients

This part of the thesis contains additional figures and tables for the manuscript "Transfusion of ever-pregnant donor red blood cells and mortality of male patients".

Contents

Supplemental methods

Exposure

Information was collected on the date of birth of all offspring and the sex of the biological offspring. If the date of birth preceded the date of transfusion, and the child was determined to be biological offspring (which was determined by comparing the date of birth with the date of start of the family relation), the donor was classified as 'ever-pregnant', with sons and/or daughters, respectively. Three comparisons were performed (outlined in *Figure 1*). Comparison 3 acts as a control comparison for the study hypothesis, because exposure to blood products from female donors with daughters was not expected to be associated with mortality. All exposure information was obtained from the BRP at the date of donation for every female donor, and from the blood bank information system for the male donors.

Comparison 1 can be considered a comprehensive reproduction study of the earlier found association between ever-pregnant donors and mortality, as it uses the same exposure and outcome as have been previously reported in a partially overlapping cohort (period January 1st 2005-September 1st 2015).[1] Comparisons 2 and 3 pertain to different exposures that have not been described elsewhere previously and should therefore be viewed as an independent analysis.[1,2] Analyses were also performed separately for the population aged ≥18 years to have a study population that is comparable with other studies.

Outcome

The study outcome was all-cause mortality. Mortality data were obtained from the hospital administration at the hospital's end of data collection or the administrative end of study (1/1/2019).[1,2]

Covariates

Although the MATER study is an observational study, we expected that the potential for confounding in this study was small. As the information about donor sex and pregnancy is not available to treating physicians, in practice red blood cell units are allocated independently of donor characteristics (notably, sex and parity of the donor).

However, the logistics of the distribution of blood products depend on a number of factors that we consider to be potential confounders (*Figure S1*). Hospital (cat-

egorical, six levels) is considered a potential confounder, because it is associated with mortality and can become associated with exposure through geographical differences in product distribution. Year (continuous) is a potential confounder, because (1) mortality risk following transfusions varies over time due to more restrictive transfusion policies becoming the norm, and (2) characteristics of the donor population vary over time[3,4]. Blood group (categorical variable, 9 levels) is another potential confounder, because it is associated with mortality and because some blood groups are rare, the distribution of donor factors can differ

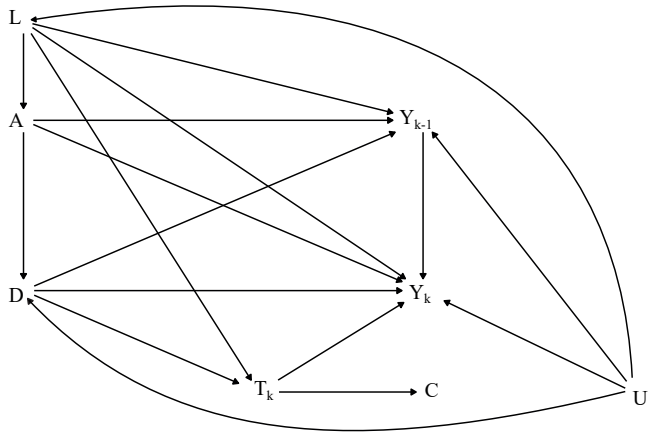


Figure S1. Directed acyclic graph of the effect of product characteristics (donor pregnancy and sex of the offspring) on mortality

between blood groups. All information on potential confounders was obtained from hospital administration and the R-FACT study at baseline.[2,5]

In Figure S1, A represents assignment to study arm at time *k-1*. L represents the set of 'center' variables consisting of year of transfusion, hospital and patient blood group. These center variables together influence the receival of a next transfusion and the risk of mortality of the patient, and are therefore a sufficient set for adjustment of the confounding at study start. D is a mediator, here influenced by treatment arm A and on the causal path of A to Y, and stands for the dose of hemoglobin received by the patient after the transfusion at time *k-1*. T_k represents the receival of a next transfusion. C stands for censoring of the patient following receival of the transfusion, and in the population where follow-up is limited to time until mixture of arms, C is conditioned on by design. This conditioning is removed by weighing the population by the inverse probability of censoring weights estimated with T_k. Y_{k-1} and Y_k represent mortality at timepoints *k-1* and *k*, respectively. U is a vector containing all unmeasured covariates that could influence mortality (e.g. disease severity of patients at *k-1* and

k), hemoglobin dose received (blood bank logistic factors) and center variables (patient population differences between centers).

Follow-up

Follow-up started with the first receipt of a transfusion during study period (starting 1/1/2005) and ended when patients were censored, which was at the time of death, time of transfusion from different exposure group, or administrative end of study (1/1/2019), whichever came first. Patients could only contribute follow-up to the analyses if they received all their transfusions from the same exposure category on their first day.

Statistical analysis

To be able to compare the effect of the abovementioned different exposure categories, patients were censored at the time they received a transfusions from a different category than their previously received transfusions. This resulted in patients receiving more transfusions (and thus more likely to have a worse prognosis) being more likely to be censored, a phenomenon known as *informative censoring.*[6] Furthermore, the possibility exists that *treatment-confounder feedback* by hemoglobin present in the blood product further exacerbates the already existing bias in any analysis not adjusted for informative censoring.[7] This is because blood products from female donors have a consistently lower hemoglobin content compared to male donors, and this difference is not adjusted during the production process of red blood cell units in the Netherlands.[8] If chosen as exposure, any variable which affects the hemoglobin dose of the product may lead to bias if not accounted for correctly, because the hemoglobin dose of the product affects (in part) the time to next transfusion, and the number of transfusions is associated with underlying disease severity. As women have a lower normal level of hemoglobin compared to men, treatment-confounder feedback should be accounted for in the analyses.

To correct for both confounding at baseline, and the informative censoring during follow-up and treatment-confounder feedback, inverse probability weighting was applied in three steps.

First, a propensity score was estimated based on the identified potential confounders using a logistic model with exposure (i.e., assignment to either exposure arm or reference arm) as the dependent variable. Second, to correct for the censoring upon receiving a transfusion from a different exposure category, a propensity-score weighted pseudo-population was created in which further inverse probability of censoring weights (IPCW) were estimated. Weights were

constructed per transfusion day for the first 28 days, and per 4-weekly interval thereafter, using a Cox model with the cumulative number of transfusions as continuous covariate. The IPCW estimator (predicted probability of censoring) corrects for censored subjects by redistributing weights of similar censored and uncensored patients when used to calculate the survival probabilities. As censoring, due to reaching the end of follow-up at the reference date of the hospital, is not influenced by patient characteristics, this information was not included in the censoring model. Instead, we developed a censoring model for time to non-administrative censoring only. Third, the propensity score was multiplied with the censoring score to obtain the final weights.[9-11] Weights were trimmed at a fixed level of 10, to reduce instability of the IPW estimator. Weighted marginal structural Cox models were fitted using the R packages *ipw* and *survey*.[11]

Analyses were stratified by patient sex and age, in line with previous studies.[1,12] We consider age as a proxy for transfusion indication, with young male patients more often receiving transfusions for trauma and massive transfusion.[13] Age categories were defined as 0-17, 18-50, 51-70 and over 70 years of age. This analysis was repeated in the independent cohort of data collected after 1st of September 2015 to the 1st of January 2019, and can be viewed as an effort to independently replicate the previous findings of Caram-Deelder et al. which included data up to 1st of September 2015.[1]

In sensitivity analysis I, hazard ratios were calculated using standard Cox PH survival analysis. This analysis was performed to compare with previous work[1,12] and to empirically assess the necessity of accounting for treatment-confounder feedback. Three ways of specifying the included study population were analyzed (*Figure S2*). In the full cohort analysis, exposures from the concerned reference and exposure could be mixed, and censoring took place when a patient received an exposure from a different exposure category. In the no-mixture cohort, patients were censored when they received a transfusion from a different exposure category than the one of their first transfusion. In the single transfusion cohort, patients were censored when they received a second transfusion. Cox proportional hazards models were fitted, adjusted for:

- cumulative number of transfusions [time-varying, restricted cubic spline with five knots];
- hospital [fixed];
- blood group [fixed];
- calendar year [fixed];
- age of the donor [time-varying, cumulative number of units from donors aged ≥50 years];

- interaction term for cumulative number of transfusions and hospital [time-varying].

In sensitivity analysis II, when products from female donors with uncertainty about their offspring (due to BRP records being less complete before 1958) were transfused, patients were censored. Sensitivity analysis III was repeated for the independent cohort of patients included after 1st of September 2015 to the 1st of January 2019, and can be viewed as an effort to independently replicate the previous findings of Caram-Deelder et al.[1] Other sensitivity analyses included censoring at the time a product from a donor with both sons and daughters was given (sensitivity analysis IV.), and censoring for both the donor with sons and daughters and the exclusion of never-pregnant women from the exposure groups (sensitivity analysis V.).

Supplemental results

Additional results for the manuscript are presented here. In brief, Table S2 contains donor and patient characteristics for the cohorts used in the sensitivity analyses. Table S3 contains donor and patient characteristics of the study population aged ≥18 years. Table S4 contains the results for the main analysis for the study population aged ≥18 years.

Results for the analysis stratified by patient age for female patients are reported in Table S5.

Results for the analysis in the independent cohort included after 1st of September 2015 stratified by patient age for male patients are reported in Table S6. Results for the analysis in the independent cohort included after 1st of September 2015 stratified by patient age for female patients are reported in Table S7.

Results for the sensitivity analyses are reported in Tables S8-11. The following figure provides a visual aid for the content of tables S8-11:

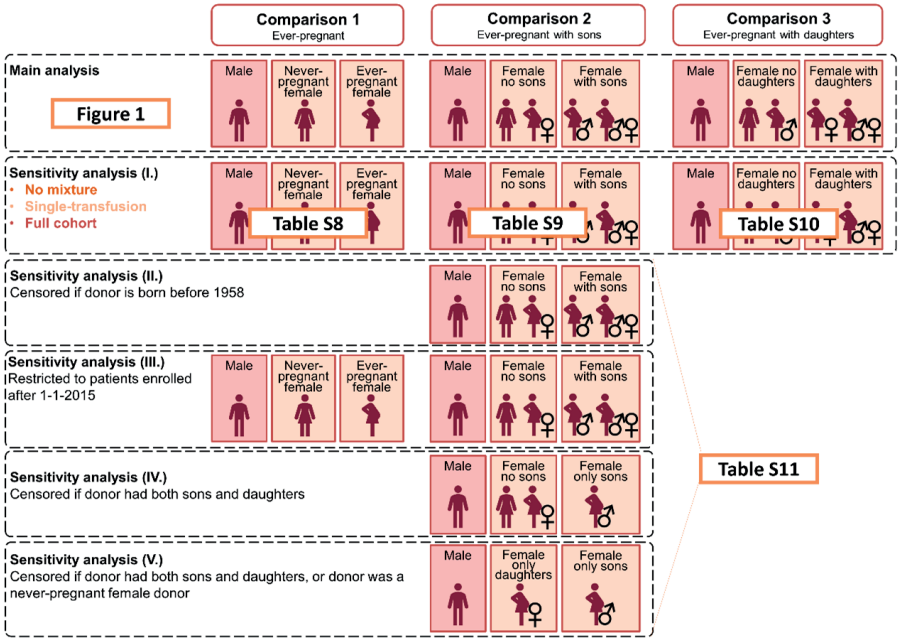


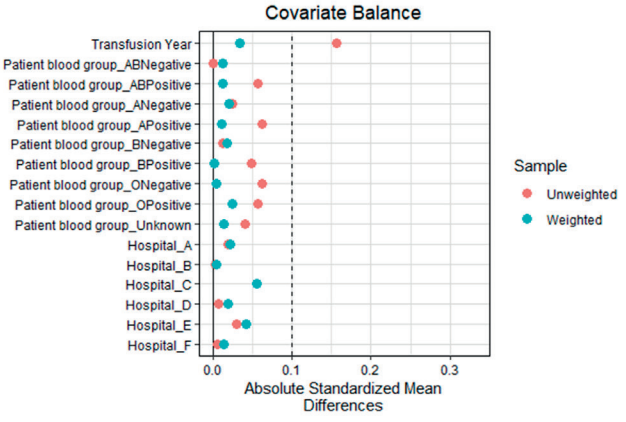
Figure S2. Schematic representation of exposure definition in sensitivity analyses and corresponding tables

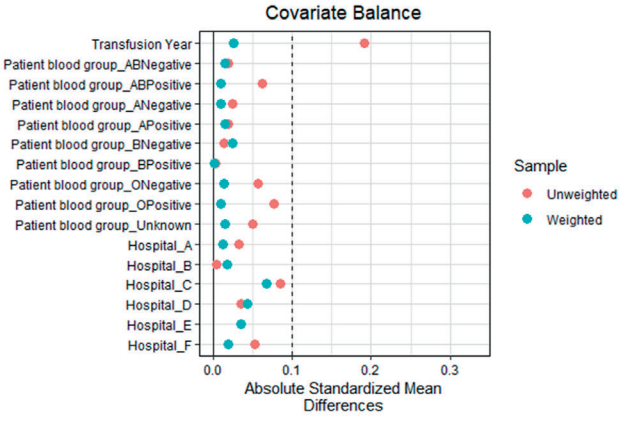
Table S12 contains results for the comparison of exposure categories as assigned on the first day for the complete study population. TableS13 contains the distribution of the weights prior to truncation, for the population of male and female patients in the primary analysis, comparison 1.

Male patients

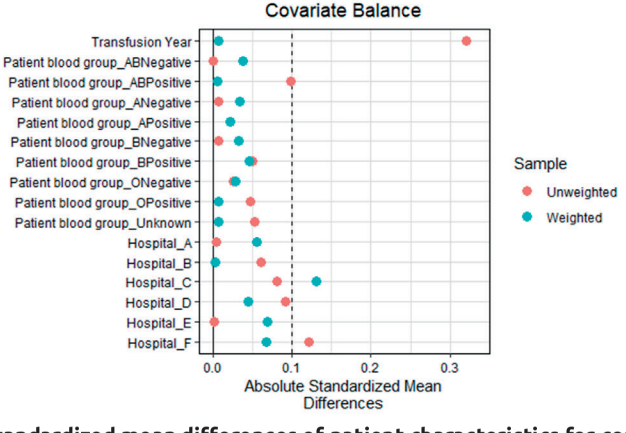
Female patients

Exposure: ever-pregnant donor





Exposure: never-pregnant donor



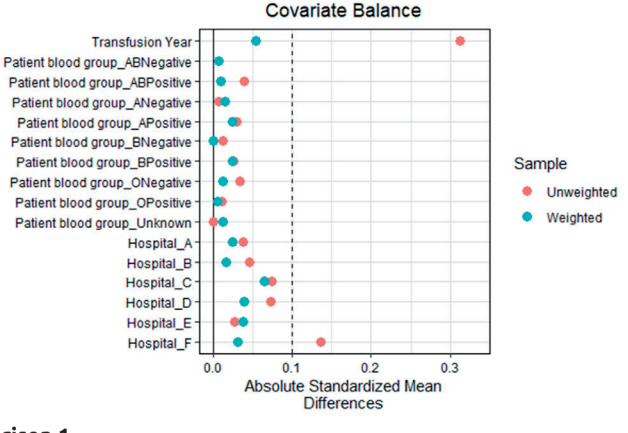


Figure S3. Absolute standardized mean differences of patient characteristics for comparison 1

Sensitivity analyses I-V

Sensitivity analyses were performed to verify the previously described assumptions about the data and the used methods, and results can be found in Tables S8-11.

Sensitivity analysis I was performed on the full cohort, the no-mixture of exposure cohort and the single-transfusion cohort, which are reported on in *Table S8* (comparison 1), *Table S9* (comparison 2) and *Table S10* (comparison 3). Of these, exposure to ever-pregnant donors, ever-pregnant donors with sons and ever-pregnant donors with daughters was not associated with mortality in the full cohort (comparison 1: HR 1.02 (1.00-1.05); comparison 2: HR 1.01 (95% CI 0.98-1.05); comparison 3: HR 1.03 (95% CI 0.99-1.06)). In the no-mixture cohort, exposure to ever-pregnant donors was significantly associated with mortality (HR 1.05 (95% CI 1.00-1.09), but exposure to ever-pregnant donors with sons and ever-pregnant donors with daughters was not (comparison 2: HR 1.04 (95% CI 0.98-1.10); comparison 3: HR 1.04 (95% CI 0.98-1.11)). The single-transfusion cohort had the comparatively largest effect sizes for exposure to ever-pregnant donors, ever-pregnant donors with sons and ever-pregnant donors with daughters (comparison 1: HR 1.14 (95% CI 1.02-1.28); comparison 2: HR 1.11 (95% CI 0.98-1.26); comparison 3: HR 1.13 (95% CI 0.99-1.28)). Of note, these analyses are performed with exposure as a continuous variable as opposed to the main analysis, and the HRs should be interpreted as the HR for a one-unit increase in the exposure category, compared to reference.

Results for sensitivity analyses II-V can be found in *Table S11*. Exposure to ever-pregnant donors with sons born after 1958 was not associated with mortality (HR 0.87 (95% CI 0.76-1.00)). Exposure to ever-pregnant donors and ever-pregnant donor with sons was not associated with mortality in the study population included after September 1st 2015 (comparison 1: HR 0.87 (95% CI 0.68-1.11)); comparison 2: HR 0.83 (95% CI 0.63-1.10)). Exposure to ever-pregnant donors with sons, without daughters, was not associated with mortality (HR 0.94 (95% CI 0.78-1.13)).

Supplemental tables

Table S1. Censored patients and follow-up of patients in the complete dataset and primary analysis, by exposure group

	Complete dataset*		Primary analysis	
Characteristics	**Male patients**	**Female patients**	**Male patients**	**Female patients**
Number of patients	N=48,538	N=50,138	N=28,115	N=28,710
Arm: male	36,439†	37,762†	18,367	18,964
Arm: ever-pregnant	20,905†	21,219†	6,274	6,218
Arm: never-pregnant	14,347†	14,712†	3,474	3,528
Number of patients censored on day 1, (%)	-	-	20,423 (42%)	21,428 (43%)
Number of patients censored during follow-up, (%)	-	-	8,790 (31%)	8,633 (30%)
Arm: male, (%)	-	-	3,447 (39%)	3,376 (39%)
Arm: ever-pregnant, (%)	-	-	2,874 (33%)	2,722 (32%)
Arm: never-pregnant, (%)	-	-	2,469 (28%)	2,535 (29%)
Follow-up, median (IQR), days†	1,081 (230-2,415)	1,372 (373-2,662)	151 (6-1,597)	434 (11-2,007)
Arm: male	1,380 (337-2,691)	1,609 (496-2,849)	244 (9-1,817)	617 (22-2,227)
Arm: ever-pregnant	1,142 (298-2,388)	1,383 (427-2,499)	48 (4-1,208)	170 (4-1,592)
Arm: never-pregnant	1,064 (308-2,221)	1,111 (348-2,260)	33 (3-1,020)	120 (3-1,247)

*In the complete dataset, all follow-up from patients is included and no censoring takes place
†In the complete dataset, patients could receive different exposures on day 1, and these can therefore classified into multiple arms.

Table S2. Patient and transfusion characteristics for the Sensitivity Analyses

	Full cohort		No-donor mixture cohort*		Single-transfusion cohort†	
Characteristics	**Male patients**	**Female patients**	**Male patients**	**Female patients**	**Male patients**	**Female patients**
Number of patients	N=42,996	N=44,850	N=28,115	N=28,710	N=17,403	N=16,705
Number of deaths, (%)	15,817 (37%)	13,557 (30%)	4,280 (15%)	4,008 (14%)	1,610 (9%)	1,420 (9%)
Follow-up, median (IQR), days‡	606 (40-2,078)	978 (112-2,421)	151 (6-1,597)	434 (11-2,007)	18 (2-1,142)	28 (2-1,326)
Person-time, sum in years	137,590	171,123	69,558	85,898	34,037	35,343
Age of patients, median (IQR), years	65 (49-75)	65 (41-77)	64 (39-75)	65 (36-77)	62 (2-74)	63 (11-77)
0 to 17	6,490 (15%)	5,246 (12%)	5,931 (21%)	4,819 (17%)	5,386 (31%)	4,345 (26%)
18 to 50	4,726 (11%)	8,888 (20%)	2,644 (9%)	4,865 (17%)	1,278 (7%)	1,983 (12%)
51 to 70	16,086 (37%)	12,921 (29%)	9,687 (34%)	7,787 (27%)	5,058 (29%)	4,064 (24%)
≥71	15,694 (37%)	17,795 (40%)	9,853 (35%)	11,239 (39%)	5,681 (33%)	6,313 (38%)
Transfusions of red blood cell units per patient, median (IQR)	2 (2-4)	2 (2-4)	2 (1-2)	2 (1-2)	1 (1-1)	1 (1-1)
Units of red blood cells transfused, Number (%)§	136,586	130,552	49,992	51,052	17,403	16,705
female donor, never-pregnant	15,404 (11%)	15,480 (12%)	4,467 (9%)	4,648 (9%)	2,776 (16%)	2,704 (16%)
female donor, ever-pregnant, male offspring	24,226 (18%)	22,892 (18%)	6,602 (13%)	6,721 (13%)	3,382 (19%)	3,292 (20%)
female donor, ever-pregnant, no male offspring	23,114 (17%)	22,762 (17%)	6,644 (13%)	6,749 (13%)	3,930 (23%)	3,730 (22%)
male donor	88,779 (65%)	84,438 (65%)	36,662 (73%)	37,447 (73%)	10,028 (58%)	9,622 (58%)

* Consists of all the follow-up time during which patients either received all their red blood cell transfusions exclusively from one exposure category: female donors without a history of pregnancy (never-pregnant donors), female donors with a history of pregnancy (ever-pregnant donors, with or without sons), or male donors. The main analysis uses this cohort definition.

† Consists of patients with only a single red blood cell transfusion during the period in which they were followed up. Follow-up time will be censored at the time this inclusion criterion was violated.

‡ Median follow-up time is defined as the longest time any patient is in one of the comparisons. Exposure categories are: female donors without a history of pregnancy (never-pregnant donors), female donors with a history of pregnancy (ever-pregnant donors, with or without sons), male donors.

§ Includes units from female donors with offspring of unknown sex

Table S3. Patient and transfusion characteristics for the analysis with patients aged ≥18 years

	Complete dataset		Main analysis*	
Characteristics	**Male patients**	**Female patients**	**Male patients**	**Female patients**
Number of patients	41,857	44,743	22,184	23,891
Number of deaths, (%)	17,482 (42%)	14,709 (33%)	3,993 (18%)	3,777 (16%)
Follow-up, median (IQR), days†	956 (193-2,299)	1,309 (349-2,626)	95 (5-1,305)	393 (9-1,907)
Person-time, sum in years	157,340	195,710	49,169	69,219
Age of patients, median (IQR), y	68 (58-76)	68 (52-78)	69 (59-77)	69 (55-79)
18 to 50	5,626 (13%)	10,295 (23%)	2,644 (12%)	4,865 (20%)
51 to 70	18,412 (44%)	14,636 (33%)	9,687 (44%)	7,787 (33%)
≥71	17,819 (43%)	19,812 (44%)	9,853 (44%)	11,239 (47%)
Transfusions of red blood cell units per patient, median (IQR)	3 (2-7)	3 (2-5)	2 (1-2)	2 (1-2)
Units of red blood cells transfused, Number (%)‡	276,985	224,547	41,175	43,851
female donor, never-pregnant	46,566 (17%)	37,771 (17%)	3,719 (9%)	3,951 (9%)
female donor, ever-pregnant, male offspring	53,957 (19%)	43,375 (19%)	5,146 (12%)	5,503 (13%)
female donor, ever-pregnant, no male offspring	16,902 (6%)	13,822 (6%)	1,730 (4%)	1,721 (4%)
male donor	157,658 (57%)	127,954 (57%)	30,492 (74%)	32,561 (74%)

* Consists of all the follow-up time during which patients either received all their red blood cell transfusions exclusively from one exposure category: female donors without a history of pregnancy (never-pregnant donors), female donors with a history of pregnancy (ever-pregnant donors, with or without sons), or male donors. The main analysis uses this cohort definition.

† Median follow-up time is defined as the longest time any patient is in one of the comparisons. Exposure categories are: female donors without a history of pregnancy (never-pregnant donors), female donors with a history of pregnancy (ever-pregnant donors, with or without sons), male donors.

‡ Includes units from female donors with offspring of unknown sex.

Table S4. Mortality Hazard Ratio of Male and Female Transfusion Recipients in the Analysis with Patients Aged ≥18 Years, Comparisons 1, 2 and 3

	Male recipients			Female recipients		
Donor category	**No. of Deaths**	**No. of Recipients**	**HR (95% CI)**	**No. of Deaths**	**No. of Recipients**	**HR (95% CI)**
Comparison 1						
Male (reference)	2,881	14,665	1 (reference)	2,752	16,028	1 (reference)
Female, ever-pregnant	744	4,696	0.99 (0.92-1.09)	636	4,935	0.95 (0.87-1.05)
Female, never-pregnant	368	2,823	0.87 (0.78-0.98)	389	2,928	1.03 (0.92-1.16)
Comparison 2						
Male (reference)	2,881	14,665	1 (reference)	2,752	16,028	1 (reference)
Female, ever-pregnant with sons	468	3,135	0.98 (0.88-1.08)	423	3,353	0.99 (0.89-1.11)
Female, never-pregnant with sons	604	4,173	0.95 (0.86-1.04)	577	4,315	0.98 (0.89-1.08)
Comparison 3						
Male (reference)	2,881	14,665	1 (reference)	2,752	16,028	1 (reference)
Female, ever-pregnant with daughters	465	3,191	0.99 (0.89-1.10)	396	3,292	0.91 (0.82-1.02)
Female, never-pregnant with daughters	607	4,154	0.93 (0.85-1.02)	591	4,398	1.00 (0.90-1.10)

Table S5. Mortality Hazard Ratio of Female Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) vs Male Donors Stratified by Patient Age

	0-17 y			18-50 y			51-70 y			≥71 y			p value for interaction
Donor category	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	
Comparison 1													
Male (reference)	152	2,936	1 (reference)	180	3,425	1 (reference)	858	5,232	1 (reference)	1,714	7,371	1 (reference)	
Female, ever-pregnant	50	1,283	0.76 (0.54-1.06)	37	895	1.02 (0.71-1.48)	188	1,647	0.86 (0.72-1.02)	411	2,393	0.94 (0.84-1.06)	0.6797
Female, never-pregnant	29	600	1.33 (0.73-2.40)	24	545	1.36 (0.85-2.16)	104	908	0.85 (0.68-1.03)	261	1,475	1.01 (0.87-1.18)	0.0150
Comparison 2													
Male (reference)	152	2,936	1 (reference)	180	3,425	1 (reference)	858	5,232	1 (reference)	1,714	7,371	1 (reference)	
Female, ever-pregnant with sons	34	955	0.69 (0.47-1.03)	28	592	1.28 (0.84-1.96)	121	1,118	0.87 (0.71-1.08)	274	1,643	0.97 (0.84-1.11)	0.2440
Female, never-pregnant with sons	43	899	1.10 (0.75-1.62)	38	827	1.26 (0.87-1.83)	167	1,383	0.88 (0.73-1.06)	372	2,105	0.94 (0.83-1.06)	0.0686
Comparison 3													
Male (reference)	152	2,936	1 (reference)	180	3,425	1 (reference)	858	5,232	1 (reference)	1,714	7,371	1 (reference)	
Female, ever-pregnant with daughters	30	936	0.64 (0.43-0.97)	20	586	0.88 (0.54-1.43)	125	1,150	0.84 (0.68-1.03)	251	1,556	0.90 (0.78-1.04)	0.8156
Female, never-pregnant with daughters	46	907	1.15 (0.75-1.77)	31	833	1.07 (0.71-1.60)	163	1,374	0.89 (0.74-1.07)	397	2,191	1.00 (0.88-1.13)	0.1313

Table S6. Mortality Hazard Ratio of Male Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female (Never-Pregnant With or Ever-Pregnant) vs Male Donors Stratified by Patient Age for Patients included after 1st of September 2015

	0-17 y			18-50 y			51-70 y			≥71 y			p value for interaction
Donor category	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	
Comparison 1													
Male (reference)	36	626	1 (reference)	24	321	1 (reference)	138	1,207	1 (reference)	243	1,325	1 (reference)	
Female, ever-pregnant	14	321	0.77 (0.40-1.47)	14	107	2.45 (1.13-5.30)	39	453	0.81 (0.54-1.20)	78	534	1.01 (0.75-1.36)	0.0027
Female, never-pregnant	4	135	0.93 (0.29-3.02)	3	94	0.92 (0.25-3.40)	29	382	0.73 (0.46-1.14)	64	398	1.21 (0.88-1.64)	0.2249
Comparison 2													
Male (reference)	36	626	1 (reference)	24	321	1 (reference)	138	1,207	1 (reference)	243	1,325	1 (reference)	
Female, ever-pregnant with sons	11	243	0.83 (0.40-1.72)	10	72	2.44 (1.04-5.70)	26	309	0.88 (0.55-1.41)	50	374	0.90 (0.63-1.29)	0.0155
Female, never-pregnant with sons	7	213	0.86 (0.34-2.13)	7	122	1.28 (0.52-3.15)	44	542	0.91 (0.59-1.39)	92	559	1.16 (0.89-1.52)	0.5243
Comparison 3													
Male (reference)	36	626	1 (reference)	24	321	1 (reference)	138	1,207	1 (reference)	243	1,325	1 (reference)	
Female, ever-pregnant with daughters	14	253	0.98 (0.51-1.89)	8	77	2.22 (0.94-5.27)	26	316	0.76 (0.48-1.21)	58	382	1.12 (0.82-1.55)	0.0342
Female, never-pregnant with daughters	5	205	0.81 (0.28-2.32)	7	122	0.97 (0.37-2.54)	40	507	0.80 (0.54-1.20)	78	551	1.01 (0.76-1.35)	0.7276

Table S7. Mortality Hazard Ratio of Female Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) vs Male Donors Stratified by Patient Age for Patients included after 1st of September 2015

	0-17 y			18-50 y			51-70 y			≥71 y			p value for interaction
Donor category	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)	
Comparison 1													
Male (reference)	19	483	1 (reference)	18	571	1 (reference)	113	1,008	1 (reference)	163	1,304	1 (reference)	
Female, ever-pregnant	9	276	0.88 (0.25-3.09)	4	187	0.72 (0.24-2.22)	42	408	1.26 (0.85-1.88)	53	530	1.06 (0.73-1.55)	0.7541
Female, never-pregnant	9	139	2.10 (0.66-6.72)	4	146	1.31 (0.40-4.27)	23	275	0.89 (0.54-1.47)	50	412	1.10 (0.74-1.62)	0.0174
Comparison 2													
Male (reference)	19	483	1 (reference)	18	571	1 (reference)	113	1,008	1 (reference)	163	1,304	1 (reference)	
Female, ever-pregnant with sons	6	194	0.87 (0.23-3.27)	3	121	1.00 (0.28-3.64)	27	275	1.30 (0.80-2.10)	38	370	1.25 (0.82-1.93)	0.9437
Female, never-pregnant with sons	13	218	1.67 (0.60-4.63)	9	206	1.69 (0.71-4.03)	33	393	0.80 (0.48-1.31)	63	562	1.05 (0.67-1.65)	0.0192
Comparison 3													
Male (reference)	19	483	1 (reference)	18	571	1 (reference)	113	1,008	1 (reference)	163	1,304	1 (reference)	
Female, ever-pregnant with daughters	7	208	0.98 (0.28-3.44)	3	121	0.87 (0.25-3.08)	28	304	1.15 (0.72-1.83)	31	360	0.81 (0.52-1.27)	0.5285
Female, never-pregnant with daughters	12	203	1.85 (0.65-5.25)	5	214	1.03 (0.36-2.92)	33	279	0.91 (0.58-1.43)	69	563	1.08 (0.76-1.54)	0.0395

Table S8. Comparison 1: Mortality Hazard Ratio of Male and Female Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) Donors vs Male Donors (Sensitivity Analysis I.)

	Male recipients			Female recipients		
Donor category	**No. of Deaths**	**No. of Recipients**	**HR (95% CI)**	**No. of Deaths**	**No. of Recipients**	**HR (95% CI)**
Full cohort						
Ever-pregnant female analysis						
Male (reference)	7,203	29,879	1 (reference)	6,517	30,916	1 (reference)
Female, ever-pregnant	4,958	19,771	1.02 (1.00-1.05)	4,299	19,726	1.02 (1.00-1.05)
Never-pregnant female analysis						
Male (reference)	4,850	26,162	1 (reference)	4,850	26,162	1 (reference)
Female, never-pregnant	2,403	11,467	1.02 (0.97-1.06)	2,364	11,888	1.03 (0.99-1.07)
No mixture of exposure						
Male (reference)	3,068	18,367	1 (reference)	2,904	18,964	1 (reference)
Female, ever-pregnant	823	6,274	1.05 (1.00-1.09)	686	6,218	1.00 (0.95-1.04)
Female, never-pregnant	389	3,474	1.00 (0.93-1.08)	418	3,528	1.08 (1.01-1.16)
Single-transfusion						
Male (reference)	911	10,028	1 (reference)	823	9,622	1 (reference)
Female, ever-pregnant	447	4,599	1.14 (1.02-1.28)	353	4,379	1.01 (0.89-1.15)
Female, never-pregnant	252	2,776	1.03 (0.90-1.19)	244	2,704	1.13 (0.98-1.31)

Table S9. Comparison 2: Mortality Hazard Ratio of Male and Female Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female (Never-Pregnant With Male Offspring or Ever-Pregnant With Male Offspring) Donors vs Male Donors (Sensitivity Analysis I.)

	Male recipients			Female recipients		
Donor category	**No. of Deaths**	**No. of Recipients**	**HR (95% CI)**	**No. of Deaths**	**No. of Recipients**	**HR (95% CI)**
Full cohort						
Ever-pregnant female, sons analysis						
Male (reference)	5,698	26,426	1 (reference)	5,245	27,433	1 (reference)
Female, ever-pregnant with sons	3,149	14,006	1.01 (0.98-1.05)	2,798	14,019	1.04 (1.00-1.08)
Never-pregnant female, no sons analysis						
Male (reference)	6,266	28,032	1 (reference)	5,871	29,281	1 (reference)
Female, never-pregnant with sons	3,843	16,560	1.03 (1.00-1.07)	3,587	17,017	1.02 (0.98-1.05)
No mixture of exposure						
Male (reference)	3,068	18,367	1 (reference)	2,904	18,964	1 (reference)
Female, ever-pregnant with sons	519	4,301	1.04 (0.98-1.10)	457	4,308	1.02 (0.96-1.09)
Female, never-pregnant with sons*	645	5,209	1.04 (0.98-1.10)	620	5,214	1.03 (0.98-1.09)
Single-transfusion						
Male (reference)	911	10,028	1 (reference)	823	9,622	1 (reference)
Female, ever-pregnant with sons	320	3,382	1.11 (0.98-1.26)	261	3,292	1 (0.87-1.15)
Female, never-pregnant with sons*	371	3,930	1.08 (0.96-1.22)	329	3,730	1.11 (0.97-1.26)

* Combined category of products from never-pregnant female donors and ever-pregnant female donors without sons.

Table S10. Comparison 3: Mortality Hazard Ratio of Male and Female Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female (Never-Pregnant With Female Offspring or Ever-Pregnant With Female Offspring) Donors vs Male Donors (Sensitivity Analysis I.)

	Male recipients			Female recipients		
Donor category	No. of Deaths	No. of Recipients	HR (95% CI)	No. of Deaths	No. of Recipients	HR (95% CI)
Full cohort						
Ever-pregnant female, daughters analysis						
Male (reference)	5,663	26,336	1 (reference)	5,209	27,337	1 (reference)
Female, ever-pregnant with daughters	3,120	13,902	1.03 (0.99-1.06)	2,731	13,913	1.01 (0.98-1.05)
Female, no daughters analysis						
Male (reference)	6,301	28,083	1 (reference)	5,889	29,375	1 (reference)
Female, never-pregnant with daughters*	3,877	16,691	1.02 (0.99-1.05)	3,622	17,144	1.02 (0.99-1.05)
No mixture of exposure						
Male (reference)	3,068	18,367	1 (reference)	2,904	18,964	1 (reference)
Female, ever-pregnant with daughters	525	4,367	1.04 (0.98-1.11)	426	4,228	0.96 (0.89-1.02)
Female, never-pregnant with daughters*	644	5,183	1.03 (0.98-1.08)	637	5,305	1.04 (0.99-1.09)
Single-transfusion						
Male (reference)	911	10,028	1 (reference)	823	9,622	1 (reference)
Female, ever-pregnant with daughters	333	3,460	1.13 (0.99-1.28)	262	3,234	0.99 (0.87-1.14)
Female, never-pregnant with daughters*	358	3,852	1.07 (0.95-1.21)	328	3,788	1.11 (0.97-1.26)

* Combined category of products from never-pregnant female donors and ever-pregnant female donors without daughters.

Table S11. Mortality Hazard Ratio of Male and Female Transfusion Recipients Exposed to Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) Donors vs Male Donors (Sensitivity Analyses II. to VI.)

	Male recipients			Female recipients		
Donor category	**No. of Deaths**	**No. of Recipients**	**HR (95% CI)**	**No. of Deaths**	**No. of Recipients**	**HR (95% CI)**
II. Comparison 2, censored if donor born before 1958						
Male (reference)	3,068	18,367	1 (reference)	2,904	18,964	1 (reference)
Female, ever-pregnant with sons	247	2,399	0.87 (0.76-1.00)	225	2,385	0.88 (0.76-1.03)
Female, never-pregnant with sons*	465	4,054	0.88 (0.78-0.99)	474	4,052	0.99 (0.88-1.12)
III. Comparison 1, patients enrolled after 1-9-2015						
Male (reference)	441	3,479	1 (reference)	313	3,366	1 (reference)
Female, ever-pregnant	145	1,415	0.87 (0.68-1.11)	108	1,401	1.10 (0.79-1.51)
Female, never-pregnant	100	1,009	1.06 (0.76-1.46)	86	972	1.12 (0.82-1.53)
Comparison 2, patients enrolled after 1-9-2015						
Male (reference)	441	3,479	1 (reference)	313	3,366	1 (reference)
Female, ever-pregnant with sons	97	998	0.83 (0.63-1.10)	74	960	1.22 (0.82-1.81)
Female, never-pregnant with sons*	150	1,436	0.97 (0.75-1.25)	118	1,379	1.21 (0.83-1.79)
IV. Comparison 2, censored if donor had both sons and daughters						
Male (reference)	3,068	18,367	1 (reference)	2,904	18,964	1 (reference)
Female, ever-pregnant, only sons	127	1,161	0.94 (0.78-1.13)	106	1,190	0.88 (0.71-1.09)
Female, never-pregnant with sons*	645	5,209	0.94 (0.86-1.03)	620	5,214	0.97 (0.88-1.06)
V. Comparison 2, censored if donor was never-pregnant female or if donor had both sons and daughters						
Male (reference)	3,068	18,367	1 (reference)	2,904	18,964	1 (reference)
Female, ever-pregnant, only sons	127	1,161	0.94 (0.78-1.13)	106	1,190	0.88 (0.71-1.09)
Female, ever-pregnant, only daughters	143	1,235	1.00 (0.83-1.21)	98	1,125	0.78 (0.63 -0.97)

* Combined category of products from never-pregnant female donors and ever-pregnant female donors without sons.

Table S12. Exposure Group Assignment of Transfusion Recipients on Day 1 for Comparison 1 Stratified by Patient Age and Sex

Exposure category	0-17 y		18-50 y		51-70 y		≥71 y	
(day 1 assignment)	Male	Female	Male	Female	Male	Female	Male	Female
	N=6,679	N=5,392	N=5,621	N=10,291	N=18,409	N=14,636	N=17,813	N=19,810
Male	3,701 (55%)	2,935 (54%)	1,802 (32%)	3,424 (33%)	6,408 (35%)	5,232 (36%)	6,453 (36%)	7,369 (37%)
Female, ever-pregnant	1,577 (24%)	1,283 (24%)	518 (9%)	895 (9%)	2,045 (11%)	1,647 (11%)	2,130 (12%)	2,393 (12%)
Female, never-pregnant	651 (10%)	598 (11%)	323 (6%)	545 (5%)	1,232 (7%)	908 (6%)	1,268 (7%)	1,475 (7%)
Mixture	750 (11%)	576 (11%)	2,978 (53%)	5,427 (53%)	8,724 (47%)	6,849 (47%)	7,962 (45%)	8,573 (43%)

Table S13. Weights distribution of primary analysis, comparison 1

Population	Min.	Max.	0.5th percentile	99.5th percentile
Male patients, female ever-pregnant exposure	0,487292	51,05472	0,627831	1,692817
Male patients, female never-pregnant exposure	0,308522	4,166463	0,504654	1,687214
Female patients, female ever-pregnant exposure	0,456485	958,0321	0,587501	2,263018
Female patients, female never-pregnant exposure	0,316219	21132,81	0,504492	2,502784

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