Out for blood: causal inference in clinical transfusion research

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Chapter 3

Donor pregnancies and transfusion recipient mortality: a role for red blood cell storage?

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Abstract

Background and objectives

Donor characteristics have been implicated in transfusion-related adverse events. Uncertainty remains whether sex, and specifically pregnancy history of the blood donor, could affect patient outcomes. Whether storage duration of the blood product could be important for patient outcomes has also been investigated, and a small detrimental effect of fresh products remains a possibility. Here, we hypothesize fresh red blood cell products donated by ever-pregnant donors are associated with mortality in male patients.

Materials and methods

We used data from a cohort study of adult patients receiving a first transfusion between 2005 and 2015 in the Netherlands. The risk of death after receiving a transfusion from one of five exposure categories (female never-pregnant stored ≤10 days, female never-pregnant stored >10 days, female ever-pregnant stored ≤10 days, female ever-pregnant stored >10 days, male stored ≤10 days), compared to receiving a unit donated by a male donor that was stored >10 days (reference), was calculated using a Cox proportional hazards model.

Results

The study included 42,456 patients who contributed 88,538 person-years in total, of whom 13,948 died during the follow-up of the study (33%). Fresh units (stored ≤10 days) from ever-pregnant donors were associated with mortality in male patients, but the association was not statistically significant (hazard ratio 1.39, 95% confidence interval 0.97 to 1.99). Sensitivity analyses did not corroborate this finding.

Conclusion

These findings do not consistently support the notion that the observed association between ever-pregnant donor units and mortality is mediated by blood product storage.

Keywords

erythrocyte transfusion, mortality, RBC storage lesion, blood donor

Highlights

- The association between exposure to ever-pregnant donors and mortality in young men may be modified by product storage
- Studying parameters related to blood product hemoglobin requires careful consideration of statistical methods

Introduction

Although transfusions can be a necessary life-saving medical intervention, they are also associated with adverse events.[2] Some of these are attributable to certain donor characteristics, such as the passive infusion of leukocyte and neutrophil antibodies in Transfusion Related Acute Lung Injury (TRALI)[3] and the transfer of plasma containing IgA and IgE antibodies in allergic transfusion reactions.[4] Notwithstanding, the influence of blood donor characteristics on long term patient outcomes is incompletely understood. Uncertainty remains about whether sex and pregnancy history of the blood donor could influence recipient outcomes, beyond an increased risk of TRALI. In two earlier large-scale cohort studies, we identified an association between transfusions of red blood cells from female donors and increased mortality in male recipients under 50 years of age.[5, 6] The association was shown to be limited to female donors with a history of pregnancy, with an estimated impact of one death per day.[6, 7] In contrast, another large cohort study on this topic did not support these findings.[8] This lack of agreement between studies could be explained by differences in country-specific production methods, patient populations, and statistical methods. Although these studies constitute observational research, associations are interpreted causally.[9]

Whether 'fresh' or 'old' red blood cell transfusions are better for clinical outcomes has long been subject of debate, a question complicated by the widely varying ways this contrast has been defined in the transfusion research field. A systematic review and meta-analysis including evidence from randomized controlled trials up to 2017 did not find a benefit of using fresh red blood cell products in hospitalized patients, combining evidence from studies using different definitions of fresh and old red blood cell transfusions.[10] However, the authors could not exclude a small detrimental effect of fresh blood products on mortality, as confidence intervals included the potential for 1-2% benefit and up to 9% harm. Our research group previously investigated the association between storage time and mortality, and found, when comparing blood products that were stored <10 days with products stored >24 days, longer stored blood was associated with a lower risk of mortality (hazard ratio (HR) 0.56, 95% CI 0.32-0.97).[11]

Here, we quantified the association between storage time of the red cell product, donor sex and pregnancy history, and mortality of patients in a large observational cohort in the Netherlands. We hypothesize mortality will be highest in male patients who received fresh units from ever-pregnant donors.

Methods

Source database

In this observational cohort study, the analyses were performed as a post-hoc analysis on a combined cohort that has previously been described in the publications by Middelburg *et al.* and Caram-Deelder *et al.*[1, 5, 6] The cohort includes adult (≥18 years) first-ever transfusion recipients from six hospitals in the Netherlands between 2005 and 2015. Information was collected on donor, product, and patient characteristics. Data has been collected to the 'R-FACT study,' (CCMO-NL29563.058.09; clinicaltrials.gov: NCT01616329), and the study design for the cohort has been previously described.[6, 12, 13] The statistical analysis plan was specified prior to data analysis, and was reviewed and approved by the Scientific Committee of the Department of Clinical Epidemiology, Leiden University Medical Center (LUMC). The database is available at the Department of Clinical Epidemiology at the LUMC. All analyses were performed in Stata.[14]

Statistical analysis

We quantified the association between product characteristics and mortality using a Cox proportional hazards model. As can be seen in Figure 1, patients were classified as either having received blood products from ever-pregnant, never-pregnant or male donors, and storage was defined as fresh or old (Figure 1). Results were stratified by patient sex to be consistent with previous publications, where no association between mortality and previous pregnancy of the donor was observed in female patients.[6]

We defined *fresh* products as red cell products stored for 1 to 10 days, and compared those to *old* products, with a storage duration of 11 to 36 days. Results for exposure defined as 0-7 days for fresh products, and old products defined as products stored 8-36 days, are provided in the Supplemental materials to be consistent with the initial study protocol, which was adapted to allow for more balanced comparison groups. Exposure categories were further defined according to the sex and pregnancy history of the donors, sourced from the questionnaire about pregnancy status since last donation, at the time of donation at the blood bank. For this study, the patients receiving units donated by never-pregnant female donors act as a `negative control'. The reference category constitutes of old units donated by male donors, unless otherwise specified. We hypothesize female patients are not affected by blood products from ever-pregnant donors, and thereby view this patient group as a negative control for the research question. Hazard ratios were estimated to quantify the risk of mortality per trans-

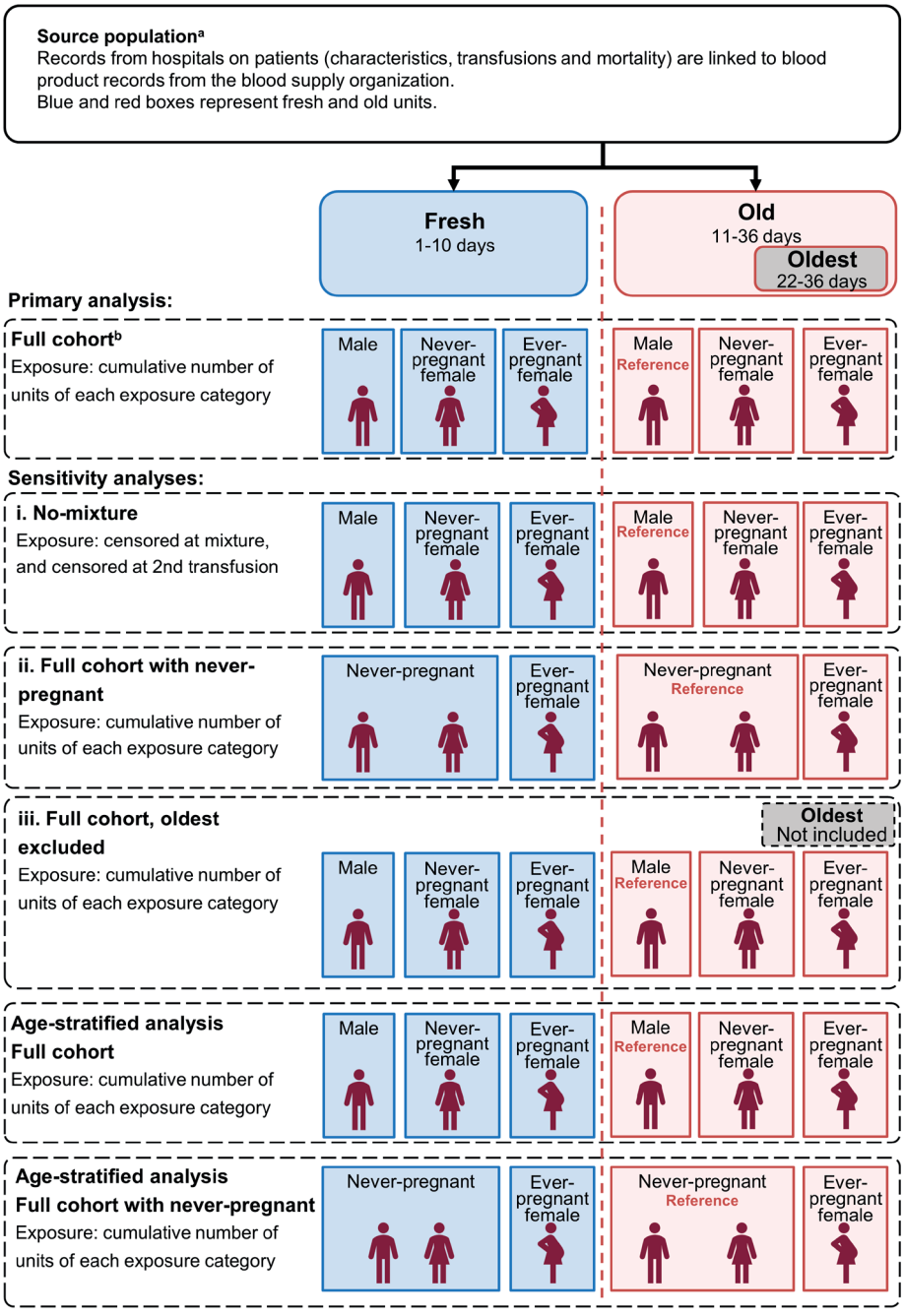

Figure 1

The figure contains a visual representation of the different exposure and reference groups for the primary and sensitivity analyses.

a Products donated by female donors with unknown pregnancy history were not assessed in this analysis.

b For sensitivity analysis iv. the same exposure and reference groups were used.

fused unit from the exposure category, compared with receiving a unit from the reference category.

Reference and exposure were included in the model as the time-varying cumulative number of units. For all analyses, HRs were not presented if a subgroup experienced less than 5 events.[15] Follow-up was in all analyses limited to a maximum of 15 transfusions, to maintain a homogeneous population of patients. Follow-up was accordingly defined as the time from inclusion up until the 16th transfusion (after which follow-up was censored), the first subsequent transfusion from an exposure category other than the categories included in the comparison (after which follow-up was censored), death, or administrative censoring due to reaching final hospital follow-up date.

Confounding

As sex and pregnancy history of the donor is unknown at the time a blood product is requested or transfused by the patient's treating physician, this exposure can be considered to be randomly distributed. Yet, the storage duration of red blood cell products is known. In neonates and younger patients who require massive transfusion, transfusion of fresh products (i.e. ≤5 days stored) is indicated. Also, irradiation (of predominantly fresh products) is indicated following intra-uterine transfusion, in premature neonates, and patients with severe combined immunodeficiency syndrome (SCID).[16, 17]. Thereby, in this patient group, short storage duration is associated with poorer clinical outcomes. For this reason, only adult patients were included in the cohort. Additionally, the probability of exposure with respect to storage is tied to the cumulative number of transfusions received, and blood product distribution factors. Based on these considerations, the following confounders for the study research question were identified and included in the models: number of transfusions [time-varying]; calendar year [time-varying]; blood group [fixed]; donor age [time-varying]; hospital [fixed]. Additional information about confounders can be found in the Supplemental methods (Figure S2). A restricted cubic spline with five knots was used for the time-varying cumulative number of transfusions. An interaction term for hospital and cumulative number of transfusions [time-varying] was included in the model to account for differences in transfusion practices between hospitals.

Primary analysis

The primary analysis was performed in the cohort of all patients, stratified by recipient sex, and this analysis is referred to as the full cohort. Here, follow-up was limited to the time during which the patient received units from the concerned exposure category and reference category only; the patient's follow-up

was censored as soon as they received units from a different exposure category. This means, a patient could receive units from both the exposure and reference category without being censored, with this patient then contributing follow-up time to both arms.[18] However, the patient's follow-up is censored upon receiving transfusions from another category, e.g. after any other exposure than male old and ever-pregnant fresh for the comparison male old vs. ever-pregnant fresh, such as a male fresh transfusion (for examples, see Figure S1).

Sensitivity analyses
Four sensitivity analyses were performed:

i) **No-mixture:** In the full cohort, more than one product category (exposure and reference) can be attributed to a single patient, which we expect might result in the underestimation of the association. Thus, we performed a sensitivity analysis where patients were censored upon receiving a transfusion from a different exposure category (*no-mixture*) and where patients who receive multiple transfusions were censored at their second transfusion (*single-transfusion*). Although censoring at the moment a product from a different exposure category is received is a type of informative censoring, it can be used to study the effect of transfusion exposures when patients receive multiple transfusions.[18]

ii) **Full cohort with reference group of never-pregnant donors:** To increase the subgroup size, within the full cohort an alternative reference category was introduced, combining all male and never-pregnant female donors into the category *never-pregnant donors*. The reference category for this analysis therefore constitutes both female and male donor products.

iii) **Full cohort, oldest excluded:** This sensitivity analysis was performed in the full cohort, and a comparison was made between fresh (less than or equal to 10 days storage) and intermediate (between 11 and 21 days of storage) products. The cutoff of 21 days was chosen to rule out a possible detrimental effect of long storage, which could then have concealed associations in our comparisons. These storage-induced blood product changes, such as hemolysis, oxidative stress and micro-vesicle formation, are collectively called the red blood cell storage lesion.[17] Units in the fourth and last week of storage are still generally considered safe, but evidence for safety of end-of-storage (28-36 days stored) red blood cell units is limited, as is evidence for use in vulnerable patient populations.[18-20]

iv) **No-mixture, first exposure only:** This sensitivity analysis was performed in the no-mixture cohort and only the first exposure was used, after which the com-

plete follow-up was included in the analysis. Patients for whom it was not possible to determine which transfusion was their first (i.e. patients who received multiple transfusions on their first transfusion day) were excluded. This analysis was performed to assess potential misspecification of the models that censored patients upon receiving multiple transfusion.

Age-stratified analysis

The *primary analysis* and *sensitivity analysis ii.* were stratified by patient sex and age to study effect-measure modification by age.[6, 8] Age categories were defined as 18-50, 51-70 and over 70 years of age. Effect-measure modification was formally quantified by adding an interaction term for patient age to the final model (p-value for interaction trend between patient age and exposure), as described previously.[6]

Results

Population

Patient and transfusion characteristics for three cohorts included in the primary and sensitivity analyses (*full cohort, no-mixture* and *single transfusion*), are presented, stratified by recipient sex (*Table 1*). In total, 42,456 patients contributed 88,538 person-years. From the total population, 53% (n=22,412) were female. During follow-up 13,948 (33%) patients died, with a median follow-up of 405 days (IQR 36-1,269) for the total population. The median age of all patients was 68 (IQR 55-77) years. The study population received a total of 127,687 transfusions, with a median of 2 transfusions per patient (IQR 2-4). The large majority of red cell products were stored >10 days. When the storage cutoff of 7 days was used, fewer patients could be included for the product categories ever-pregnant, *fresh,* never-pregnant, *fresh* and male, *fresh* (see *Table S1*).

Primary analysis

A total of 42,456 patients were included in this analysis, 22,412 female and 20,044 male (*Figure 2*). No statistically significant associations between exposure categories and mortality were observed among male patients. Male patients receiving fresh blood from ever-pregnant donors may have had higher mortality after transfusions, but this association was not statistically significant (HR 1.39 (95% CI 0.97-1.99)). No association was present when the units donated by ever-pregnant female donors were old (HR 1.05 (95% CI 0.99-1.12)).

All HRs for female patients were around or below 1, suggesting a smaller risk, when compared to the reference category of old male units. Receiving fresh

Table 1. Patient and transfusion characteristics

	Full cohort		No-donor mixture cohort[a]		Single-transfusion cohort[b]	
	Male patients	Female patients	Male patients	Female patients	Male patients	Female patients
Characteristics						
Number of patients	20,044	22,412	13,319	14,925	6,473	6,978
Number of deaths, (%)	7,465 (37%)	6,483 (29%)	2,155 (16%)	2,096 (14%)	655 (10%)	604 (9%)
Follow-up, median (IQR), days[c]	282 (22-1,098)	514 (59 -1,400)	91 (5-937)	309 (11-1303)	8 (2-547)	15 (2-744)
Person-time, sum in years	37,037	51,501	21,561	30,746	7,519	9,546
Age of patients, median (IQR), years	68 (58-76)	68 (52-79)	69 (59-77)	69 (54-79)	70 (60-77)	71 (57-80)
18 to 50 years	7,889 (13%)	5,202 (23%)	1,665 (13%)	3,276 (22%)	702 (11%)	1,309 (19%)
51 to 70 years	15,877 (44%)	7,097 (32%)	5,762 (43%)	4,654 (31%)	2,660 (41%)	2,148 (31%)
≥71 years	18,690 (43%)	10,113 (45%)	5,892 (44%)	6,995 (47%)	3,111 (48%)	3,521 (50%)
Transfusions of red blood cell units per patient, median (IQR)	2 (2-4)	2 (2-3)	2 (1-2)	2 (1-2)	1 (1-1)	1 (1-1)
Red blood cells transfusions, n (%)						
Total	63,837	63,850	26,032	28,626	6,473	6,978
female donor, never-pregnant, fresh	581 (1%)	632 (1%)	73 (1%)	120 (1%)	48 (1%)	86 (1%)
female donor, never-pregnant, old	8,646 (14%)	8,380 (13%)	1,378 (5%)	1,419 (5%)	863 (13%)	860 (12%)
female donor, ever-pregnant, fresh	601 (1%)	665 (1%)	82 (1%)	115 (1%)	49 (1%)	75 (1%)
female donor, ever-pregnant, old	8,850 (14%)	8,369 (13%)	1,463 (6%)	1,461 (5%)	903 (14%)	876 (13%)
male donor, fresh	3,501 (5%)	3,852 (6%)	1,416(5%)	1,736 (6%)	286 (4%)	539 (8%)
male donor, old	41,658 (65%)	41,952 (66%)	21,620 (83%)	23,775 (83%)	4,324 (67%)	4,542 (65%)

Abbreviation: IQR, interquartile range. Storage time definition: *fresh* refers to storage from 0 to 10 days; and *old* refers to storage from 11 to 36 days.

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

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

c 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, fresh or old), female donors with a history of pregnancy (ever-pregnant donors, fresh or old), male donors (fresh or old).

Full cohort[a]	Deaths/Recipients (exposure)	Deaths/Recipients (reference)[b]	HR per unit[c]
Male patients			
Ever-pregnant female old	922/4,560	2,551/13,078	1.05 (0.99-1.12)
Never-pregnant female old	908/4,420	2,561/13,025	1.05 (0.98-1.12)
Male fresh	174/1,049	1,840/10,506	0.93 (0.86-1.01)
Ever-pregnant female fresh	18/101	1,783/10,232	1.39 (0.97-1.99)
Never-pregnant female fresh	9/93	1,779/10,239	0.61 (0.33-1.11)
Female patients			
Ever-pregnant female old	784/4,664	2,424/14,569	0.99 (0.92-1.06)
Never-pregnant female old	820/4,759	2,461/14,655	0.95 (0.89-1.02)
Male fresh	187/1,410	1,846/11,905	0.86 (0.79-0.93)
Ever-pregnant female fresh	13/140	1,764/11,545	0.83 (0.52-1.30)
Never-pregnant female fresh	11/150	1,760/11,544	0.68 (0.42-1.11)

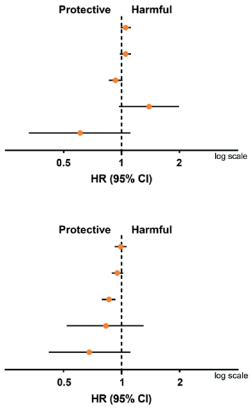

Figure 2

Forest plot containing the HRs from the primary analysis, stratified by sex. Reference category consists of patients exposed to units donated by male donors, stored >10 days (old). HRs are shown as orange dots, along with 95% confidence intervals.

Abbreviation: HR, hazard ratio.

a All models adjusted for calendar year, blood group (ABO-RhD), age of donor, hospital, cumulative number of transfusions, and an interaction term for hospital and cumulative number of transfusions.

b Recipients in the full cohort could receive mixed blood from both the exposure of interest and the reference category; therefore, the number of recipients receiving blood from male donors (old) is different for the different comparisons (see also Supplemental methods).

c Hazard ratios per transfused unit compared with receiving a stored unit from a male blood donor (reference group: male old)

units from ever-pregnant donors was not associated with mortality in female patients (HR 0.83 (95% CI 0.52-1.30)). For female patients, receiving fresh male units was associated with a small survival benefit (HR 0.86 (95% CI 0.79-0.93)).

Due to small sample size, the HR for exposure to ever-pregnant units stored for a short duration could not be shown when the cutoff of 7 days was used in both male and female patients (*Table S2*).

Sensitivity analyses

We only present sensitivity analyses with implications for the interpretation of the primary analysis here, and refer to the Supplemental materials for further information (Table S3-S4).

In sensitivity analysis iv. **(No-mixture, no censoring, Table S3)**, which is the analysis where follow-up was not censored, results differed from the primary

analysis in both direction and magnitude of the effect of exposure. The HR was 0.87 (95% CI 0.54-1.42) when comparing fresh ever-pregnant donor red blood cell units with the reference group (male, stored >10 days) for male patients. For female patients, the HR was 0.78 (95% CI 0.47-1.28) for ever-pregnant donor red blood cell units that were fresh compared to units that was stored >10 days, donated by male donors.

Age-stratified analysis

For the comparisons stratified by age, for male patients, the number of included patients was small (*Table 2*). Therefore, the analysis was only carried out for the **Full cohort** and the full cohort with the combined category of male donors and never-pregnant female donors **(Full cohort with never-pregnant)**.

For the full cohort analysis, the HR for the age group of 18-50 years was not shown due to the low number of events, the HR for the age group of 51-70 years was 1.36 (95% CI 0.77-2.40) for the comparison ever-pregnant fresh to male, old. The HR for the age group of 71 years and older could not be computed due to zero events in this age group after exposure to fresh red blood cell units from ever-pregnant donors. The p-value for the trend for the interaction between age and exposure was 0.316. The low event numbers suggest considerable uncertainty regarding the interaction between age and exposure. The interaction between age and exposure was significant in other comparisons (never-pregnant female old, never-pregnant female old, male fresh).

The results for fresh ever-pregnant units, now compared to the reference of the combined category of male donors and never-pregnant female donors (stored >10 days; old) for male patients were similar to those presented above (*Table 2;* 18-50 years, HR not shown, 51-70 years, HR 1.38 (95% CI 0.85-2.23), 70 and older, HR 1.32 (0.82-2.14)), with no significant interaction with patient age (p=0.179).

No noteworthy associations were present between product characteristics and mortality in female patients in the stratified analysis, with effect sizes around 1 for all comparisons, and small group sizes (*Table S5*).

Results for the storage cutoff of 7 days can be found in the Supplemental materials (*Table S6, S7*).

Table 2. Mortality Hazard Ratio of Male Patients Exposed to fresh or old Red Blood Cell Transfusions From Female Ever-Pregnant Donors vs Male Donors in the Full Cohort, Stratified by Patient Age[a]

Donor category	18-50 y			51-70 y			≥71 y			p value for interaction[c]
	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]	
Full cohort										
Male old (reference)[d]	161	1,632	1 (reference)	949	5,631	1 (reference)	1,441	5,815	1 (reference)	0.000
Ever-pregnant female old	73	572	1.38 (1.09-1.74)	363	1,996	1.02 (0.93-1.13)	486	1,992	1.02 (0.93-1.12)	
Male old (reference)[d]	160	1,659	1 (reference)	922	5,603	1 (reference)	1,479	5,763	1 (reference)	0.000
Never-pregnant female old	62	618	0.97 (0.75-1.26)	327	1,919	1.01 (0.90-1.12)	519	1,883	1.08 (0.99-1.18)	
Male old (reference)[d]	100	1,244	1 (reference)	642	4,393	1 (reference)	1,041	4,595	1 (reference)	0.316
Ever-pregnant female fresh	2	16	-	7	45	1.36 (0.77-2.40)	9	38	1.36 (0.81-2.27)	
Male old (reference)[d]	100	1,245	1 (reference)	639	4,394	1 (reference)	1,040	4,600	1 (reference)	0.069
Never-pregnant female fresh	1	19	-	3	46	-	5	37	1.01 (0.46-2.25)	
Male old (reference)[d]	103	1,294	1 (reference)	674	4,531	1 (reference)	1,063	4,681	1 (reference)	0.000
Male fresh	12	193	0.94 (0.68-1.32)	87	512	0.96 (0.85-1.07)	75	344	0.96 (0.85-1.09)	
Full cohort with never-pregnant										
Never-pregnant old (reference)[d]	273	2,320	1 (reference)	1,507	7,691	1 (reference)	2,245	7,739	1 (reference)	0.000
Ever-pregnant female old	123	845	1.18 (0.99-1.41)	594	2,758	1.03 (0.95-1.11)	771	2,650	1.00 (0.93-1.07)	
Never-pregnant old (reference)[d]	163	1,756	1 (reference)	972	5,988	1 (reference)	1,562	6,102	1 (reference)	0.179
Ever-pregnant female fresh	3	23	-	10	54	1.38 (0.85-2.23)	11	42	1.32 (0.82-2.14)	
Never-pregnant old (reference)[d]	174	1,835	1 (reference)	1,040	6,208	1 (reference)	1,606	6,242	1 (reference)	0.000
Never-pregnant female fresh	22	275	0.93 (0.74-1.17)	138	724	0.97 (0.89-1.06)	109	489	0.91 (0.83-0.99)	

Abbreviation: HR, hazard ratio. Storage time definition: *fresh* refers to storage from 0 to 10 days; and *old* refers to storage from 11 to 36 days.

a All models are adjusted for calendar year, blood group (ABO-RhD), hospital, age of donor, cumulative number of transfusions, and an interaction term for hospital and cumulative number of transfusions.

b Hazard ratios per transfused unit compared with receiving a unit from the reference category.

c For the trend in interaction across the continuous variable patient age.

d Recipients in the full cohort could receive mixed blood from both the exposure of interest and the reference category; therefore, the number of recipients receiving blood from male donors (old) or never-pregnant donors (old) is different for the different comparisons (see also *Supplemental methods*).

Discussion

In this study, a large database of patient and transfusion data was used for an in-depth analysis of multiple aspects of the 'transfusion continuum', namely sex and pregnancy history of the donor and storage of blood products.[23] Although these parameters have been studied in great detail separately, blood product storage has not yet been studied together with sex of the donor and whether the donor was previously pregnant. The findings did not consistently support the notion that storage plays a role in modifying the association between donor characteristics and patient survival.

Recent publications have rightly criticized aspects of previous work investigating the effect of sex (and pregnancy history) of the donor, specifically that Cox regression may not be appropriate.[23, 25] Bias due to treatment-confounder feedback could lead to biased hazard ratio's obtained with Cox regression. Female donors have lower hemoglobin concentrations and this could lead to more, or earlier, additional transfusions. This issue could be further exacerbated by looking at 'fresh' and 'older' units, as storage also affects red blood cell viability and subsequent hemoglobin measurements. However, the small subgroup sizes for the various storage contrasts did not allow for data-intensive approaches like g-methods. Alternatively, we performed an analysis in which patients were studied according to their first transfusion independent of additional transfusions, thereby avoiding the problem of treatment-confounder feedback. The results of the latter analysis did not corroborate the results from the primary analysis, suggesting that the observed association did not reflect a causal effect.

Furthermore, we did not have access to the indication of the transfusion or disease severity of the patient. The indication of the transfusion is associated with both the number of transfusions a patient will receive, and the risk of mortality, but is not directly associated with the probability of receiving transfusions with certain donor and product characteristics. However, transfusion indication could still be an effect modifier, with subpopulations of patients potentially being 'sensitive' to an effect of exposure. Exploring outcomes of subgroups of patients could be a way to help us understand biological mechanisms of harm when an effect is present.[26, 27] It is also important to note that patients who are transfused at a young age are inherently different from adults with regards to blood product distribution policy and prognosis. For neonates and young children, units stored shorter than five days are prescribed to decrease the exposure to blood products with an increased potassium and decreased 2,3-diphosphoglycerate (2,3-DPG) content. Because we do not know which patients were prescribed these fresh units, all children were excluded from the study (see *Supplemental methods*).

[16] Importantly, blood products are frequently irradiated and subsequently administered in the first week of storage.[16] The inclusion of irradiated products potentially biases the effect estimates, because irradiated products are more likely to be prescribed to patients with a poor prognosis. These products are requested for preterm neonates but are also prescribed for other immunologically impaired patients. We postulated previously that the associations between transfusion of products from ever-pregnant donors and mortality are mediated by a cellular component.[28] If lymphocyte proliferation-dependent effects are inhibited by irradiation in a subset of products included in this study, the estimates could be an underestimation of the effect of exposure, although these patients tend to have a poor prognosis. It is therefore difficult to predict the direction and magnitude of confounding by the request of irradiated products. Assessing the exposure of interest in context with other conditions where an effect should be absent (negative controls, e.g. never-pregnant exposure or female patients) alleviates this relevant concern. Lastly, as the data collection for this study spanned several years, minor changes were implemented regarding blood product processing and transfusion guidelines during the study period. [16, 29] However, during this period no changes were made to leukoreduction filter types.

In summary, blood products from ever-pregnant donors stored for a short storage duration were associated with increased mortality in male patients in the primary analysis of this study, but this was not corroborated in sensitivity analyses. The validity of studies on donor- and blood product characteristics relies on strong assumptions about the data, which should be thoroughly verified, especially when treatment-confounder feedback is suspected.

REFERENCES

1 Valk SJ, Caram-Deelder C, Evers D, De Vooght KMK, Van de Kerkhof D, Wondergem MJ, et al. Donor pregnancies and transfusion recipient mortality: a role for red blood cell storage? 2019; Conference: 29th Regional Congress of the ISBT, Basel.

2 Kato H, Uruma M, Okuyama Y, Fujita H, Handa M, Tomiyama Y, et al. Incidence of transfusion-related adverse reactions per patient reflects the potential risk of transfusion therapy in Japan. Am J Clin Pathol. 2013; 140: 219-24.

3 Porretti L, Cattaneo A, Coluccio E, Mantione E, Colombo F, Mariani M, et al. Implementation and outcomes of a transfusion-related acute lung injury surveillance programme and study of HLA/HNA alloimmunisation in blood donors. Blood Transfus. 2012; 10: 351-9.

4 Johansson SG, Nopp A, van Hage M, Olofsson N, Lundahl J, Wehlin L, et al. Passive IgE-sensitization by blood transfusion. Allergy. 2005; 60: 1192-9.

5 Middelburg RA, Briet E, van der Bom JG. Mortality after transfusions, relation to donor sex. Vox Sang. 2011; 101: 221-9.

6 Caram-Deelder C, Kreuger AL, Evers D, de Vooght KMK, van de Kerkhof D, Visser O, et al. Association of Blood Transfusion From Female Donors With and Without a History of Pregnancy With Mortality Among Male and Female Transfusion Recipients. JAMA. 2017; 318: 1471-8.

7 Altman DG, Andersen PK. Calculating the number needed to treat for trials where the outcome is time to an event. BMJ. 1999; 319: 1492-5.

8 Edgren G, Murphy EL, Brambilla DJ, Westlake M, Rostgaard K, Lee C, et al. Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. Jama. 2019; 321: 2183-92.

9 Hernán MA. The C-Word: Scientific Euphemisms Do Not Improve Causal Inference From Observational Data. Am J Public Health. 2018; 108: 616-9.

10 McQuilten ZK, French CJ, Nichol A, Higgins A, Cooper DJ. Effect of age of red cells for transfusion on patient outcomes: a systematic review and meta-analysis. Transfus Med Rev. 2018; 32: 77-88.

11 Middelburg RA, van de Watering LM, Briet E, van der Bom JG. Storage time of red blood cells and mortality of transfusion recipients. Transfus Med Rev. 2013; 27: 36-43.

12 Evers D, Middelburg RA, de Haas M, Zalpuri S, de Vooght KM, van de Kerkhof D, et al. Red-blood-cell alloimmunisation in relation to antigens' exposure and their immunogenicity: a cohort study. Lancet Haematol. 2016; 3: e284-92.

13 Zalpuri S, Zwaginga JJ, Bom JGvd. Risk Factors for Alloimmunisation after red blood Cell Transfusions (R-FACT): a case cohort study. BMJ. 2012. https://doi.org/.

14 StataCorp. Stata Statistical Software: Release 16. College Station, TX, StataCorp LLC, 2019.

15 Vittinghoff E, McCulloch CE. Relaxing the Rule of Ten Events per Variable in Logistic and Cox Regression. Am J Epidemiol. 2006; 165: 710-8.

16 CBO. Dutch Guideline Bloodtransfusion. Retrieved October 22, 2018, from: https://nvicnl/ sites/nvicnl/files/CBO%20Richtlijn%20Bloedtransfusiepdf. 2011

17 Kreuger A. Adenine metabolism during and after exchange transfusions in newborn infants with CPD-adenine blood. Transfusion. 1976; 16: 249-52.

18 Middelburg RA, Le Cessie S, Briët E, Vandenbroucke JP, Van Der Bom JG. A solution to the problem of studying blood donor -related risk factors when patients have received multiple transfusions. 2010; 50: 1959-66.
19 García-Roa M, Del Carmen Vicente-Ayuso M, Bobes AM, Pedraza AC, González-Fernández A, Martín MP, et al. Red blood cell storage time and transfusion: current practice, concerns and future perspectives. Blood Transfus. 2017; 15: 222-31.
20 Prudent M, Tissot J-D, Lion N. In vitro assays and clinical trials in red blood cell aging: lost in translation. Transfus Apher Sci. 2015; 52: 270-6.
21 Goel R, Johnson DJ, Scott AV, Tobian AA, Ness PM, Nagababu E, et al. Red blood cells stored 35 days or more are associated with adverse outcomes in high- risk patients. Transfusion. 2016; 56: 1690-8
22 Ng MS, David M, Middelburg RA, Ng AS, Suen JY, Tung J-P, et al. Transfusion of packed red blood cells at the end of shelf life is associated with increased risk of mortality–a pooled patient data analysis of 16 observational trials. Haematologica. 2018; 103: 1542.
23 Ning S, Heddle NM, Acker JP. Exploring donor and product factors and their impact on red cell post-transfusion outcomes. Transfus Med Rev. 2018; 32: 28-35.
24 Zhao J, Sjölander A, Edgren G. Mortality Among Patients Undergoing Blood Transfusion in Relation to Donor Sex and Parity: A Natural Experiment. JAMA Intern Med. 2022; 182: 747-56.
25 Bruun-Rasmussen P, Andersen PK, Banasik K, Brunak S, Johansson PI. 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. eClinicalMedicine. 2022; 51.
26 Middelburg RA, Caram-Deelder C, van der Bom JG. Ever-Pregnant Female Blood Donors and Mortality Risk in Male Recipients—Reply. JAMA. 2018; 319: 1048-9.
27 Ali O, Wasfi M, Uzoigwe C. Ever-pregnant female blood donors and mortality risk in male recipients. JAMA. 2018; 319: 1048-9.
28 Valk SJ, Caram-Deelder C, Zwaginga JJ, van der Bom JG, Middelburg RA. Donor sex and recipient outcomes. ISBT Sci Ser. 2019. https://doi.org/10.1111/voxs.12528.
29 Bontekoe IJ, van der Meer PF, Mast G, de Korte D. Separation of centrifuged whole blood and pooled buffy coats using the new CompoMat G5: 3 years experience. Vox Sang. 2014; 107: 140-7.

Supplementary materials - Donor pregnancies and transfusion recipient mortality: a role for red blood cell storage?

Content

Supplementary materials

The Supplementary materials contain additional clarification regarding the study methods (Supplemental methods), supplementary results for analyses with alternative storage cutoff (Supplemental results) and additional references.

Supplemental methods

Pregnancy of female blood donors

At their first donation, female blood donors self-reported any previous pregnancy. At all subsequent donations, they reported if they have been pregnant since the previous donation. However, since some female donors had their first ever donation prior to the establishment of the current electronic recording system at the Sanquin blood bank, data on pregnancy history is incomplete for a subset of the donors. We therefore adopted a conservative strategy in coding of pregnancy data. If a female donor ever answered '*yes*' to the question if she had been pregnant, all subsequent donations were considered to be from an ever-pregnant female donor. If she never answered yes, we assumed pregnancy status to be unknown, rather than negative. Similarly all donations before the first recorded pregnancy were considered unknown, rather than negative, unless we could positively confirm our data also included the first ever transfusion from this donor. Therefore, only when the first donation was registered and answered as never-pregnant the pregnancy status was considered never-pregnant until the first donation at which a pregnancy was reported. For our analyses of exposure to red cells of ever-pregnant donors, all patients receiving one or more red cell transfusions from donors of unknown pregnancy status were excluded from the analyses. Some examples are given below:

Female donor A

Donation record	1st donation	2nd donation	3rd donation	4th donation	5th donation
Pregnancy question	Ever been pregnant	Pregnant since the last donation	Pregnant since the last donation	Pregnant since the last donation	Pregnant since the last donation
Pregnancy Answer	No	No	No	Yes	Yes
Status of the donation	Never-pregnant	Never-pregnant	Never-pregnant	Ever-pregnant	Ever-pregnant

Female donor B

Donation record	1st donation	2nd donation	3rd donation	4th donation
Pregnancy question	Ever been pregnant	Pregnant since the last donation	Pregnant since the last donation	Pregnant since the last donation
Pregnancy Answer	Yes	No	No	Yes
Status of the donation	Ever-pregnant	Ever-pregnant	Ever-pregnant	Ever-pregnant

Female donor C

Donation record	1st donation	2nd donation	3rd donation
Pregnancy question	Ever been pregnant	Pregnant since the last donation	Pregnant since the last donation
Pregnancy Answer	Missing	No	No
Status of the donation	Unknown	Unknown	Unknown

Female donor D

Donation record	1st donation	2nd donation	3rd donation	4th donation	5th donation
Pregnancy question	Ever been pregnant	Pregnant since the last donation	Pregnant since the last donation	Pregnant since the last donation	Pregnant since the last donation
Pregnancy Answer	Missing	No	No	Yes	No
Status of the donation	Unknown	Unknown	Unknown	Ever-pregnant	Ever-pregnant

Reference and exposure groups in the full cohort

As a result of the choice to accept units from both exposure and reference groups in the full cohort, patients would sometimes be included as contributing to the reference group and sometimes be excluded, resulting in different numbers of patients for each comparison contributing to the reference group. *Figure S1* shows five examples which illustrate this.

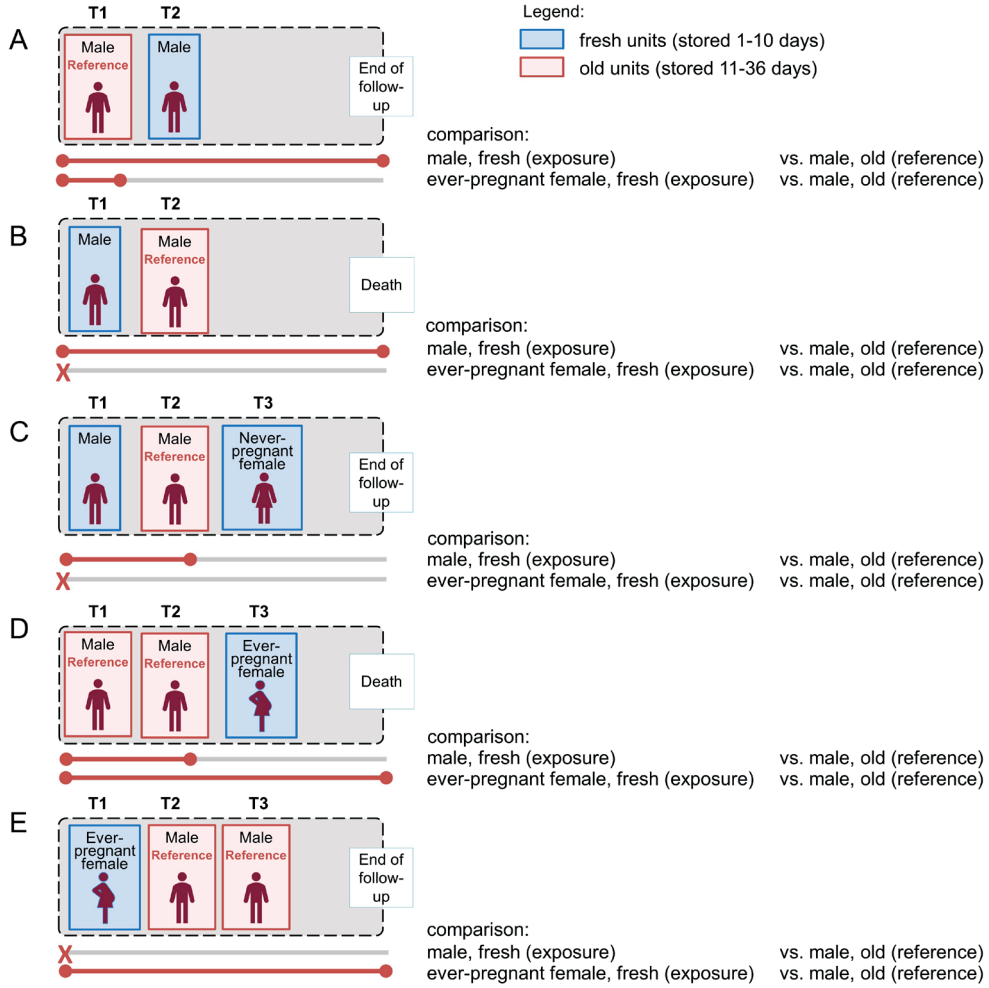

Figure S1. Explanatory graphic with five patient examples for reference and exposure groups in the full cohort

This figure contains a visual representation of five patients and their inclusion in the full cohort, here depicted for two comparisons for illustrative purposes. Colors represent the two product storage groups: red (old units) and blue (fresh units). Transfusions are numbered consecutively (T1, T2, T3), and patient follow-up is included in the database until either end of hospital follow-up (patient A, C, E) or death (patient B, D).

In Figure S1, patient A received two transfusions, the first one from a male donor (storage: old) and the second one from a male donor (storage: fresh). For the comparison male, fresh (exposure) to male, old (reference), the full follow-up of the patient contributes to the analysis. For the comparison of ever-pregnant

female donor, fresh (exposure) to male, old (reference), only the time up to the second transfusion is included, as this is the time during which the patient adhered to the conditions of the comparison.

Similarly, patient B received two transfusions, the first one from a male donor (storage: fresh) and the second one from a male donor (storage: old). For the comparison male, fresh (exposure) to male, old (reference), the full follow-up of the patient contributes to the analysis. In contrast, for the comparison of ever-pregnant female donor, fresh (exposure) to male, old (reference), the patient is excluded because they received a transfusion that did not adhere to the comparison conditions.

Patient C received three transfusions in total: first, one from a male donor (fresh), the second one from a male donor (storage: old), and the third one from a female donor that was never pregnant (storage: fresh). For the comparison male, fresh (exposure) to male, old (reference), the follow-up is included up to the third transfusion, because up until that point the patient adheres to the conditions of the cohort. For the comparison of ever-pregnant female donor, fresh (exposure) to male, old (reference), the patient is excluded because they received a transfusion outside the comparison first.

Patient D also received three transfusions: two units from a male donor (storage: old) and one unit from an ever-pregnant female donor (storage: fresh). For the comparison male, fresh (exposure) to male, old (reference), the follow-up is included up to the third transfusion, because up until that point the patient adheres to the conditions of the comparison. For the comparison of ever-pregnant female donor, fresh (exposure) to male, old (reference), the full follow-up is included.

Lastly, patient E received three transfusions, one unit donated by an ever-pregnant blood donor (storage: fresh) and two units from a male donor (storage: old). The patient is not included for the comparison male, fresh (exposure) to male, old (reference) because the first transfusion is not part of the comparison. For the comparison of ever-pregnant female donor, fresh (exposure) to male, old (reference), the full follow-up is included.

Confounders

The following confounders were included in the model:

- number of transfusions;
- year;
- blood group;
- donor age;
- hospital;
- an interaction term for hospital and cumulative number of transfusions.

The number of transfusions is an important confounder (*Figure S2*), because more severely ill patients receive more transfusions.[1] And, as the number of transfusions increases, the chance of receiving a blood product from an ever-pregnant donor increases. The cumulative number of transfusions also varies by year, as transfusion practices have changed[2] , and by hospital. The cumulative number of transfusions was modelled as a time-varying variable, as a continuous variable with a restricted cubic spline with five knots.[3] This allows for modelling of the potential non-linear relation between the confounder and the outcome.[4]

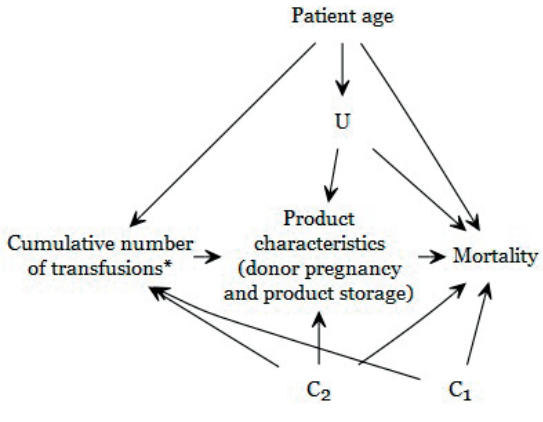

Figure S2. Directed Acyclic Graph of the Effect of Product Characteristics (Donor Sex, Pregnancy and Product Storage) on Mortality

*The cumulative number of transfusions both influences exposure and is a proxy for disease severity; an arrow between disease severity and mortality is also present (variable not shown)

U: unmeasured confounding by indication in age group 0-17;

c_1: patient sex, hospital;

c_2: calendar year, blood group

The probabilities of receiving units stored >7/>10 days and blood from ever-pregnant donors, mortality, and the total number of transfusions, can vary over time. The proportion of female donors and female donors with a history of preg-

nancy varies by year. Thus, the calendar year of each transfusion was modelled as a time-varying categorical variable.

Some blood groups are rarer and therefore products with these blood groups will, on average, have an increased shelf time. Blood groups are also potentially associated with the total number of transfusions received, and blood groups are associated with mortality. We adjusted for categories of recipient blood group in the model.

Donor age might be associated with mortality and is associated with the probability of a positive pregnancy history.[5] We adjusted for donor age with the number of units from donors aged 50 years or older as a time-varying continuous variable.

There could be differences in policy regarding the storage time of red blood cells at distribution in the different centers. If there are any centers which allow the selection of fresh red cells on the request of the physician, this can result in confounding. Therefore, hospital was added to the model as a categorical variable. We also added an interaction term between hospital and number of transfusions, which further accounts for the variation in patient populations between hospitals.

Several aspects of blood product distribution make studying product characteristics challenging in children. Due to the increased potassium and decreased 2,3-DPG-concentration in blood products with extended storage, blood products for neonates can be requested to have a short (≤5 days) storage duration, in certain clinical situations (depicted by U, *Figure S1*). For example, when these patients are expected to receive a large transfusion volume (>80 ml/kg in <24 hours, >40 ml/kg in 3 hours or a transfusion rate >5 ml/kg/hr) only fresh units are used.[6] Additionally, a request can be made for the blood product to be irradiated prior to the transfusion – especially for premature neonates – to abrogate the risk of transfusion-associated graft-versus-host disease (ta-GVHD).[7] Products are more often irradiated in the first week of storage, because irradiation affects the expiration date of the product negatively. Donor exposure has also been limited to protect this patient group from adverse events, with neonates preferably receiving blood transfusions split from the same unit up to the expiration date of the unit, or from a single donor, if they are available.[8] However, the actual transfusion strategy followed will vary considerably and is dictated by patient specific conditions and more or less optimal product choices by urgency versus availability. We do not have information about which subgroups of

children were actually prescribed irradiated or very fresh units. Because storage time cannot be considered to be independent from patient age – due to children preferentially getting shorter stored blood products – and because patient age is associated with mortality, children were excluded from the analysis.

Differences between statistical analysis plan and final analysis

In the original statistical analysis plan, the cutoff for storage time was set at 7 days. As this cutoff of 7 days did not yield sufficiently large groups to perform all planned analyses, we extended the storage time cutoff to 10 days. Results for the original 7-day cutoff analysis are provided in the Supplemental materials (Table S1-S5).

In addition, we excluded patients aged 0-17 years from all analyses, due to possible unmeasured confounding by indication, since the clinical situation necessitating the transfusion is likely (strongly) associated to the clinical outcome. In this patient population, requesting products stored for shorter storage durations (i.e. five days or less) was an option available to physicians.[9] We expect the request of fresh units to especially have been made for children with a worse prognosis. This could have resulted in unmeasured confounding by indication (see *Supplemental materials*, *Figure S2*), and therefore we decided to restrict all analyses to adults. Results for the complete population (including children), as well as the statistical analysis plan, are available on request.

Lastly, we added an analysis (Sensitivity analysis iv.) which does not censor patients and only uses information from the first transfusion as exposure category. In brief, the inclusion of complete follow-up from patients while only taking their first transfusion as exposure group 'assignment' should allow for a crude comparison of effects of exposures without interference of any post-transfusion treatment-confounder feedback. Because mixing of exposure does occur after this initial assignment, the effect estimate is expected to be less extreme, but should still follow the same direction (compared to the primary analysis and other sensitivity analyses) if these methods are unbiased.

Supplemental results

Sensitivity analysis i. **(Censored at mixture)** included a total of 28,228 patients (14,920 female patients and 13,308 male patients) in the no-mixture cohort (*Table S3*). A total of 13,420 patients were included in the single-transfusion cohort **(Censored at second transfusion)**, of which 6,950 were female and 6,470

were male. In male patients in the no-mixture cohort, confidence intervals for the HR for receiving one fresh unit of blood from an ever-pregnant female donor are wide (HR 1.34 (0.85-2.10)). This HR for the single-transfusion cohort is not shown due to lack of events in the exposed subgroup of male patients.

In the no-mixture cohort, when comparing ever-pregnant donor units that were fresh with the reference group in female patients, the association was similar (HR 0.62 (95% CI 0.31-1.25)), and the association in the single transfusion cohort was also similar but less precise (HR 0.59 (95% CI 0.22-1.60)). Of note, in female patients, receiving male fresh units was associated with a survival benefit in the no-mixture cohort (HR 0.81 (0.73-0.90)) and the single-transfusion cohort (HR 0.33 (95% CI 0.22-0.50)).

The HR for exposure to ever-pregnant units stored for a short duration in sensitivity analysis i. (with a storage cutoff of 7 days) could not be shown due to small sample size (*Table S4*).

In sensitivity analysis ii. **(Full cohort with never-pregnant)**, 53,487 patients were included, 27,877 female and 25,610 male. In the cohort of male patients, receiving fresh ever-pregnant units was associated with mortality, but the estimate was less precise (HR 1.32 (95% CI 0.96-1.82)). When comparing the extended reference group with never-pregnant fresh units, receiving these units was associated with a small survival advantage (HR 0.92 (95% CI 0.87-0.98)).

For female patients, the HR was 1.08 (95% CI 0.88-1.33) for fresh ever-pregnant units compared to reference. No notable associations were present in the other comparisons.

In sensitivity analysis iii. **(Full cohort with intermediate)**, 30,268 patients were included (15,873 female patients, 14,395 male patients) and patients were censored when they received units stored longer than 21 days. The results were comparable to previous comparisons, with a HR of 1.50 (95% CI 1.05-2.16) when comparing fresh ever-pregnant donor red blood cell units with reference (male, intermediate) for male patients.

For female patients, the HR was 0.84 (95% CI 0.52-1.34) for ever-pregnant donor red blood cell units that were fresh compared to units that was stored 11-21 days, donated by male donors. Here, female patients who received fresh units donated by male donors had a lower risk of mortality during follow-up, compared to patients who received units from the reference category (HR 0.86

(95% CI 0.79-0.94)). The HR for exposure to ever-pregnant units stored for 7 days or shorter in sensitivity analysis iii. could not be shown due to small sample size (*Table S4*).

Table S5 contains results for the stratified analysis for female patients and is described in the manuscript: "No noteworthy associations were present between product characteristics and mortality in female patients in the stratified analysis, with effect sizes around 1 for all comparisons, and small group sizes (*Table S5*)."

Results for the stratified analysis with the storage cutoff of 7 days were not shown for exposure to ever-pregnant, fresh units due to small sample size in all age groups for both male (*Table S6*) and female patients (*Table S7*).

Table S1. Patient and transfusion characteristics – Cutoff 7 days, only adults

	Full cohort		No-donor mixture cohort[a]		Single-transfusion cohort[b]	
	Male patients	Female patients	Male patients	Female patients	Male patients	Female patients
Characteristics						
Number of patients	20,044	22,412	13,319	14,925	6,473	6,978
No. of deaths, (%)	7,465 (37%)	6,483 (29%)	2,155 (16%)	2,096 (14%)	655 (10%)	604 (9%)
Follow-up, median (IQR), d[c]	282 (22-1,098)	514 (59 -1,400)	91 (5-937)	309 (11-1303)	8 (2-547)	15 (2-744)
Person-time, sum in years	37,037	51,501	21,561	30,746	7,519	9,546
Age of patients, median (IQR), years	68 (58-76)	68 (52-79)	69 (59-77)	69 (54-79)	70 (60-77)	71 (57-80)
2,687 (13%)	2,687 (13%)	5,202 (23%)	1,680 (13%)	3,340 (22%)	702 (11%)	1,309 (19%)
8,780 (44%)	8,780 (44%)	7,097 (32%)	5,811 (43%)	4,691 (31%)	2,660 (41%)	2,148 (31%)
8,577 (43%)	8,577 (43%)	10,113 (45%)	5,921 (44%)	7,044 (47%)	3,111 (48%)	3,521 (50%)
Transfusions of red blood cell units per patient, median (IQR)	2 (2-4)	2 (2-3)	2 (1-2)	2 (1-2)	1 (1-1)	1 (1-1)
Red blood cells transfusions No. (%)						
Total	63,837	63,850	26,032	28,626	6,473	6,978
female donor, never-pregnant, fresh	581 (1%)	632 (1%)	73 (1%)	120 (1%)	48 (1%)	86 (1%)
female donor, never-pregnant, old	8,646 (14%)	8,380 (13%)	1,378 (5%)	1,419 (5%)	863 (13%)	860 (12%)
female donor, ever-pregnant, fresh	601 (1%)	665 (1%)	82 (1%)	115 (1%)	49 (1%)	75 (1%)
female donor, ever-pregnant, old	8,850 (14%)	8,369 (13%)	1,463 (6%)	1,461 (5%)	903 (14%)	876 (13%)
male donor, fresh	3,501 (5%)	3,852 (6%)	1,416(5%)	1,736 (6%)	286 (4%)	539 (8%)
male donor, old	41,658 (65%)	41,952 (66%)	21,620 (83%)	23,775 (83%)	4,324 (67%)	4,542 (65%)

Abbreviation: IQR, interquartile range. Storage time definition: *fresh* refers to storage from 0 to 7 days; and *old* refers to storage from 8 to 36 days.

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

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

c 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, fresh or old), female donors with a history of pregnancy (ever-pregnant donors, fresh or old), male donors (fresh or old).

Table S2. Mortality Hazard Ratio of Male and Female Transfusion Recipients Exposed to fresh or old Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) Donors vs Male Donors in the Primary Analyses – Cutoff 7 days, only adults[a]

Donor category	Male recipients			Female recipients		
	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]
Full cohort, only adult patients[c]						
Ever-pregnant female old analysis						
Male old (reference)[c]	2,734	13,832	1 (reference)	2,615	15,471	1 (reference)
Ever-pregnant female old	993	4,830	1.07 (1.00-1.13)	847	4,998	0.98 (0.92-1.05)
Never-pregnant female old analysis						
Male old (reference)[c]	2,745	13,798	1 (reference)	2,656	15,536	1 (reference)
Never-pregnant female old	976	4,708	1.05 (1.00-1.11)	888	5,073	0.96 (0.90-1.03)
Male fresh analysis						
Male old (reference)[c]	1,917	10,949	1 (reference)	1,917	12,359	1 (reference)
Male fresh	54	373	0.86 (0.74-1.00)	54	630	0.62 (0.52-0.73)
Ever-pregnant female fresh analysis						
Male old (reference)[c]	1,897	10,819	1 (reference)	1,892	12,203	1 (reference)
Ever-pregnant female fresh	4	30	-	3	47	-
Never-pregnant female fresh analysis						
Male old (reference)[c]	1,897	10,831	1 (reference)	1,892	12,204	1 (reference)
Never-pregnant female fresh	5	40	0.64 (0.27-1.49)	5	67	0.58 (0.28-1.22)

Abbreviation: HR, hazard ratio. Storage time definition: *fresh* refers to storage from 0 to 7 days; and *old* refers to storage from 8 to 36 days.

a All models adjusted for calendar year, blood group (ABO-RhD), age of donor, age of the patient, hospital, cumulative number of transfusions, and an interaction term for hospital and cumulative number of transfusions.

b Hazard ratios per transfused unit compared with receiving a stored unit from a male blood donor (reference group: male stored >7 days).

c Recipients in the full cohort could receive mixed blood from both the exposure of interest and the reference category; therefore, the number of recipients receiving blood from male donors (stored >7 days) is different for the different comparisons.

Table S3. Mortality Hazard Ratio of Male and Female Patients Exposed to fresh or old Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) Donors vs Male Donors in the Sensitivity Analyses – Cutoff 10 days, only adults[a]

	Male recipients			Female recipients		
Donor category	**Deaths**	**Recipients**	**HR (95% CI)[b]**	**Deaths**	**Recipients**	**HR (95% CI)[b]**
i) No-mixture						
Censored at mixture[d]						
Male old	1,773	10,203	1 (reference)	1,756	11,499	1 (reference)
Ever-pregnant female old	144	1,148	1.09 (0.97-1.23)	116	1,136	0.99 (0.87-1.13)
Never-pregnant female old	120	1,090	0.96 (0.84-1.09)	115	1,111	1.00 (0.87-1.14)
Male fresh	107	753	0.93 (0.85-1.02)	97	983	0.81 (0.73-0.90)
Ever-pregnant female fresh	8	57	1.34 (0.85-2.10)	5	88	0.62 (0.31-1.25)
Never-pregnant female fresh	3	57	-	7	103	0.80 (0.46-1.38)
Censored at second transfusion[e]						
Male old	442	4,324	1 (reference)	416	4,542	1 (reference)
Ever-pregnant female old	96	903	1.09 (0.87-1.37)	82	876	1.07 (0.84-1.37)
Never-pregnant female old	82	863	0.90 (0.71-1.16)	74	860	0.96 (0.74-1.23)
Male fresh	30	286	0.82 (0.56-1.22)	25	539	0.33 (0.22-0.50)
Ever-pregnant female fresh	2	49	-	4	75	-
Never-pregnant female fresh	3	48	-	3	86	-
ii) Full cohort with never-pregnant[f]						
Ever-pregnant female old analysis						
Never-pregnant old (reference)[c]	1,488	17,750	1 (reference)	3,679	19,600	1 (reference)
Ever-pregnant female old	1,488	6,253	1.02 (0.97-1.07)	1,219	6,235	1.00 (0.95-1.06)
Never-pregnant fresh analysis						
Never-pregnant old (reference)[c]	2,820	14,285	1 (reference)	2,722	16,083	1 (reference)
Never-pregnant fresh	269	1,488	0.92 (0.87-0.98)	287	1,879	1.08 (0.88-1.33)
Ever-pregnant female fresh analysis						
Never-pregnant old (reference)[c]	2,697	13,846	1 (reference)	2,585	15,542	1 (reference)
Ever-pregnant female fresh	24	119	1.32 (0.96-1.82)	14	163	0.74 (0.48-1.16)
iii) Full cohort with intermediate[g]						
Ever-pregnant female intermediate analysis						
Male intermediate (reference)[c]	1,444	8,001	1 (reference)	1,320	8,763	1 (reference)
Ever-pregnant female intermediate	466	2,579	1.01 (0.92-1.11)	383	2,611	0.98 (0.88-1.08)
Never-pregnant female intermediate analysis						

Table S3. Mortality Hazard Ratio of Male and Female Patients Exposed to fresh or old Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) Donors vs Male Donors in the Sensitivity Analyses – Cutoff 10 days, only adults[a] (*continued*)

	Male recipients			Female recipients		
Donor category	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]
Male intermediate (reference)[c]	1,461	8,055	1 (reference)	1,392	8,873	1 (reference)
Never-pregnant female intermediate	468	2,588	0.97 (0.88-1.06)	463	2,801	0.99 (0.90-1.10)
Male fresh analysis						
Male intermediate (reference)[c]	1,116	6,621	1 (reference)	1,080	7,346	1 (reference)
Male fresh	160	984	0.94 (0.86-1.02)	170	1,317	0.86 (0.79-0.94)
Ever-pregnant female fresh analysis						
Male intermediate (reference)[c]	1,073	6,389	1 (reference)	1,014	7,047	1 (reference)
Ever-pregnant female fresh	18	95	1.50 (1.05-2.16)	12	130	0.84 (0.52-1.34)
Never-pregnant female fresh analysis						
Male intermediate (reference)[c]	1,069	6,392	1 (reference)	1,011	7,051	1 (reference)
Never-pregnant female fresh	9	94	0.70 (0.38-1.27)	11	141	0.70 (0.43-1.13)
iv) No-mixture, no censoring						
Male old	1,486	4,324	1 (reference)	1,275	4,542	1 (reference)
Ever-pregnant female old	306	903	1.08 (0.95-1.22)	230	876	0.97 (0.84-1.12)
Never-pregnant female old	264	863	0.93 (0.81-1.07)	265	860	1.15 (1.00-1.32)
Male fresh	99	286	0.92 (0.74-1.13)	97	539	0.48 (0.38-0.60)
Ever-pregnant female fresh	17	49	0.87 (0.54-1.42)	16	75	0.78 (0.47-1.28)
Never-pregnant female fresh	18	48	1.28 (0.79-2.07)	13	86	0.43 (0.25-0.75)

Abbreviation: HR, hazard ratio. Storage time definition: *fresh* refers to storage from 0 to 10 days; and *old* refers to storage from 11 to 36 days.

a All models adjusted for calendar year, blood group (ABO-RhD), age of donor, hospital, cumulative number of transfusions, and an interaction term for hospital and cumulative number of transfusions.

b Hazard ratios per transfused unit compared with receiving a stored unit from a male blood donor or a never-pregnant donor (reference group: male old or never-pregnant old).

c Recipients in the full cohort could receive mixed blood from both the exposure of interest and the reference category; therefore, the number of recipients receiving blood from male donors (old) or never-pregnant donors (old) is different for the different comparisons (see also *Supplemental methods*).

d Consists of all the follow-up time during which patients received all their red blood cell transfusions exclusively from female donors without a history of pregnancy (never-pregnant donors, fresh or old), from female donors with a history of pregnancy (ever-pregnant donors, fresh or old), or male donors (fresh or old).

e Consists of participants who received only a single red blood cell transfusion during the period of follow-up. Follow-up time was censored at the time this inclusion criterion was violated.

f In this comparison, never-pregnant exposure is defined as the exposure to either units donated by male donors and or by never-pregnant female donors. Recipients in this cohort could receive units from both reference (never-pregnant, old) and exposure (ever-pregnant donors, fresh or old, or male donors fresh) categories.

g Consists of follow-up time during which patients received only units stored 21 days or less. Recipients in this cohort could receive units from both reference (male, intermediate) and exposure (never-pregnant donors, fresh or intermediate, ever-pregnant donors, fresh or intermediate, or male donors, fresh) categories.

Table S4. Mortality Hazard Ratio of Male and Female Transfusion Recipients Exposed to fresh or old Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) Donors vs Male Donors in the Sensitivity Analyses – Cutoff 7 days, only adults[a]

	Male recipients			Female recipients		
Donor category	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]
i) No-mixture						
Censored at mixture[c]						
Male old (reference)	1,893	10,811	1 (reference)	1,889	12,190	1 (reference)
Ever-pregnant female old	152	1,193	1.11 (0.99-1.24)	121	1,200	0.98 (0.86-1.12)
Never-pregnant female old	124	1,140	0.96 (0.84-1.09)	121	1,170	1.00 (0.89-1.15)
Male fresh	30	236	0.91 (0.75-1.11)	26	437	0.55 (0.44-0.69)
Ever-pregnant female fresh	0	18	-	0	33	-
Never-pregnant female fresh	1	14	-	2	45	-
Censored at second transfusion[d]						
Male old (reference)	459	4,518	1 (reference)	433	4,762	1 (reference)
Ever-pregnant female old	98	939	1.06 (0.85-1.33)	86	924	1.09 (0.86-1.38)
Never-pregnant female old	84	899	0.86 (0.69-1.13)	77	905	0.96 (0.75-1.23)
Male fresh	13	92	0.95 (0.54-1.69)	8	319	0.14 (0.07-0.28)
Ever-pregnant female fresh	0	13	-	0	27	-
Never-pregnant female fresh	1	12	-	0	41	-
ii) Full cohort with never-pregnant[e]						
Ever-pregnant female old analysis						
Never-pregnant old (reference)[f]	4,346	18,766	1 (reference)	3,997	20,807	1 (reference)
Ever-pregnant female old	1,629	6,667	1.03 (0.98-1.08)	1,341	6,742	0.99 (0.95-1.05)
Never-pregnant fresh analysis						
Never-pregnant old (reference)[f]	2,919	14,816	1 (reference)	2,829	16,640	1 (reference)
Never-pregnant fresh	81	534	0.84 (0.74-0.95)	86	812	0.86 (0.58-1.29)
Ever-pregnant female fresh analysis						
Never-pregnant old (reference)[f]	2,876	14,623	1 (reference)	2,781	16,414	1 (reference)
Ever-pregnant female fresh	7	38	1.15 (0.58-2.30)	4	54	-
iii) Full cohort with intermediate storage[g]						
Ever-pregnant female intermediate storage analysis						
Male intermediate storage (reference)[f]	1,602	8,726	1 (reference)	1,490	9,610	1 (reference)

Table S4. Mortality Hazard Ratio of Male and Female Transfusion Recipients Exposed to fresh or old Red Blood Cell Transfusions From Female (Never-Pregnant or Ever-Pregnant) Donors vs Male Donors in the Sensitivity Analyses – Cutoff 7 days, only adults[a] (*continued*)

Donor category	Male recipients			Female recipients		
	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]
Ever-pregnant female intermediate storage	521	2,821	1.03 (0.95-1.13)	435	2,904	0.97 (0.88-1.07)
Never-pregnant female intermediate storage analysis						
Male intermediate storage (reference)[f]	1,627	8,794	1 (reference)	1,567	9,701	1 (reference)
Never-pregnant female intermediate storage	527	2,850	0.98 (0.90-1.07)	521	3,074	1.00 (0.91-1.09)
Male fresh analysis						
Male intermediate storage (reference)[f]	1,193	7,076	1 (reference)	1,151	7,814	1 (reference)
Male fresh	49	349	0.85 (0.72-1.00)	47	598	0.59 (0.49-0.71)
Ever-pregnant female fresh analysis						
Male intermediate storage (reference)[f]	1,178	6,959	1 (reference)	1,133	7,680	1 (reference)
Ever-pregnant female fresh	4	29	-	3	46	-
Never-pregnant female fresh analysis						
Male intermediate storage (reference)[f]	1,178	6,968	1 (reference)	1,133	7,682	1 (reference)
Never-pregnant female fresh	5	37	0.70 (0.29-1.67)	5	64	0.57 (0.27-1.20)

Abbreviation: HR, hazard ratio. Storage time definition: *fresh* refers to storage from 0 to 7 days; and *old* refers to storage from 8 to 36 days.

a All models adjusted for calendar year, blood group (ABO-RhD), age of donor, hospital, cumulative number of transfusions, and an interaction term for hospital and cumulative number of transfusions.

b Hazard ratios per transfused unit compared with receiving a stored unit from a male blood donor or a never-pregnant donor (reference group: male old or never-pregnant old).

c Consists of all the follow-up time during which patients received all their red blood cell transfusions exclusively from female donors without a history of pregnancy (never-pregnant donors, fresh or old), from female donors with a history of pregnancy (ever-pregnant donors, fresh or old), or male donors (fresh or old).

d Consists of participants who received only a single red blood cell transfusion during the period of follow-up. Follow-up time was censored at the time this inclusion criterion was violated.

e In this comparison, never-pregnant exposure is defined as the exposure to either units donated by male donors and or by never-pregnant female donors. Recipients in this cohort could receive units from both reference (never-pregnant, old) and exposure (ever-pregnant donors, fresh or old, or male donors fresh) categories.

f Recipients in the full cohort could receive mixed blood from both the exposure of interest and the reference category; therefore, the number of recipients receiving blood from male donors (old) or never-pregnant donors (old) is different for the different comparisons.

g Consists of follow-up time during which patients received only units stored 21 days or less. Recipients in this cohort could receive units from both reference (male, intermediate storage) and exposure (never-pregnant donors, fresh or intermediate storage, ever-pregnant donors, fresh or intermediate storage, or male donors fresh) categories.

Table S5. Mortality Hazard Ratio of Female Transfusion Recipients Exposed to fresh or old Red Blood Cell Transfusions From Female Ever-Pregnant Donors vs Male Donors in the Full Cohort, Stratified by Patient Age – Cutoff 10 days, only adults[a]

	18-50 y			51-70 y			≥71 y			p value for interaction[c]
Donor category	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]	
Full cohort										
Male old (reference)[d]	177	3,061	1 (reference)	715	4,653	1 (reference)	1,532	6,855	1 (reference)	0.000
Ever-pregnant female old	58	970	1.18 (0.91-1.53)	235	1,555	0.88 (0.78-1.01)	491	2,139	1.01 (0.92-1.11)	
Male old (reference)[d]	184	3,149	1 (reference)	746	4,670	1 (reference)	1,531	6,836	1 (reference)	0.000
Never-pregnant female old	67	1,051	1.06 (0.82-1.37)	264	1,524	0.96 (0.84-1.09)	489	2,184	0.92 (0.84-1.01)	
Male old (reference)[d]	127	2,379	1 (reference)	512	3,653	1 (reference)	1,125	5,513	1 (reference)	0.011
Ever-pregnant female fresh	1	38	-	5	50	0.74 (0.34-1.61)	7	52	1.09 (0.60-2.00)	
Male old (reference)[d]	126	2,382	1 (reference)	513	3,656	1 (reference)	1,121	5,506	1 (reference)	0.001
Never-pregnant female fresh	0	56	-	4	46	-	7	48	0.98 (0.57-1.69)	
Male old (reference)[d]	142	2,514	1 (reference)	534	3,756	1 (reference)	1,170	5,635	1 (reference)	0.000
Male fresh	27	603	0.91 (0.72-1.14)	56	387	0.96 (0.84-1.10)	104	420	1.02 (0.91-1.15)	
Full cohort with never-pregnant										
Never-pregnant old (reference)[d]	287	4,223	1 (reference)	1,122	6,270	1 (reference)	2,270	9,107	1 (reference)	0.000
Ever-pregnant female old	101	1,312	1.09 (0.89-1.33)	378	2,076	0.90 (0.82-0.99)	740	2,847	1.05 (0.98-1.13)	
Never-pregnant old (reference)[d]	194	3,313	1 (reference)	777	4,935	1 (reference)	1,614	7,294	1 (reference)	0.018
Ever-pregnant female fresh	1	45	-	6	60	0.72 (0.35-1.48)	7	58	1.02 (0.56-1.88)	
Never-pregnant old (reference)[d]	217	3,518	1 (reference)	818	5,090	1 (reference)	1,687	7,475	1 (reference)	0.000
Never-pregnant female fresh	38	761	0.87 (0.73-1.05)	85	543	1.00 (0.90-1.10)	164	575	1.11 (1.01-1.21)	

Abbreviation: HR, hazard ratio. Storage time definition: *fresh* refers to storage from 0 to 10 days; and *old* refers to storage from 11 to 36 days.

a All models are adjusted for calendar year, blood group (ABO-RhD), hospital, age of donor, cumulative number of transfusions, and an interaction term for hospital and cumulative number of transfusions.

b Hazard ratios per transfused unit compared with receiving a unit from the reference category.

c For the trend in interaction across the continuous variable patient age.

d Recipients in the full cohort could receive mixed blood from both the exposure of interest and the reference category; therefore, the number of recipients receiving blood from male donors (old) or never-pregnant donors (old) is different for the different comparisons (see also *Supplemental methods*)

Table S6. Mortality Hazard Ratio of Male Transfusion Recipients Exposed to fresh or old Red Blood Cell Transfusions From Female Ever-Pregnant Donors vs Male Donors in the Full Cohort, Stratified by Patient Age – Cutoff 7 days, only adults[a]

	18-50 y			51-70 y			≥71 y			p value for interaction[c]
Donor category	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]	
Full cohort										
Male old (reference)[d]	176	1,763	1 (reference)	1,036	5,997	1 (reference)	1,522	6,072	1 (reference)	0.000
Ever-pregnant female old	79	615	1.37 (1.10-1.71)	392	1,727	1.03 (0.94-1.14)	522	2,096	1.03 (0.95-1.13)	
Male old (reference)[d]	176	1,621	1 (reference)	1,019	5,969	1 (reference)	1,550	6,032	1 (reference)	0.000
Never-pregnant female old	68	667	0.98 (0.77-1.26)	364	2,053	1.03 (0.93-1.14)	544	1,988	1.06 (0.97-1.16)	
Male old (reference)[d]	110	1,350	1 (reference)	702	4,681	1 (reference)	1,085	4,788	1 (reference)	0.624
Ever-pregnant female fresh	1	6	-	3	16	-	0	8	-	
Male old (reference)[d]	109	1,242	1 (reference)	701	4,688	1 (reference)	1,087	4,792	1 (reference)	0.170
Never-pregnant female fresh	0	7	-	3	22	-	2	11	-	
Male old (reference)[d]	110	1,375	1 (reference)	713	4,035	1 (reference)	1,094	4,826	1 (reference)	0.000
Male fresh	2	72	-	26	187	0.86 (0.67-1.09)	26	114	0.96 (0.77-1.19)	
Full cohort with never-pregnant										
Never-pregnant old (reference)[d]	302	2,495	1 (reference)	1,067	6,357	1 (reference)	2,384	8,103	1 (reference)	0.000
Ever-pregnant female old	137	907	1.18 (1.00-1.40)	652	2,953	1.03 (0.96-1.11)	840	2,807	1.01 (0.95-1.08)	
Never-pregnant old (reference)[d]	179	1,892	1 (reference)	1,067	6,357	1 (reference)	1,630	6,374	1 (reference)	0.992
Ever-pregnant female fresh	2	10	-	4	19	-	1	9	-	
Never-pregnant old (reference)[d]	182	11,928	1 (reference)	1,090	6,464	1 (reference)	1,647	6,424	1 (reference)	0.000
Never-pregnant female fresh	6	110	0.74 (0.47-1.16)	40	270	0.83 (0.69-1.00)	35	154	0.91 (0.76-1.08)	

Abbreviation: HR, hazard ratio. Storage time definition: *fresh* refers to storage from 0 to 7 days; and *old* refers to storage from 8 to 36 days.

a All models are adjusted for calendar year, blood group (ABO-RhD), hospital, age of donor, cumulative number of transfusions, and an interaction term for hospital and cumulative number of transfusions.

b Hazard ratios per transfused unit compared with receiving a unit from the reference category.

c For the trend in interaction across the continuous variable patient age.

d Recipients in the full cohort could receive mixed blood from both the exposure of interest and the reference category; therefore, the number of recipients receiving blood from male donors (old) or never-pregnant donors (old) is different for the different comparisons.

Table S7. Mortality Hazard Ratio of Female Transfusion Recipients Exposed to fresh or old Red Blood Cell Transfusions From Female Ever-Pregnant Donors vs Male Donors in the Full Cohort, Stratified by Patient Age – Cutoff 7 days, only adults[a]

	18-50 y			51-70 y			≥71 y			p value for interaction[c]
Donor category	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]	Deaths	Recipients	HR (95% CI)[b]	
Full cohort										
Male old (reference)[d]	201	3,332	1 (reference)	783	4,939	1 (reference)	1,631	7,200	1 (reference)	0.000
Ever-pregnant female old	67	1,060	1.12 (0.88-1.41)	262	1,671	0.89 (0.79-1.00)	518	2,267	1.01 (0.93-1.10)	
Male old (reference)[d]	204	3,423	1 (reference)	810	4,938	1 (reference)	1,642	7,175	1 (reference)	0.000
Never-pregnant female old	72	1,137	1.00 (0.78-1.28)	284	1,624	0.93 (0.82-1.05)	532	2,312	0.96 (0.87-1.04)	
Male old (reference)[d]	143	2,589	1 (reference)	555	3,852	1 (reference)	1,194	5,762	1 (reference)	0.189
Ever-pregnant female fresh	2	24	-	1	15	-	0	7	-	
Male old (reference)[d]	141	2,589	1 (reference)	556	3,853	1 (reference)	1,195	5,762	1 (reference)	0.010
Never-pregnant female fresh	0	38	-	3	19	-	2	10	-	
Male old (reference)[d]	149	2,643	1 (reference)	557	3,903	1 (reference)	1,211	5,813	1 (reference)	0.000
Male fresh	12	361	0.76 (0.53-1.10)	12	149	0.70 (0.52-0.95)	30	120	0.96 (0.75-1.24)	
Full cohort with never-pregnant										
Never-pregnant old (reference)[d]	325	4,584	1 (reference)	1,236	6,653	1 (reference)	1,756	7,699	1 (reference)	0.000
Ever-pregnant female old	119	1,461	1.07 (0.90-1.27)	431	2,253	0.82 (0.67-1.00)	47	165	1.11 (0.93-1.32)	
Never-pregnant old (reference)[d]	215	3,582	1 (reference)	840	5,201	1 (reference)	1,726	7,631	1 (reference)	0.094
Ever-pregnant female fresh	2	25	-	2	20	-	0	9	-	
Never-pregnant old (reference)[d]	225	3,668	1 (reference)	848	5,273	1 (reference)	2,436	9,570	1 (reference)	0.000
Never-pregnant female fresh	18	440	0.82 (0.62-1.08)	21	207	0.90 (0.83-0.99)	791	3,028	1.05 (0.98-1.12)	

Abbreviation: HR, hazard ratio. Storage time definition: *fresh* refers to storage from 0 to 7 days; and *old* refers to storage from 8 to 36 days.

a All models are adjusted for calendar year, blood group (ABO-RhD), hospital, age of donor, cumulative number of transfusions, and an interaction term for hospital and cumulative number of transfusions.

b Hazard ratios per transfused unit compared with receiving a unit from the reference category.

c For the trend in interaction across the continuous variable patient age.

d Recipients in the full cohort could receive mixed blood from both the exposure of interest and the reference category; therefore, the number of recipients receiving blood from male donors (old) or never-pregnant donors (old) is different for the different comparisons.

1 van de Watering L, Lorinser J, Versteegh M, Westendord R, Brand A. Effects of storage time of red blood cell transfusions on the prognosis of coronary artery bypass graft patients. Transfusion. 2006; 46: 1712-8.
2 van Hoeven LR, Koopman MM, Koffijberg H, Roes KC, Janssen MP. Historical time trends in red blood cell usage in the Netherlands. International journal of clinical transfusion medicine. 2016; 4: 67-77.
3 Groenwold RHH, Klungel OH, Altman DG, van der Graaf Y, Hoes AW, Moons KGM, et al. Adjustment for continuous confounders: an example of how to prevent residual confounding. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne. 2013; 185: 401-6.
4 Groenwold RH, Klungel OH, Altman DG, van der Graaf Y, Hoes AW, Moons KG. Adjustment for continuous confounders: an example of how to prevent residual confounding. Cmaj. 2013; 185: 401-6.
5 Chasse M, McIntyre L, English SW, Tinmouth A, Knoll G, Wolfe D, et al. Effect of Blood Donor Characteristics on Transfusion Outcomes: A Systematic Review and Meta-Analysis. Transfus Med Rev. 2016; 30: 69-80.
6 CBO. Dutch Guideline Bloodtransfusion. Retrieved September 17th, 2021, from: https://wwwnvognl/wp-content/uploads/2018/02/Bloedtransfusie-20-11-11-2011 pdf. https://doi.org/.
7 Kopolovic I, Ostro J, Tsubota H, Lin Y, Cserti-Gazdewich CM, Messner HA, et al. A systematic review of transfusion-associated graft-versus-host disease. Blood. 2015; 126: 406-14.
8 Sanquin. Guideline Blood Products. Retrieved September 17th, 2021. https://doi.org/.
9 CBO. Dutch Guideline Bloodtransfusion. Retrieved October 22, 2018, from: https://nvicnl/sites/nvicnl/files/CBO%20Richtlijn%20Bloedtransfusiepdf. 2011. https://doi.org/.