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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 about 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 that 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 and male stored for ≤10 days), compared to receiving a unit donated by a male donor, which was

stored for >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 for ≤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 0.97–1.99). 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

blood donor, erythrocyte transfusion, mortality, RBC storage lesion

Highlights

- We hypothesize that the transfusion of fresh red blood cell products donated by ever-pregnant donors to male patients might increase mortality.
- The association between exposure, via transfusion, to ever-pregnant donor units and mortality in young men may be modified by product storage.
- Studying parameters related to blood product haemoglobin requires careful consideration of statistical methods.

INTRODUCTION

Although transfusions can be a necessary life-saving medical intervention, they are also associated with adverse events [1]. Some of these are attributable to certain donor characteristics, such as the passive infusion of leucocyte and neutrophil antibodies in transfusion-related acute lung injury (TRALI) [2] and the transfer of plasma containing IgA and IgE antibodies in allergic transfusion reactions [3]. Nevertheless, 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 [4, 5]. The association was shown to be limited to female donors with a history of pregnancy, with an estimated impact of one death per day [5, 6]. In contrast, another large cohort study on this topic did not support these findings [7]. This lack of agreement between studies could be explained by differences among country-specific production methods, patient populations and statistical methods. Although these studies constitute observational research, associations are interpreted causally [8].

Whether 'fresh' or 'old' red blood cell transfusions are better for clinical outcomes has long been a 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 any 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 [9]. However, the authors could not exclude a small detrimental effect of fresh blood products on mortality, as confidence intervals (CIs) 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 that, when comparing blood products that were stored for <10 days with products stored for >24 days, longer stored blood was associated with a lower risk of mortality (hazard ratio [HR] 0.56, 95% CI 0.32–0.97) [10].

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 that mortality will be highest in male patients who received fresh units from ever-pregnant donors.

METHODS

Source database

In this observational cohort study, 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. [4, 5, 11]. 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 have been collected for the 'R-FACT study' (CCMO-NL29563.058.09; clinicaltrials.gov: NCT01616329), and the study design for the cohort has been previously described [5, 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 [5].

We defined *fresh* products as red cell products stored for 1–10 days and compared those to *old* products, with a storage duration of 11–36 days. Results for exposure defined as 0–7 days for *fresh* products, and *old* products defined as products stored for 8–36 days, are provided in the Supplementary 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 the 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 old units donated by male donors, unless otherwise specified. We hypothesize that 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. HRs were estimated to quantify the risk of mortality per transfused 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 five events [15]. Follow-up in all analyses was 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 are unknown, at the time a blood product is requested or transfused by the patient's treating physician—this exposure can be considered 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 in patients with severe combined immunodeficiency syndrome [16, 17]. Therefore, 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); and 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. For example, after any other exposure than *male*, *old* and *ever-pregnant fresh* for the comparison *male old* versus *ever-pregnant fresh*, the patients's follow-up time is no longer included (see Figure S1 for visual representation of this example).

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

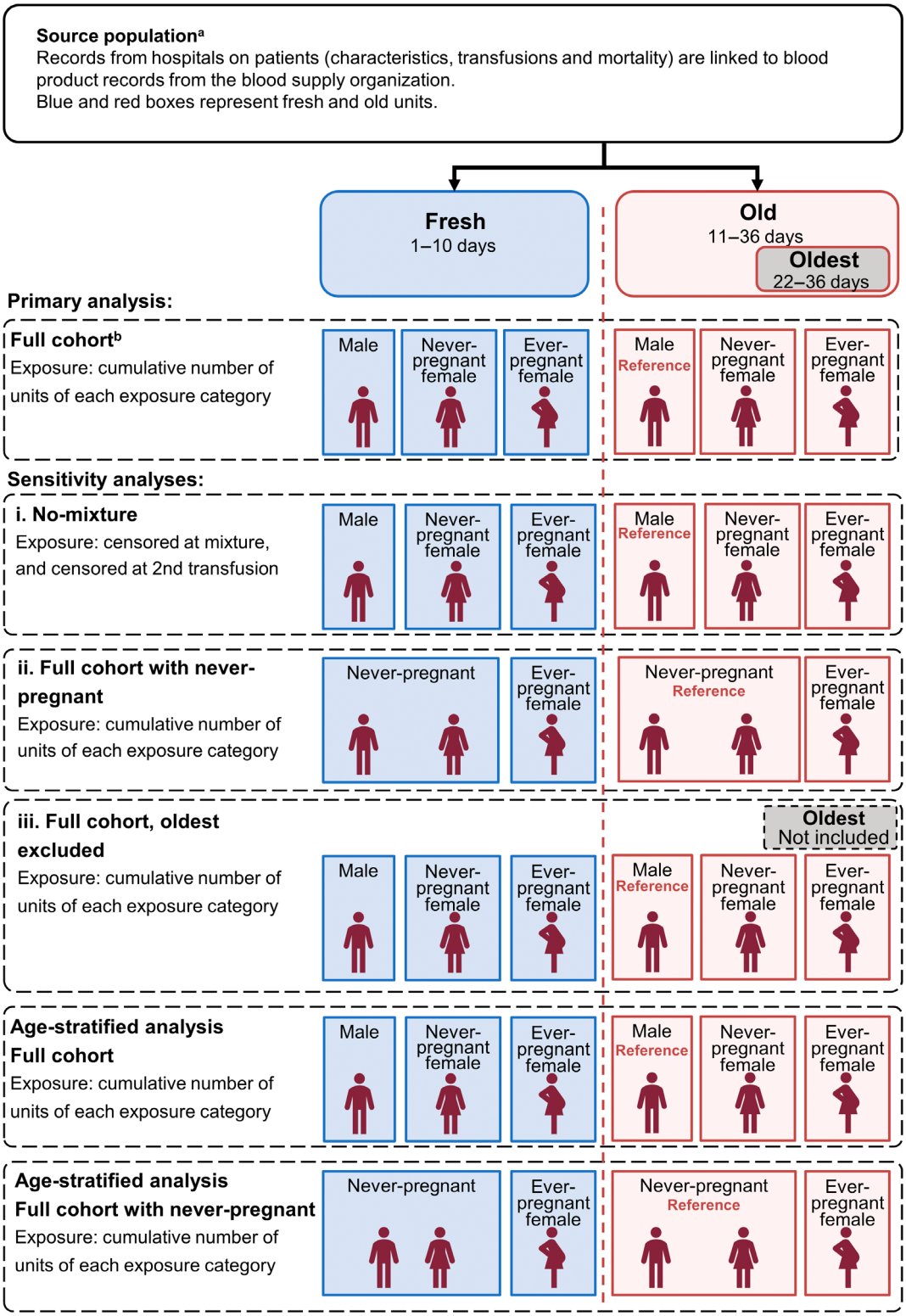

FIGURE 1 The figure contains a visual representation of the different exposure and reference groups for the primary and sensitivity analyses. [a]Products donated by female donors with unknown pregnancy history were not assessed in this analysis. [b]For sensitivity analysis (iv), the same exposure and reference groups were used.

were censored upon receiving a transfusion from a different exposure category (*no mixture*) and where patients who received 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, a no-mixture analysis 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 by 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 of storage) and intermediate (between 11 and 21 days of storage) products. The cut-off 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 haemolysis, oxidative stress and micro-vesicle formation, are collectively called the red blood cell storage lesion [19]. Units in the fourth and last week of storage are still generally considered safe, but evidence for the safety of end-of-storage (stored for 28–36 days) red blood cell units is limited, as is evidence for use in vulnerable patient populations [20–22].
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 complete 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 transfusions.

Age-stratified analysis

The *primary analysis* and *sensitivity analysis* (ii) were stratified by patient sex and age to study the effect measure modification by age [5, 7]. 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 [5].

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 patients. During follow-up, 13,948 (33%) patients died, with a median follow-up of 405 days (IQR 36–1269) 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 for >10 days. When the storage cut-off 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 patients (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 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 cut-off 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, so refer to the Supplementary Materials for further information (Tables S3 and 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 were stored >10 days and 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)**.

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	6473	6978
Number of deaths (%)	7465 (37%)	6483 (29%)	2155 (16%)	2096 (14%)	655 (10%)	604 (9%)
Follow-up, median (IQR), days[c]	282 (22–1098)	514 (59–1400)	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	7519	9546
Age of patients, median (IQR), years	68 (58–76)	68 (52–79)	69 (59–77)	69 (54–79)	70 (60–77)	71 (57–80)
18–50 years	7889 (13%)	5202 (23%)	1665 (13%)	3276 (22%)	702 (11%)	1309 (19%)
51–70 years	15,877 (44%)	7097 (32%)	5762 (43%)	4654 (31%)	2660 (41%)	2148 (31%)
≥71 years	18,690 (43%)	10,113 (45%)	5892 (44%)	6995 (47%)	3111 (48%)	3521 (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	6473	6978
Female donor, never-pregnant, fresh	581 (1%)	632 (1%)	73 (1%)	120 (1%)	48 (1%)	86 (1%)
Female donor, never-pregnant, old	8646 (14%)	8380 (13%)	1378 (5%)	1419 (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	8850 (14%)	8369 (13%)	1463 (6%)	1461 (5%)	903 (14%)	876 (13%)
Male donor, fresh	3501 (5%)	3852 (6%)	1416 (5%)	1736 (6%)	286 (4%)	539 (8%)
Male donor, old	41,658 (65%)	41,952 (66%)	21,620 (83%)	23,775 (83%)	4324 (67%)	4542 (65%)

Note: Storage time definition: *fresh* refers to storage from 0 to 10 days; and *old* refers to storage from 11 to 36 days.
Abbreviation: IQR, interquartile range.
[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) and 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 as follows: 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) and male donors (fresh or old).

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, and the HR for the age group of 51–70 years was 1.36 (95% CI 0.77–2.40) for the ever-pregnant, fresh in comparison with 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; and 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 for >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; and 70 and older, HR 1.32, 95% CI 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 cut-off of 7 days can be found in the Tables S6 and S7.

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

Full cohort[a]	Deaths/Recipients (exposure)	Deaths/Recipients (reference)[b]	HR per unit[c]
Male patients			
Ever-pregnant female old	922/4560	2551/13,078	1.05 (0.99–1.12)
Never-pregnant female old	908/4420	2561/13,025	1.05 (0.98–1.12)
Male fresh	174/1049	1840/10,506	0.93 (0.86–1.01)
Ever-pregnant female fresh	18/101	1783/10,232	1.39 (0.97–1.99)
Never-pregnant female fresh	9/93	1779/10,239	0.61 (0.33–1.11)
Female patients			
Ever-pregnant female old	784/4664	2424/14,569	0.99 (0.92–1.06)
Never-pregnant female old	820/4759	2461/14,655	0.95 (0.89–1.02)
Male fresh	187/1410	1846/11,905	0.86 (0.79–0.93)
Ever-pregnant female fresh	13/140	1764/11,545	0.83 (0.52–1.30)
Never-pregnant female fresh	11/150	1760/11,544	0.68 (0.42–1.11)

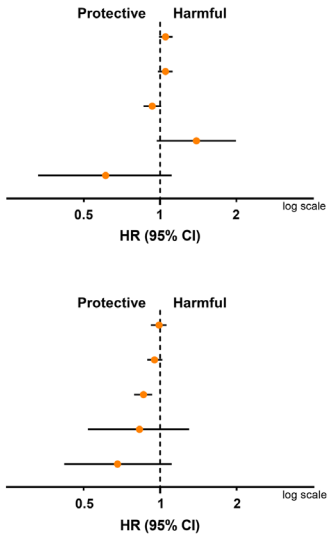

FIGURE 2 Forest plot containing the hazard ratios (HRs) from the primary analysis, stratified by sex. Reference category consists of patients exposed to units donated by male donors, stored for >10 days (old). HRs are shown as orange dots, along with 95% confidence intervals. [a]All models adjusted for 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]HRs per transfused unit compared with receiving a stored unit from a male blood donor (reference group: male, old).

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 [24, 25]. Bias due to treatment-confounder feedback could lead to biased HRs obtained with Cox regression. Female donors have lower haemoglobin 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 haemoglobin 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 the 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 regard to blood product distribution policy and prognosis. For neonates and young children, units stored shorter than 5 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 not only 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.

TABLE 2 Mortality hazard ratio (HR) of male patients exposed to fresh or old red blood cell transfusions from female ever-pregnant donors versus male donors in the full cohort, stratified by patient age.[a]

Donor category	18–50 years			51–70 years			≥71 years			*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	1632	1 (reference)	949	5631	1 (reference)	1441	5815	1 (reference)	0.000
Ever-pregnant female, old	73	572	1.38 (1.09–1.74)	363	1996	1.02 (0.93–1.13)	486	1992	1.02 (0.93–1.12)	
Male, old (reference)[d]	160	1659	1 (reference)	922	5603	1 (reference)	1479	5763	1 (reference)	0.000
Never-pregnant female, old	62	618	0.97 (0.75–1.26)	327	1919	1.01 (0.90–1.12)	519	1883	1.08 (0.99–1.18)	
Male, old (reference)[d]	100	1244	1 (reference)	642	4393	1 (reference)	1041	4595	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	1245	1 (reference)	639	4394	1 (reference)	1040	4600	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	1294	1 (reference)	674	4531	1 (reference)	1063	4681	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	2320	1 (reference)	1507	7691	1 (reference)	2245	7739	1 (reference)	0.000
Ever-pregnant female, old	123	845	1.18 (0.99–1.41)	594	2758	1.03 (0.95–1.11)	771	2650	1.00 (0.93–1.07)	
Never pregnant, old (reference)[d]	163	1756	1 (reference)	972	5988	1 (reference)	1562	6102	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	1835	1 (reference)	1040	6208	1 (reference)	1606	6242	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)	

Note: Storage time definition: *fresh* refers to storage from 0 to 10 days; and *old* refers to storage from 11 to 36 days.
Abbreviation: CI, confidence interval.
[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]HRs 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).

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 [29, 30]. However, during this period, no changes were made to leucoreduction filter types.

In summary, blood products from ever-pregnant donors stored for a short 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.

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S.J.V., C.C.-D., R.A.M. and J.G.v.d.B. designed the study. D.E., K.M.K.d.V., D.v.d.K., M.J.W., N.C.V.P., F.H., J.J.Z. and J.G.v.d.B. collected the data. S.J.V., C.C.-D. and J.G.v.d.B. analysed and interpreted the data and wrote the manuscript. D.d.K. and L.M.G.v.d.W. provided subject-specific content knowledge. All authors revised and approved the final manuscript. We thank the Scientific Committee at the Department of Clinical Epidemiology of the LUMC for their methodological support. A conference abstract has previously been published on the same dataset as described here [11].

CONFLICT OF INTEREST STATEMENT

J.J.Z. is in the scientific advisory council of Novartis/Amgen/Sanofi and received a speaker's fee. The other authors declare that they have no conflict of interest relevant to the work presented in this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available for inspection upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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