Out for blood: causal inference in clinical transfusion research

Valk, S.J.

Out for blood:

causal inference in clinical transfusion research

Sarah Juanita Valk

Cover: S.J. Valk
Layout/ print: ProefschriftMaken, www.proefschriftmaken.nl
ISBN: 978-94-6469-694-3

Out for blood: causal inference in clinical transfusion research

The research described in this thesis was performed at the Centre for Clinical Transfusion Research of Sanquin Research and Department of Clinical Epidemiology at the Leiden University Medical Center, Leiden, The Netherlands.

Financial support for printing of this thesis was kindly provided by Leiden University, the Department of Clinical Epidemiology of the Leiden University Medical Center and Sanquin.

Out for blood:

causal inference in clinical transfusion research

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op donderdag 1 februari 2024
klokke 13.45

door

Sarah Juanita Valk
geboren te Woerden in 1994

Promotoren	Prof. J. G. van der Bom
	Prof. J.J. Zwaginga
Copromotor	Dr. C. Caram-Deelder
Leden promotiecommissie	Prof. M. de Haas
	Prof. R.H.H. Groenwold
	Prof. E.A.B. Beckers (Maastricht University)
	Dr. G. Edgren (Karolinska Institutet)

Table of contents

1

Chapter 1

General introduction and outline of the thesis

Part 1. Introduction

Blood transfusions have been performed since the early 1800's in order to reduce complications due to bleeding and anemia. Their life-saving potential, ultimately improving tissue oxygenation, is well-established in the acutely bleeding patient seen in the trauma and surgery setting. For the non-acute anemic patient, benefits of transfusions are less clear.[1] Importantly, these benefits need to be weighed against increasing healthcare expenses, the limited availability of blood, and both avoidable and unavoidable risks. These risks – admittedly – have become relatively small but can still lead to severe, life-threatening side effects, e.g. anaphylaxis, transfusion associated circulatory overload and transfusion-associated Graft-Vs-Host disease.[2] In this context, it is understandable that a restrictive transfusion strategy is the default in many settings[3], and an increased interest in potentially avoidable risks has developed. When referring to avoidable risks associated with transfusions, an example which comes to mind is plasma from female donors being associated with increased risk of transfusion-related acute lung injury (TRALI).[4] Using observational data, the 'culprit' was found to be the transfer of antibodies against human leukocyte antigens (HLA), which are more abundant in parous women. This observation prompted a policy change – in the Netherlands – where use of male-only plasma or pooled plasma was made the standard, which resulted in a 33% reduction in TRALI cases.[5, 6] There are still more uncertainties about avoidable harms from transfusions, e.g. other risks relating to sex of the donor[7, 8] and the impact of storage of blood products[9]. The role of clinical transfusion research using observational data to investigate such potential risk factors is evident: at first sight, observational data seems exceptionally suited to investigate a large number of patients exposed to a specific blood product characteristic, and to follow these patients for the occurrence of relatively rare outcomes (such as mortality) within a large time frame. There are, however, a number of caveats when analysing data originating from such uncontrolled settings. In this thesis, characteristics of red blood cell products and their connection with patient outcomes are carefully described, in order to achieve an understanding of the intricate relationship between product, and patient. Ultimately, the goal of the research described here is to contribute to the further development of clinical transfusion practice, by performing epidemiological studies that can estimate causal effects, and thereby discerning ways to mitigate avoidable transfusion risks.

First, to better define the field of clinical transfusion research, the different types of blood products and their uses need to be introduced (*Figure 1*). When the first transfusions were performed, these consisted of whole blood, a product which

is used today but has limited applications.[10] The development of centrifugation and filtration made the individualized transfusion of leukocyte-reduced blood components possible.[11] Red blood cell units, or erythrocyte transfusions, are used to treat anemia and bleeding, and are indicated for symptomatic anemia with pre-transfusion hemoglobin thresholds ranging from 4 to 6 mmol/L.[11] Fresh frozen plasma is used to treat patients with massive bleeding-associated co-

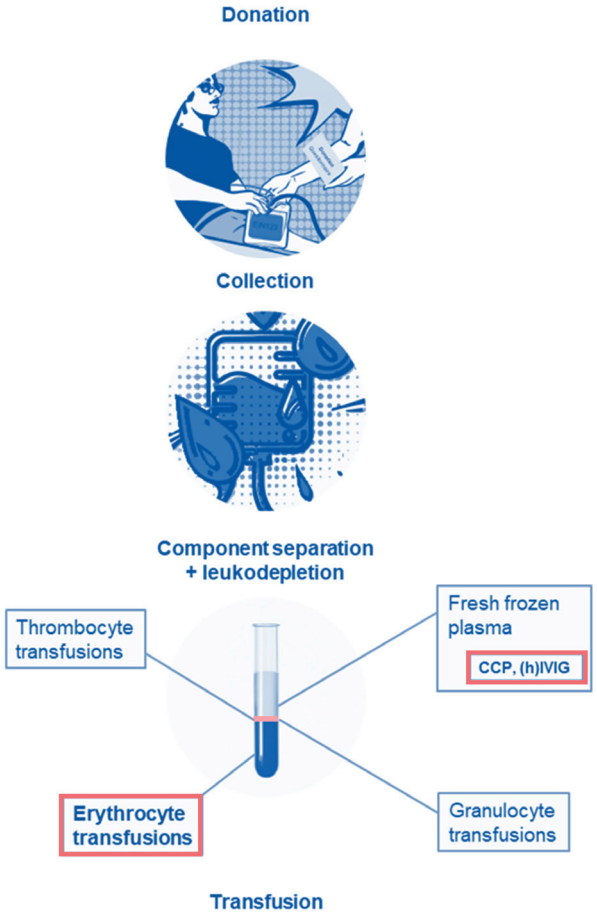


Figure 1

The process from donation to transfusion in the Netherlands consists of multiple steps. First, the donation is performed in one of the blood collection centers from a voluntary non-remunerated donor, and a self-reported questionnaire on risk behaviour and health status is taken. Each donation is assigned a unique number (unit identification number, or "EIN"). The central collection of whole blood is performed in two locations, and component separation by centrifugation is performed, including a leukodepletion filtration step. Whole blood separates into erythrocytes (45%, in dark blue), buffy coat (<1%, in pink) and plasma (55%, in light blue) during the centrifugation process. The separation of whole blood into the blood components for transfusion is further facilitated by the addition of different types of storage media and the use of advanced separation techniques. At multiple timepoints, donors and products are tested for infectious risks to ensure safety of blood products. In this thesis, the safety and efficacy of erythrocyte transfusions, or red blood cell units, and convalescent plasma for COVID-19 and hyperimmune immunoglobulin (hIVIG) are the main focus.

agulopathy and for therapeutic plasma exchange. From plasma, other products can be prepared, such as clotting factor concentrates, albumin and intravenous immunoglobulin (IVIG). Thrombocyte transfusions (platelets) are prepared from the buffy coat of the centrifugated whole blood, by pooling platelet-rich plasma, or by single-donor apheresis and used to treat and prevent thrombocytopenia-associated bleeding. Other blood products have limited indications. One of them is the granulocyte transfusion, derived from granulocyte colony-stimulating factor' treated donors by apheresis, or from pooled buffy coats. These granulocytes are sometimes prescribed for severely neutropenic patients with active infections or prophylactically for patients entering a neutropenic period. Other less common products are convalescent plasma and hyperimmune immunoglobulin (hIVIG) obtained from plasma of individuals who have recovered from a specific infection or that are recently vaccinated, and have been primarily studied in the context of H1N1 influenza and Coronavirus Disease 2019 (COVID-19). These products contain antibodies developed by the donor immune system as a response to infections, and can be used to treat other individuals who are currently infected with the same pathogen.

Clinical transfusion research of blood product characteristics: two perspectives

Blood product characteristics research borders on various fields in the scientific spectrum, and the considerations for researchers in this field can be viewed from two perspectives: the biological perspective and the epidemiological perspective. From the biological perspective, understanding of the different blood products and their uses is essential. It is important to understand the pathophysiology of the various diseases in which transfusions are indicated. As opposed to pharmaceutical products, blood products are intrinsically heterogeneous: they derive from various donors, and can be produced in varying ways across the world. Whereas in pharmaceutical interventions, we intend to give the patient a single treatment, blood products can be considered to contain a multitude of active components, ranging from plasma, red blood cells, leukocytes, cytokines, and various other compounds, that can also change throughout the products' timeline from donation to transfusion. These factors all contribute to the challenging nature of blood product characteristics research from a biological perspective.[12]

From an epidemiological perspective, there are also challenges which contribute to the difficulty of blood product characteristics research.[13] Rather than comparing a single intervention in a randomized controlled clinical trial (RCT), blood product characteristics research is mostly based on observational data from

patients receiving – what can be perceived as – a range of different interventions, which commonly also includes the transfusion of multiple units, over time.

As opposed to creating a division, we want to emphasize that these two perspectives are inherently complementary. The epidemiologists' toolbox will naturally be supplied with a large dose of fundamental scientific information and, in turn, clinical research provides the rationale for laboratory investigations. These perspectives together should be kept in mind by clinical transfusion researchers when investigating blood product characteristics, as they enhance each other and thereby allow the study of complex research questions.

Donor sex and clinical transfusion research

Donor characteristics and transfusion recipient outcomes have been frequently studied. Previously, an association was observed between transfusions of red blood cells from female donors with increased mortality in male recipients under 50 years of age.[14] This association of red blood cell transfusions from female donors with decreased survival of male recipients was later confirmed in an independent cohort.[7] The association was furthermore shown to be limited to female donors with a history of pregnancy, and it was estimated that this association could be responsible for one potentially preventable death per day in the Netherlands.[7] Several studies, although performed in different populations and with a different methodology, have since been published which were not able to confirm these findings.[8, 15] Hence, there still remain a number of unanswered questions about the significance of donor sex and parity in clinical transfusion research, e.g. whether this finding is replicable, and if it is, how it can be explained (i.e. "biological plausibility"). The epidemiological perspective and the biological perspective, that were introduced earlier, coincide here.

As already suggested, for the investigation of the association between donor sex and recipient outcomes several methodological challenges should be addressed. First, due to the nature of observational data, the existence of time-varying exposures, mixed exposures, and incomplete confounder information (e.g. underlying disease severity) make analyses complex. Second, the possibility of bias due to 'treatment-confounder feedback' that will be explained in detail in this thesis, poses a newly recognized challenge. This phenomenon can occur when the exposure of interest is a time-varying product characteristic which has a relation with both the outcome, and with the ability of the product to increase the hemoglobin level of the patient (e.g. through a different Hb content of the product, or if the post-transfusion yield is affected by a changed clearance of donor cells). In this setting, treatment-confounder feedback results in differen-

tial transfusion needs for the exposure of interest, and thereby makes standard confounding adjustment prone to bias.[8] Lastly, the presence of country-specific production methods and practices, population differences of both donor and patient, and differences in analysis methods could account for disparities between studies in detecting an association between donor sex and recipient outcomes. The interpretation of the available evidence requires careful consideration of all these challenges.

The red blood cell storage lesion: lessons from the past

Whether young or old red blood cells are better for clinical outcome is still the subject of debate, with studies showing both positive and negative associations between the storage time of red blood cells and clinical outcomes, including mortality.[16-27] Earlier, our research group showed that very fresh red cells were associated with increased mortality in an observational cohort study.[28] Several randomized trials on this subject have been since published.[19, 22-27] These trials were designed expecting the transfusion of older red cells to be associated with increased mortality. Therefore it was considered unethical to transfuse very old red cells, like those during the last two weeks of the maximum allowed storage time. This resulted in less pronounced storage time contrasts between the treatment arms. Moreover, these trials were not designed and sufficiently powered to accommodate an unexpected inverse association, i.e. fresh red cells potentially being harmful. Still, blood products stored for short storage duration were not found to be superior to older units; although not statistically significant in any individual trial, harm from fresh units was more likely.[19, 22-28]

When comparing these trials to observational research, they are not in agreement.[29, 30] Although results from observational studies and RCTs need not coincide[31], in this specific situation the expectation is that they should, if the observational studies sufficiently eliminate bias due to confounding and selection. To explain this, we need to look at the causal question the observational studies were attempting to answer. In general, the observational studies answered the general question "what is the effect of receiving only 'fresher' transfusions compared to receiving only 'older' transfusions", but without taking into account the time-varying aspect of receiving additional transfusions. Because patients receive multiple transfusions over time, and their probability to receive only one of these categories consequently diminishes, analysing restricted subgroups of patients receiving "only-fresh" and "only-old" units throughout their follow-up would be subject to bias. These inconsistencies have made the scientific community aware of limitations of past studies, but still the conclusions of these studies – namely that stored blood is inferior to fresher units – persist. More

attention to methods in clinical transfusion research can shed light on the causal relations that give rise to the observed data.

Evidence sources: observational research and rapid reviews

Although observational research has a number of limitations, especially in the field of transfusion medicine there is a large role for observational research in providing answers to causal questions. Indeed, for longer term outcomes that are not determined by blood transfusion alone, the size of RCTs needed would not be feasible, and answers should be sought, and can be found, elsewhere. We argue this is especially true when investigating blood product characteristics. This is because the setting in which transfusions are administered can be considered to be similar to a "natural experiment".[8] In this setting, there is no risk of traditional confounding or confounding by indication being introduced, because particular exposure characteristics:

- can typically not be chosen or avoided,
- are not known at the time of transfusion,
- cannot influence how outcomes are reported.

This is why it is puzzling to see that implying causality in an observational study publication can lead to a swift rejection from some scientific journals. Rather than avoiding the "c-word", causality, and only describing associations, observational studies can play an important role in assessing the effects of different interventions, especially when patients are randomly exposed with respect to their prognosis.[32] However, this does not mean clinical transfusion research with observational data in this setting is similar to analysing a true randomized experiment. Rather, clinical transfusion research is complicated by patients receiving multiple transfusions over time, exponentially increasing the probability of mixed exposure. At the same time, transfusion efficacy and safety is likely varying per product, and patient disease severity and outcome effects will additionally vary throughout transfusion episodes. With proper epidemiological study designs and appropriate statistical analyses, however, these difficulties can be overcome. Hence, causality can be inferred from observational research, and importantly, observational studies can serve to inform clinical practice.

In the context of public health emergencies, the use of rapid reviews has become increasingly popular as a means of synthesizing evidence in a timely and efficient manner.[33] The accelerated nature of the review process can reduce costs and increase efficiency, but this approach can also be a limitation, as the review may not be as comprehensive or rigorous as a traditional systematic review. Evidence sources may also be limited to non-randomized studies, such as case reports and

case series. There are also advantages of performing early rapid reviews. Systematic reviews that include non-randomized studies allow researchers to identify and evaluate the full range of evidence available at that time. Indeed, valuable insights can be obtained by carefully selecting and evaluating the studies to be included in the review and assessing the risk of bias in every study. Gaps in the existing literature can be identified to guide future research efforts. Finally, RCTs, as opposed to observational research, often include a strongly selected patient group and are not representative of clinical practice. On the other hand, it is important to note that especially smaller, non-randomized studies that have not been carefully designed can be subject to biases. We hence should always assess carefully where the validity and reliability of findings of both observational studies and RCTs could be compromised.

Part 2. Aim and outline of the thesis

The research questions in this thesis relate to two themes: investigating donor- and blood product characteristics, and investigating safety and efficacy of convalescent plasma for people with Coronavirus disease 2019 (COVID-19). The research in this thesis uses observational data and thorough epidemiological methods to answer several research questions in these two areas.

In **Chapter 2**, the different biological mechanisms potentially underlying associations of donor sex and pregnancy history with mortality in the clinical transfusion field are discussed in the form of a narrative review. Past studies have drawn attention to the potential adverse effects of donor and blood product characteristics. In particular, donor sex and parity have been implicated in increased mortality among transfusion recipients.[7] Interestingly, sex mismatch has long been recognized as a risk factor in solid organ and stem cell transplantation, with the strongest association observed for multiparous female donors and male recipients of hematopoietic stem cell transplantations.[34] In this chapter, the available evidence from transfusion, solid organ transplantation, and stem cell transplantation medicine is summarized and possible biological mechanisms underlying the association between donor parity and red blood cell unit recipient mortality are discussed. A key aspect of this chapter is the possible role of cellular microchimerism in immune modulation of transfusion recipients, and how this may contribute to adverse outcomes.

At the same time, longer blood product storage is associated with mortality in randomized clinical trials, with 'fresh' blood associated with a non-significant

mortality risk.[30] We postulated that both donor pregnancy history and blood product storage may impact the safety of red blood cell transfusions, with 'fresh' units from ever-pregnant donors providing the highest risk after transfusion. This is investigated in **Chapter 3,** where we examined the association between donor pregnancy history and storage with mortality of recipients in a cohort study of first-ever transfusion recipients in the Netherlands. We proposed that both associations were mediated through residual leukocytes which decay during storage, and studied this using Cox proportional hazards models, with the number of units with each product characteristic as exposure.

In **Chapter 4**, a rapid review investigating convalescent plasma and hyperimmune immunoglobulin for treating individuals with COVID-19 is described. This review constitutes the first instalment of the systematic review series investigating these therapies using Cochrane systematic review methodology. Convalescent plasma and hyperimmune immunoglobulin are therapies that have been studied in the context of several respiratory viral infections, but it was not clear whether they were safe and effective for people with COVID-19. A thorough understanding of the most current evidence regarding their benefits and risks at the time was required. Therefore, the objective of this study was as follows: to assess the efficacy and safety of convalescent plasma and hyperimmune immunoglobulin transfusion for treating people with COVID-19 at the start of the COVID-19 pandemic. We conducted a systematic review of the literature, searching multiple databases for completed and ongoing studies as of April 2020.

Chapter 5 continues on the conflicting evidence on the effect of donor sex on outcomes after red blood cell transfusion. Some studies have suggested that transfusion of blood from female donors may increase the risk of adverse outcomes, while others found no difference between male and female donor blood.[7, 8, 15, 35] Donor pregnancy may partly explain this association, with a study from our research group showing increased risk of mortality, predominantly in younger adult male patients.[7] Whether sex of the donor's offspring (i.e., whether the donor had sons or daughters) has any impact on transfusion outcomes is unknown, but because earlier studies showed an association with mortality in especially male patients, we postulated offspring male sex could potentially mediate this association. On that account, we performed a large observational cohort study with information on donor characteristics and their offspring, and studied the association of these characteristics with mortality using inverse probability weighting (IPW). The use of IPW or other advanced statistical modelling techniques is required here, because of the presence of time-varying treatment-confounder feedback. This is due to the probability of additional transfusions

depending on donor sex, with female donors providing a red blood cell unit with lower hemoglobin concentration compared to male donors; and the relation between the number of transfusions and mortality, with patients who are more ill requiring more transfusions on average.

As shown in chapter 5, advanced statistical modelling techniques, like IPW, allow for sophisticated analysis of complex data, and these methods may be required to estimate causal effects when time-varying treatment and confounder information is of interest. There are several reasons why IPW and other similar analytical approaches have not been widely adopted in clinical transfusion research. First, these methods require an understanding of advanced statistical concepts and techniques, and close collaboration with methodological experts. Second, there may be a lack of awareness of the benefits or necessity of these techniques among researchers. Third, some of these methods can be computationally complex and may require large sample sizes, which may be a challenge in the setting of clinical transfusion research. Finally, their results are more challenging to interpret and hence appreciate by the general scientific community. In **Chapter 6,** we provide a detailed tutorial of the use of IPW in clinical transfusion research, including a sample dataset. The situations where the use of IPW is necessary are discussed in depth, and guidance for future research is provided.

In **Chapter 7** the main findings of the thesis are summarized and the implications for future research are discussed. Here, a balance is sought between the epidemiological and the biological perspectives, by combining insights from clinical and fundamental research in the interpretation of the evidence on blood product characteristics and their influence on patient health.

Reference list

1. Pape A, Stein P, Horn O, Habler O. Clinical evidence of blood transfusion effectiveness. *Blood Transfus*. Oct 2009;7(4):250-8. doi:10.2450/2008.0072-08
2. Kopolovic I, Ostro J, Tsubota H, et al. A systematic review of transfusion-associated graft-versus-host disease. *Blood*. 2015;126(3):406-414. doi:10.1182/blood-2015-01-620872
3. Salpeter SR, Buckley JS, Chatterjee S. Impact of More Restrictive Blood Transfusion Strategies on Clinical Outcomes: A Meta-analysis and Systematic Review. *The American Journal of Medicine*. 2014/02/01/ 2014;127(2):124-131.e3. doi:https://doi.org/10.1016/j.amjmed.2013.09.017
4. Middelburg RA, van Stein D, Atsma F, et al. Alloexposed blood donors and transfusion-related acute lung injury: a case-referent study. *Transfusion*. Oct 2011;51(10):2111-7. doi:10.1111/j.1537-2995.2011.03118.x
5. Vlaar APJ, Binnekade JM, Schultz MJ, Juffermans NP, Koopman MMW. Preventing TRALI: Ladies first, what follows? *Critical Care Medicine*. 2008;36(12):3283-3284. doi:10.1097/CCM.0b013e31818f2f37
6. Wiersum-Osselton JC, Middelburg RA, Beckers EA, et al. Male-only fresh-frozen plasma for transfusion-related acute lung injury prevention: before-and-after comparative cohort study. *Transfusion*. Jun 2011;51(6):1278-83. doi:10.1111/j.1537-2995.2010.02969.x
7. Caram-Deelder C, Kreuger AL, Evers D, 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*. Oct 17 2017;318(15):1471-1478. doi:10.1001/jama.2017.14825
8. Zhao J, Sjölander A, Edgren G. Mortality Among Patients Undergoing Blood Transfusion in Relation to Donor Sex and Parity: A Natural Experiment. *JAMA Internal Medicine*. 2022;182(7):747-756. doi:10.1001/jamainternmed.2022.2115
9. Koch CG, Duncan AI, Figueroa P, et al. Real Age: Red Blood Cell Aging During Storage. *The Annals of Thoracic Surgery*. 2019/03/01/ 2019;107(3):973-980. doi:https://doi.org/10.1016/j.athoracsur.2018.08.073
10. Maclennan S, Murphy MF. Survey of the use of whole blood in current blood transfusion practice. *Clinical & Laboratory Haematology*. 2001;23(6):391-396. doi:https://doi.org/10.1046/j.1365-2257.2001.00419.x
11. CBO. Dutch Guideline Bloodtransfusion. *Retrieved October 22, 2018, from: https://nvicnl/sites/nvicnl/files/CBO%20Richtlijn%20Bloedtransfusiepdf*. 2011;
12. Hillyer CD, Blumberg N, Glynn SA, Ness PM, Epidemiology ftmotNWGiTR, Research O. Transfusion recipient epidemiology and outcomes research: possibilities for the future. *Transfusion*. 2008;48(8):1530-1537. doi:https://doi.org/10.1111/j.1537-2995.2008.01807.x
13. Edgren G, Rostgaard K, Hjalgrim H. Methodological challenges in observational transfusion research: lessons learned from the Scandinavian Donations and Transfusions (SCANDAT) database. 2017;12(1):191-195. doi:10.1111/voxs.12342
14. Middelburg RA, Briet E, van der Bom JG. Mortality after transfusions, relation to donor sex. *Vox Sang*. Oct 2011;101(3):221-9. doi:10.1111/j.1423-0410.2011.01487.x

15. Edgren G, Murphy EL, Brambilla DJ, et al. Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. *Jama*. Jun 11 2019;321(22):2183-2192. doi:10.1001/jama.2019.7084
16. Desmarets M, Bardiaux L, Benzenine E, et al. Effect of storage time and donor sex of transfused red blood cells on 1-year survival in patients undergoing cardiac surgery: an observational study. *Transfusion*. May 2016;56(5):1213-22. doi:10.1111/trf.13537
17. Heddle NM, Cook RJ, Arnold DM, et al. Effect of Short-Term vs. Long-Term Blood Storage on Mortality after Transfusion. *N Engl J Med*. Nov 17 2016;375(20):1937-1945. doi:10.1056/NEJMoa1609014
18. Heddle NM, Eikelboom J, Liu Y, Barty R, Cook RJ. Exploratory studies on the age of transfused blood and in-hospital mortality in patients with cardiovascular diagnoses. *Transfusion*. Feb 2015;55(2):364-72. doi:10.1111/trf.12861
19. Lacroix J, Hébert PC, Fergusson DA, et al. Age of Transfused Blood in Critically Ill Adults. *New England Journal of Medicine*. 2015;372(15):1410-1418. doi:10.1056/NEJMoa1500704
20. Marti-Carvajal AJ, Simancas-Racines D, Pena-Gonzalez BS. Prolonged storage of packed red blood cells for blood transfusion. *Cochrane Database Syst Rev*. Jul 14 2015;(7):Cd009330. doi:10.1002/14651858.CD009330.pub2
21. Zalpuri S, Schonewille H, Middelburg R, et al. Effect of storage of red blood cells on alloimmunization. *Transfusion*. Nov 2013;53(11):2795-800. doi:10.1111/trf.12156
22. Cooper DJ, McQuilten ZK, Nichol A, et al. Age of Red Cells for Transfusion and Outcomes in Critically Ill Adults. *New England Journal of Medicine*. 2017/11/09 2017;377(19):1858-1867. doi:10.1056/NEJMoa1707572
23. Cecile Aubron GS, Alistair Nichol, Michael Bailey, Jasmin Board, Geoff Magrin, Lynnette Murray, Jeffrey Presneill, Joanne Sutton, Shirley Vallance, Siouxzy Morrison, Rinaldo Bellomo, D. Jamie Coope. A pilot feasibility trial of allocation of freshest available red blood cells versus standard care in critically ill patients. . *Transfusion Practice* 2012;52:1196-1202.
24. Dhabangi A, Ainomugisha B, Cserti-Gazdewich C, et al. Effect of Transfusion of Red Blood Cells With Longer vs Shorter Storage Duration on Elevated Blood Lactate Levels in Children With Severe Anemia: The TOTAL Randomized Clinical Trial. *Jama*. Dec 15 2015;314(23):2514-23. doi:10.1001/jama.2015.13977
25. Hebert PC, Chin-Yee I, Fergusson D, et al. A pilot trial evaluating the clinical effects of prolonged storage of red cells. *Anesth Analg*. May 2005;100(5):1433-8, table of contents. doi:10.1213/01.ane.0000148690.48803.27
26. Heddle NM, Cook RJ, Arnold DM, et al. The effect of blood storage duration on in-hospital mortality: a randomized controlled pilot feasibility trial. *Transfusion*. Jun 2012;52(6):1203-12. doi:10.1111/j.1537-2995.2011.03521.x
27. Steiner ME, Ness PM, Assmann SF, et al. Effects of red-cell storage duration on patients undergoing cardiac surgery. *N Engl J Med*. Apr 9 2015;372(15):1419-29. doi:10.1056/NEJMoa1414219
28. 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*. Jan 2013;27(1):36-43. doi:10.1016/j.tmrv.2012.06.002
29. Remy KE, Spinella PC. Red blood cell storage age – what we know from clinical trials. *Expert Review of Hematology*. 2016/11/01 2016;9(11):1011-1013. doi:10.1080/17474086.2016.1243051

30. 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. *Transfusion Medicine Reviews*. 2018/04/01/ 2018;32(2):77-88. doi:https://doi.org/10.1016/j.tmrv.2018.02.002
31. Groenwold RHH. Trial Emulation and Real-World Evidence. *JAMA Network Open*. 2021;4(3):e213845-e213845. doi:10.1001/jamanetworkopen.2021.3845
32. Hernán MA. The C-Word: Scientific Euphemisms Do Not Improve Causal Inference From Observational Data. *American Journal of Public Health*. 2018;108(5):616-619. doi:10.2105/ajph.2018.304337
33. Rehfuess EA, Burns JB, Pfadenhauer LM, et al. Lessons learnt: Undertaking rapid reviews on public health and social measures during a global pandemic. *Research Synthesis Methods*. 2022;13(5):558-572. doi:https://doi.org/10.1002/jrsm.1580
34. Ciurea SO, Champlin RE. Donor Selection in T Cell–Replete Haploidentical Hematopoietic Stem Cell Transplantation: Knowns, Unknowns, and Controversies. *Biology of Blood and Marrow Transplantation*. 2013/02/01/ 2013;19(2):180-184. doi:https://doi.org/10.1016/j.bbmt.2012.08.007
35. 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;51doi:10.1016/j.eclinm.2022.101628

2

Chapter 2

Donor sex and recipient outcomes

Authors: Sarah J Valk[1,2] , Camila Caram-Deelder[1,2] , Jaap Jan Zwaginga[1,3], Johanna G van der Bom[1,2], Rutger A Middelburg[1,2]

Affiliations

1. Center for Clinical Transfusion Research, Sanquin Research, Leiden, The Netherlands

2. Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

3. Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands

ISBT Science Series, 2020, 15: 142-150.

Abstract

Donor characteristics, such as donor sex and age, have been implicated in adverse outcomes following red blood cell transfusions. There is a vast body of evidence supporting a role for sex-mismatch in solid organ and stem cell transplantation. Most of these findings suggest the strongest effect of sex-mismatch between multiparous female donors and male recipients. In this review, we discuss the available evidence from transfusion, solid organ transplantation, and stem cell transplantation medicine. We suggest several possible biological mechanisms behind the association of donor pregnancy and transfusion recipient mortality that can be further investigated in future research. Foremost, we claim donor microchimeric cell-mediated immune modulation is the most likely explanation for the observed associations in transfusion medicine.

Key words: blood transfusion, transplantation, pregnancy, sex mismatch

Introduction

Blood products from female donors are associated with adverse outcomes after transfusion[1, 2]. Initially, the association between donor sex and transfusion recipient mortality was limited to plasma-rich products, which were implicated in causing transfusion-related acute lung injury (TRALI)[3, 4]. TRALI is caused by the transfer of donor alloantibodies that react with human neutrophil antigens (HNA) or class I or class II human leukocyte antigens (HLA)[5] of recipient cells and tissue. These antibodies are induced by exposure to alloantigens, which can occur during pregnancy, transfusion, and transplantation[6-9]. In TRALI, donor antibodies originating from leukocytes and located in the plasma fraction of the blood product cause neutrophil priming and activation in the pulmonary vasculature, resulting in edema and acute dyspnea[10]. Therefore, the use of plasma-rich products from female donors has been restricted, resulting in a reduction of the incidence of TRALI[11].

However, an association between transfusions from female donors and subsequent adverse outcomes was also seen for other blood products, which contain a limited amount of plasma[12-18]. We furthermore observed increased death rates among young male recipients of packed red blood cell transfusions from ever-pregnant female donors[16]. In search of potential biological mechanisms to explain these observations, we reviewed the literature on the role of donor and recipient sex-mismatch in outcomes in blood transfusion, solid organ and stem cell transplantation. We summarize the possible mechanisms behind the frequently seen association between female donor sex and adverse events in (predominantly male) recipients.

Donor sex and pregnancy in hematopoietic stem cell transplantation

Although allogeneic hematopoietic stem cell transplantation can be a life-saving therapy for hemato-oncologic malignancies, serious complications frequently occur[19]. Graft-versus-host disease (GVHD) is a potentially lethal complication which is caused by the attack of the host by T-cells originating from the allogeneic graft[20]. However, the occurrence of GVHD is also associated with a graft-versus-leukemia or graft-versus-tumor effect, with lower relapse incidence in patients with this condition[21-23]. Lower relapse and increased GVHD risk go hand in hand: the outcome of allogeneic transplantation depends heavily on HLA and minor histocompatibility antigen (miHA) mismatches between donor

and recipient, and the amount of functional and mismatch reactive T-cells within the transplant[24, 25].

Sex-mismatch has been studied in the context of allogeneic stem cell transplantation in aplastic anemia[26], acute myeloid leukemia[27, 28], acute lymphoblastic leukemia[27, 28], chronic myeloid leukemia[28, 29], and multiple myeloma[30]. Female-to-male allogeneic transplantations were associated with increased risk of death in allogeneic stem cell transplant recipients, due to a higher rate of acute and chronic GVHD, and increased non-relapse mortality[26-29, 31]. However, the increase in chronic GVHD related to female donors was also observed in female recipients[32].

Non-relapse mortality in male patients receiving a hematopoietic stem cell transplantation from a female donor was associated with pregnancy history of the female donor, and particularly with a prior pregnancy with a male child[33]. During pregnancy, there is exchange of fetal and maternal cells across the placenta[34-36]. After pregnancy, allogeneic cells can thus persist in the host, leading to microchimerism[37]. Parous women can mount an immune response against these chimeric cells through the inherited paternal HLA antigens (IPA) or paternal miHA, and in the case of a pregnancy with a boy through the Y-chromosome encoded miHA (HY-antigens)[38-40]. The introduction of HY-specific donor T-cells[41] via the stem cell transplant is associated with both acute and chronic GVHD in male allogeneic stem cell transplant recipients[41]. Next to HY-specific helper T cells and cytotoxic T cells, also anti-HY antibodies involved in antibody-dependent cellular cytotoxicity could be demonstrated in females with male children.

Donor sex and pregnancy in solid organ transplant

In solid organ transplantation medicine, the role of donor sex on allograft engraftment and function has been extensively described[42]. Overall, a worse graft outcome has been identified for female donor allografts[42]. This association has been observed in both cadaveric and living-donor liver transplantation[43, 44]. A decreased overall survival was observed in male recipients receiving a female donor heart, compared to a male donor heart[45]. Overall, renal allografts from female donors are associated with poor survival both in male and female recipients[42, 46].

Several biological mechanisms were postulated to explain these findings. First, the increased mortality in recipients of female liver allografts has been ascribed to deprivation of estrogen, which provides protection to ischemic injury, and promotes cholangiocyte proliferation in the liver[43, 44]. Second, increased mortality among recipients of heart transplants from female donors could be due to graft under sizing, commonly attributed to sex mismatching[47, 48]. This effect could be further exacerbated by a progressive loss of 1g of myocytes per year partially compensated by a reactive hypertrophic response, which has been observed in healthy male hearts, but not in females[49, 50]. Finally, increased mortality among male recipients of female kidneys has been attributed to the lower nephron mass of female donor kidneys, and higher functional demand of male recipients, resulting in allograft hyperfiltration injury[51, 52].

However, kidney allografts from male donors in female recipients, compared to all other donor-recipient combinations, were also associated with increased adverse outcomes[53-57]. These adverse effects of sex-mismatch in kidney transplantation are postulated to again relate to higher antibody titers against HY-antigens observed in female recipients[58, 59]. HY-antigen mismatch is hypothesized to lead to sensitization, allogeneic transplant rejection, and ultimately transplant failure[56]. Some studies have also shown a detrimental effect of HY-antigen mismatch on acute immunological rejection in corneal transplantation[60], lung[61], liver[62] kidney[63] and heart transplantation[64, 65]. Overall, these findings suggest a role for HY-antigens in solid organ transplantation, through an immunological female-anti-male H-Y effect[53].

Donor sex and pregnancy and red blood cell recipient mortality

The first study reporting an association between donor sex and transfusion recipient mortality after transfusion of plasma poor, leukoreduced red blood cell products was published in 2011[17]. This study noted an especially strong association of young male transfusion recipient mortality and female donor transfusions. Since then, several other studies have also observed this association[12-15, 18].

Figure 1 shows the results of all studies reporting the association of donor sex with transfusion recipient mortality for male and female transfusion recipients separately (adapted from[66]). The pooled hazard ratio for mortality of male transfusion recipients after red blood cell transfusions from female donors,

compared to male donors was 1.15 (95% confidence interval (CI): 1.01 to 1.30). For female recipients, this hazard ratio was 1.01 (95% CI: 0.94 to 1.08).

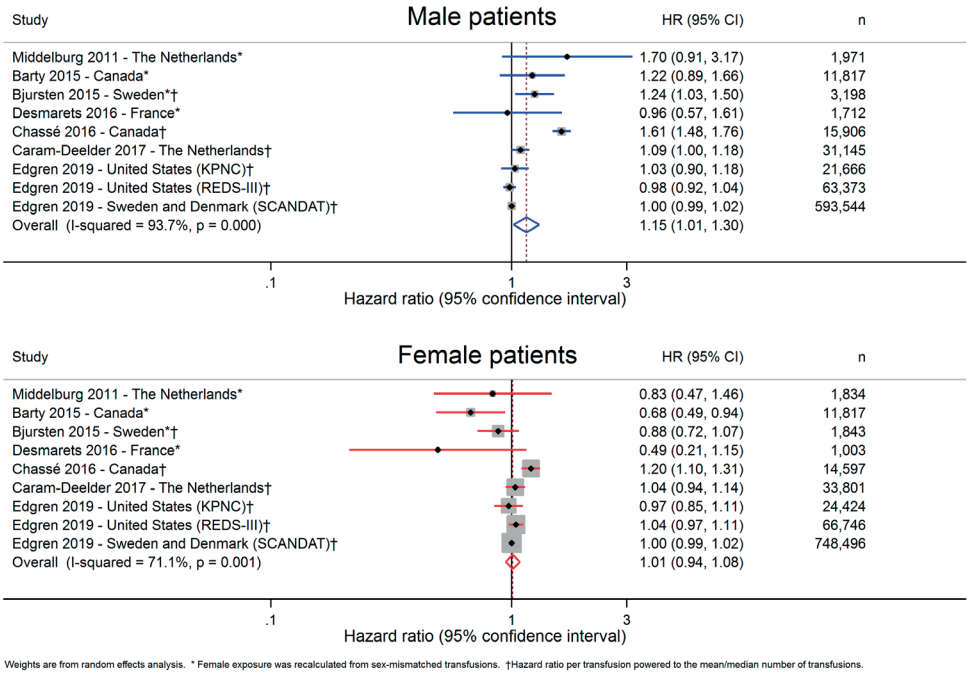


Figure 1 - Publications on the association of recipient mortality female blood donors, stratified by recipient sex

Some studies did not find this association between donor sex and adverse outcomes following transfusion[67-69]. Differences in study population, chosen comparisons, and production methods of blood products could explain these differences and potentially modify the risk associated with receiving blood products from female donors. Namely, one of these studies investigated cardiovascular disease patients only[68]. Furthermore, a recent publication reported a positive association between red cell transfusions from ever-pregnant donors and mortality of young male recipients[16]. Although this finding is tentative and was not corroborated by another more recent study, it is consistent with the observation that female donors are associated with adverse outcomes in male transfusions recipients[69]. It could also explain why some studies did not find an association between female donors and mortality; the donor populations in different countries have different demographics. Different statistical analysis techniques could further explain why not all studies showed an effect of sex-mismatched transfusions. The methods used to adjust for confounding variables, such as the

total number of transfusions, were theorized to explain some of these differences[69]. We also cannot rule out that the length of follow-up, which varied widely between studies, may have influenced the observed point estimates.

Transfusion-associated microchimerism (TA-MC) has been proposed as a possible explanation for higher mortality after sex-mismatched transfusions[16, 70, 71]. Donor cells have been detected in transfusion recipients up to 60 years after transfusions[72]. Interestingly, transfused trauma patients have been shown to be significantly more sensitive to persistent microchimerism[73]. Trauma patients receiving transfusions are often males (84%), and relatively young (77% under 44 years of age)[74]. It is therefore plausible, that the increased tendency of young male transfusion recipients to develop long-lasting microchimerism might be implicated in the apparent susceptibility of this patient group to sex-mismatched blood transfusions.

Universal leukoreduction of donated blood products is thought to reduce the risks associated with blood transfusion, and has been indicated to reduce post-operative mortality after open-heart surgery[75, 76]. Strikingly, the occurrence of TA-MC remained unchanged after the introduction of universal leukoreduction[77]. Also, the number of transfusions did not determine whether microchimeric cells persist[78]. Finally, prolonged storage of the blood product had no apparent effect on the occurrence of TA-MC, even though the leukocyte content in some blood products decreased to undetectable levels during storage[79]. These findings indicate, TA-MC may not be leukocyte dose dependent.

Summarizing the findings from observed associations in transfusion and transplantation research, a compelling theory emerges. Mortality after transfusion from female donors is related to pregnancy history of the donor and age and sex of the recipients[16]. Parity is known to be associated both with cellular and humoral HY-immunity in women[38], while this transferred immunity is associated with GVHD in male recipients[40, 41, 80]. After transfusion, microchimerism can be detected more often in trauma patients[77], which are predominantly young and male[74]. Thus, we hypothesize HY- and other Y coded (minor) antigen-directed alloimmunity is unintentionally transferred with parous female donor blood products, and may play a role in causing mortality and morbidity in male transfusion recipients.

Other raised mechanisms for the association between donor sex and transfusion recipient mortality

Hemoglobin

Lower hemoglobin concentrations of female donors may also affect transfusion recipient mortality[81]. Less hemoglobin in the product could result in the need for more transfusions; donor and recipient sex are significant predictors of hemoglobin increments[82]. However, a higher number of transfusions does not explain why blood products from ever-pregnant female donors could be harmful, or why this association should be limited to young male transfusion recipients. Although hemoglobin levels are affected by pregnancy, these effects are transient and hemoglobin levels return to normal after childbirth[83].

The higher levels of hemoglobin of red blood cell units from male donors are actually postulated to be harmful to female transfusion recipients[84]. The excess hemoglobin, in the form of toxic free hemoglobin, might overwhelm the scavenging capacity of female haptoglobin, resulting in a temporary depletion of nitric oxide, inducing endothelial dysfunction, platelet aggregation and oxidative injury[85-87]. Also, this free hemoglobin may trigger pro-inflammatory effects through toll-like receptor 4[88].

Cell-free DNA

Blood products with short storage duration are possibly associated with post-transfusion mortality[89-91]. As the blood product ages, less cell-free DNA is present in the product[92], possibly due to degradation by DNases[93]. Different blood product production methods also resulted in different concentrations of cell-free DNA[92], with the main differences being the timing of the leukoreduction procedure. Cell-free DNA is known to be released by neutrophils in neutrophil extracellular traps[94]. Increased cell-free DNA levels have been associated with impaired fibrinolysis in septic patients[95]. Pro-coagulant, platelet-stimulating and pro-inflammatory properties all have been ascribed to cell-free DNA[93, 96-98]. Thus, a role for cell-free DNA in the adverse events linked to very fresh blood products is conceivable. This principally applies to products which were manufactured using the whole-blood filtration method, which were shown to contain high cell-free DNA concentrations[92].

However, no link to donor pregnancy history and presence of cell-free DNA in blood products has been established. A novel, yet unknown effect of cell-free DNA from (ever-pregnant) female donors on mortality could be studied by in-

vestigating effect modification by storage time on the effect of ever-pregnant donors on mortality. However, preliminary investigations into this subject suggested that older units may actually potentiate the effect of ever-pregnant donors[99].

Hormones

There are indications that hormones act differently on red blood cells in men and in women[100, 101]. The membrane rigidity of female erythrocytes was shown to increase following adrenaline stimulation, while in male erythrocytes it decreases[100]. Increased membrane rigidity was shown to reduce white blood cell adhesion to an inflamed endothelium, potentially inducing a susceptibility to infection[102]. Furthermore, higher membrane deformability was observed during the luteal phase of menstruation, which is known for higher estrogen and progesterone levels[101].

Although the impact of hormones on red blood cell deformability and membrane rigidity has been demonstrated, it is unclear whether these findings have clinical implications. No research has been performed on the effect of female fertility on outcomes after transfusions. However, although the differential effects of hormones could play a role in the short-term effects of blood transfusions, it is unlikely these would play a role in long-term outcomes of transfusion recipients.

Conclusions and clinical implications

In transfusion medicine, donor sex is associated with recipient outcomes; not only for alloantibodies containing plasma products but also for plasma-poor products. We hypothesized that HY-directed immunity is unintentionally transferred to male transfusion recipients. This hypothesis is fueled by findings in transplantation medicine, where HY-mismatch is a bad prognostic factor for chronic GVHD in allogeneic stem cell transplantation and for solid organ transplant rejection. Alternatively, immunity against other antigens could be implicated. Pregnancy primes for IPA and paternal miHA[39]. If trauma is capable of inducing a 'susceptibility' to persistent microchimerism, any fetal antigen recognizing cells could potentially engraft in a trauma patient, regardless of patient sex. (Table 1)

Table 1 - Matrix combining observations from the fields of transfusion and transplantation medicine with possible corresponding mechanisms suspected to influence mortality after transfusion

Field Observations	Possible mechanisms				
	Cellular immunity to HY-antigens	Cellular immunity to child paternal antigens	Low Hb	Cell-free DNA	Hormones
Stem cell transplantation					
Increased mortality in female-to-male hematopoietic stem cell transplantation	✓	✓			
Higher GVHD when female-to-male hematopoietic stem cell transplantation	✓	✓			
Lower relapse incidence when female-to-male hematopoietic stem cell transplantation	✓	✓			
HY-specific donor T-cells observed in acute and chronic GVHD in men	✓				
Transplantation medicine					
Acute immunological reaction in women who receive male donor allografts	✓	✓			
Transfusion medicine					
Sex mismatch and mortality after transfusion	✓	✓	✓	✓	✓
Donor pregnancy history and mortality after transfusion, specific to young male recipients	✓	✓			
Transfusion-associated microchimerism in trauma patients					
No association female donor sex and mortality in cardiovascular disease patients					
Transfusion-associated microchimerism establishes independent of leukocyte dose					

We proposed donor microchimeric cell-mediated immune modulation as the most likely explanation for the observed association between donor pregnancy history and adverse outcomes in transfusion medicine. Other mechanisms that could explain the association between donor sex and recipient mortality were also discussed. However, none of these explain how parity of female donors would influence recipient outcomes. In order to provide guidance for blood banking, improve safety, and maintain the continuity of the blood supply, it is necessary to first specify which donors and patients are implicated in these adverse events. Future research investigating donor characteristics on a molecular and cellular level should be encouraged, in addition to well-designed randomized clinical trials to determine the clinical impact of sex-mismatched red blood cell transfusions. Ultimately, this can pave the way for personalized transfusion strategies that will minimize both side effects and associated mortality of recipients of transfusions, while still maintaining a blood inventory that is as flexible and broad as possible.

Acknowledgements

This research was funded by Sanquin Research (grant PPOC-18-03).

Disclosure

The authors declare no conflict of interests regarding the publication of this paper.

References

1 Tynell E, Andersson TML, Norda R, et al.: Should plasma from female donors be avoided? A population-based cohort study of plasma recipients in Sweden from 1990 through 2002. *Transfusion* 2010; 50: 1249-56.

2 Chasse M, McIntyre L, English SW, et al.: Effect of Blood Donor Characteristics on Transfusion Outcomes: A Systematic Review and Meta-Analysis. *Transfusion medicine reviews* 2016; 30: 69-80.

3 Palfi M, Berg S, Ernerudh J, et al.: A randomized controlled trial oftransfusion-related acute lung injury: is plasma from multiparous blood donors dangerous? *Transfusion* 2001; 41: 317-22.

4 Middelburg RA, Van Stein D, Zupanska B, et al.: Female donors and transfusion-related acute lung injury: A case-referent study from the International TRALI Unisex Research Group. *Transfusion* 2010; 50: 2447-54.

5 Middelburg RA, van Stein D, Briet E, et al.: The role of donor antibodies in the pathogenesis of transfusion-related acute lung injury: a systematic review. *Transfusion* 2008; 48: 2167-76.

6 Densmore TL, Goodnough LT, Ali S, et al.: Prevalence of HLA sensitization in female apheresis donors. *Transfusion* 1999; 39: 103-6.

7 Payne R: The development and persistence of leukoagglutinins in parous women. *Blood* 1962; 19: 411-24.

8 Triulzi DJ, Kleinman S, Kakaiya RM, et al.: The effect of previous pregnancy and transfusion on HLA alloimmunization in blood donors: implications for a transfusion-related acute lung injury risk reduction strategy. *Transfusion* 2009; 49: 1825-35.

9 Boulton-Jones R, Norris A, O'Sullivan A, et al.: The impact of screening a platelet donor panel for human leucocyte antigen antibodies to reduce the risk of transfusion-related acute lung injury. *Transfusion medicine (Oxford, England)* 2003; 13: 169-70.

10 Bux J, Sachs UJH: The pathogenesis of transfusion-related acute lung injury (TRALI). *British Journal of Haematology* 2007; 136: 788-99.

11 Lin Y, Saw C-L, Hannach B, et al.: Transfusion-related acute lung injury prevention measures and their impact at Canadian Blood Services. *Transfusion* 2012; 52: 567-74.

12 Bjursten H, Dardashti A, Bjork J, et al.: Transfusion of sex-mismatched and non-leukocyte-depleted red blood cells in cardiac surgery increases mortality. *The Journal of thoracic and cardiovascular surgery* 2016; 152: 223-32.e1.

13 Chasse M, Tinmouth A, English SW, et al.: Association of Blood Donor Age and Sex With Recipient Survival After Red Blood Cell Transfusion. *JAMA internal medicine* 2016; 176: 1307-14.

14 Barty RL, Cook RJ, Liu Y, et al.: Exploratory analysis of the association between donor sex and in hospital mortality in transfusion recipients. *Transfusion* 2015; Conference: AABB Annual Meeting 2015 Anaheim:September 2015.

15 Heddle NM, Eikelboom J, Liu Y, et al.: Exploratory studies on the age of transfused blood and in-hospital mortality in patients with cardiovascular diagnoses. *Transfusion* 2015; 55: 364-72.

16 Caram-Deelder C, Kreuger AL, Evers D, 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.

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

18 Heddle NM, Cook RJ, Liu Y, et al.: The association between blood donor sex and age and transfusion recipient mortality: an exploratory analysis. *Transfusion* 2019; 59: 482-91.

19 Jaing T-H: Complications of haematopoietic stem cell transplantation. *ISBT Science Series* 2011; 6: 332-6.

20 Ferrara JLM, Levine JE, Reddy P, et al.: Graft-versus-host disease. *Lancet (London, England)* 2009; 373: 1550-61.

21 Weiden PL, Flournoy N, Thomas ED, et al.: Antileukemic Effect of Graft-versus-Host Disease in Human Recipients of Allogeneic-Marrow Grafts. *New England Journal of Medicine* 1979; 300: 1068-73.

22 Weiden PL, Sullivan KM, Flournoy N, et al.: Antileukemic Effect of Chronic Graft-versus-Host Disease. *New England Journal of Medicine* 1981; 304: 1529-33.

23 Horowitz M, Gale R, Sondel P, et al.: Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; 75: 555-62.

24 Goulmy E, Schipper R, Pool J, et al.: Mismatches of Minor Histocompatibility Antigens between HLA-Identical Donors and Recipients and the Development of Graft-Versus-Host Disease after Bone Marrow Transplantation. *New England Journal of Medicine* 1996; 334: 281-5.

25 Petersdorf EW, Gooley TA, Anasetti C, et al.: Optimizing Outcome After Unrelated Marrow Transplantation by Comprehensive Matching of HLA Class I and II Alleles in the Donor and Recipient. *Blood* 1998; 92: 3515-20.

26 Stern M, Passweg JR, Locasciulli A, et al.: Influence of donor/recipient sex matching on outcome of allogeneic hematopoietic stem cell transplantation for aplastic anemia. *Transplantation* 2006; 82: 218-26.

27 Frassoni F, Labopin M, Gluckman E, et al.: Results of allogeneic bone marrow transplantation for acute leukemia have improved in Europe with time--a report of the acute leukemia working party of the European group for blood and marrow transplantation (EBMT). *Bone marrow transplantation* 1996; 17: 13-8.

28 Randolph SS, Gooley TA, Warren EH, et al.: Female donors contribute to a selective graft-versus-leukemia effect in male recipients of HLA-matched, related hematopoietic stem cell transplants. *Blood* 2004; 103: 347-52.

29 Gratwohl A, Hermans J, Niederwieser D, et al.: Female donors influence transplant-related mortality and relapse incidence in male recipients of sibling blood and marrow transplants. *The hematology journal : the official journal of the European Haematology Association* 2001; 2: 363-70.

30 Gahrton G, Iacobelli S, Apperley J, et al.: The impact of donor gender on outcome of allogeneic hematopoietic stem cell transplantation for multiple myeloma: reduced relapse risk in female to male transplants. *Bone marrow transplantation* 2005; 35: 609-17.

31 Kim HT, Zhang M-J, Woolfrey AE, et al.: Donor and recipient sex in allogeneic stem cell transplantation: what really matters. *Haematologica* 2016; 101: 1260-6.

32 Kollman C, Howe CW, Anasetti C, et al.: Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood* 2001; 98: 2043-51.

33 Shinohara A, Inamoto Y, Kurosawa S, et al.: High non-relapse mortality and low relapse incidence in gender-mismatched allogeneic hematopoietic stem cell transplantation from a parous female donor with a male child. *Leukemia & lymphoma* 2017; 58: 578-85.
34 Bianchi DW, Shuber AP, DeMaria MA, et al.: Fetal cells in maternal blood: determination of purity and yield by quantitative polymerase chain reaction. *American journal of obstetrics and gynecology* 1994; 171: 922-6.
35 Herzenberg LA, Bianchi DW, Schroder J, et al.: Fetal cells in the blood of pregnant women: detection and enrichment by fluorescence-activated cell sorting. *Proceedings of the National Academy of Sciences of the United States of America* 1979; 76: 1453-5.
36 Iverson GM, Bianchi DW, Cann HM, et al.: Detection and isolation of fetal cells from maternal blood using the flourescence-activated cell sorter (FACS). *Prenatal diagnosis* 1981; 1: 61-73.
37 Bianchi DW, Zickwolf GK, Weil GJ, et al.: Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proceedings of the National Academy of Sciences of the United States of America* 1996; 93: 705-8.
38 James E, Chai JG, Dewchand H, et al.: Multiparity induces priming to male-specific minor histocompatibility antigen, HY, in mice and humans. *Blood* 2003; 102: 388-93.
39 Verdijk RM, Kloosterman A, Pool J, et al.: Pregnancy induces minor histocompatibility antigen-specific cytotoxic T cells: implications for stem cell transplantation and immunotherapy. *Blood* 2004; 103: 1961-4.
40 Miklos DB, Kim HT, Miller KH, et al.: Antibody responses to H-Y minor histocompatibility antigens correlate with chronic graft-versus-host disease and disease remission. *Blood* 2005; 105: 2973-8.
41 Toubai T, Tawara I, Sun Y, et al.: Induction of acute GVHD by sex-mismatched H-Y antigens in the absence of functional radiosensitive host hematopoietic–derived antigen-presenting cells. *Blood* 2012; 119: 3844-53.
42 Zeier M, Dohler B, Opelz G, et al.: The effect of donor gender on graft survival. *Journal of the American Society of Nephrology : JASN* 2002; 13: 2570-6.
43 Croome KP, Segal D, Hernandez-Alejandro R, et al.: Female donor to male recipient gender discordance results in inferior graft survival: a prospective study of 1,042 liver transplants. *J Hepatobiliary Pancreat Sci* 2014; 21: 269-74.
44 Yoshizumi T, Shirabe K, Taketomi A, et al.: Risk Factors That Increase Mortality After Living Donor Liver Transplantation. *Transplantation* 2012; 93: 93-8.
45 Khush KK, Kubo JT, Desai M: Influence of donor and recipient sex mismatch on heart transplant outcomes: analysis of the International Society for Heart and Lung Transplantation Registry. *The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation* 2012; 31: 459-66.
46 Kwon OJ, Kwak JY: The impact of sex and age matching for long-term graft survival in living donor renal transplantation. *Transplantation proceedings* 2004; 36: 2040-2.
47 Reed RM, Netzer G, Hunsicker L, et al.: Cardiac size and sex-matching in heart transplantation : size matters in matters of sex and the heart. *JACC Heart failure* 2014; 2: 73-83.
48 Trieber FA, McCaffrey F, Pflieger K, et al.: Determinants of Left Ventricular Mass in Normotensive Children. *American journal of hypertension* 1993; 6: 505-13.

49 Al-Khaldi A, Oyer PE, Robbins RC: Outcome Analysis of Donor Gender in Heart Transplantation. *The Journal of Heart and Lung Transplantation* 2006; 25: 461-8.

50 Olivetti G, Giordano G, Corradi D, et al.: Gender differences and aging: effects on the human heart. *Journal of the American College of Cardiology* 1995; 26: 1068-79.

51 Douverny JB, Baptista-Silva JC, Pestana JOM, et al.: Importance of renal mass on graft function outcome after 12 months of living donor kidney transplantation. *Nephrology Dialysis Transplantation* 2007; 22: 3646-51.

52 Poggio ED, Hila S, Stephany B, et al.: Donor Kidney Volume and Outcomes Following Live Donor Kidney Transplantation. *American Journal of Transplantation* 2006; 6: 616-24.

53 Gratwohl A, Döhler B, Stern M, et al.: H-Y as a minor histocompatibility antigen in kidney transplantation: a retrospective cohort study. *The Lancet* 2008; 372: 49-53.

54 Zukowski M, Kotfis K, Biernawska J, et al.: Donor-recipient gender mismatch affects early graft loss after kidney transplantation. *Transplantation proceedings* 2011; 43: 2914-6.

55 McGee J, Magnus JH, Islam TM, et al.: Donor-recipient gender and size mismatch affects graft success after kidney transplantation. *J Am Coll Surg* 2010; 210: 718-25. e1, 25-6.

56 Kim SJ, Gill JS: H-Y Incompatibility Predicts Short-Term Outcomes for Kidney Transplant Recipients. *Journal of the American Society of Nephrology* 2009; 20: 2025-33.

57 Tan JC, Kim JP, Chertow GM, et al.: Donor-recipient sex mismatch in kidney transplantation. *Gender medicine* 2012; 9: 335-47.e2.

58 Scott DM, Ehrmann IE, Ellis PS, et al.: Why do some females reject males? The molecular basis for male-specific graft rejection. *Journal of molecular medicine (Berlin, Germany)* 1997; 75: 103-14.

59 Tan JC, Wadia PP, Coram M, et al.: H-Y antibody development associates with acute rejection in female patients with male kidney transplants. *Transplantation* 2008; 86: 75-81.

60 Bohringer D, Spierings E, Enczmann J, et al.: Matching of the minor histocompatibility antigen HLA-A1/H-Y may improve prognosis in corneal transplantation. *Transplantation* 2006; 82: 1037-41.

61 Roberts DH, Wain JC, Chang Y, et al.: Donor-recipient gender mismatch in lung transplantation: impact on obliterative bronchiolitis and survival. *The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation* 2004; 23: 1252-9.

62 Candinas D, Gunson BK, Nightingale P, et al.: Sex mismatch as a risk factor for chronic rejection of liver allografts. *Lancet (London, England)* 1995; 346: 1117-21.

63 Goulmy E, Bradley BA, Lansbergen Q, et al.: The importance of H-Y incompatibility in human organ transplantation. *Transplantation* 1978; 25: 315-9.

64 Kawauchi M, Gundry SR, de Begona JA, et al.: Male donor into female recipient increases the risk of pediatric heart allograft rejection. *The Annals of thoracic surgery* 1993; 55: 716-8.

65 Prendergast TW, Furukawa S, Beyer AJ, 3rd, et al.: The role of gender in heart transplantation. *The Annals of thoracic surgery* 1998; 65: 88-94.

66 Caram-Deelder C: The bright and the dark side of blood transfusion : turning data into knowledge. *Thesis* 2017.

67 Edgren G, Ullum H, Rostgaard K, et al.: Association of Donor Age and Sex With Survival of Patients Receiving Transfusions. *JAMA internal medicine* 2017; 177: 854-60.
68 Desmarets M, Bardiaux L, Benzenine E, et al.: Effect of storage time and donor sex of transfused red blood cells on 1-year survival in patients undergoing cardiac surgery: an observational study. *Transfusion* 2016; 56: 1213-22.
69 Edgren G, Murphy EL, Brambilla DJ, et al.: Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. *Jama* 2019; 321: 2183-92.
70 Lapierre V, Auperin A, Robinet E, et al.: Immune modulation and microchimerism after unmodified versus leukoreduced allogeneic red blood cell transfusion in cancer patients: results of a randomized study. *Transfusion* 2007; 47: 1691-9.
71 Claas FH, Roelen DL, van Rood JJ, et al.: Modulation of the alloimmune response by blood transfusions. *Transfusion clinique et biologique : journal de la Societe francaise de transfusion sanguine* 2001; 8: 315-7.
72 Utter GH, Lee TH, Rivers RM, et al.: Microchimerism decades after transfusion among combat-injured US veterans from the Vietnam, Korean, and World War II conflicts. *Transfusion* 2008; 48: 1609-15.
73 Utter GH, Reed WF, Lee T-H, et al.: Transfusion-associated microchimerism. *Vox sanguinis* 2007; 93: 188-95.
74 Roberts I, Shakur H, Afolabi A, et al.: The importance of early treatment with tranexamic acid in bleeding trauma patients: an exploratory analysis of the CRASH-2 randomised controlled trial. *Lancet (London, England)* 2011; 377: 1096-101, 101.e1-2.
75 Vamvakas EC: WBC-containing allogeneic blood transfusion and mortality: a meta-analysis of randomized controlled trials. *Transfusion* 2003; 43: 963-73.
76 CBO: Dutch Guideline Bloodtransfusion. *Retrieved October 22, 2018, from: https://nvicnl/sites/nvicnl/files/CBO%20Richtlijn%20Bloedtransfusiepdf*.
77 Utter GH, Nathens AB, Lee TH, et al.: Leukoreduction of blood transfusions does not diminish transfusion-associated microchimerism in trauma patients. *Transfusion* 2006; 46: 1863-9.
78 Lee T-H, Paglieroni T, Ohto H, et al.: Survival of Donor Leukocyte Subpopulations in Immunocompetent Transfusion Recipients: Frequent Long-Term Microchimerism in Severe Trauma Patients. *Blood* 1999; 93: 3127-39.
79 Flesland O, Ip LS, Storlien AS, et al.: Microchimerism in immune competent patients related to the leukocyte content of transfused red blood cell concentrates. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis* 2004; 31: 173-80.
80 Sahaf B, Yang Y, Arai S, et al.: H-Y antigen-binding B cells develop in male recipients of female hematopoietic cells and associate with chronic graft vs. host disease. *Proceedings of the National Academy of Sciences of the United States of America* 2013; 110: 3005-10.
81 Kratz A, Ferraro M, Sluss PM, et al.: Normal Reference Laboratory Values. *New England Journal of Medicine* 2004; 351: 1548-63.
82 Roubinian N, Plimier C, Woo J, et al.: Effect of donor, component and recipient characteristics on hemoglobin increments following red blood cell transfusion. *Blood* 2019: blood.2019000773.

83 Van Eijk HG, Kroos MJ, Hoogendoorn GA, et al.: Serum ferritin and iron stores during pregnancy. *Clin Chim Acta* 1978; 83: 81-91.

84 Zeller MP, Rochwerg B, Jamula E, et al.: Sex-mismatched red blood cell transfusions and mortality: A systematic review and meta-analysis. *Vox sanguinis* 2019; 114: 505-16.

85 Minneci PC, Deans KJ, Zhi H, et al.: Hemolysis-associated endothelial dysfunction mediated by accelerated NO inactivation by decompartmentalized oxyhemoglobin. *The Journal of clinical investigation* 2005; 115: 3409-17.

86 Donadee C, Raat NJH, Kanias T, et al.: Nitric Oxide Scavenging by Red Blood Cell Microparticles and Cell-Free Hemoglobin as a Mechanism for the Red Cell Storage Lesion. *Circulation* 2011; 124: 465-U294.

87 Gladwin MT, Kanias T, Kim-Shapiro DB: Hemolysis and cell-free hemoglobin drive an intrinsic mechanism for human disease. *The Journal of clinical investigation* 2012; 122: 1205-8.

88 Belcher JD, Chen C, Nguyen J, et al.: Heme triggers TLR4 signaling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease. *Blood* 2014; 123: 377-90.

89 Lacroix J, Hébert PC, Fergusson DA, et al.: Age of Transfused Blood in Critically Ill Adults. *New England Journal of Medicine* 2015; 372: 1410-8.

90 Aubron C, Syres G, Nichol A, et al.: A pilot feasibility trial of allocation of freshest available red blood cells versus standard care in critically ill patients. *Transfusion* 2012; 52: 1196-202.

91 Heddle NM, Arnold DM, Acker JP, et al.: Red blood cell processing methods and in-hospital mortality: a transfusion registry cohort study. *The Lancet Haematology* 2016; 3: e246-54.

92 Shih AW, Bhagirath VC, Heddle NM, et al.: Quantification of Cell-Free DNA in Red Blood Cell Units in Different Whole Blood Processing Methods. *Journal of blood transfusion* 2016; 2016: 9316385-.

93 Gould TJ, Vu TT, Swystun LL, et al.: Neutrophil extracellular traps promote thrombin generation through platelet-dependent and platelet-independent mechanisms. *Arterioscler Thromb Vasc Biol* 2014; 34: 1977-84.

94 Brinkmann V, Reichard U, Goosmann C, et al.: Neutrophil extracellular traps kill bacteria. *Science (New York, NY)* 2004; 303: 1532-5.

95 Gould TJ, Vu TT, Stafford AR, et al.: Cell-Free DNA Modulates Clot Structure and Impairs Fibrinolysis in Sepsis. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2015; 35: 2544-53.

96 Bhagirath VC, Dwivedi DJ, Liaw PC: Comparison of the Proinflammatory and Procoagulant Properties of Nuclear, Mitochondrial, and Bacterial DNA. *Shock (Augusta, Ga)* 2015; 44: 265-71.

97 Semeraro F, Ammollo CT, Morrissey JH, et al.: Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood* 2011; 118: 1952-61.

98 Zhang Q, Raoof M, Chen Y, et al.: Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 2010; 464: 104-7.

99 Valk SJ, Caram-Deelder C, Evers D, et al.: Donor pregnancies and transfusion recipient mortality: a role for red blood cell storage? 2019; Conference: 29th Regional Congress of the ISBT, Basel.

100 Hilario S, Saldanha C, Martins e Silva J: An in vitro study of adrenaline effect on human erythrocyte properties in both gender. *Clinical hemorheology and microcirculation* 2003; 28: 89-98.
101 Pehlivanoglu B, Dikmenoglu N, Balkanci DZ: Effect of stress on erythrocyte deformability, influence of gender and menstrual cycle. *Clinical hemorheology and microcirculation* 2007; 37: 301-8.
102 Gutierrez M, Fish MB, Golinski AW, et al.: Presence of Rigid Red Blood Cells in Blood Flow Interferes with the Vascular Wall Adhesion of Leukocytes. *Langmuir* 2018; 34: 2363-72.

3

Chapter 3

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

Authors: Sarah J Valk[1,2], Camila Caram-Deelder[2], Dorothea Evers[3], Karen MK de Vooght[4], Daan van de Kerkhof[5], Marielle J Wondergem[6], Nathalie CV Péquériaux[7], Francisca Hudig[8], Jaap Jan Zwaginga[1,9], Dirk de Korte[10,11], Leo MG van de Watering[1,12], Rutger A Middelburg[2,13], Johanna G van der Bom[2]

1. Jon J van Rood Center for Clinical Transfusion Research, Sanquin/LUMC, Leiden, The Netherlands,
2. Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands,
3. Department of Haematology, Radboudumc, Nijmegen, The Netherlands,
4. Central Diagnostic Laboratory, University Medical Center Utrecht, Utrecht, The Netherlands,
5. Department of Clinical Chemistry and Haematology, Catharina Hospital, Eindhoven, The Netherlands,
6. Department of Haematology, Amsterdam UMC, location VUmc, Amsterdam, The Netherlands,
7. Department of Clinical Chemistry and Haematology, Jeroen Bosch Hospital, 's Hertogenbosch, The Netherlands,
8. LabWest, Haga Teaching Hospital, The Hague, The Netherlands,
9. Department of Haematology, Leiden University Medical Center, Leiden, The Netherlands,
10. Department of Product and Process Development, Sanquin Blood Bank, Amsterdam, The Netherlands,
11. Department of Blood Cell Research, Sanquin Research, and Landsteiner Laboratory, University of Amsterdam, Amsterdam, The Netherlands,
12. Department of Transfusion Medicine, Sanquin Blood Bank, Amsterdam, The Netherlands
13. Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, The Netherlands.

Vox Sanguinis 2023, published online

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-

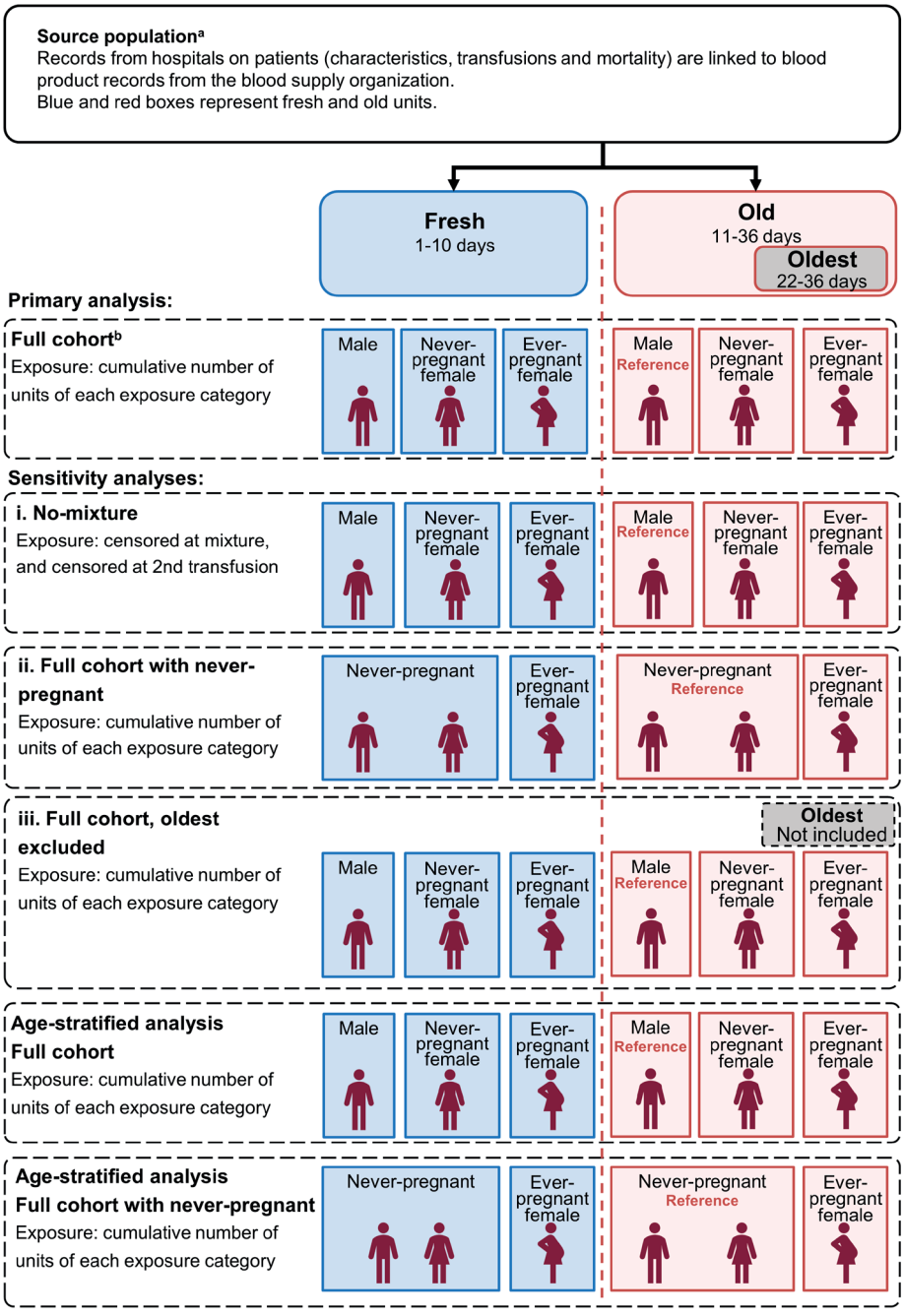


Figure 1

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)

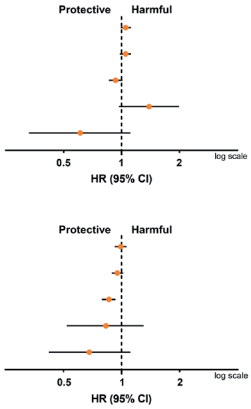


Figure 2

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.

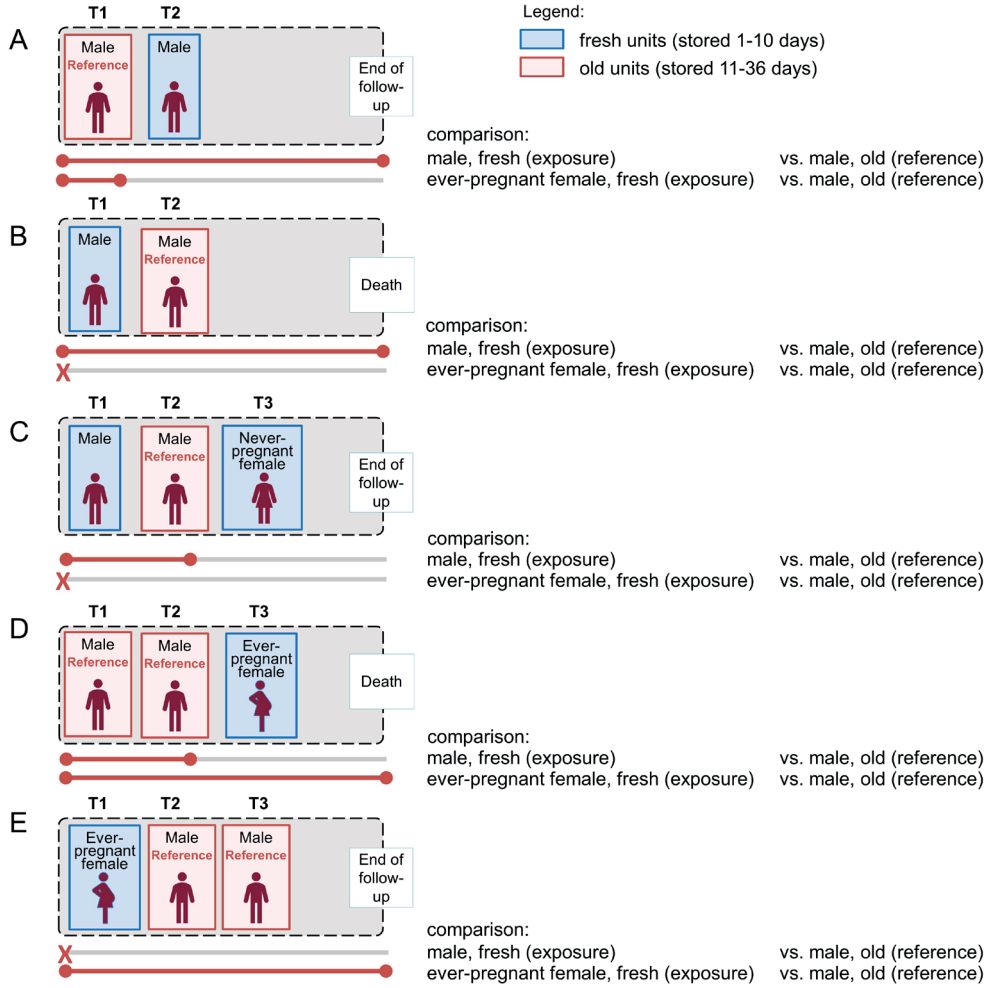


Figure S1. Explanatory graphic with five patient examples for reference and exposure groups in the full cohort

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]

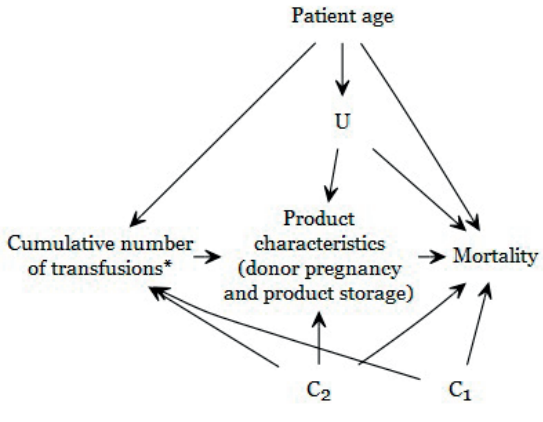


Figure S2. Directed Acyclic Graph of the Effect of Product Characteristics (Donor Sex, Pregnancy and Product Storage) on Mortality

*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/.

4

Chapter 4

Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a rapid review

Authors: Sarah J Valk[1,2,†], Vanessa Piechotta[3,‡] , Khai Li Chai[4,a] , Carolyn Doree[5], Ina Monsef[3] , Erica M Wood[4], Abigail Lamikanra[6], Catherine Kimber[5], Zoe McQuilten[4], Cynthia So-Osman[7,8], Lise J Estcourt[9], Nicole Skoetz[10]

1. Jon J van Rood Center for Clinical Transfusion Research, Sanquin/Leiden University Medical Center, Leiden, Netherlands

2. Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands

3. Cochrane Haematology, Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

4. Transfusion Research Unit, School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia

5. Systematic Review Initiative, NHS Blood and Transplant, Oxford, UK

6. Clinical, Research and Development, NHS Blood and Transplant, Oxford, UK

7. Sanquin Blood Bank, Amsterdam, Netherlands

8. Erasmus Medical Centre, Rotterdam, Netherlands

9. Haematology/Transfusion Medicine, NHS Blood and Transplant, Oxford, UK

10. Cochrane Cancer, Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

Cochrane Database Syst Rev. 2020 May 14;5(5):CD013600

Abstract

Background

Convalescent plasma and hyperimmune immunoglobulin may reduce mortality in patients with respiratory virus diseases, and are currently being investigated in trials as a potential therapy for coronavirus disease 2019 (COVID-19). A thorough understanding of the current body of evidence regarding the benefits and risks is required.

Objectives

To assess whether convalescent plasma or hyperimmune immunoglobulin transfusion is effective and safe in the treatment of people with COVID-19.

Search methods

The protocol was pre-published with the Center for Open Science and can be accessed here: osf.io/dwf53

We searched the World Health Organization (WHO) COVID-19 Global Research Database, MEDLINE, Embase, Cochrane COVID-19 Study Register, Centers for Disease Control and Prevention COVID-19 Research Article Database and trials registries to identify ongoing studies and results of completed studies on 23 April 2020 for case-series, cohort, prospectively planned, and randomised controlled trials (RCTs).

Selection criteria

We followed standard Cochrane methodology and performed all steps regarding study selection in duplicate by two independent review authors (in contrast to the recommendations of the Cochrane Rapid Reviews Methods Group).

We included studies evaluating convalescent plasma or hyperimmune immunoglobulin for people with COVID-19, irrespective of disease severity, age, gender or ethnicity.

We excluded studies including populations with other coronavirus diseases (severe acute respiratory syndrome (SARS) or Middle East respiratory syndrome (MERS)) and studies evaluating standard immunoglobulins.

Data collection and analysis

We followed recommendations of the Cochrane Rapid Reviews Methods Group regarding data extraction and assessment.

To assess bias in included studies, we used the assessment criteria tool for observational studies, provided by Cochrane Childhood Cancer. We rated the certainty of evidence using the GRADE approach for the following outcomes: all-cause mortality at hospital discharge, improvement of clinical symptoms (7, 15, and 30 days after transfusion), grade 3 and 4 adverse events, and serious adverse events.

Main results

We included eight studies (seven case-series, one prospectively planned, single-arm intervention study) with 32 participants, and identified a further 48 ongoing studies evaluating convalescent plasma (47 studies) or hyperimmune immunoglobulin (one study), of which 22 are randomised.

Overall risk of bias of the eight included studies was high, due to: study design; small number of participants; poor reporting within studies; and varied type of participants with different severities of disease, comorbidities, and types of previous or concurrent treatments, including antivirals, antifungals or antibiotics, corticosteroids, hydroxychloroquine and respiratory support.

We rated all outcomes as very low certainty, and we were unable to summarise numerical data in any meaningful way. As we identified case-series studies only, we reported results narratively.

Effectiveness of convalescent plasma for people with COVID-19

The following reported outcomes could all be related to the underlying natural history of the disease or other concomitant treatment, rather than convalescent plasma.

All-cause mortality at hospital discharge

All studies reported mortality. All participants were alive at the end of the reporting period, but not all participants had been discharged from hospital by the end of the study (15 participants discharged, 6 still hospitalised, 11 unclear). Follow-up ranged from 3 days to 37 days post-transfusion. We do not know whether convalescent plasma therapy affects mortality (very low-certainty evidence).

Improvement of clinical symptoms (assessed by respiratory support)
Six studies, including 28 participants, reported the level of respiratory support required; most participants required respiratory support at baseline. All studies reported improvement in clinical symptoms in at least some participants. We do not know whether convalescent plasma improves clinical symptoms (very low-certainty evidence).

Time to discharge from hospital
Six studies reported time to discharge from hospital for at least some participants, which ranged from four to 35 days after convalescent plasma therapy.

Admission on the intensive care unit (ICU)
Six studies included patients who were critically ill. At final follow-up the majority of these patients were no longer on the ICU or no longer required mechanical ventilation.

Length of stay on the ICU
Only one study (1 participant) reported length of stay on the ICU. The individual was discharged from the ICU 11 days after plasma transfusion.

Safety of convalescent plasma for people with COVID-19

Grade 3 or 4 adverse events
The studies did not report the grade of adverse events after convalescent plasma transfusion. Two studies reported data relating to participants who had experienced adverse events, that were presumably grade 3 or 4. One case study reported a participant who had moderate fever (38.9 °C). Another study (3 participants) reported a case of severe anaphylactic shock. Four studies reported the absence of moderate or severe adverse events (19 participants). We are very uncertain whether or not convalescent plasma therapy affects the risk of moderate to severe adverse events (very low-certainty evidence).

Serious adverse events
One study (3 participants) reported one serious adverse event. As described above, this individual had severe anaphylactic shock after receiving convalescent plasma. Six studies reported that no serious adverse events occurred. We are very uncertain whether or not convalescent plasma therapy affects the risk of serious adverse events (very low-certainty evidence).

Authors' conclusions

We identified eight studies (seven case-series and one prospectively planned single-arm intervention study) with a total of 32 participants (range 1 to 10). Most studies assessed the risks of the intervention; reporting two adverse events (potentially grade 3 or 4), one of which was a serious adverse event. We are very uncertain whether convalescent plasma is effective for people admitted to hospital with COVID-19 as studies reported results inconsistently, making it difficult to compare results and to draw conclusions. We identified very low-certainty evidence on the effectiveness and safety of convalescent plasma therapy for people with COVID-19; all studies were at high risk of bias and reporting quality was low.

No RCTs or controlled non-randomised studies evaluating benefits and harms of convalescent plasma have been completed. There are 47 ongoing studies evaluating convalescent plasma, of which 22 are RCTs, and one trial evaluating hyperimmune immunoglobulin. We will update this review as a living systematic review, based on monthly searches in the above mentioned databases and registries. These updates are likely to show different results to those reported here.

Plain language summary

Plasma from people who have recovered from COVID-19 to treat individuals with COVID-19

Background

Coronavirus (COVID-19) is a highly infectious respiratory illness caused by a new strain of virus. The outbreak has spread rapidly on a global scale. People infected with this virus may not show signs of the disease, others may develop symptoms, including fever, cough, shortness of breath and sore throat. In some people the infection is more severe and can cause severe breathing difficulties, leading to hospitalisation, admission to intensive care or death. Currently, no vaccine or specific treatment is available.

People who have recovered from COVID-19 develop natural defences to the disease in their blood (antibodies). Antibodies are found in part of the blood called plasma. Plasma from blood donated from recovered patients, which contains COVID-19 antibodies, can be used to make two preparations. Firstly,

convalescent plasma, which is plasma that contains these antibodies. Secondly, hyperimmune immunoglobulin, which is more concentrated, and therefore contains more antibodies.

Convalescent plasma and hyperimmune immunoglobulin have been used successfully to treat other respiratory viruses. These treatments (given by a drip or injection) are generally well-tolerated, but unwanted effects can occur.

What did we want to find?

We wanted to know whether plasma from people who have recovered from COVID-19 is an effective treatment for people with COVID-19, and whether this treatment causes any unwanted effects.

Our methods

We searched major medical databases for clinical studies on treatment with convalescent plasma or hyperimmune immunoglobulin for people with COVID-19. Studies could be conducted anywhere in the world and include participants of any age, gender or ethnicity, with mild, moderate or severe COVID-19.

COVID-19 is spreading rapidly, so we needed to answer this question quickly. This meant that we shortened some steps of the normal Cochrane Review process - only one review author extracted data from studies and assessed study quality; normally two review authors would do this.

Key results

We included eight completed studies, with 32 participants who received convalescent plasma. None of the studies randomly allocated participants to different treatments (randomised trials produce the best evidence). None of the studies included a group of people who did not receive convalescent plasma, as a comparison group.

All participants in the studies were alive at the end of follow-up, but not all had been discharged from hospital. Follow-up varied from 3 to 37 days after treatment with convalescent plasma.

Six studies used the level of breathing support that participants required as a measure of recovery. Breathing support included oxygen therapy, mechanical ventilation and the need for a special machine that oxygenates the blood. All six studies reported clinical improvement in at least some of their participants,

but it remains uncertain whether this improvement was related to convalescent plasma, another treatment, or the natural progression of the disease.

Six studies reported time to discharge from hospital for some of their participants, all of whom received convalescent plasma. The time to discharge ranged from 4 to 35 days after convalescent plasma treatment.

Six studies included participants with severe COVID-19. Most had improved at final follow-up, but this improvement may have been due to another treatment, the natural progression of the disease or convalescent plasma treatment.

Two participants reported unwanted effects related to convalescent plasma. One participant developed a fever, and a second participant experienced anaphylactic shock (severe allergic reaction) early on in the transfusion.

Certainty of the evidence

Our certainty (confidence) in the evidence was very limited because the studies were not randomised and did not use reliable methods to measure their results. Furthermore, they had only a small number of participants, who received various treatments alongside convalescent plasma, and some had underlying health problems.

Conclusion

We are very uncertain whether plasma from people who have recovered from COVID-19 is an effective treatment for people with COVID-19. The completed studies we found were poor quality and their results could be related to the natural progression of the disease, other treatments that the participants received, or to convalescent plasma. However, our searches found 48 ongoing studies: 47 evaluating convalescent plasma and 1 evaluating hyperimmune immunoglobulin, of which 22 are randomised. We will update this review with their results when these studies are completed.

Background

Description of the condition

The clinical syndrome coronavirus disease 2019 (COVID-19) is a new, rapidly emerging zoonotic infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; WHO 2020a). On 11 March 2020, the World Health

Organization (WHO) declared the current COVID-19 outbreak a pandemic, with the outbreak resulting in almost 3.5 million cases and over 239,000 deaths worldwide (WHO 2020b; WHO 2020c). Although there are similarities with historic coronavirus epidemics, with severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) responsible for 813 and 858 deaths respectively, the scale and impact of the COVID-19 pandemic presents unprecedented challenges to health facilities and healthcare workers all over the world (WHO 2007; WHO 2019).

With a preliminary hospitalisation rate of 12.3 patients per 100,000 population in the USA, COVID-19 has taken a toll on healthcare capacity, and especially on intensive care unit (ICU) capacity (CDC 2020a). Early reports of the case fatality rate suggest that it ranges between of 0.7% to 4%, with higher rates also reported (WHO 2020a; WHO 2020c). However, these numbers should be interpreted with great care due to the data pertaining to the early emergency response, which due to shortage of test kits has led to selective testing of people with severe disease, underreporting of cases and delays from confirmation of a case to time of death (Kim 2020). The median incubation period of SARS-CoV-2 was reported to be five days, with 97.5% of cases developing symptoms within 11.5 days of infection (Lauer 2020). Common signs and symptoms can include fever, dry cough, fatigue and sputum production (WHO 2020a). Other, less commonly reported signs and symptoms are shortness of breath, sore throat, headache, myalgia or arthralgia, chills, nausea or vomiting, nasal congestion, diarrhoea, haemoptysis and conjunctival congestion (WHO 2020a). Of the reported cases, 80% are estimated to have a mild or asymptomatic course of infection, and an estimated 5% of cases are admitted to the ICU with acute respiratory distress syndrome (ARDS), septic shock or multiple organ failure, or both (Team 2020; WHO 2020a). A risk factor for developing infection and progressing to severe disease is old age, with people aged over 80 years at highest risk of mortality. Other risk factors are cardiovascular disease, obesity, hypertension, diabetes, chronic respiratory disease, cancer and compromised immune status (Chen 2020; Huang 2020; Liang 2020; WHO 2020a; Wu 2020a).

SARS-CoV-2 is a positive-sense, single-stranded RNA (ribonucleic acid) virus with a large RNA genome. Although not much is known about the specific mechanisms underlying severe disease in COVID-19, there are indications that the virus is capable of inducing an excessive immune reaction in the host, with highly activated but decreased numbers of $CD4^+$ and $CD8^+$ T cells detected in the peripheral blood of people with COVID-19 (Xu 2020). Early reports also showed that people critically ill with COVID-19 frequently exhibit a hypercoagulable state and endothelial inflammation, which is hypothesised to lead to the high burden of thromboembolic

events seen in this population (Driggin 2020). Preliminary reports into the pathophysiology of SARS-CoV-2 have further indicated that the observed decrease in human angiotensin-converting enzyme 2 (ACE2) activity may play a role in causing the rapid deterioration of patient lung function (Tolouian 2020; Van de Veerdonk 2020). ACE2 is a protein that functions as the receptor facilitating entry of SARS-CoV-2 into the host cell, and is most abundant on type II alveolar cells in the lungs.

Description of the intervention

Convalescent plasma, convalescent serum and hyperimmune immunoglobulin prepared from convalescent plasma, are interventions that have been used in the past to treat conditions when no vaccine or pharmacological interventions were available. Diphtheria, pneumococcal pneumonia, hepatitis A and B, mumps, polio, measles and rabies are conditions where convalescent plasma has been shown to be effective (Eibl 2008).

A systematic review has shown that convalescent plasma may have clinical benefit for people with influenza and SARS (Mair-Jenkins 2015). This systematic review included observational studies and randomised controlled trials (RCTs) investigating the use of convalescent plasma, serum or hyperimmune immunoglobulin for treating severe acute respiratory infections of laboratory-confirmed or suspected viral aetiology, and included investigations with patients of any age and sex. Control interventions consisted of sham, or placebo, therapy and no therapy. The authors concluded that, although the included studies were generally small and of low quality, with a moderate to high risk of bias, the use of convalescent plasma may reduce mortality and appears safe (Mair-Jenkins 2015). The authors also suggested that the effectiveness of convalescent plasma in reducing hospital length of stay is dependent on early administration of the therapy, and use as prophylaxis is more likely to be beneficial than treating severe disease. However, the optimal timing and dosage of convalescent plasma therapy is unknown.

There is conflicting evidence about the effect of convalescent plasma or hyperimmune immunoglobulin for treating severe acute respiratory infections. Studies investigating the effectiveness of hyperimmune immunoglobulin for influenza have been contradictory, with some RCTs showing effectiveness (Hung 2013), whereas others show no benefit (Beigel 2017; Beigel 2019; Davey 2019).

Although convalescent plasma is generally thought to be a safe and well-tolerated therapy, adverse events can occur. Limited information is available about specific adverse events related to convalescent plasma therapy, but symptoms that have been reported are similar to those for other types of plasma blood

components, including fever or chills, allergic reactions, and transfusion-related acute lung injury (TRALI; Beigel 2019; Chun 2016; Luke 2006). Furthermore, the transfer of coagulation factors present in plasma products is potentially harmful for people with COVID-19, who are already at an increased risk of thromboembolic events (Driggin 2020). Plasma transfusions are also known to cause transfusion-associated circulatory overload (TACO). TACO and TRALI are especially important to consider, because COVID-19 patients with comorbidities, who might be eligible for experimental treatment with convalescent plasma therapy, are at an increased risk of these adverse events. There are risk-mitigation strategies that can be implemented to prevent TRALI. These include limiting donations from female donors, especially those with a history of pregnancy, and screening of donors for antibodies that are implicated in TRALI (Otrock 2017). In addition to the aforementioned adverse events, transfusion-transmitted infections, red blood cell alloimmunisation and haemolytic transfusion reactions have also been described following plasma transfusion, although they are less common (Pandey 2012). Pathogen inactivation can be implemented to decrease the risk of transmitting infections by transfusion (Rock 2011).

When compared to convalescent plasma, hyperimmune immunoglobulin has the advantage of preventing transfer of potentially harmful coagulation factors that are present in plasma products. The amount and antibody concentration can be more accurately dosed compared to convalescent plasma, and hyperimmune immunoglobulin can be prepared in a consistent manner (Hung 2013). Not many studies have reported on adverse events of hyperimmune immunoglobulin, but the safety profile of standard intravenous immunoglobulin is known and the adverse events reported here are also likely to occur in hyperimmune immunoglobulin therapy. Common adverse events of intravenous immunoglobulin that occur immediately after administration are: infusion site pain; swelling and erythaema; and immediate systemic reactions, such as head and body aches, chills and fever (Stiehm 2013). Other, less common early adverse reactions to immunoglobulin therapy are pulmonary complications, such as pulmonary embolism, pulmonary oedema and pleural effusion, with TRALI also reported (Baudel 2020; Stiehm 2013). Anaphylactic and anaphylactoid reactions to immunoglobulin therapy are rare (Brennan 2003; Stiehm 2013). Delayed adverse events of immunoglobulin therapy, which occur within hours to days of initiation of immunoglobulin therapy, are persistent headaches (common), aseptic meningitis, renal failure, thromboembolic events, and haemolytic reactions (Sekul 1994; Stiehm 2013). Transmission of infectious agents has been described after administration of intravenous immunoglobulin, but this risk is considered to be low (Stiehm 2013). Other, severe adverse events that occur late after administration are lung disease, enteritis and dermatological disorders (Stiehm 2013).

A theoretical risk related to virus-specific antibodies, which are transferred with convalescent plasma and hyperimmune immunoglobulin administration, is antibody-dependent enhancement of infection (Morens 1994). Here, virus-binding antibodies facilitate the entry and replication of virus particles into monocytes, macrophages and granulocytic cells and thereby increase the risk of more severe disease in the infected host. Although antibody-dependent enhancement has not been demonstrated in COVID-19, it has been seen with previous coronavirus infections when the antibodies given targeted a different serotype of the virus (Wan 2020; Wang 2014). A mechanism for antibody-dependent enhancement in COVID-19 has recently been proposed, with non-neutralising antibodies to variable S domains potentially enabling an alternative infection pathway via Fc receptor-mediated uptake (Ricke 2020). Antibody-dependent enhancement is therefore a potentially harmful consequence of convalescent plasma and hyperimmune immunoglobulin therapy for COVID-19.

In summary, the benefits of the intervention, both for convalescent plasma or hyperimmune immunoglobulin, should be carefully considered in view of the risks of adverse events.

How the intervention might work

Convalescent plasma contains pathogen-specific neutralising antibodies, which can neutralise viral particles, and treatment with convalescent plasma or hyperimmune immunoglobulins confers passive immunity to recipients. The duration of conferred protection can differ depending on the timing of administration, ranging from weeks to months after treatment (Casadevall 2020).

By neutralising SARS-CoV-2 particles, early treatment with convalescent plasma is postulated to increase the patient's own capacity to clear the initial inoculum (Casadevall 2020; Robbins 1995). This could lead to a reduction in mortality and fewer hospitalised patients progressing to the ICU. Furthermore, convalescent plasma may reduce the length of ICU stay in critically ill patients (Mair-Jenkins 2015), thus helping to lift pressure from global healthcare systems and increasing ICU capacity.

Preliminary evidence in humans and rhesus macaques has shown that reinfection with SARS-CoV-2 is not likely, with most (but not all) patients who recovered from COVID-19 producing sufficient amounts of neutralising antibodies to protect against reinfection (Bao 2020; Wu 2020b). This implies that convalescent plasma from people who have recovered from SARS-CoV-2 infection is capable of conferring passive immunity. A recently reported case series also indicated

sufficient neutralising antibody titres in convalescent plasma to neutralise SARS-CoV-2 in five COVID-19 patients, who all recovered after treatment (Shen 2020). It is important to note, however, that research in other coronavirus species has shown that immunity may not be long-lasting, with two to three years of protection estimated from work with SARS and MERS (Mo 2006; Payne 2016). Furthermore, there are indications that the severity of infection has an impact on antibody titres, with less severe disease leading to lower neutralising antibody response in people with SARS and COVID-19 (Ho 2005; Zhao 2020).

Why it is important to do this review

There is a clear, urgent need for more information to guide clinical decision-making for COVID-19 patients. Pharmacological interventions have not yet proven to be effective, and current treatment consists of supportive care with extracorporeal membrane oxygenation in severe cases and oxygen supply in mild cases (CDC 2020b; WHO 2020d). A vaccine could aid in inducing immunity in the population and preventing transmission to those who are at risk for severe disease, but no vaccine is currently available, although multiple candidate vaccines are in development. Until these vaccines are available and distributed, convalescent plasma is a potential therapy for COVID-19 patients. Convalescent plasma, and hyperimmune immunoglobulin to a certain extent, can be prepared and made rapidly available by blood banks and hospitals when enough potential donors have recovered from the infection, using readily available materials and methods (Bloch 2020). However, its safety and efficacy are not well characterised, and there are costs associated with pursuing the use of convalescent plasma for treatment of COVID-19.

A multitude of clinical trials investigating the safety and effectiveness of convalescent plasma or hyperimmune immunoglobulins have been announced, and their results will need to be interpreted with care. Thus, there needs to be a thorough understanding of the current body of evidence regarding the use of convalescent plasma for people with COVID-19, and an extensive review of the available literature is required.

Objectives

To assess whether convalescent plasma or hyperimmune immunoglobulin transfusion is effective and safe in the treatment of people with COVID-19.

Methods

Criteria for considering studies for this review

Types of studies

The protocol for this review was registered with the Center for Open Science (Piechotta 2020).

As planned at the protocol stage, we included prospective non-comparative study designs (e.g. case series), because there was no evidence from randomised controlled trials (RCTs), non-randomised studies of interventions (NRSIs), and only one prospective observational study available (please find further explanations in Appendix 1). We followed the suggestions specified in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2019a), as far as possible, and applied the methodology outlined in the following sections. We considered studies including one or more participant(s) with coronavirus disease 2019 (COVID-19).

We included full-text publications, abstract publications, and results published in trials registries, if sufficient information was available on study design, characteristics of participants, interventions and outcomes. We did not apply any limitation with respect to the length of follow-up.

Types of participants

We included individuals with a confirmed diagnosis of COVID-19, with no age, gender or ethnicity restrictions.

We excluded studies including populations with other coronavirus diseases (severe acute respiratory syndrome (SARS) or Middle East respiratory syndrome (MERS)). We also excluded studies including populations with mixed virus diseases (e.g. influenza), unless the trial authors provided subgroup data for people with COVID-19.

Types of interventions

We included the following interventions.

- Convalescent plasma from people who recovered from SARS-CoV-2 infection
- Hyperimmune immunoglobulin therapy

We did not include studies on standard immunoglobulin.

In future updates we plan to include the following comparisons for studies with a control arm.

- Convalescent plasma versus standard care or placebo

- Convalescent plasma therapy versus control treatment, for example, drug treatments (including but not limited to hydroxychloroquine, remdesivir). Co-interventions will be allowed, but must be comparable between intervention groups.
- Convalescent plasma therapy versus hyperimmune immunoglobulin
- Hyperimmune immunoglobulin versus standard care or placebo
- Hyperimmune immunoglobulin versus control treatment, for example, drug treatments (including but not limited to hydroxychloroquine, remdesivir). Co-interventions will be allowed, but must be comparable between intervention groups.

Types of outcome measures

We evaluated core outcomes as pre-defined by the Core Outcome Measures in Effectiveness Trials Initiative for Covid-19 patients (COMET 2020).

Primary outcomes

Effectiveness of convalescent plasma for people with COVID-19

- All-cause mortality at hospital discharge
- Time to death

Secondary outcomes

Effectiveness of convalescent plasma for people with COVID-19

- Improvement of clinical symptoms, assessed by need for respiratory support at up to 7 days; 8 to 15 days; 16 to 30 days:
  - oxygen by mask or nasal prongs
  - oxygen by non-invasive ventilation (NIV) or high-flow
  - intubation and mechanical ventilation
  - mechanical ventilation plus high-flow oxygen
  - extracorporeal membrane oxygenation (ECMO)
- 30-day and 90-day mortality
- Time to discharge from hospital
- Admission on the ICU
- Length of stay on the ICU

Safety of convalescent plasma for people with COVID-19

- Number of participants with grade 3 and grade 4 adverse events, including potential relationship between intervention and adverse reaction (e.g. TRALI,

transfusion-transmitted infection, TACO, transfusion-associated dyspnoea (TAD), acute transfusion reactions)
- Number of participants with serious adverse events

Timing of outcome measurement

For time-to-event outcomes, such as mortality, discharge from hospital, and improvement of clinical symptoms, we included outcome measures representing the longest follow-up time available.

We included all other outcome categories for the observational periods that the study publications reported. We included those adverse events occurring during active treatment and had planned to include long-term adverse events as well. If sufficient data had been available, we planned to group the measurement time points of eligible outcomes, for example, adverse events and serious adverse events, into those measured directly after treatment (up to seven days after treatment), medium-term outcomes (15 days after treatment) and longer-term outcomes (over 30 days after treatment).

Search methods for identification of studies

We searched for studies in all languages in order to limit language bias. However, we prioritised articles in languages that our review team could accommodate (these are English, Dutch, German, French, Italian, Malay and Spanish). We did not seek translators for this version of the review. We tagged all references in additional languages as 'awaiting classification' and will seek translators via Cochrane TaskExchange in an update of this review.

Electronic searches

We designed and tested search strategies for electronic databases according to methods suggested in the *Cochrane Handbook for Systematic Reviews of Interventions* (Lefebvre 2019), CD developed them and Cochrane Haematology's Information Specialist (IM) peer reviewed them. In this emerging field, we expected that at least the abstract would be in English. If studies are published in other languages than those our review team could accommodate (English, Dutch, German, French, Italian, Malay and Spanish), we plan to involve Cochrane TaskExchange to identify people within Cochrane to translate these studies for an update of this review.

As publication bias might influence all subsequent analyses and conclusions, we searched all potential relevant trials registries in detail to detect ongoing as well as completed studies, but not yet published studies. Nowadays, it is mandatory

to provide results at least in the trials registry. In case results were not published elsewhere, we had planned to extract and analyse these data. However, no outcome data have yet been added to the trials registries (also stated in Differences between protocol and review).

We searched the following databases and sources, from 1 January 2019 to 23 April 2020.

- Databases of medical literature
  - WHO COVID-19 Global Research Database (search.bvsalud.org/global-research-on-novel-coronavirus-2019-ncov/advanced/?lang=en), searched 23 April 2020; Appendix 2
  - MEDLINE (Ovid, 1 January 2019 to 23 April 2020), Appendix 3
  - Embase (Ovid, 1 January 2019 to 23 April 2020), Appendix 4
  - PubMed (for epublications ahead of print only; searched 23 April 2020), Appendix 5
  - Center for Disease Control and Prevention COVID-19 Research Article Database (www.cdc.gov/library/researchguides/2019novelcoronavirus/databasesjournals.html; downloaded 22 April 2020), Appendix 6
  - Cochrane COVID-19 Study Register (covid-19.cochrane.org; searched 23 April 2020), Appendix 7
- Trials registries and registry platforms to identify ongoing studies and results of completed studies
  - ClinicalTrials.gov - COVID-19 Subset (clinicaltrials.gov/ct2/results?cond=COVID-19; searched 23 April 2020), Appendix 8
  - WHO International Clinical Trials Registry Platform (ICTRP) - COVID-19 Subset (www.who.int/ictrp/en); searched 23 April 2020), Appendix 9

Searching other resources

In an update of this rapid review we plan to:

- handsearch the reference lists of all identified studies, relevant review articles and current treatment guidelines for further literature; and
- contact experts in the field, drug manufacturers and regulatory agencies in order to retrieve information on unpublished studies.

Data collection and analysis

Selection of studies

Two out of four review authors (SJV, KLC, VP, NS) independently screened the results of the search strategies for eligibility for this review by reading the abstracts using Covidence software. We coded the abstracts as either 'retrieve'

or 'do not retrieve'. In the case of disagreement or if it was unclear whether we should retrieve the abstract or not, we obtained the full-text publication for further discussion. Two review authors assessed the full-text articles of selected studies. If the two review authors were unable to reach a consensus, they consulted a third review author to reach a final decision.

We documented the study selection process in a flow chart, as recommended in the PRISMA statement (Moher 2009), and show the total numbers of retrieved references and the numbers of included and excluded studies. We list all articles that we excluded after full-text assessment and the reasons for their exclusion in the Characteristics of excluded studies table.

Data extraction and management

One review author (SJV or KLC) performed all data extractions and assessments. Two other review authors (VP, NS) verified the accuracy and (where applicable) the plausibility of extractions and assessment.

One review author (VP or NS) assessed eligible studies obtained in the process of study selection (as described above) for methodological quality and risk of bias, the other review author verified the 'Risk of bias' assessment.

One review author (SJV or KLC) extracted data using a customised data extraction form developed in Microsoft Excel (Microsoft Corporation 2018); please see Differences between protocol and review). Another review author (NS) verified the accuracy and (where applicable) the plausibility of extractions and assessment. We conducted data extraction according to the guidelines proposed by Cochrane (Li 2019). If the review authors were unable to reach a consensus, we consulted a third review author (VP).

We collated multiple reports of one study so that the study, and not the report, is the unit of analysis.

We extracted the following information.

- General information: author, title, source, publication date, country, language, duplicate publications
- Quality assessment: study design, confounding, definition of risk estimates, selection bias, attrition bias, detection bias, reporting bias
- Study characteristics: trial design, setting and dates, source of participants, inclusion/exclusion criteria, comparability of groups, treatment cross-overs, compliance with assigned treatment, length of follow-up

- Participant characteristics: age, gender, ethnicity, number of participants recruited/allocated/evaluated, disease, severity of disease, additional diagnoses, previous treatments (e.g. experimental drug therapies, oxygen therapy, ventilation)
- Interventions: convalescent plasma therapy or hyperimmune immunoglobulin therapy, concomitant therapy, duration of follow-up
  - For studies including a control group: comparator (type)
- Outcomes
  - Effectiveness of convalescent plasma for people with COVID-19:
    - all-cause mortality at hospital discharge
    - time to death
    - improvement of clinical symptoms, assessed through need for respiratory support at up to 7 days; 8 to 15 days; 16 to 30 days
    - 30-day and 90-day mortality
    - time to discharge from hospital
    - admission on the ICU
    - length of stay on the ICU
  - Safety of convalescent plasma for people with COVID-19:
    - number of participants with grade 3 and grade 4 adverse events, including potential relationship between intervention and adverse reaction (e.g. TRALI, transfusion-transmitted infection, TACO, TAD, acute transfusion reactions)
    - number of participants with serious adverse events

Assessment of risk of bias in included studies

If RCT data had been available, we had planned to use the Risk of Bias 2.0 (RoB 2) tool to analyse the risk of bias in the underlying study results (Sterne 2019). If non-randomised studies of interventions (NRSIs) data had been available, we had planned to use the Risk Of Bias in Non-randomised Studies - of Interventions (ROBINS-I) tool (Sterne 2016). Please refer to Appendix 1 for detailed information regarding how we had planned to assess the risk of bias of RCTs and NRSIs.

Non-controlled, prospectively planned studies

As specified in the Types of studies section we only included non-controlled prospective studies because we did not identify any controlled studies.

One review author (VP or NS) assessed eligible studies for methodological quality and risk of bias (using the 'Risk of bias' assessment criteria for observational studies tool provided by Cochrane Childhood Cancer (see Table 1; Mulder 2019). A second review author (VP or NS) verified the accuracy and the plausibility. Any 'Risk of bias' judgements were performed and presented per outcome per study.

The quality assessment strongly depends upon information on the design, conduct and analysis of the trial. The two review authors (VP, NS) resolved any disagreements regarding the quality assessments by discussion, in case of disagreement they would have consulted a third review author (SJV or KLC).

We assessed the following domains of bias.

- Internal validity
  - Unrepresentative study group (selection bias)
  - Incomplete outcome assessment/follow-up (attrition bias)
  - Outcome assessors unblinded to investigated determinant (detection bias)
  - Important prognostic factors or follow-up not taken adequately into account (confounding)
- External validity
  - Poorly defined study group (reporting bias)
  - Poorly defined follow-up (reporting bias)
  - Poorly defined outcome (reporting bias)
  - Poorly defined risk estimates (analyses)

For every criterion, we made a judgement using one of three response options.

- High risk of bias
- Low risk of bias
- Unclear risk of bias

Measures of treatment effect

Please refer to Appendix 1 for information regarding how we had planned to measure the treatment effects of RCTs and NRSIs.

Uncontrolled studies

For uncontrolled studies we did not carry out an analysis using quantitative data from indirect controls, as we are aware of the difficulties of indirect comparisons of participant groups with varying baseline characteristics, especially in the absence of individual patient data. Because authors of one-arm, non-comparative studies, often discuss their findings using information from other intervention and observational studies as implicit controls, we discussed our findings extensively in the context of what is known about the outcome of 'comparable' patients receiving other experimental treatments but not convalescent plasma therapy or hyperimmune immunoglobulin therapy. We did not meta-analyse the data but provided information from individual studies within tables.

Unit of analysis issues

As we identified uncontrolled studies only, meta-analysis was not appropriate. Instead, we narratively described and presented results per study in tables.

Please refer to Appendix 1 for information regarding how we had planned to combine studies with multiple treatment groups.

Dealing with missing data

Chapter 6 of the *Cochrane Handbook for Systematic Reviews of Interventions* suggests a number of potential sources for missing data, which we will need to take into account: at study level, at outcome level and at summary data level (Higgins 2019b). In the first instance, it is of the utmost importance to differentiate between data 'missing at random' and 'not missing at random'.

We will request missing data from the study authors in an update of this review. If, after this, data are still missing, we will have to make explicit assumptions of any methods the included studies used. For example, we will assume that the data were missing at random or we will assume that missing values had a particular value, such as a poor outcome.

Assessment of heterogeneity

As we identified uncontrolled studies only, meta-analysis was not appropriate. Instead, we narratively described and presented results per study in tables.

Please refer to Appendix 1 for information regarding how we had planned to assess heterogeneity.

Assessment of reporting biases

As mentioned above, we searched trials registries to identify completed studies that have not been published elsewhere, to minimise or determine publication bias.

In an update of this review, we intend to explore potential publication bias by generating a funnel plot and statistically testing this by conducting a linear regression test (Sterne 2019), for meta-analyses involving at least 10 studies. We will consider $P < 0.1$ as significant for this test.

Data synthesis

Please refer to Appendix 1 for information regarding how we had planned to synthesise data from RCTs and NRSIs.

We did not meta-analyse data from uncontrolled trials, as there might be no additional benefit in meta-analysing data without a control group. We reported outcome data of each included trial within tables.

As data did not allow quantitative assessment, we presented outcome data individually per study within tables.

Subgroup analysis and investigation of heterogeneity

In an update of this review, we plan to perform subgroup analyses of the following characteristics.

- Age of participants (divided into applicable age groups, e.g. children; 18 to 65 years, 65 years and older)
- Severity of condition
- Pre-existing conditions (diabetes, respiratory disease, hypertension, immunosuppression)

We will use the tests for interaction to test for differences between subgroup results.

Sensitivity analysis

In an update of this review, we will perform only one sensitivity analysis for the following.

- 'Risk of bias' assessment components (low risk of bias versus high risk of bias)

To assess the influence of study quality on an outcome, we will perform sensitivity analyses per outcome, comparing studies with at least one domain of high risk of bias to those without high risk of bias.

- Influence of completed, but not published studies
- Influence of premature termination of studies

Summary of findings and assessment of the certainty of the evidence

We used the GRADE approach to assess the certainty of the evidence for the following outcomes (please find the rationale for the amendment of graded outcomes in the Differences between protocol and review).

- All-cause mortality at hospital discharge
- Time to death
- Clinical improvement (assessed by need for respiratory support) at the following time points
  - 7 days post-convalescent plasma transfusion
  - 15 days post-convalescent plasma transfusion

- 30 days post-convalescent plasma transfusion
- Grade 3 and 4 adverse events
- Serious adverse events

We used GRADEpro GDT software to create an 'evidence profile'. We will also use the GRADEpro GDT software to create a 'Summary of findings' table, as suggested in the *Cochrane Handbook for Systematic Reviews of Interventions* when results of controlled trials are available (Schünemann 2019).

Results

Description of studies

Results of the search

We identified 1267 potentially relevant references. After removing duplicates, we screened 1039 references based on their titles and abstracts, and we excluded 956 references that were irrelevant because they did not meet the prespecified inclusion criteria. We evaluated the remaining 83 references and screened the full texts, or, if these were not available, abstract publications or trials registry entries. Of these, we classified two studies as awaiting classification for this review (Qiu 2020; Tu 2020).

We identified 56 potentially eligible studies within 57 citations: eight completed studies (Ahn 2020; Duan 2020; Pei 2020; Shen 2020; Tan 2020; Ye 2020; Zhang 2020a; Zhang 2020b), and 48 ongoing studies (ChiCTR2000029757; ChiCTR2000029850; ChiCTR2000030010; ChiCTR2000030039; ChiCTR2000030179; ChiCTR2000030627; ChiCTR2000030702; ChiCTR2000030841; ChiCTR2000030929; ChiCTR2000031501; EUCTR2020-001310-38; IRCT20151228025732N53; IRCT20200310046736N1; IRCT20200325046860N1; IRCT20200404046948N1; IRCT20200409047007N1; IRCT20200413047056N1; NCT04264858; NCT04292340; NCT04321421; NCT04327349; NCT04332380; NCT04332835; NCT04333251; NCT04333355; NCT04338360; NCT04340050; NCT04342182; NCT04343261; NCT04343755; NCT04344535; NCT04345289; NCT04345523; NCT04345679; NCT04345991; NCT04346446; NCT04346589; NCT04347681; NCT04348656; NCT04348877; NCT04352751; NCT04353206; NCT04354831; NCT04355767; NCT04355897; NCT04356482; NCT04356534; NCT04357106). See PRISMA flow diagram (Figure 1; Moher 2009).

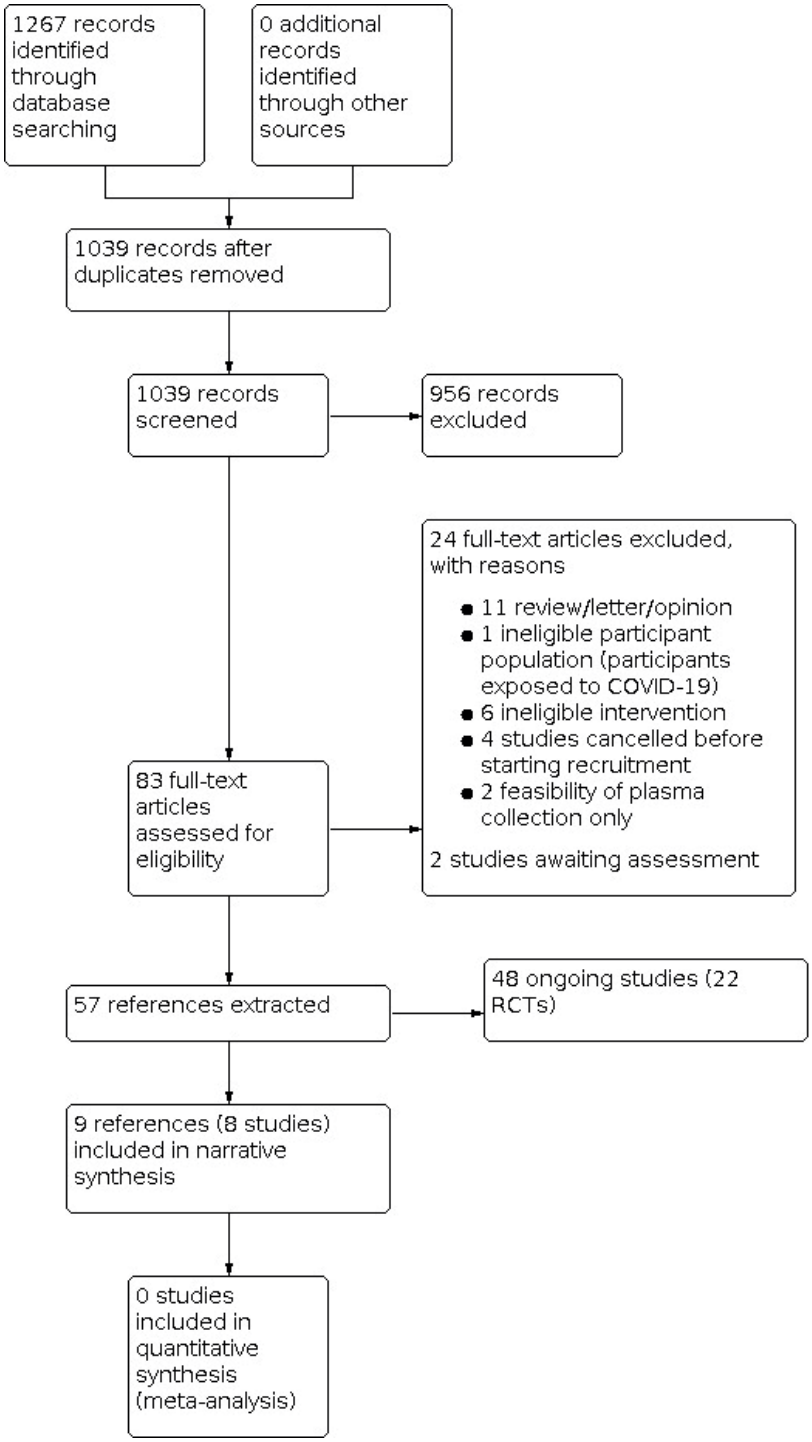


Figure 1.

Study flow diagram

Included studies

We included eight studies describing 32 participants in this review (Ahn 2020; Duan 2020; Pei 2020; Shen 2020; Tan 2020; Ye 2020; Zhang 2020a; Zhang 2020b). The eight included studies were all uncontrolled studies, seven studies were case series (Ahn 2020; Pei 2020; Shen 2020; Tan 2020; Ye 2020; Zhang 2020a; Zhang 2020b), and one was a prospectively registered single-arm intervention study (Duan 2020). Of the eight included studies, seven originated from China (Duan 2020; Pei 2020; Shen 2020; Tan 2020; Ye 2020; Zhang 2020a; Zhang 2020b), and one from South Korea (Ahn 2020). In seven of the eight studies, convalescent plasma was transfused in critically ill individuals (Ahn 2020; Duan 2020; Pei 2020; Shen 2020; Ye 2020; Zhang 2020a; Zhang 2020b). One study described a person with moderate disease severity (Pei 2020), and one study described a hospitalised participant with moderate disease severity (Tan 2020).

The dose, volume and timing of convalescent plasma varied greatly between studies. The total volume of convalescent plasma transfused varied between 200 mL and 2400 mL, with participants receiving between one to eight doses of plasma. Antibody titres were reported in four studies (Duan 2020; Pei 2020; Shen 2020; Zhang 2020b). Characteristics of the donors of convalescent plasma also varied between studies, although reporting was not complete. Out of the eight studies, only six reported information on plasma donors (Ahn 2020; Duan 2020; Pei 2020; Shen 2020; Ye 2020; Zhang 2020b). Most donors were male, but Pei 2020 included a female donor with a previous history of pregnancy. The age of the donors varied: Ahn 2020 included donors in their twenties; Shen 2020 included donors aged between 18 and 60 years; Duan 2020 included donors with a median age of 42 years; and Zhang 2020b included donors aged between 30 and 50. Some studies provided information on previously reported symptoms and disease severity of convalescent plasma donors (Ahn 2020; Duan 2020; Zhang 2020b). Ahn 2020 reported that the two included donors had been admitted to hospital with fever, cough and pneumonia. Duan 2020 reported that donors had been admitted to hospital, but no other information on severity of illness was available. Zhang 2020b reported that all six donors had fever and cough during the course of disease and were admitted to the hospital. In the five studies that reported assessment of donor recovery, all donors were symptom-free and completely recovered from coronavirus disease 2019 (COVID-19) prior to donating plasma (Ahn 2020; Duan 2020; Shen 2020; Ye 2020; Zhang 2020b). Four studies required a negative SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) test prior to convalescent plasma donation (Duan 2020; Shen 2020; Ye 2020; Zhang 2020b), with three studies requiring two consecutive negative results as a requirement for donation (Duan 2020; Ye 2020; Zhang 2020b). Four

studies used an enzyme-linked immunosorbent assay (ELISA) to quantify neutralising antibodies (Duan 2020; Pei 2020; Shen 2020; Zhang 2020b), with limited information available on the type of ELISA that was used. One study additionally used a plaque reduction neutralisation assay to assess the neutralising activity of the plasma (Duan 2020).

We had also planned to include studies on hyperimmune immunoglobulin therapy in this rapid review. However we did not identify any eligible studies.

Please refer to the Characteristics of included studies for more detailed information.

Ongoing studies

Of the 48 ongoing studies, 22 are RCTs (ChiCTR2000029757; ChiCTR2000030010; ChiCTR2000030179; ChiCTR2000030627; ChiCTR2000030702; ChiCTR2000030929; EUCTR2020-001310-38; IRCT20200310046736N1; IRCT20200404046948N1; IRCT20200409047007N1; IRCT20200413047056N1; NCT04332835; NCT04333251; NCT04342182; NCT04344535; NCT04345289; NCT04345991; NCT04345523; NCT04355767; NCT04346446; NCT04348656; NCT04356534).

Of these, 16 are expected to be completed in 2020 (ChiCTR2000030010; ChiCTR2000030179; ChiCTR2000030627; ChiCTR2000030702; ChiCTR2000030929; IRCT20200310046736N1; IRCT20200404046948N1; IRCT20200409047007N1; IRCT20200413047056N1; NCT04332835; NCT04342182; NCT04345523; NCT04345991; NCT04346446; NCT04348656; NCT04356534), and plan to evaluate between 15 and 1200 participants.

Two further large RCTs are planned to be completed in 2021: NCT04344535, randomising 500 participants and NCT04345289, evaluating 1500 participants.

Please refer to Characteristics of ongoing studies for more detailed information.

Excluded studies

We excluded 24 studies that did not match our inclusion criteria:

- 11 were a review of the literature, a letter or an opinion (Bloch 2020; Casadevall 2020; Chen 2020; Jawhara 2020; Roback 2020; Syal 2020; Tanne 2020; Tiberghien 2020; Wong 2020; Yoo 2020; Zhao 2020b);

- six studies were performed with an intervention other than convalescent plasma or hyperimmune immunoglobulin (Cao 2020; Díez 2020; Hu 2020; NCT04261426; Shi 2020; Xie 2020);
- four studies were cancelled by the investigator before recruiting participants into the study (ChiCTR2000030312; ChiCTR2000030381; ChiCTR2000030442; NCT04325672);
- two studies pertained to feasibility of collection of convalescent plasma only (NCT04344015; NCT04344977);
- one study included the wrong participant population (participants exposed to COVID-19; NCT04323800).

Risk of bias in included studies

Overall, we rated the risk of bias within and across studies to be serious. In addition to the high risk of bias due to the non-randomised study design, we assessed the internal and external validity as outlined in the 'Risk of bias' assessment criteria for observational studies tool provided by the Cochrane Childhood Cancer Group (see Additional Table 1; Mulder 2019). The full judgement per trial and category is presented in Figure 2 and the support for judgement in the Characteristics of included studies.

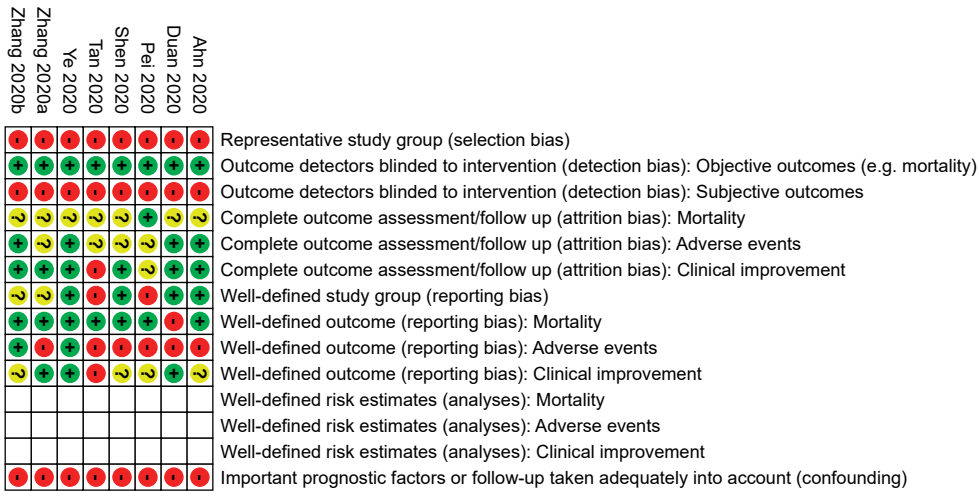


Figure 2.

'Risk of bias' summary: review authors' judgements about each 'Risk of bias' item for each included study

Allocation

All studies were at high risk of selection bias. We considered study groups not to be representative, as all studies included low numbers of participants (1 to 10 participants) with no control groups.

Blinding

All studies were unblinded and therefore at high risk of performance and detection bias for subjective outcomes. All outcomes apart from all-cause mortality are subjective to a greater or lesser extent and therefore at risk of bias.

Incomplete outcome data

We assessed attrition bias in terms of whether studies (equally) assessed outcomes for all participants. We evaluated attrition bias for three outcome categories.

Mortality

All studies assessed this outcome until discharge from hospital or the latest point of follow-up. We judged the risk for attrition bias to be unclear for seven studies (Ahn 2020; Duan 2020; Shen 2020; Tan 2020; Ye 2020; Zhang 2020a; Zhang 2020b), because some participants were either still hospitalised or it was unclear whether participants had been discharged. Therefore the outcome for these participants is unknown.

We judged the risk for attrition bias to be low for one study (Pei 2020), as all participants had been free of disease and were discharged from the hospital.

Adverse events

We judged the risk of attrition bias to be low for four studies (Ahn 2020; Duan 2020; Ye 2020; Zhang 2020b), because they assessed and reported adverse events for all participants.

We judged the risk of attrition bias to be unclear for the other four studies (Pei 2020; Shen 2020; Tan 2020; Zhang 2020a), because it was unclear whether they had assessed adverse events for all participants or whether they had selectively reported outcomes. Pei 2020 reported one serious adverse event occurring in one participant, however did not report whether they had assessed or observed other adverse events. Shen 2020 did not provide any information regarding the safety of plasma transfusion. Tan 2020 reported that their participant experienced moderate fever after the transfusion, however did not report whether other adverse events occurred. Zhang 2020a described that they had observed no adverse events for one of their participants after plasma transfusion, but did not provide any information regarding the occurrence of adverse events for the other participants. They stated in the conclusions that they had not observed any serious adverse events.

Clinical improvements
We judged the risk of bias to be low for six studies (Ahn 2020; Duan 2020; Shen 2020; Ye 2020; Zhang 2020a; Zhang 2020b), because they assessed and reported clinical improvements for all participants.

We judged the risk of attrition bias to be high for one study (Tan 2020), because it was unclear why the participant was still hospitalised and they did not report clinical improvements.

Pei 2020 did not report the course of disease after convalescent plasma transfusion so we judged it at unclear risk of bias for this domain.

Selective reporting

We assessed reporting bias in terms of whether the study group and intervention were well-defined and whether the outcomes were equally reported for all participants and the length of follow-up was mentioned.

Well-defined study group and intervention
We judged the risk of reporting bias to be low for four studies (Ahn 2020; Duan 2020; Shen 2020; Ye 2020), because both the study population and intervention were well described.

Zhang 2020a described the study population, but reported only limited information on the intervention. Zhang 2020b provided clear information on the intervention, but scarcely described the participant. We therefore judged the risk of reporting bias to be unclear for these two studies.

We judged the risk of bias to be high for two studies (Pei 2020; Tan 2020), which only reported limited information on the study population and the intervention. However, Pei 2020 was a preprint only, and claimed that the patient characteristics would be provided in the supplementary material once published.

Well-defined outcomes
We evaluated reporting bias for three outcome categories.

Mortality
We judged the risk for reporting bias to be low for seven studies (Ahn 2020; Pei 2020; Shen 2020; Tan 2020; Ye 2020; Zhang 2020a; Zhang 2020b), because all reported information for this outcome per participant until discharge from hospital or the latest point of follow-up.

We judged the risk for reporting bias to be high for Duan 2020 because the follow-up was unclear and it was unclear whether all participants were free of disease and discharged.

Adverse events

We judged the risk of reporting bias to be low for two studies (Ye 2020; Zhang 2020b), because observation period and results were reported for all participants.

We judged the risk of reporting bias to be high for the other six studies (Ahn 2020; Duan 2020; Pei 2020; Shen 2020; Tan 2020; Zhang 2020a), because it was unclear whether adverse events had not been (equally) assessed for all participants or whether outcomes were selectively reported. Pei 2020 reported one serious adverse event occurring in one participant, however did not report whether they had assessed or observed other adverse events. Shen 2020 did not provide any information regarding the safety of plasma transfusion. Tan 2020 reported that their participant experienced moderate fever after the transfusion, however did not report whether other adverse events occurred. Zhang 2020a described they had not observed any adverse events for one of their participants after plasma transfusion, but did not provide any information regarding the occurrence of adverse events for the other participants. They stated in the conclusions that they had not observed any serious adverse events.

Clinical improvements

Reporting of clinical improvements was very heterogeneous across studies.

We judged the risk of reporting bias to be low for three studies (Duan 2020; Ye 2020; Zhang 2020a), which clearly described clinical improvements and periods of follow-up per participant.

We judged the risk of reporting bias to be unclear for three studies (Ahn 2020; Shen 2020; Zhang 2020b), because of the following reasons. Reporting and follow-up was unclear for one participant of Ahn 2020, two participants of Shen 2020 probably were still on the intensive care unit (ICU) but it was unclear, and Zhang 2020b did not provide details but the participant was transferred to another ward.

We judged the risk of reporting bias to be high for Tan 2020 because neither clinical symptoms nor clinical improvement were reported in detail, but the participant was still in hospital.

Pei 2020 did not report the course of disease after convalescent plasma transfusion so we judged it at unclear risk of bias for this domain.

Other potential sources of bias

We further considered confounding and poorly-defined risk estimates as potential sources of bias.

Confounding

All studies were at high risk of confounding because none of the studies adjusted for confounding factors, including concomitant treatments.

Poorly-defined risk estimates

None of the studies performed any analyses.

Effects of interventions

In the 'Evidence profile' (Additional Table 2), we present certainty of the evidence for the outcomes that were prioritised in the protocol (Piechotta 2020).

Effectiveness of convalescent plasma for people with COVID-19

As no RCTs or well conducted non-randomised studies evaluating benefits and harms of convalescent plasma have yet been completed, we are not sure if the following results are related to convalescent plasma therapy; they could also be related to the underlying natural history of the disease or other concomitant treatments.

All-cause mortality at hospital discharge

All-cause mortality at hospital discharge cannot be fully evaluated, as not all of the participants had been discharged at the end of follow-up. None of the studies reported any deaths during their study periods, meaning that all 32 participants were alive at the end of follow-up. Participants were followed until discharge from hospital or from three (Duan 2020), to 37 days (Shen 2020), after transfusion. Two participants of Shen 2020 and one participant each of Ahn 2020, Ye 2020, Zhang 2020a, and Zhang 2020b were still hospitalised. The participant in Zhang 2020a still remained on the ICU. Further, it was unclear, whether all 11 participants of Duan 2020 and Tan 2020 had been discharged from hospital.

Time to death

All participants were alive at the end of follow-up (3 to 37 days).

Improvement of clinical symptoms (assessed by need for respiratory support)
The effect of convalescent plasma on improvement of clinical symptoms was reported in six included studies (Ahn 2020; Duan 2020; Shen 2020; Ye 2020; Zhang 2020a; Zhang 2020b), including 24 participants on respiratory support at baseline, and four participants who did not require respiratory support. The results of these studies can be found in Additional Table 3. We grouped them according to the prespecified time points; day 7, day 15, and day 30 after the plasma transfusion, and summarised baseline information and clinical status at the longest time of follow-up for each study.

Six studies reported on improvement of clinical symptoms, but we could not extract all information about timing of improvement and types of respiratory support from all the studies.

Ahn 2020 described two critically ill people with COVID-19 requiring intubation and mechanical ventilation. The two participants received a tracheotomy and one participant was reportedly successfully weaned from the ventilator by day 18 after convalescent plasma therapy. For the other participant, the date of cessation of respiratory support was not evident from the publication, but tracheotomy and weaning from mechanical ventilation were reported during the study period.

Duan 2020 reported decreased need for respiratory support in four out of 10 participants within three days of convalescent plasma transfusion. One other participant was reported to require only intermittent oxygenation after previously receiving continuous low-flow oxygenation via nasal cannula. The study also reported on two individuals who required no respiratory support preceding convalescent plasma therapy. No information on improvement of clinical symptoms for other time points was available.

Shen 2020 reported a case series that included five participants who were described as critically ill at baseline, with four participants in need of mechanical ventilation and intubation and one participant receiving extracorporeal membrane oxygenation (ECMO). Of these five participants, three were discharged from hospital at the end of the study period, and two were in a stable condition, intubated and receiving mechanical ventilation.

Ye 2020 included six participants, four of whom required oxygen at baseline (one via nasal cannula, with the other modes not specified in the publication). Two individuals did not require respiratory support before convalescent plasma

was administered. All four participants previously requiring respiratory support experienced alleviation of symptoms after convalescent plasma therapy, with none of them requiring respiratory support at the end of the study follow-up. The study reports information on respiratory support but lacks information on the type of support received by the participants, and the timing of this outcome is not part of the presented data for all participants.

Zhang 2020a reported in detail the clinical characteristics and timing of convalescent plasma therapy for four people with COVID-19. One participant was on non-invasive ventilation (NIV) and high-flow oxygenation, one participant was mechanically ventilated and intubated at baseline, and two participants received ECMO. Three out of the four participants were discharged at the end of the study period, and all participants were reported to have recovered from the infection eventually. For one participant it was unclear whether oxygen support was still required by the end of the study period.

Zhang 2020b described one participant who was mechanically ventilated and intubated before receiving convalescent plasma therapy. At day 11 after convalescent plasma therapy, the participant was removed from mechanical ventilation. Whether the participant required other types of respiratory support was not reported.

30-day and 90-day mortality

All participants were alive at the end of follow-up. Participants were followed until discharge from hospital or three (Duan 2020), to 37 days (Shen 2020), after transfusion.

Time to discharge from hospital

The time to discharge was reported for at least some of the participants in six studies (Ahn 2020; Pei 2020; Shen 2020; Ye 2020; Zhang 2020a; Zhang 2020b). The day of discharge after convalescent plasma therapy ranged from 4 days to 35 days. Only one study (3 participants) reported time to discharge from hospital for all participants (Pei 2020). Please refer to Additional Table 4 for further information regarding each trial and participant.

It was unclear, whether all participants of Duan 2020 and Tan 2020 had been discharged from the hospital.

Admission on the ICU

This outcome was not reported in a consistent way in the included studies. Ye 2020, Zhang 2020a and Zhang 2020b reported the number of participants on the ICU at baseline (Additional Table 5). These were none of six (Ye 2020), four of four (Zhang 2020a), and one of one (Zhang 2020b), respectively. The other studies did not report the number of participants on the ICU at baseline, however Ahn 2020, Duan 2020, Pei 2020, and Shen 2020 reported the number of participants that were mechanically ventilated, and so presumably on the ICU (please see Additional Table 5). The participant reported in Tan 2020 presented with moderate symptoms only, and so presumably was not on the ICU.

Length of stay on the ICU

We could not evaluate the length of stay on the ICU as none of the included studies reported this outcome in a consistent way. Zhang 2020a reported that one participant was still on the ICU at the end of follow-up, the other three participants had been discharged from the ICU. Zhang 2020b reported that their participant could be released from the ICU 11 days after plasma transfusion to a general ward; 18 days after admission on the ICU. Based on the reported clinical course of disease presumably one participant of Ahn 2020 and two participants of Shen 2020 were also still on the ICU at the end of follow-up (please see Table 5). However, this was not clearly reported.

Safety of convalescent plasma for people with COVID-19

Number of participants with adverse events of possibly grade 3 or grade 4 severity

Seven studies reported assessment of adverse events (Ahn 2020; Duan 2020; Pei 2020; Tan 2020; Ye 2020; Zhang 2020a; Zhang 2020b), however, Zhang 2020a only reported for one of their participants that no adverse event had been observed. It was unclear whether the other three participants did or did not experience any adverse events.

Six studies therefore reported the presence or absence of adverse events for all participants. Two studies reported adverse events that were possibly grade 3 or 4 severity but they did not report the degree of severity (see Additional Table 6). Tan 2020, a case study, reported that their participant experienced moderate fever (38.9 °C) after convalescent plasma transfusion. One of the three participants in Pei 2020 had severe anaphylactic shock after receiving 30 mL of plasma from a female donor with a history of pregnancy. Four other studies reported no

adverse events that were possibly of grade 3 or grade 4 severity (19 participants; Ahn 2020; Duan 2020; Ye 2020; Zhang 2020b).

Number of participants with serious adverse events
Seven studies assessed and reported serious adverse events (Ahn 2020; Duan 2020; Pei 2020; Tan 2020; Ye 2020; Zhang 2020a; Zhang 2020b). One participant in Pei 2020 (3 participants) experienced a serious adverse event (see Additional Table 7). As described above, this individual had severe anaphylactic shock after receiving convalescent plasma from a female donor with a history of pregnancy. No serious adverse events occurred in six studies (24 participants).

Discussion

Summary of main results
The aim of this review was to assess the effectiveness and safety of convalescent plasma and hyperimmune immunoglobulin in the treatment of coronavirus disease 2019 (COVID-19) illness.

We included eight studies in this review - seven case-series and one prospectively planned, single-arm intervention study, all evaluating convalescent plasma (32 participants in total). There were no completed studies evaluating hyperimmune immunoglobulin. We identified 47 ongoing studies evaluating convalescent plasma and one ongoing study evaluating hyperimmune immunoglobulin. Twenty-two of the ongoing studies on convalescent plasma are randomised.

Effectiveness of convalescent plasma for people with COVID-19

As no RCTs or high-quality, non-randomised studies evaluating benefits and harms of convalescent plasma are completed yet, we do not know whether the following results are related to the underlying natural history of the disease, other concomitant treatment, or convalescent plasma.

All-cause mortality at hospital discharge
All studies reported mortality, and all participants were alive at the end of reporting, but not all of the participants had been discharged from hospital at the end of follow-up. We do not know whether convalescent plasma has any effect on all-cause mortality (very low-certainty evidence).

Improvement of clinical symptoms (as assessed by respiratory support)
Six studies reported on the level of respiratory support required in participants; most participants required respiratory support at baseline. All studies reported improvement in clinical symptoms in at least some of their participants. We do not know whether convalescent plasma improves clinical symptoms or whether this improvement was due to other interventions, or the natural history of the disease (very low-certainty evidence).

Time to discharge from the hospital
Six studies reported time to discharge from hospital for at least some of their participants. The day of discharge after convalescent plasma therapy ranged from 4 to 35 days.

Admission on the intensive care unit (ICU)
Six studies included participants who were critically ill. The majority of these participants were no longer on the ICU or no longer required mechanical ventilation at final follow-up.

Length of stay on the ICU
None of the studies clearly reported this outcome.

Safety of convalescent plasma for people with COVID-19

Adverse events
Two studies reported participants who had experienced adverse events, presumably of grade 3 or 4 (they did not report degree of severity). One case study reported a participant who had moderate fever (38.9 °C) after the transfusion of convalescent plasma. The other study (3 participants) reported a case of severe anaphylactic shock after convalescent plasma transfusion. Four studies reported that no participants experienced moderate or severe adverse events (19 participants). We are very uncertain whether convalescent plasma therapy affects the risk of moderate to severe adverse events (very low-certainty evidence).

Serious adverse events
One study with three participants reported one serious adverse event. This participant had severe anaphylactic shock after receiving convalescent plasma. Six studies reported that no serious adverse events occurred. We are very uncertain whether convalescent plasma therapy affects the risk of serious adverse events (very low-certainty evidence).

Overall completeness and applicability of evidence

We found eight published non-randomised, uncontrolled studies (seven case-series, one prospectively planned study) evaluating convalescent plasma in adults, most with severe COVID-19. These studies included 32 participants (ranging from 1 to 10 participants). Most of these participants had already received different treatment options either solely or in combination. These included antivirals, antifungals or antibiotics, corticosteroids, hydroxychloroquine and respiratory support (extracorporeal membrane oxygenation (ECMO), mechanical ventilation or oxygen). Therefore, the participant population might have been too small and heterogeneous to generalise results.

We identified 48 ongoing studies, of which 22 are designed as RCTs. Of these ongoing studies, 47 evaluate convalescent plasma and one evaluates hyperimmune immunoglobulin. Sixteen RCTs are planned to be completed in 2020. The publication of the results of these studies will necessitate an update of this review. The conclusions of the updated review could differ from those of the present review, and may allow for a better judgement regarding the effectiveness and safety of convalescent plasma therapy.

Certainty of the evidence

It is important to note that the outcome measures are heterogeneous with wide variation in reporting across the included studies. Only one study was prospectively planned (Duan 2020), a non-randomised and uncontrolled study, evaluating 10 participants. However, this study reported a very short follow-up only (three days after convalescent plasma was given). The other seven small case-series studies were not registered. These study designs lead to high risk of bias, both in terms of selection and detection bias. Studies were not adjusted for potential confounders (e.g. severity of disease, comorbidities, previous or concomitant COVID-19 treatment). Currently, there is no standard instrument available to assess risk of bias for this type of study. We used the form developed by the Cochrane Childhood Cancer Group (Additional Table 1; Mulder 2019).

As we included eight small observational studies only (32 participants altogether), the results are very imprecise and very inconsistent, with very high risk of bias. Therefore, the certainty of the evidence is very low for all prioritised outcomes.

Potential biases in the review process

To avoid potential bias in the review, we had planned to include the best available evidence. However, as COVID-19 is a novel disease, results from RCTs and non-

RCTs are not yet available. In fact, we could only identify uncontrolled studies, reporting on a small number of participants. To increase the informative value of our review, we are tracking all registered trials and will update this review on a monthly basis as more evidence becomes available.

Two experienced Information Specialists developed a sensitive search strategy, to identify all ongoing and completed studies. We searched all relevant databases and trials registries, and in contrast to the recommendations of the Cochrane Rapid Reviews Methods Group, we decided to conduct all review steps regarding the study selection in duplicate by two independent review authors. We are confident that we identified all relevant published and ongoing studies and will monitor them closely in the future.

Unlike standard Cochrane methodology, only one review author performed data extraction and 'Risk of bias' and GRADE assessments for this rapid review. To minimise bias in these steps, at least one other review author verified the accuracy and (where applicable) the plausibility of extractions and assessment.

Although we have very limited confidence in the available evidence, we are not aware of any deficiencies in our review process. However, we are certain that the results are likely to be substantially different and conclusions may change as soon as high-certainty evidence becomes available.

Agreements and disagreements with other studies or reviews

This systematic review identified very low-certainty evidence on the safety and effectiveness of convalescent plasma for people with COVID-19.

A recent systematic review and meta-analysis found low-certainty evidence for the use of convalescent plasma for treating people with infections with different aetiologies (Mair-Jenkins 2015). The authors reported a systematic review and meta-analysis of the literature on the use of convalescent plasma and hyperimmune immunoglobulin in treating severe acute respiratory infections of viral aetiology, and found that this treatment is likely to be both safe and effective in preventing mortality. The study identified a 75% reduction in the odds of mortality in their exploratory post hoc meta-analysis across all viral aetiologies. The studies included in this review were performed with people treated with convalescent plasma for severe acute respiratory syndrome (SARS) and influenza. The limited number of identified studies and the low quality of included, mainly uncontrolled studies restricted the authors' ability to analyse extensively the risks and benefits of convalescent plasma therapy. Recommendations from the

authors were to investigate the use of convalescent plasma and hyperimmune immunoglobulin in large, well-designed clinical trials or other formal evaluations to obtain better-certainty evidence, and to evaluate the optimal treatment regimen.

Results from several large RCTs on the use of convalescent plasma and hyperimmune immunoglobulin in treating severe influenza have recently been made public (Beigel 2017; Beigel 2019; Davey 2019; Hung 2013). However, the results from these studies are inconsistent, with some studies showing a beneficial effect of convalescent plasma for treating people with severe influenza, whereas other studies show no benefit. The studies were well designed and reported in detail the timing of the intervention and relevant outcomes. One trial reported effectiveness of hyperimmune immunoglobulin, but only in a post hoc analysis of a subgroup of participants treated within five days of symptom onset (Hung 2013). In a different trial, for the subgroup analysis of people with influenza B, the effect of hyperimmune immunoglobulin also resulted in a demonstrable clinical and virological benefit (Davey 2019). Different mechanisms in the human immune system and their role in responding to different circulating influenza strains might further explain why the results of clinical trials of convalescent plasma and hyperimmune immunoglobulin for influenza varied (Davey 2019). Influenza A immunity is reported to carry over to the next years, known as heterosubtypic immunity (Kreijtz 2011), and the current outbreak of COVID-19 can, in that sense, not be compared with seasonal influenza. Notwithstanding these dissimilarities which might explain why the aforementioned influenza studies were not successful in clearly demonstrating benefit, the possibility of a null effect of convalescent plasma over a suitable comparator cannot be ruled out with the currently available evidence on COVID-19.

The adverse events associated with plasma transfusions are well characterised. Critically ill patients receiving plasma transfusions have an especially high risk of TACO, which is the leading cause of transfusion-related mortality (Pandey 2012). Many countries have now introduced risk mitigation strategies to decrease the risk of TRALI. In the UK in 2018 there was only one confirmed case of TRALI.

In this systematic review of the literature, which mainly identified studies that included people with COVID-19 with critical illness, we identified one potentially grade 3 adverse event and one potentially grade 4 adverse event (which also qualified as a serious adverse event). With the information available at this moment from published trials registry entries, it is apparent that the majority of clinical trials are enrolling people with COVID-19 who have progressed to moder-

ate or severe disease. Despite there being some evidence from other infectious diseases that early therapy might be more effective (Mair-Jenkins 2015), targeting this population is justifiable given the evident lack of effective interventions for COVID-19. The population that is eligible for treatment in these trials with convalescent plasma is potentially at high risk of transfusion reactions, and when treating critically ill people with COVID-19, their status should be carefully monitored.

Authors' conclusions

Implications for practice

The currently available evidence on the safety and effectiveness of convalescent plasma and hyperimmune immunoglobulin for treatment of people with COVID-19 is of very low certainty. Thus, any conclusions that are drawn based on these data are of limited value and these conclusions are subject to change as more reliable results become available. For the primary outcomes, the included studies reported that all participants were alive at the end of follow-up. Clinical improvement assessed through the need for respiratory support was reported by most studies, but details on timing and type of respiratory support were not clear for all studies. Other outcomes that were reported in a subset of the included studies were length of stay on the intensive care unit (ICU) and time to discharge from hospital, but reporting of these outcomes was not complete. Two studies reported adverse events that were potentially grade 3 and grade 4, of which one was a serious adverse event. More thorough investigations, preferably well-designed clinical trials, are needed in order to assess the benefits and risks of convalescent plasma therapy for people with COVID-19.

Implications for research

In this systematic review of the literature, we identified seven case-series studies and one prospectively planned, single-arm intervention study. We encountered difficulties while extracting data from these studies because there were major differences in the way these studies reported participant characteristics, details on the intervention, and outcomes. Future publications could benefit from more standardised reporting, especially of timing of intervention and clinically relevant outcomes, like days until discharge from hospital and improvement of clinical symptoms. We support the adoption of a reporting guideline in this rapidly evolving field of research.

Randomised controlled trials (RCTs) or at least non-randomised trials with a control group are needed to confirm the findings of this review. As there are 47 ongoing studies evaluating convalescent plasma and one ongoing study evaluating hyperimmune immunoglobulin, of which 22 are randomised, we will screen search results monthly and publish updates as a living systematic review in the near future. It might well be that this update will show different results than those published in this rapid review.

Acknowledgements

This review was published in collaboration with the Cochrane Editorial and Methods Department. We particularly thank Sarah Hodgkinson (Associate Editor, Editorial and Methods Dept), Analysis of Review Group Output (ARGO) for their comments on the Abstract, Clare Dooley (Managing Editor), and Denise Mitchell (Copy Editor) for their excellent support. Thanks also to the Cochrane Editorial and Methods Department team, for their valuable comments on the review and timely management of the editorial process.

We thank Robin Featherstone (Information Specialist, Editorial and Methods Department) for commenting on the search strategy and Gerald Gartlehner and Adrienne Stevens for their advice on rapid review methodology. We thank Susan J Brunskill for her support in identifying included and ongoing studies.

We thank all external peer reviewers who read and commented on this review. We thank Miquel Lozano (MD, PhD Clinic University Hospital, University of Barcelona, Spain), and Michael James Ankcorn (Virology Department, Northern General Hospital, Sheffield Teaching Hospitals NHS Foundation Trust, UK), who greatly helped to improve this review.

History

Review first published: Issue 5, 2020

Contributions of authors

SJV: clinical expertise and conception and writing of the review
VP: methodological expertise and conception and writing of the review

KLC: clinical expertise and conception and writing of the review
CD: development of the search strategy
IM: development of the search strategy
EMW: clinical expertise and advice
AL: clinical expertise and advice
CK: clinical expertise and advice
ZM: clinical expertise and advice
CS-O: clinical expertise and advice
LJE: clinical and methodological expertise and conception and writing of the review
NS: methodological expertise and advice and conception and writing of the review

Declarations of interest

SJV: none known
VP: none known
KLC: HSANZ Leukaemia Foundation PhD scholarship to support studies at Monash University. This is not related to the work in this review.
CD: none known
IM: none known
EMW: I have sought funding support from Australian Medical Research Future Fund for a trial of convalescent plasma. I will not be involved in bias assessment, data extraction or interpretation, but will serve as a content expert.
AL: none known
CK: none known
ZM: I have sought funding support from Australian Medical Research Future Fund for a trial of convalescent plasma. I will not be involved in bias assessment, data extraction or interpretation, but will serve as a content expert.
CS-O: is a member of the BEST Collaborative Clinical Study Group. I will not be involved in bias assessment, data extraction or interpretation, but will serve as a content expert.
LJE: co-lead of the COVID-19 immunoglobulin domain of the REMAP-CAP trial. I will not be involved in bias assessment, data extraction or interpretation, but will serve as a content expert.
NS: none known

Sources of support

Internal sources
· Sanquin Blood Supply, Netherlands
Center for Clinical Transfusion Research
· University Hospital of Cologne, Germany
Cochrane Cancer, Department I of Internal Medicine
· Monash University, Australia
Transfusion Research Unit, Department of Epidemiology and Preventive Medicine
· NHS Blood and Transplant, UK
NHS Blood and Transplant

External sources
· No sources of support supplied

Differences between protocol and review

Types of outcome measures
We revised the secondary outcome 'Improvement of clinical symptoms, assessed through need for respiratory support at up to 7 days; 8 to 15 days; 16 to 30 days' and added to the fourth bullet point: 'plus high-flow oxygen', to differentiate from the third bullet point. It now reads:

Improvement of clinical symptoms, assessed by need for respiratory support at up to 7 days; 8 to 15 days; 16 to 30 days:
o oxygen by mask or nasal prongs
o oxygen by NIV (non-invasive ventilation) or high flow
o intubation and mechanical ventilation
o mechanical ventilation plus high-flow oxygen
o extracorporeal membrane oxygenation (ECMO)

Electronic searches
As publication bias might influence all subsequent analyses and conclusions, we searched all potential relevant trials registries in detail to detect ongoing as well as completed studies, but not yet published studies. Nowadays, it is mandatory to provide results at least in the trials registry. In case results were not published

elsewhere, we had planned to extract and analyse these data. However, no outcome data had yet been added to the trials registries.

Data extraction and management

We had planned to extract data using a standardised data extraction form developed in Covidence. However, we could not adapt the standardised form to our needs. Therefore we generated a customised data extraction form in Microsoft Excel (Microsoft Corporation 2018).

Summary of findings and assessment of the certainty of the evidence

At protocol stage we had planned to assess the certainty of the evidence for our primary outcomes (all-cause mortality at hospital discharge and time to death), only. However, as none of the included studies reported any deaths during their study periods, we decided to assess the certainty of the evidence also for prioritised secondary outcomes (clinical improvement, grade 3 and 4 adverse events, and serious adverse events) to increase the informative value on effectiveness and safety of convalescent plasma therapy.

Some passages in this protocol, especially in the methods section, are from the standard template of Cochrane Haematology.

References

References to studies included in this review

Ahn 2020 {published data only}

Ahn JY , Sohn Y , Lee SH , Cho Y , Hyun JH , Baek YJ , et al. Use of convalescent plasma therapy in two COVID-19 patients with acute respiratory distress syndrome in Korea. Journal of Korean Medical Science 2020;35(14):e149.

Duan 2020 {published data only}

Duan K , Liu B , Li C , Zhang H , Yu T , Qu J , et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. In: National Academy of Sciences of the United States of America. Vol. 202004168. 2020. [DOI: 10.1073/pnas.2004168117]

Duan K , Liu B , Li C , Zhang H , Yu T , Qu J , et al. The feasibility of convalescent plasma therapy in severe COVID-19 patients: a pilot study. medRxiv 2020. [DOI: 101/2020.03.16.20036145]

Pei 2020 {published data only}

Pei S , Yuan X , Zhimin ZZ , Run YR , Xie Y , Minxue SM , et al. Convalescent plasma to treat COVID-19: Chinese strategy and experiences. medRxiv 2020. [DOI: 10.1101/2020.04.07.20056440]

Shen 2020 {published data only}

Shen C , Wang Z , Zhao F , Yang Y , Li J , Yuan J , et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA 2020. [DOI: 10.1001/jama.2020.4783]

Tan 2020 {published data only}

Tan L , Kang X , Zhang B , Zheng S , Liu B , Yu T , et al. A special case of COVID-19 with long duration of viral shedding for 49 days. medRxiv 2020. [DOI: 10.1101/2020.03.22.20040071]

Ye 2020 {published data only}

Ye M , Fu D , Ren Y , Wang F , Wang D , Zhang F , et al. Treatment with convalescent plasma for COVID-19 patients in Wuhan, China. Journal of Medical Virology 2020. [DOI: 10.1002/jmv.25882]

Zhang 2020a {published data only}

Zhang B , Liu S , Tan T , Huang W , Dong Y , Chen L , et al. Treatment with convalescent plasma for critically ill patients with SARS-CoV-2 infection. Chest 2020 Mar 31 [Epub ahead of print]. [DOI: 10.1016/j.chest.2020.03.039]

Zhang 2020b {published data only}

Zhang L , Pang R , Xue X , Bao J , Ye S , Dai Y , et al. Anti-SARS-CoV-2 virus antibody levels in convalescent plasma of six donors who have recovered from COVID-19. Aging 2020 Apr 22 [Epub ahead of print];12. [DOI: 10.18632/aging.103102]

References to studies excluded from this review

Bloch 2020 {published data only}

Bloch EM , Shoham S , Casadevall A , Sachais BS , Shaz B , Winters JL , et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. Journal of Clinical Investigation 2020. [DOI: 10.1172/JCI138745]

Cao 2020 {published data only}

Cao W , Liu X , Bai T , Fan H , Hong K , Song H , et al. High-dose intravenous immunoglobulin as a therapeutic option for deteriorating patients with coronavirus disease 2019. Open Forum Infectious Diseases 2020;7(3):ofaa102.

Casadevall 2020 {published data only}

Casadevall A , Pirofski L A . The convalescent sera option for containing COVID-19. Journal of Clinical Investigation 2020;130(4):1545-8.

Chen 2020 {published data only}

Chen L , Xiong J , Bao L , Shi Y . Convalescent plasma as a potential therapy for COVID-19. Lancet Infectious Diseases 2020;27:27.

ChiCTR2000030312 {published data only}

ChiCTR2000030312 . Cancelled, due to modify the protocol A single-center, open-label and single arm trial to evaluate the efficacy and safety of anti-SARS-CoV-2 inactivated convalescent plasma in the treatment& [A single-center, open-label and single arm trial to evaluate the efficacy and safety of anti-SARS-CoV-2 inactivated convalescent plasma in the treatment of novel coronavirus pneumonia (COVID-19) patient]. www.chictr.org.cn/showproj.aspx?proj=50258 (first received 23 April 2020).

ChiCTR2000030381 {published data only}

ChiCTR2000030381 . Cancelled by investigator A randomized, open-label, controlled and single-center trial to evaluate the efficacy and safety of anti-SARS-CoV-2 inactivated convalescent plasma in the treatment of novel coronavirus pneumonia (COVID-19) patient [A randomized, open-label, controlled and single-center trial to evaluate the efficacy and safety of anti-SARS-CoV-2 inactivated convalescent plasma in the treatment of novel coronavirus pneumonia (COVID-19) patient]. www.chictr.org.cn/showproj.aspx?proj=50290 (first received 23 April 2020).

ChiCTR2000030442 {published data only}

ChiCTR2000030442 . Combination of tocilizumab, IVIG and CRRT in severe patients with novel coronavirus pneumonia (COVID-19). www.chictr.org.cn/showproj.aspx?proj=50380 (first received 23 April 2020).

Díez 2020 {published data only}

Díez JM , Romero C , Gajardo R . Currently available intravenous immunoglobulin (Gamunex-C and Flebogamma© DIF) contains antibodies reacting against SARS-CoV-2 antigens. bioRxiv 2020. [DOI: 10.1101/2020.04.07.029017]

Hu 2020 {published data only}

Hu H , Ma F , Wei X , Fang Y . Coronavirus fulminant myocarditis saved with glucocorticoid and human immunoglobulin. European Heart Journal 2020;16:16.

Jawhara 2020 {published data only}

Jawhara S . Could intravenous immunoglobulin collected from recovered coronavirus patients protect against COVID-19 and strengthen the immune system of new patients? International Journal of Molecular Sciences 2020;21(7):2272. [DOI: 10.3390/ijms21072272]

NCT04261426 {published data only}

NCT04261426 . The efficacy of intravenous immunoglobulin therapy for severe 2019-nCoV infected pneumonia. clinicaltrials.gov/ct2/show/NCT04261426 (first received 23 April 2020).

NCT04323800 {published data only}

NCT04323800 . Convalescent plasma to stem coronavirus: a randomized, blinded phase 2 study comparing the efficacy and safety human coronavirus immune plasma (HCIP)

vs. control (SARS-CoV-2 non-immune plasma) among adults exposed to COVID-19. clinicaltrials.gov/show/NCT04323800 (first received 23 April 2020).

NCT04325672 {published data only}

NCT04325672 . Convalescent plasma to limit coronavirus associated complications: an open label, phase 2A study of high-titer anti-SARS-CoV-2 plasma in hospitalized patients with COVID-19. clinicaltrials.gov/show/NCT04325672 (first received 23 April 2020).

NCT04344015 {published data only}

NCT04344015 . COVID-19 plasma collection. ClinicalTrials.gov/show/NCT04344015 (first received 23 April 2020).

NCT04344977 {published data only}

NCT04344977 . COVID-19 plasma collection. clinicaltrials.gov/ct2/show/NCT04344977 (first received 23 April 2020).

Roback 2020 {published data only}

Roback JD , Guarner J . Convalescent plasma to treat COVID-19: possibilities and challenges. JAMA 2020. [DOI: 10.1001/jama.2020.4940]

Shi 2020 {published data only}

Shi H , Zhou C , He P , Huang S , Duan Y , Wang X , et al. Successful treatment of plasma exchange followed by intravenous immunoglobulin in a critically ill patient with 2019 novel coronavirus infection. International Journal of Antimicrobial Agents 2020:105974.

Syal 2020 {published data only}

Syal K . COVID-19: herd immunity and convalescent plasma transfer therapy. Journal of Medical Virology 2020;13:13.

Tanne 2020 {published data only}

Tanne JH . Covid-19: FDA approves use of convalescent plasma to treat critically ill patients. BMJ 2020;368:m1256.

Tiberghien 2020 {published data only}

Tiberghien P , de Lambalerie X , Morel P , Gallian P , Lacombe K , Yazdanpanah Y . Collecting and evaluating convalescent plasma for COVID-19 treatment: why and how. Vox Sanguinis 2020. [DOI: 10.1111/vox.12926]

Wong 2020 {published data only}

Wong HK , Lee CK . Pivotal role of convalescent plasma in managing emerging infectious diseases. Vox Sanguinis 2020. [DOI: 10.1111/vox.12927]

Xie 2020 {published data only}

Xie Y , Cao S , Li Q , Chen E , Dong H , Zhang W , et al. Effect of regular intravenous immunoglobulin therapy on prognosis of severe pneumonia in patients with COVID-19. Journal of Infection 2020:S0163-4453(20)30172-9.

Yoo 2020 {published data only}

Yoo JH . Convalescent plasma therapy for corona virus disease 2019: a long way to go but worth trying. Journal of Korean Medical Science 2020;35(14):e150.

Zhao 2020b {published data only}

Zhao Q , He Y . Challenges of convalescent plasma therapy on COVID-19. Journal of Clinical Virology 2020;127:104358.

References to studies awaiting assessment

Qiu 2020 {published data only}

Qiu T , Wang J , Zhou J , Zou J , Chen Z , Ma X , et al. The report of two cases infection with novel coronavirus (2019-ncov) after kidney transplantation and the association literature analyzation. Chinese Journal of Organ Transplantation 2020;41(0):E004.

Tu 2020 {published data only}

Tu Y , Wu X , Liu F , Wang J , Luo Y , Cai Z , et al. Two clinical cases of novel coronavirus pneumonia (NCP) in renal transplant recipients. Chinese Journal of Organ Transplantation 2020;41(0):E005.

References to ongoing studies

ChiCTR2000029757 {published data only}

ChiCTR2000029757 . Convalescent plasma for the treatment of severe and critical novel coronavirus pneumonia (COVID-19): a prospective randomized controlled trial. www.chictr.org.cn/showproj.aspx?proj=49081 (first received 12 February 2020).

ChiCTR2000029850 {published data only}

ChiCTR2000029850 . Effecacy and safety of convalescent plasma treatment for severe patients with novel coronavirus pneumonia (COVID-19): a prospective cohort study. www.chictr.org.cn/showproj.aspx?proj=49533 (first received 15 February 2020).

ChiCTR2000030010 {published data only}

ChiCTR2000030010 . A randomized, double-blind, parallel-controlled, trial to evaluate the efficacy and safety of anti-SARS-CoV-2 virus inactivated plasma in the treatment of severe novel coronavirus pneumonia patients (COVID-19). www.chictr.org.cn/showproj.aspx?proj=49777 (first received 19 February 2020).

ChiCTR2000030039 {published data only}

ChiCTR2000030039 . Clinical study for infusing convalescent plasma to treat patients with new coronavirus pneumonia (COVID-19). www.chictr.org.cn/showproj.aspx?proj=49544 (first received 21 February 2020).

ChiCTR2000030179 {published data only}

ChiCTR2000030179 . Experimental study of novel coronavirus pneumonia rehabilitation plasma therapy severe novel coronavirus pneumonia (COVID-19). www.chictr.org.cn/showproj.aspx?proj=50059 (first received 24 February 2020).

ChiCTR2000030627 {published data only}

ChiCTR2000030627 . Study on the application of convalescent plasma therapy in severe COVID-19. www.chictr.org.cn/showproj.aspx?proj=50727 (first received 8 March 2020).

ChiCTR2000030702 {published data only}

ChiCTR2000030702 . Convalescent plasma for the treatment of common COVID-19: a prospective randomized controlled trial. www.chictr.org.cn/showproj.aspx?proj=50537 (first received 10 March 2020).

ChiCTR2000030841 {published data only}

ChiCTR2000030841 . Treatment of acute severe COVID-19 with immunoglobulin from cured COVID-19 patients. www.chictr.org.cn/showproj.aspx?proj=51072 (first received 15 March 2020).

ChiCTR2000030929 {published data only}

ChiCTR2000030929 . A randomized, double-blind, parallel-controlled trial to evaluate the efficacy and safety of anti-SARS-CoV-2 virus inactivated plasma in the treatment of severe novel coronavirus pneumonia (COVID-19). www.chictr.org.cn/showproj.aspx?proj=50696 (first received 17 March 2020).

ChiCTR2000031501 {published data only}

ChiCTR2000031501 . The efficacy of convalescent plasma in patients with critical novel coronavirus pneumonia (COVID-19): a pragmatic, prospective cohort study. www.chictr.org.cn/showproj.aspx?proj=50254 (first received 2 April 2020).

EUCTR2020-001310-38 {published data only}

EUCTR2020-001310-38 . A randomized, prospective, open label clinical trial on the use of convalescent plasma compared to best supportive care in patients with severe COVID-19. www.clinicaltrialsregister.eu/ctr-search/search?query=eudract_number:2020-001310-38 (first received 23 April 2020).

IRCT20151228025732N53 {published data only}

IRCT20151228025732N53 . Therapeutic effects of plasma of recovered people from COVID-19 on hospitalized patients with this disease. en.irct.ir/trial/46931 (first received 10 April 2020).

IRCT20200310046736N1 {published data only}

IRCT20200310046736N1 . Comparison of the therapeutic effect of convalescent plasma and plasma-derived immunoglobulin-enriched solution on COVID-19 patients. en.irct.ir/trial/46424 (first received 1 April 2020).

IRCT20200325046860N1 {published data only}

IRCT20200325046860N1 . Convalescent plasma therapy for COVID-19 patients. en.irct.ir/trial/46759 (first received 30 March 2020).

IRCT20200404046948N1 {published data only}

IRCT20200404046948N1 . Efficacy and safety of convalescent plasma in the treatment of COVID-19. en.irct.ir/trial/46973 (first received 15 April 2020).

IRCT20200409047007N1 {published data only}

IRCT20200409047007N1 . Effect of COVID 19 survivors plasma in COVID 19 patients with ARDS. en.irct.ir/trial/47058 (first received 12 April 2020).

IRCT20200413047056N1 {published data only}

IRCT20200413047056N1 . Comparison between the efficacy of intravenous immunoglobulin and convalescent plasma in COVID-19. en.irct.ir/trial/47212 (first received 17 April 2020).

NCT04264858 {published data only}

NCT04264858 . An exploratory clinical study on the treatment of acute severe 2019-nCoV pneumonia with immunoglobulin from cured 2019-nCoV pneumonia patients. clinicaltrials.gov/show/NCT04264858 (first received 11 February 2020).

NCT04292340 {published data only}

NCT04292340 . The efficacy and safety of anti-SARS-CoV-2 inactivated convalescent plasma in the treatment of novel coronavirus pneumonia patient (COVID-19): an observational study. clinicaltrials.gov/show/NCT04292340 (first received 3 March 2020).

NCT04321421 {published data only}

NCT04321421 . Plasma from donors recovered from new coronavirus 2019 as therapy for critical patients with COVID-19. clinicaltrials.gov/show/NCT04321421 (first received 25 March 2020).

NCT04327349 {published data only}
NCT04327349 . Investigating effect of convalescent plasma on COVID-19 patients outcome: a clinical trial. clinicaltrials.gov/show/NCT04327349 (first received 31 March 2020).
NCT04332380 {published data only}
NCT04332380 . Convalescent plasma for patients with COVID-19: a pilot study. clinicaltrials.gov/show/NCT04332380 (first received 2 April 2020).
NCT04332835 {published data only}
NCT04332835 . Convalescent plasma for patients with COVID-19: a randomized, open label, parallel, controlled clinical study. clinicaltrials.gov/show/NCT04332835 (first received 3 April 2020).
NCT04333251 {published data only}
NCT04333251 . Evaluating convalescent plasma to decrease coronavirus associated complications. A phase I study comparing the efficacy and safety of high-titer anti-Sars-CoV-2 plasma vs best supportive care in hospitalized patients with interstitial pneumonia due to COVID-19. clinicaltrials.gov/show/NCT04333251 (first received 3 April 2020).
NCT04333355 {published data only}
NCT04333355 . Phase 1 study to evaluate the safety of convalescent plasma as an adjuvant therapy in patients with SARS-CoV-2 infection. clinicaltrials.gov/show/NCT04333355 (first received 3 April 2020).
NCT04338360 {published data only}
NCT04338360 . Expanded access to convalescent plasma for the treatment of patients with COVID-19. ClinicalTrials.gov/show/NCT04338360 (first received 8 April 2020).
NCT04340050 {published data only}
NCT04340050 . COVID-19 convalescent plasma. ClinicalTrials.gov/show/NCT04340050 (first received 9 April 2020).
NCT04342182 {published data only}
NCT04342182 . Convalescent plasma as therapy for Covid-19 severe SARS-CoV-2 disease (CONCOVID Study) (ConCoVid-19). ClinicalTrials.gov/show/NCT04342182 (first received 10 April 2020).
NCT04343261 {published data only}
NCT04343261 . Convalescent plasma in the treatment of COVID 19. ClinicalTrials.gov/show/NCT04343261 (first received 13 April 2020).
NCT04343755 {published data only}
NCT04343755 . Convalescent plasma as treatment for hospitalized subjects with COVID-19 infection. ClinicalTrials.gov/show/NCT04343755 (first received 13 April 2020).
NCT04344535 {published data only}
NCT04344535 . Convalescent plasma vs. standard plasma for COVID-19. ClinicalTrials.gov/show/NCT04344535 (first received 14 April 2020).
NCT04345289 {published data only}
EUCTR2020-001367-88-DK . Efficacy and safety of novel treatment options for adults with COVID-19 pneumonia. apps.who.int/trialsearch/Trial2.aspx?TrialID=EUCTR2020-001367-88-DK (first received 14 April 2020).
NCT04345289 . Efficacy and safety of novel treatment options for adults with COVID-19 pneumonia (CCAP). ClinicalTrials.gov/show/NCT04345289 (first received 14 April 2020).

NCT04345523 {published data only}
NCT04345523 . Convalescent plasma therapy vs. SOC for the treatment of COVID19 in hospitalized patients (ConPlas-19). ClinicalTrials.gov/show/NCT04345523 (first received 14 April 2020).
NCT04345679 {published data only}
NCT04345679 . Anti COVID-19 convalescent plasma therapy. ClinicalTrials.gov/show/NCT04345679 (first received 14 April 2020).
NCT04345991 {published data only}
NCT04345991 . Efficacy of convalescent plasma to treat COVID-19 patients, a nested trial in the CORIMUNO-19 cohort. ClinicalTrials.gov/show/NCT04345991 (first received 15 April 2020).
NCT04346446 {published data only}
NCT04346446 . Efficacy of convalescent plasma therapy in severely sick COVID-19 patients. ClinicalTrials.gov/show/NCT04346446 (first received 15 April 2020).
NCT04346589 {published data only}
NCT04346589 . Convalescent antibodies infusion in critically ill COVID 19 patients. clinicaltrials.gov/ct2/show/NCT04346589 (first received 15 April 2020).
NCT04347681 {published data only}
NCT04347681 . Potential efficacy of convalescent plasma to treat severe COVID-19 and patients at high risk of developing severe COVID-19. ClinicalTrials.gov/show/NCT04347681 (first received 15 April 2020).
NCT04348656 {published data only}
NCT04348656 . Convalescent plasma for hospitalized adults with COVID-19 respiratory illness (CONCOR-1). ClinicalTrials.gov/show/NCT04348656 (first received 16 April 2020).
NCT04348877 {published data only}
NCT04348877 . Plasma rich antibodies from recovered patients from COVID19. ClinicalTrials.gov/show/NCT04348877 (first received 16 April 2020).
NCT04352751 {published data only}
NCT04352751 . Experimental use of convalescent plasma for passive immunization in current COVID-19 pandemic in Pakistan in 2020. ClinicalTrials.gov/show/NCT04352751 (first received 20 April 2020).
NCT04353206 {published data only}
NCT04353206 . Convalescent plasma in ICU patients with COVID-19-induced respiratory failure. ClinicalTrials.gov/show/NCT04353206 (first received 20 April 2020).
NCT04354831 {published data only}
NCT04354831 . A study evaluating the efficacy and safety of high-titer anti-SARS-CoV-2 plasma in hospitalized patients with COVID-19 infection. clinicaltrials.gov/ct2/show/NCT04354831 (first received 21 April 2020).
NCT04355767 {published data only}
NCT04355767 . Convalescent plasma vs. placebo in emergency room patients with COVID-19. clinicaltrials.gov/ct2/show/NCT04355767 (first received 21 April 2020).
NCT04355897 {published data only}
NCT04355897 . CoVID-19 plasma in treatment of COVID-19 patients. clinicaltrials.gov/ct2/show/NCT04355897 (first received 21 April 2020).
NCT04356482 {published data only}

NCT04356482 . Convalescent plasma for ill patients by COVID-19. ClinicalTrials.gov/show/NCT04356482 (first received 22 April 2020).

NCT04356534 {published data only}

NCT04356534 . Convalescent plasma trial in COVID -19 patients. ClinicalTrials.gov/show/NCT04356534 (first received 22 April 2020).

NCT04357106 {published data only}

NCT04357106 . COPLA study: treatment of severe forms of coronavirus infection with convalescent plasma. ClinicalTrials.gov/show/NCT04357106 (first received 22 April 2020).

Additional references

Bao 2020

Bao L , Deng W , Gao H , Xiao C , Liu J , Xue J , et al. Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. bioRxiv (accessed 13 April 2020). [DOI: 10.1101/2020.03.13.990226]

Baudel 2020

Baudel JL , Vigneron C , Pras-Landre V , Joffre J , Marjot F , Ait-Oufella H , et al. Transfusion-related acute lung injury (TRALI) after intravenous immunoglobulins: French multicentre study and literature review. Clinical Rheumatology 2020;39(2):541-6.

Beigel 2017

Beigel JH , Tebas P , Elie-Turenne MC , Bajwa E , Bell TE , Cairns B , et al. Immune plasma for the treatment of severe influenza: an open-label, multicentre, phase 2 randomised study. Lancet Respiratory Medicine 2017;5(6):500-11. [DOI: 10.1016/S2213-2600(17)30174-1]

Beigel 2019

Beigel JH , Aga E , Elie-Turenne M-C, Cho J , Tebas P , Clark CL , et al. Anti-influenza immune plasma for the treatment of patients with severe influenza A: a randomised, double-blind, phase 3 trial. Lancet Respiratory Medicine 2019;7(11):941-50. [DOI: 10.1016/S2213-2600(19)30199-7]

Brennan 2003

Brennan VM , Salomé-Bentley NJ , Chapel HM , Immunology Nurses Study . Prospective audit of adverse reactions occurring in 459 primary antibody-deficient patients receiving intravenous immunoglobulin. Clinical and Experimental Immunology 2003;133(2):247-51.

CDC 2020a

Centers for Disease Control and Prevention (CDC) . Coronavirus Disease 2019 (COVID-19). www.cdc.gov/coronavirus/2019-ncov/covid-data/covidview/index.html (accessed 13 April 2020).

CDC 2020b

Centers for Disease Control and Prevention (CDC) . Interim clinical guidance for management of patients with confirmed coronavirus disease (COVID-19). www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html (last accessed 26 April 2020).

Chun 2016

Chun S , Chung CR , Ha YE , Han TH , Ki CS , Kang ES , et al . Possible transfusion-related acute lung injury following convalescent plasma transfusion in a patient with Middle East respiratory syndrome. Annals of Laboratory Medicine 2016;36(4):393-5. [DOI: 10.3343/alm.2016.36.4.393]

COMET 2020

Core outcome set developers' response to COVID-19 (2nd April 2020). www.comet-initiative.org/Studies/Details/1538 (accessed 9 April 2020).

Covidence [Computer program]

Covidence. Version accessed 20 April 2020. Melbourne, Australia: Veritas Health Innovation. Available at covidence.org.

Davey 2019

Davey RT Jr, Fernández-Cruz E , Markowitz N , Pett S , Babiker AG , Wentworth D , et al. Anti-influenza hyperimmune intravenous immunoglobulin for adults with influenza A or B infection (FLU-IVIG): a double-blind, randomised, placebo-controlled trial. Lancet Respiratory Medicine 2019;7(11):951-63. [DOI: 10.1016/S2213-2600(19)30253-X]

Deeks 2019

Deeks JJ , Higgins JP , Altman DG , editor(s) . Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.

Driggin 2020

Driggin E , Madhavan MV , Bikdeli B , Chuich T , Laracy J , Biondi-Zoccai G , et al. Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. Journal of the American College of Cardiology 2020;75(18):2352-71.

Eibl 2008

Eibl MM . History of immunoglobulin replacement. Immunology and Allergy Clinics of North America 2008;28(4):737-64. [DOI: 10.1016/j.iac.2008.06.004]

EPOC 2017

Cochrane Effective Practice and Organisation of Care (EPOC) . What study designs can be considered for inclusion in an EPOC review and what should they be called? EPOC Resources for review authors. Available from epoc.cochrane.org/resources/epoc-resources-review-authors (accessed 23 April 2019).

GRADEpro GDT [Computer program]

GRADEpro GDT. Version accessed 20 April 2020. Hamilton (ON): McMaster University (developed by Evidence Prime). Available at gradepro.org.

Higgins 2003

Higgins JP , Thompson SG , Deeks JJ , Altman DG . Measuring inconsistency in meta-analyses. BMJ 2003;327:557-60.

Higgins 2019a

Higgins JP , Thomas J , Chandler J , Cumpston M , Li T , Page MJ , et al, editor(s) . Cochrane Handbook for Systematic Reviews of Interventions Version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.

Higgins 2019b

Higgins JP , Deeks JJ , editor(s) . Chapter 6: Choosing effect measures and computing estimates of effect. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page

MJ, et al, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.

Ho 2005

Ho MS , Chen WJ , Chen HY , Lin SF , Wang MC , Di J , et al. Neutralizing antibody response and SARS severity. Emerging Infectious Diseases 2005;11(11):1730-7.

Huang 2020

Huang C , Wang Y , Li X , Ren L , Zhao J , Hu Y , et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395(10223):497-506. [DOI: 10.1016/S0140-6736(20)30183-5]

Hung 2013

Hung IF , To KK , Lee CK , Lee KL , Yan WW , Chan K , et al. Hyperimmune IV immunoglobulin treatment: a multicenter double-blind randomized controlled trial for patients with severe 2009 influenza A(H1N1) infection. Chest 2013;144(2):464-73. [DOI: 10.1378/chest.12-2907]

Kim 2020

Kim D-H, Choe YJ , Jeong J-Y . Understanding and interpretation of case fatality rate of coronavirus disease 2019. Journal of Korean Medical Science 2020;35(12):e137. [DOI: 10.3346/jkms.2020.35.e137]

Kreijtz 2011

Kreijtz JH , Fouchier RA , Rimmelzwaan GF . Immune responses to influenza virus infection. Virus Research 2011;162(1-2):19-30. [DOI: 10.1016/j.virusres.2011.09.022]

Lauer 2020

Lauer SA , Grantz KH , Bi Q , Jones FK , Zheng Q , Meredith HR , et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Annals of Internal Medicine 2020. [DOI: 10.7326/M20-0504]

Lefebvre 2019

Lefebvre C , Glanville J , Briscoe S , Littlewood A , Marshall C , Metzendorf MI , et al. Chapter 4: Searching for and selecting studies. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.

Li 2019

Li T , Higgins JP , Deeks JJ , editor(s) . Chapter 5: Collecting data. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.

Liang 2020

Liang W , Guan W , Chen R , Wang W , Li J , Xu K , et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncology 2020;21(3):335-7. [DOI: 10.1016/S1470-2045(20)30096-6]

Luke 2006

Luke TC , Kilbane EM , Jackson JL , Hoffman SL . Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? Annals of Internal Medicine 2006;145(8):599-609.

Mair-Jenkins 2015

Mair-Jenkins J , Saavedra-Campos M , Baillie JK , Cleary P , Khaw FM , Lim WS , et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. Journal of Infectious Diseases 2015;211(1):80-90. [DOI: 10.1093/infdis/jiu396]

Microsoft Corporation 2018 [Computer program]

Mircosoft Excel. Microsoft Corporation . Microsoft Corporation, available at: office.microsoft.com/excel, 2018.

Mo 2006

Mo H , Zeng G , Ren X , Li H , Ke C , Tan Y , et al. Longitudinal profile of antibodies against SARS-coronavirus in SARS patients and their clinical significance. Respirology 2006;11(1):49-53. [DOI: 10.1111/j.1440-1843.2006.00783.x]

Moher 2009

Moher D , Liberati A , Tetzlaff J , Altman DG . Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Journal of Clinical Epidemiology 2009;62(10):1006-12.

Morens 1994

Morens DM . Antibody-dependent enhancement of infection and the pathogenesis of viral disease. Clinical Infectious Diseases 1994;19(3):500-12.

Mulder 2019

Mulder RL , Bresters D , Van den Hof M , Koot BG , Castellino SM , Loke YK , et al. Hepatic late adverse effects after antineoplastic treatment for childhood cancer. Cochrane Database of Systematic Reviews 2019, Issue 4. Art. No: CD008205. [DOI: 10.1002/14651858.CD008205.pub3]

Otrock 2017

Otrock ZK , Liu C , Grossman BJ . Transfusion-related acute lung injury risk mitigation: an update. Vox Sanguinis 2017;112(8):694-703.

Pandey 2012

Pandey S , Vyas GN . Adverse effects of plasma transfusion. Transfusion 2012;52 Suppl 1:65S-79S.

Parmar 1998

Parmar MK , Torri V , Stewart L . Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Statistics in Medicine 1998;17(24):2815-34.

Payne 2016

Payne DC , Iblan I , Rha B , Algasrawi S , Hin A , Al Nsour M , et al. Persistence of antibodies against Middle East respiratory syndrome coronavirus. Emerging Infectious Disease Journal 2016;22(10):1824-6. [DOI: 10.3201/eid2210.160706]

Reeves 2019

Reeves BC , Deeks JJ , Higgins JP , Shea B , Tugwell P , Wells GA . Chapter 24: Including non-randomized studies on intervention effects. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al, editor(s). Cochrane Handbook for Systematic Reviews of Interventions *V*ersion 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.

Review Manager Web [Computer program]

Review Manager Web (RevMan Web). The Cochrane Collaboration, 2019. Available from revman.cochrane.org.

Ricke 2020
Ricke D , Malone R . Medical countermeasures analysis of 2019-nCoV and vaccine risks for antibody-dependent enhancement (ADE). www.preprints.org/manuscript/202003.0138/v1 2020;2020030138. [DOI: 10.20944/preprints202003.0138.v1]
Robbins 1995
Robbins JB , Schneerson R , Szu SC . Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. Journal of Infectious Diseases 1995;171(6):1387-98.
Rock 2011
Rock G . A comparison of methods of pathogen inactivation of FFP. Vox Sanguinis 2011;100(2):169-78.
Schünemann 2019
Schünemann HJ , Vist GE , Higgins JP , Santesso N , Deeks JJ , Glasziou P , et al. Chapter 15: Interpreting results and drawing conclusions. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al, editor(s) Cochrane Handbook for Systematic Reviews of Interventions Version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook.
Sekul 1994
Sekul EA , Cupler EJ , Dalakas MC . Aseptic meningitis associated with high-dose intravenous immunoglobulin therapy: frequency and risk factors. Annals of Internal Medicine 1994;121(4):259-62.
Sterne 2016
Sterne JA , Hernán MA , Reeves BC , Savović J , Berkman ND , Viswanathan M , et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ 2016;355:i4919.
Sterne 2019
Sterne JA , Savovic J , Page MJ , Elbers RG , Blencowe NS , Boutron I , et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ 2019;366:l4898.
Stiehm 2013
Stiehm ER . Adverse effects of human immunoglobulin therapy. Transfusion Medicine Reviews 2013;27(3):171-8.
Team 2020
Team NCPERE . Vital surveillances: the epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) – China. China CDC Weekly 2020;2(8):113-22. [DOI: 10.3760/cma.j.issn.0254-6450.2020.02.003]
Tierney 2007
Tierney JF , Stewart LA , Ghersi D , Burdett S , Sydes MR . Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16.
Tolouian 2020
Tolouian R , Vahed SZ , Ghiyasvand S , Tolouian A , Ardalan M . COVID-19 interactions with angiotensin-converting enzyme 2 (ACE2) and the kinin system; looking at a potential treatment. Journal of Renal Injury Prevention 2020;9(2):e19. [DOI: 10.34172/jrip.2020.19]
Van de Veerdonk 2020
Van de Veerdonk F , Netea MG , Van Deuren M , Van der Meer JW , De Mast Q , Bruggemann RJ , et al. Kinins and cytokines in COVID-19: a comprehensive pathophysiological

approach. Preprints 2020 (accessed 13 April 2020);2020040023. [DOI: 10.20944/preprints202004.0023.v1]

Wan 2020

Wan Y , Shang J , Sun S , Tai W , Chen J , Geng Q , et al. Molecular mechanism for antibody-dependent enhancement of coronavirus entry. Journal of Virology 2020;94(5):e02015-19.

Wang 2014

Wang S-F, Tseng S-P, Yen C-H, Yang J-Y, Tsao C-H, Shen C-W, et al. Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins. Biochemical and Biophysical Research Communications 2014;451(2):208-14.

WHO 2007

World Health Organization (WHO) . Cumulative number of reported probable cases of SARS. www.who.int/csr/sars/country/2003_07_11/en/ (accessed 13 April 2020).

WHO 2019

World Health Organization (WHO) . Middle East respiratory syndrome coronavirus (MERS-CoV). www.who.int/emergencies/mers-cov/en/ (accessed 13 April 2020).

WHO 2020a

World Health Organization (WHO) . Report of the WHO-China Joint Mission on coronavirus disease 2019 (COVID-19); February 2020. Available at www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.

WHO 2020b

World Health Organization (WHO) . Rolling updates on coronavirus diseases (COVID-19). www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen (accessed 13 April 2020).

WHO 2020c

World Health Organization (WHO) . Coronavirus disease (COVID-2019) situation report-105; 4 May 2020. Available at www.who.int/docs/default-source/coronaviruse/situation-reports/20200504-covid-19-sitrep-105.pdf.

WHO 2020d

World Health Organization (WHO) . Clinical management of severe acute respiratory infection (SARI) when COVID19 disease is suspected: interim guidance. World Health Organization 2020;WHO/2019-nCoV/clinical/2020.4.

Wu 2020a

Wu C , Chen X , Cai Y , Xia J , Zhou X , Xu S , et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Internal Medicine 2020. [DOI: 10.1001/jamainternmed.2020.0994]

Wu 2020b

Wu F , Wang A , Liu M , Wang Q , Chen J , Xia S , et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv (accessed 13 April 2020). [DOI: 110.1101/2020.03.30.2004736]

Xu 2020

Xu Z , Shi L , Wang Y , Zhang J , Huang L , Zhang C , et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respiratory Medicine 2020;8(4):420-2. [DOI: 10.1016/S2213-2600(20)30076-X]

Zhao 2020

Zhao J , Yuan Q , Wang H , Liu W , Liao X , Su Y , et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. medRxiv 2020. [DOI: 10.1101/2020.03.02.20030189]

References to other published versions of this review

Piechotta 2020

Piechotta V , Valk SJ , Chai KL , Wood EM , Lamikanra A , Kimber C , et al. Safety and effectiveness of convalescent plasma or hyperimmune globulin for people with COVID-19: a rapid review. available at: doi.org/10.17605/OSF.IO/DWF53.

The Characteristics of included, excluded and ongoing studies and studies awaiting classification can be found here: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013600/references#characteristicStudies

The Additional Tables and Appendices for this chapter can be found here: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013600/appendices#CD013600-sec-0097"

5

Chapter 5

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

Authors: Sarah J Valk[1,2], Camila Caram-Deelder[2], Rolf HHGroenwold[2], Dorothea Evers[3], Karen MK de Voogt[4], Daan van de Kerkhof[5], Marielle J Wondergem[6], Nathalie CV Péquériaux[7], Francisca Hudig[8], Jaap Jan Zwaginga[1,9], Rutger A Middelburg[2,10], Johanna G van der Bom[2]

1. Jon J van Rood Center for Clinical Transfusion Research, Sanquin/LUMC, Leiden, The Netherlands,
2. Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands,
3. Department of Haematology, Radboudumc, Nijmegen, The Netherlands,
4. Central Diagnostic Laboratory, University Medical Center Utrecht, Utrecht, The Netherlands,
5. Department of Clinical Chemistry and Haematology, Catharina Hospital, Eindhoven, The Netherlands,
6. Department of Haematology, Amsterdam UMC, location VUmc, Amsterdam, The Netherlands,
7. Department of Clinical Chemistry and Haematology, Jeroen Bosch Hospital, 's Hertogenbosch, The Netherlands,
8. LabWest, Haga Teaching Hospital, The Hague, The Netherlands,
9. Department of Haematology, Leiden University Medical Center, Leiden, The Netherlands,
10. Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, The Netherlands.

Abstract

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

Introduction

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

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

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

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

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

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

Methods

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

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

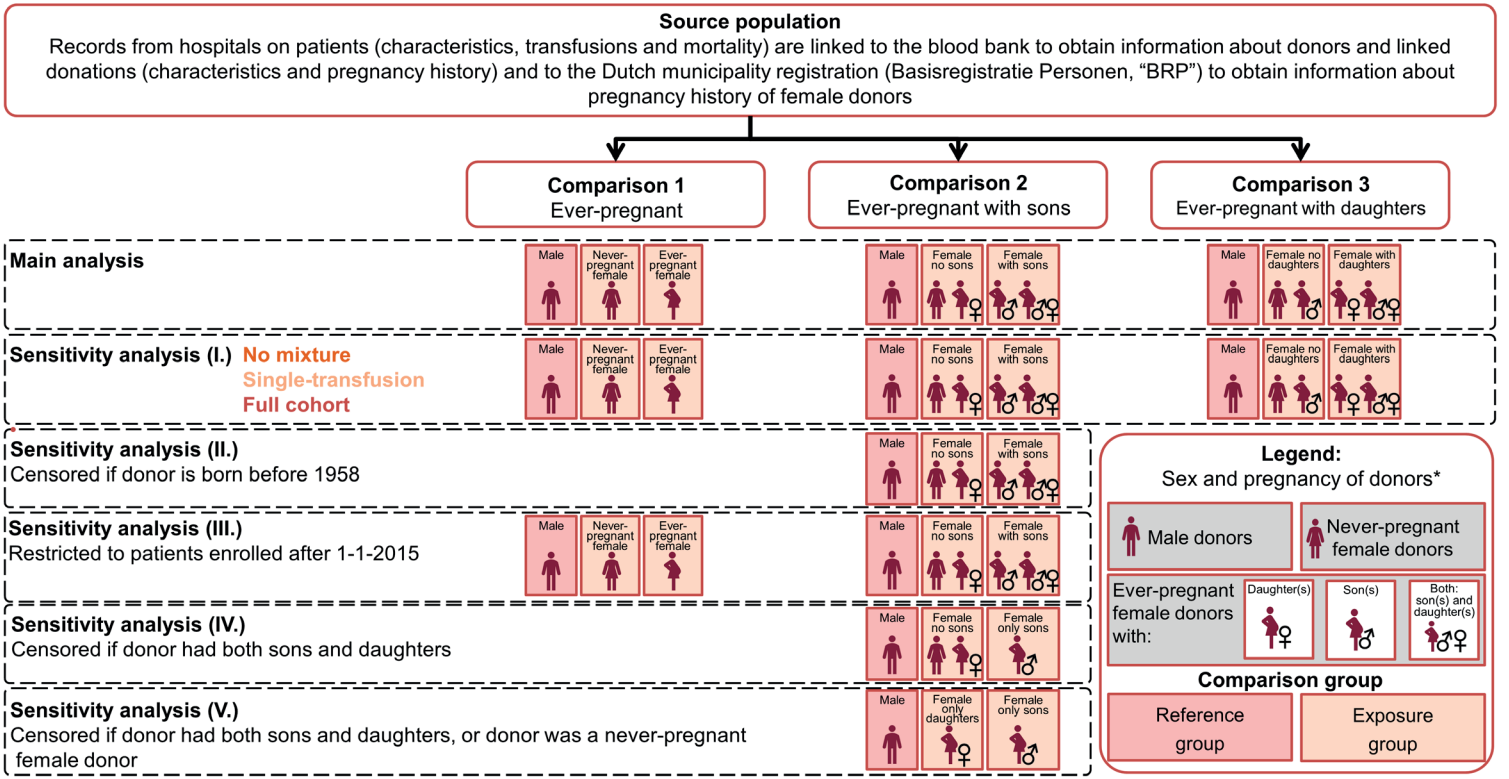


Figure 1. Schematic representation of exposure groups in main analysis and sensitivity analyses

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

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

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

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

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

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

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

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

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

Results

Population

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

Table 1. Patient and transfusion characteristics

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

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

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

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

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

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

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

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

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

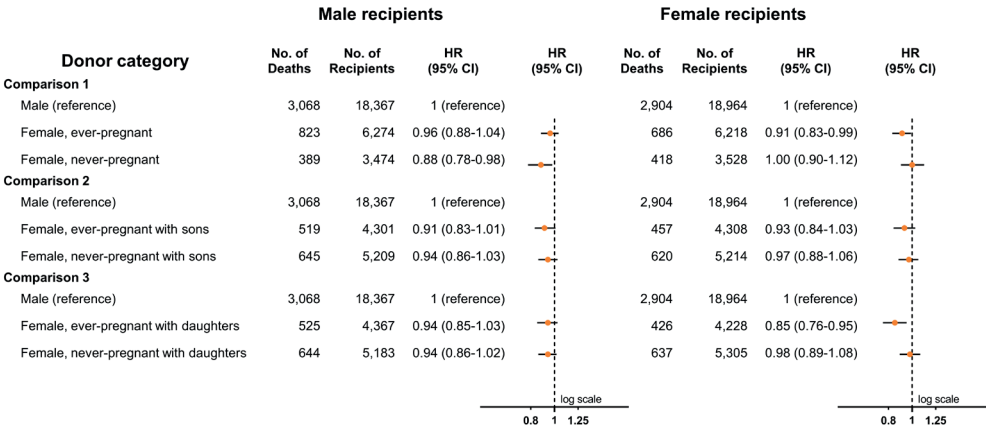


Figure 2. Mortality Hazard Ratio Of Male and Female Transfusion Recipients of Male, Ever-Pregnant (with Sons or Daughters) and Never-Pregnant Female Donor Red Blood Cell Products

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

Abbreviation: HR, hazard ratio.

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

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

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

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

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

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

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

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

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

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

Sensitivity analyses

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

Discussion

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

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

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

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

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

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

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

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

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

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

Acknowledgements

This research was funded by Sanquin Research (grant PPOC-18-03, www.sanquin.nl). We thank the Scientific Committee at the Department of Clinical Epidemiology of the LUMC for their methodological support. We thank the blood donors at Sanquin Blood Supply and the patients from the six hospitals who contributed their data to the study.

Disclosure of Conflicts of Interest

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

REFERENCES

1. Shander A, Fink A, Javidroozi M, et al. Appropriateness of Allogeneic Red Blood Cell Transfusion: The International Consensus Conference on Transfusion Outcomes. *Transfusion Medicine Reviews*. 2011;25(3):232-246.e253.
2. Carson JL, Triulzi DJ, Ness PM. Indications for and Adverse Effects of Red-Cell Transfusion. *New England Journal of Medicine*. 2017;377(13):1261-1272.
3. Middelburg RA, Briet E, van der Bom JG. Mortality after transfusions, relation to donor sex. *Vox Sang*. 2011;101(3):221-229.
4. Caram-Deelder C, Kreuger AL, Evers D, 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(15):1471-1478.
5. Altman DG, Andersen PK. Calculating the number needed to treat for trials where the outcome is time to an event. 1999;319(7223):1492-1495.
6. Zhao J, Sjölander A, Edgren G. Mortality Among Patients Undergoing Blood Transfusion in Relation to Donor Sex and Parity: A Natural Experiment. *JAMA Internal Medicine*. 2022;182(7):747-756.
7. Edgren G, Murphy EL, Brambilla DJ, et al. Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. *Jama*. 2019;321(22):2183-2192.
8. Valk SJ, Caram-Deelder C, Zwaginga JJ, van der Bom JG, Middelburg RA. Donor sex and recipient outcomes. *ISBT Science Series*. 2019.
9. Faist E, Schinkel C, Zimmer S. Update on the Mechanisms of Immune Suppression of Injury and Immune Modulation. *World Journal of Surgery*. 1996;20(4):454-459.
10. Evers D, Middelburg RA, de Haas M, et al. Red-blood-cell alloimmunisation in relation to antigens' exposure and their immunogenicity: a cohort study. *Lancet Haematol*. 2016;3(6):e284-292.
11. Edgren G, Rostgaard K, Hjalgrim H. Methodological challenges in observational transfusion research: lessons learned from the Scandinavian Donations and Transfusions (SCANDAT) database. 2017;12(1):191-195.
12. Robins JM, Finkelstein DM. Correcting for noncompliance and dependent censoring in an AIDS Clinical Trial with inverse probability of censoring weighted (IPCW) log-rank tests. *Biometrics*. 2000;56(3):779-788.
13. Robins JM. Information recovery and bias adjustment in proportional hazards regression analysis of randomized trials using surrogate markers. Proceedings of the Biopharmaceutical Section, American Statistical Association: San Francisco CA; 1993:3.
14. van der Wal WM, Geskus RB. ipw: an R package for inverse probability weighting. *Journal of Statistical Software*. 2011;43:1-23.
15. Chassé M, Fergusson DA, Tinmouth A, et al. Effect of Donor Sex on Recipient Mortality in Transfusion. *New England Journal of Medicine*. 2023;388(15):1386-1395.
16. Hernán MA RJ. Causal Inference: What If.: Boca Raton: Chapman & Hall/CRC; 2020.
17. Kanias T, Lanteri MC, Page GP, et al. Ethnicity, sex, and age are determinants of red blood cell storage and stress hemolysis: results of the REDS-III RBC-Omics study. *Blood Adv*. 2017;1(15):1132-1141.

18. Eikmans M, van Halteren AGS, van Besien K, van Rood JJ, Drabbels JJM, Claas FHJ. Naturally acquired microchimerism: implications for transplantation outcome and novel methodologies for detection. *Chimerism*. 2014;5(2):24-39.
19. van Halteren AG, Dierselhuis MP, Netelenbos T, Fechter M. Donor parity no longer a barrier for female-to-male hematopoietic stem cell transplantation. *Chimerism*. 2014;5(2):56-58.
20. Jackman RP, Utter GH, Lee TH, et al. Lack of persistent microchimerism in contemporary transfused trauma patients. *Transfusion*. 2019;59(11):3329-3336.

Supplemental materials

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

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

Contents

Supplemental methods

Exposure

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

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

Outcome

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

Covariates

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

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

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

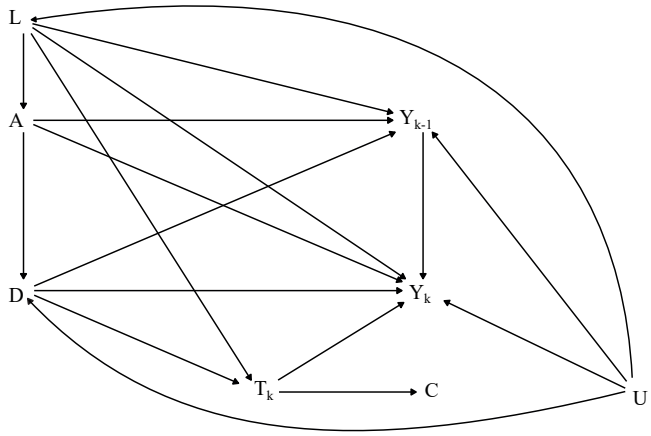


Figure S1. Directed acyclic graph of the effect of product characteristics (donor pregnancy and sex of the offspring) on mortality

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

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

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

Follow-up

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

Statistical analysis

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

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

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

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

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

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

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

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

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

Supplemental results

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

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

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

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

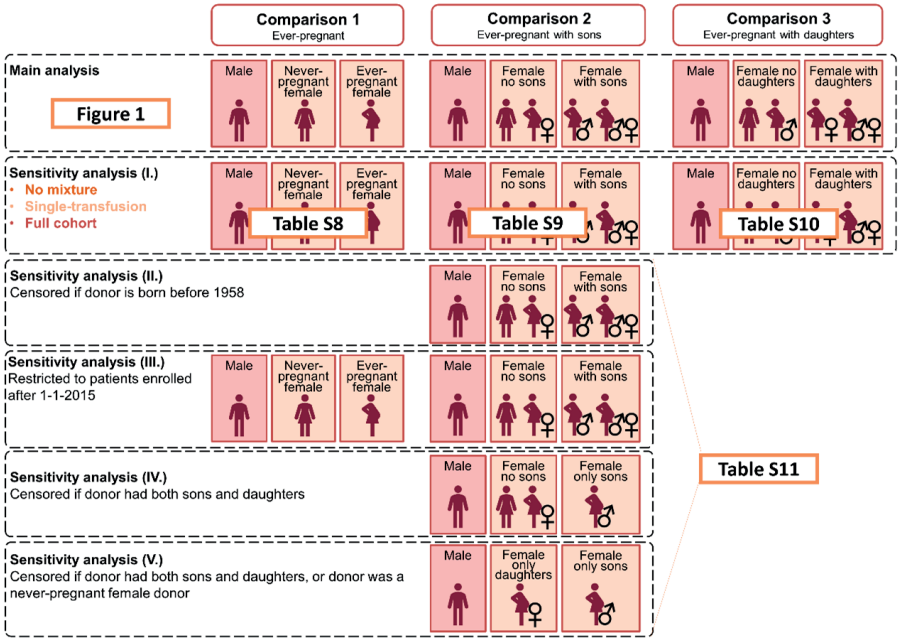


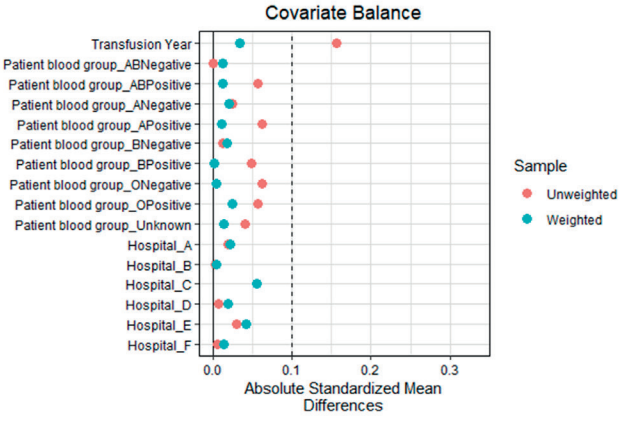
Figure S2. Schematic representation of exposure definition in sensitivity analyses and corresponding tables

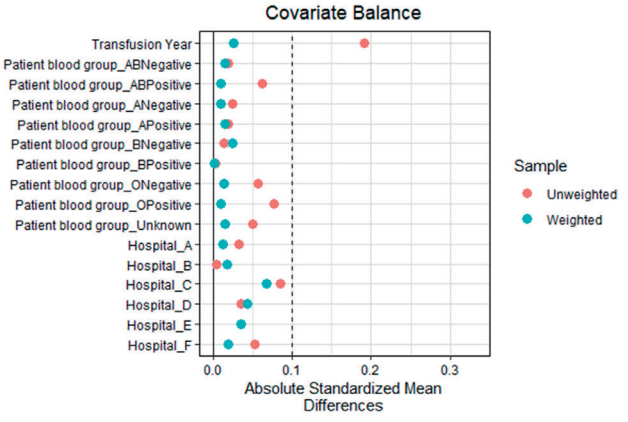
Table S12 contains results for the comparison of exposure categories as assigned on the first day for the complete study population. TableS13 contains the distribution of the weights prior to truncation, for the population of male and female patients in the primary analysis, comparison 1.

Male patients

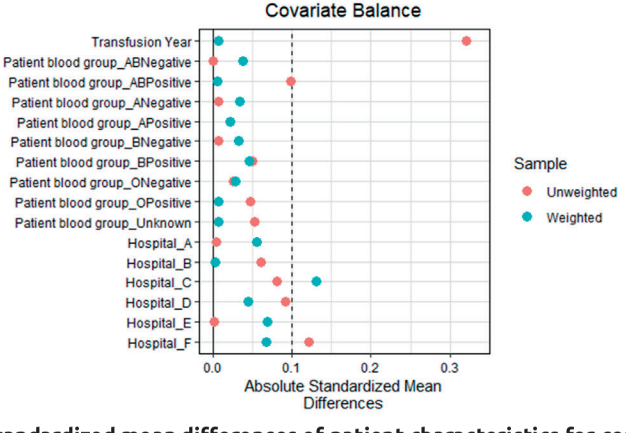
Female patients

Exposure: ever-pregnant donor





Exposure: never-pregnant donor



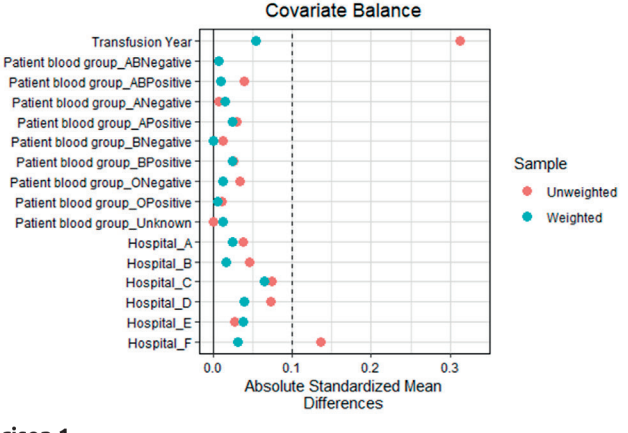


Figure S3. Absolute standardized mean differences of patient characteristics for comparison 1

Sensitivity analyses I-V

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

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

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

Supplemental tables

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

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

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

Table S2. Patient and transfusion characteristics for the Sensitivity Analyses

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

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

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

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

§ Includes units from female donors with offspring of unknown sex

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table S13. Weights distribution of primary analysis, comparison 1

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

References

1. Caram-Deelder C, Kreuger AL, Evers D, 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(15):1471-1478.
2. Middelburg RA, Briet E, van der Bom JG. Mortality after transfusions, relation to donor sex. *Vox Sang*. 2011;101(3):221-229.
3. Franchini M, Marano G, Mengoli C, et al. Red blood cell transfusion policy: a critical literature review. *Blood Transfusion*. 2017;15(4):307.
4. Langi Sasongko P, Rolink M, van den Hurk K, van Kraaij M, Janssen M. Past, present, and future: a qualitative and literature study identifying historical trends, drivers, and transformational factors for the future demand of blood supply in the Netherlands. *Transfusion*. 2019;59(11):3413-3423.
5. Evers D, Middelburg RA, de Haas M, et al. Red-blood-cell alloimmunisation in relation to antigens' exposure and their immunogenicity: a cohort study. *Lancet Haematol*. 2016;3(6):e284-292.
6. Edgren G, Rostgaard K, Hjalgrim H. Methodological challenges in observational transfusion research: lessons learned from the Scandinavian Donations and Transfusions (SCANDAT) database. 2017;12(1):191-195.
7. Zhao J, Sjölander A, Edgren G. Mortality Among Patients Undergoing Blood Transfusion in Relation to Donor Sex and Parity: A Natural Experiment. *JAMA Internal Medicine*. 2022;182(7):747-756.
8. CBO. Dutch Guideline Bloodtransfusion. *Retrieved October 22, 2018, from: https://nvicnl/sites/nvicnl/files/CBO%20Richtlijn%20Bloedtransfusiepdf*. 2011.
9. Robins JM, Finkelstein DM. Correcting for noncompliance and dependent censoring in an AIDS Clinical Trial with inverse probability of censoring weighted (IPCW) log-rank tests. *Biometrics*. 2000;56(3):779-788.
10. Robins JM. Information recovery and bias adjustment in proportional hazards regression analysis of randomized trials using surrogate markers. Proceedings of the Biopharmaceutical Section, American Statistical Association: San Francisco CA; 1993:3.
11. van der Wal WM, Geskus RB. ipw: an R package for inverse probability weighting. *Journal of Statistical Software*. 2011;43:1-23.
12. Edgren G, Murphy EL, Brambilla DJ, et al. Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. *Jama*. 2019;321(22):2183-2192.
13. Halmin M, Chiesa F, Vasan SK, et al. Epidemiology of Massive Transfusion: A Binational Study From Sweden and Denmark*. *Critical Care Medicine*. 2016;44(3):468-477.

6

Chapter 6

Clinical transfusion-outcomes research: A practical guide

Authors: Sarah J Valk[1,2], Camila Caram-Deelder[2], Rolf HHGroenwold[2], Johanna G van der Bom[2]

1. Jon J van Rood Center for Clinical Transfusion Research, Sanquin/LUMC, Leiden, The Netherlands,

2. Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands,

ABSTRACT

Clinical transfusion research on the effectiveness and safety of blood products differs greatly from other medical research fields. There are three main intricacies which are specific to research of transfusion products: 1) patients frequently require more than one unit, 2) each unit originates from a different donor, and 3) the likelihood of receiving a unit with certain characteristics depends on a variety of external circumstances, that are commonly not within the control of the investigator.

This commentary addresses methodological challenges when investigating effectiveness and safety of blood products from observational data. As an example, we focus on the association between donor sex, pregnancy history of female donors and transfusion recipient mortality. We describe the current best methodological practices and illustrate statistical analyses using an example dataset, which allows other researchers to implement these practices in their own research.

Introduction

Clinical transfusion research aims to provide insight into the benefits and harms of transfusions. Evidence from observational studies is the predominant source of information about effects of donor and product characteristics of blood transfusions on patient outcomes, as it can be used to study various factors using large datasets. In contrast, evidence from randomized controlled trials often has a more limited, specific scope, e.g. a single threshold for comparing restrictive to liberal transfusion practices[1], or comparing fresh vs. older red blood cell transfusions based on a predefined cutoff[2, 3]. Moreover, in randomized trials follow-up is often limited in duration and sample size is relatively small to avoid unnecessary burden for participants and reduce cost. However, the randomized trial design is less suited to investigate research questions about blood product characteristics. Provided observational studies are designed and executed rigorously, such that potential bias is sufficiently mitigated, evidence from observational studies can complement the evidence based on randomized trials.[4, 5]

In this commentary, we shine the spotlight on methodological aspects related to confounding and selection bias of longitudinal observational data in clinical transfusion research. The goal of this commentary is to inform readers and researchers of such studies, to provide practical guidance and to encourage discussion about these important topics. Specifically, we *(1)* discuss intricacies of observational data of blood product characteristics, *(2)* present an overview of methods used in studies of blood product characteristics, *(3)* discuss these methods, including considerations for designing and analyzing clinical transfusion studies of donor and product characteristics, and *(4)* provide a tutorial for the use of marginal structural models in investigating transfusion exposures.

1. The challenges pertaining to blood product characteristics research

There are several specific challenges that contribute to the difficulty of studying efficacy and safety in the clinical transfusion setting. First, because every transfusion is linked to a specific donor, there is a wide variation in the pool of available blood products. Depending on the research question, particular products might be very common or very rare, potentially leading to limited statistical power. Second, patients are frequently exposed to multiple transfusions. Although restrictive transfusion practices have become more common, on average patients in the Netherlands receive two transfusions per transfusion episode, with more transfusions given depending on the indication.[6] Third, external factors (e.g. calendar time, patient blood group and geographic region) influence the probability of receiving a unit with any of these different characteristics. Last, the

existence of a possible bidirectional relationship between donor characteristics and patient outcomes is a recent insight that warrants increased scrutiny.[7]

Before addressing the different statistical analyses that could be applied to accommodate these challenges, we first introduce the epidemiological concepts that are important in research addressing the clinical outcomes of patients treated with blood transfusion. From the standpoint of modern causal inference, the concepts of confounding and selection bias are barriers in the estimation of a causal effect, that can be overcome by identifying the minimally sufficient adjustment set of covariates from a directed acyclic graph (DAG).[5] A causal DAG identifies which variables to adjust for, and which not, to be able to estimate a causal effect of the exposure of interest on the outcome. Drawing the DAG can be challenging, as transfusion exposure investigations are complex studies, involving longitudinal data, often including time-varying confounding and censoring of follow-up. In contrast to single timepoint interventions, or 'point treatments', transfusions are given over time and therefore conventional approaches to adjust for confounding might not be appropriate. The causal contrast of interest in these studies is commonly defined as initiating and adhering to the initial exposure assignment, that is, the characteristics of the first received transfusion. The exposure of interest is then compared to a chosen reference category. However, in longitudinal studies, intercurrent events need to be taken into account. As patients are exposed to multiple transfusions over time, they often do not solely receive the same exposure category throughout their follow-up. The question arises, what should be done with the follow-up from these 'cross-over' patients?

The answer was generally thought to be: to adjust for the time-varying cumulative number of transfusions by censoring the follow-up time of patients when they no longer adhere to their earlier exposure category. Because the number of transfusions is associated with the exposure (a particular product characteristic), and the outcome (mortality), the causal effect of exposure to the product characteristic of interest is estimated by adjusting for the cumulative number of transfusions received over time. Follow-up should be included using time-varying approaches, because patients who receive multiple transfusions are less likely to adhere to their initial transfusion exposure, and selecting only patients who adhered to their `assigned' exposure will therefore lead to bias.[5] Thus, rather than standard adjustment for covariates at baseline, control for confounding when time-varying confounding is present requires adjustment for time-varying covariates during follow-up of individual patients and censoring of follow-up at the time of non-adherence to the initial transfusion exposure category. However, depending on assumptions about the reasons for non-adherence to the initial

transfusion exposure category, more advanced statistical modelling techniques may be required. This is because, when non-adherence is both 1. affected by prior exposure and 2. informative of the outcome, traditional methods can fail, and consequently yield biased results. This phenomenon is known as treatment-confounder feedback, which is discussed in more detail in the next section.

2. Treatment-confounder feedback in studies of transfusion exposures

When time-varying confounders are affected by prior treatment, traditional methods (e.g. stratification, matching, outcome regression) are generally not suitable for confounding adjustment, as these may adjust away part of the effect of the exposure, yet also introduce a spurious association between exposure and outcome.[5] In studying any exposures that are tied to the subsequent probability of receiving additional transfusions, i.e. exposures associated with consistent product hemoglobin increment differences, this hence becomes a problem that can no longer be solved easily. We refer to this as treatment-confounder feedback by product hemoglobin content. This concept, previously described by Zhao et al.[7], is illustrated in Figure 1.

In Figure 1, panel A shows the partial DAG for the investigation of donor characteristics and mortality. The number of transfusions received over time (L) is associated with the probability of receiving female donor-only units (A) and the underlying disease severity (U) and is therefore part of the minimally sufficient adjustment set. Adjustment for L is required to estimate the effect of A on mortality (Y); this can be done using traditional methods (not shown) or g-methods (shown). With exposure to female donor-only units, however, comes a decreased hemoglobin 'dose' and therefore an increased need for additional transfusions (panel B). This can be illustrated by creating separate timepoints for treatment A and confounder L, thereby providing the complete DAG for this research question (panel C). This DAG shows that adjustment for L using traditional methods is not appropriate when the combined effect of A_t and A_{t+1} is of interest, as L is now located in the causal path of A_t on Y, in addition to being a confounder for the effect of A_{t+1} on Y. Alternative methods, such as g-methods (which include inverse probability of treatment weighting of marginal structural models, the parametric g-formula, and g-estimation of structural nested models[5]), are required here.

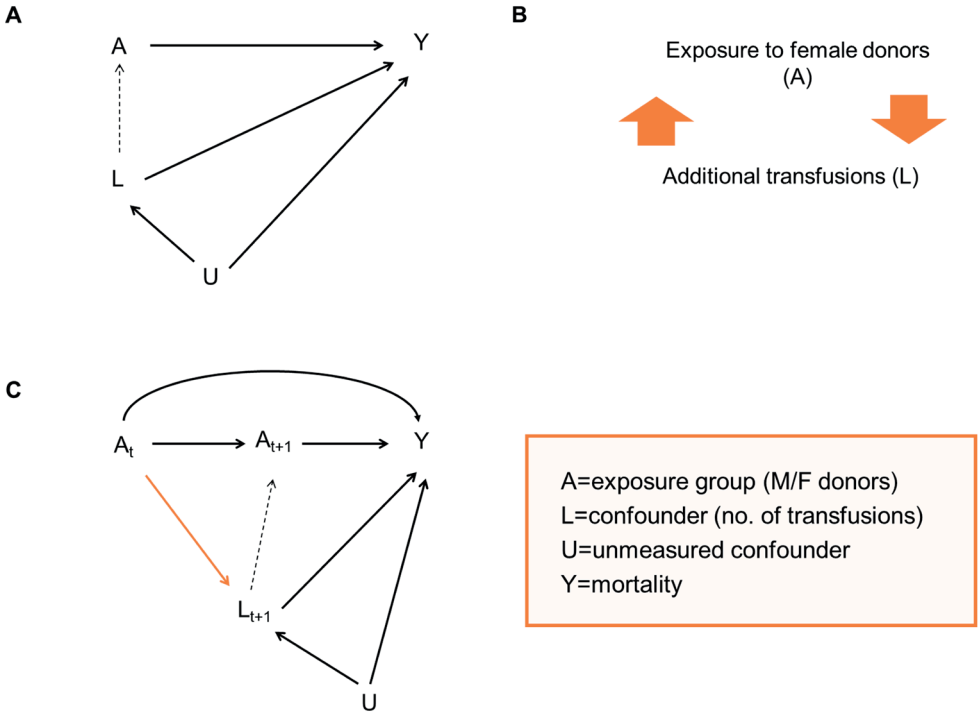


Figure 1. Different graphs to illustrate when advanced statistical modelling using g-methods is required.

A) Partial directed acyclic graph (DAG) of the effect of exposure to female donors (A) on mortality (Y) in transfusion recipients, confounded by unmeasured confounders (U, e.g. disease severity) through the cumulative number of transfusions (L). Dashed arrow represents the use of g-methods for the estimation of a causal effect of A on Y in the absence of treatment-confounder feedback, by removing the dependence of A on L.

B) Perceived bidirectionality if time is not taken into account, resulting in a cyclic graph, when assessing the effect of A on Y.

C) Complete DAG for the effect of exposure to female donor units including the treatment-confounder feedback over two timepoints (t, t+1) by lower hemoglobin concentration of units from female donors. Orange arrow represents the treatment-confounder feedback. Dashed arrow represents analysis using g-methods, removing the dependence of A_{t+1} on L, making estimation of the causal effect of A on Y possible in the presence of treatment-confounder feedback.

Specific situations where extra attention is expected to be warranted are the previously mentioned studies on donor sex, and pregnancy history of the donor. Also, storage duration of blood products can lead to smaller hemoglobin increments, and irradiation of red blood cell products would similarly require caution if chosen as exposure. Note that this is a non-exhaustive list, and researchers are encouraged to think carefully if their research question necessitates the use of alternative methods which can be used to estimate treatment effects in the presence of treatment-confounder feedback.

3. Appropriateness of methods applied in clinical transfusion research of product characteristics

Several statistical analysis methods have been applied in the field of transfusion product characteristics research (*Table 1*).

Table 1. Overview of methods used to study blood product characteristics as exposure

Methodology	Description of application in clinical transfusion research	Can handle treatment-confounder feedback	References
Traditional methods – restriction approach	Selection based on exposure classification at end of follow-up Stratification, matching, outcome regression (including propensity score regression adjustment and matching)	No	Middelburg, Alshalani[8, 9]
Traditional methods – time-varying approach	Exposure and confounder information modelled as time-varying variables Cox proportional hazards model with time-varying treatment and confounders	No	Caram-Deelder, Edgren[10-12]
G-methods – inverse-probability of censoring weighting	Time-varying exposure and confounder information used for reweighing population to mitigate bias due to treatment-confounder feedback Inverse probability-weighted marginal structural models	Yes	Zhao[7], MATER study (Chapter 5

Restriction approaches were employed, assessing the risk of exposure for groups of patients that were exposed to a single exposure type, without time-varying components.[8] This method is at risk of introducing selection bias, as the patients who only received one type of exposure throughout the follow-up period are inherently different from those who receive more transfusions, and are removed from the analysis because they did not adhere to their initial exposure at the start of follow-up. A more in-depth discussion of selection bias in cohort studies can be found elsewhere.[13] Specific for the clinical transfusion field, an example would be the selection of male-donor only and female-donor only exposure in a 'unisex' recipient cohort (i.e. selective inclusion of patients who received transfusions of single-sex donor origin Selection based on classification at the end of follow-up is not appropriate in the presence of treatment-confounder feedback, as this can lead to biased estimates of risk for transfusion characteristics.

Time-varying exposure and confounding adjustment has also been applied, with the number of units received with a specific (donor or product) characteristic included in the model as a continuous variable.[10-12] A potential pitfall in applying this method is the inclusion of continuous variables without properly taking into

account nonlinearity.[12, 14] Stratified Cox proportional hazards regression models with time-dependent exposures have recently been applied in this field.[11] The time-varying approach is not appropriate if there is treatment-confounder feedback, as it can lead to biased effect estimates.

Other possible analysis strategies include inverse probability of censoring weighting, to account for patients in certain exposure categories being more likely to receive additional transfusions and no longer being compliant to the initial blood product exposure, and therefore having to be censored.[7, 15]

Alternative approaches for defining exposure exist: binary exposure (coded as 0 or 1; female donor exposure vs. no female donor exposure) and full data utilization exposure (unexposed male vs. exposed male and female).[16] As these are either by design included in (or variations of) the methods presented here, we will not discuss them further. Lastly, methods reclassifying the exposure into a ratio have been proposed, but their complexity and computational intensity for survival data make them fall outside the scope of this commentary.[17]

4. Example dataset with applied methods illustrating that some approaches can lead to biased results

We applied the above-described methods to an example dataset to allow for a comparison of their performance in a semi-controlled setting. For this dataset, the study population consisted of male patients included in an earlier publication[10]. These male patients received transfusions in one of six included hospitals between 2005 and 2015. The complete exposure information was sourced from the Dutch municipality registration (see Chapter 5) to overcome the limitation of the original publication where 44% of the units donated by female donors had missing information about the pregnancy history. In Table S1, patient and blood product characteristics are described for this example dataset. Associations described in Table 2 apply to the patient population from the original, earlier publication and data were not altered or manipulated. This, opposed to the dataset for which the results are described in Table S2, which underwent an anonymization procedure that removed the empirical data, for the purpose of a publicly accessible tutorial.

Table 2. Results for different methods applied to the example dataset

Analysis	No. of Deaths	No. of Recipients*	HR (95% CI)
Restriction method			
Male (reference)	1,916	6,430	1 (reference)
Ever-pregnant female	207	770	1.22 (1.05-1.42)
Time-varying exposure and confounding adjustment method			
Male (reference)	1,916	10,901	1 (reference)
Ever-pregnant female	207	1,494	1.21 (1.04-1.41)
Inverse probability of censoring weighting method			
Male (reference)	1,916	10,901	1 (reference)
Ever-pregnant female	207	1,494	1.01 (0.85-1.20)

*Population included all male transfusion recipients that were identified in both datasets[10] with approx. 10% of patients not identified in the new dataset because of changes to the hospital administration records. HR hazard ratio; CI confidence interval

In Table 2, the risk for exposure to ever-pregnant donor-only units compared to the reference group of male-only unit exposure is presented for the three methods described in Section 2 applied to an example dataset. The inverse probability of treatment- and censoring-weighted analysis, estimating the average treatment effect of exposure to donors with a positive pregnancy history on mortality, is unbiased by treatment-confounder feedback present in the data (hazard ratio 1.01, 95% confidence interval 0.85-1.20). In contrast, the application of the time-varying adjustment method and restriction method give an estimate that is further away from 1, which is likely because of treatment-confounder feedback by hemoglobin increment differences between the two compared blood product exposures. In conclusion, statistical choices have considerable influence on the estimated hazard ratio in the investigation of blood product characteristics.

5. Tutorial for the application of marginal structural models as a way to estimate causal associations in the presence of treatment-confounder feedback

The use of inverse-probability weighted marginal structural models is not widespread in the field of clinical transfusion research, because their importance for studying transfusion exposures has not been recognized until recently. By providing an open-access example dataset with donor and patient characteristics, as well as concise R code, we hope to engage the scientific community, and encourage researchers to be more aware of the specific problems that arise when studying donor and product characteristics that relate to product hemoglobin content.

We provide a structured tutorial to perform the inverse probability of censoring weighting method described in Section 3 on a provided dataset (*Supplemental materials*, page 4). The dataset used in Section 4 is made available, after having applied an anonymization procedure to avoid sharing of personal patient data, and can be obtained by contacting the author (hyperlink to be added upon publication). The results for the inverse probability of censoring weighted analysis applied to the anonymized dataset can be found in Table S2. Because the original structure in the dataset was lost and treatment-confounder feedback is not present, all methods perform similar and are unbiased.

6. Conclusions

The importance of thorough epidemiological study design in clinical transfusion research cannot be overstated. In this commentary, recent insights about hemoglobin increments and their impact on blood product characteristics research were extensively discussed, and an overview including an appraisal of these methods was provided. As an example, we made use of a large observational dataset of transfusion and patient data. We applied several methods used in the past and present, from which inverse probability of censoring weighting should be considered in the presence of treatment-confounder feedback because this method can adequately account for time-varying confounding in the presence of such feedback. We also provide a detailed tutorial to guide those pursuing similar research.

Evidently, clinical transfusion outcomes research using observational data can be complex. Specifically for blood product characteristics research, these challenges include the adjustment for time-varying confounders, the censoring of follow-up time when mixed exposure occurs, and treatment-confounder feedback by product hemoglobin content. The appropriate statistical methodology can be difficult to identify, and especially when complex research questions are of interest, target trial emulation can provide useful insights. Target trial emulation, where a hypothetical randomized controlled trial is imagined and replicated with observational research, can be a useful tool to avoid both basic mistakes, and more complex analytical pitfalls.[18] With a target trial emulation approach, the hypothetical randomized trial is imagined which the observational study aims to mimic, including the specification of the start of follow-up, the exposure definition, the approach for how intercurrent events are handled, and so on. Of note, assumptions and decisions about the analysis are best specified up front, to avoid the problems associated with 'researcher degrees of freedom'.[19] When the aforementioned challenges are appropriately handled, it is possible to draw causal conclusions from observational transfusion data.

We emphasize that, while there are certainly limitations to several study designs used in the past, there is always a tradeoff between bias and precision where in some cases, a simpler method might be preferable. This can include the choice of changing the exposure of interest to single timepoint exposures, as opposed to sustained exposure over time. Researchers can and should give sufficient attention to the strengths and limitations of their chosen approach, and sensitivity analyses can be employed to test the impact of assumptions on the robustness of the estimate.

To conclude, we addressed the appropriateness of specific statistical methods in the presence of treatment-confounder feedback in the clinical transfusion research field and have provided guidance for future research. The suitability of any method depends on assumptions about the underlying causal relations in the data, and careful consideration about this is needed to ensure interpretations are valid.

Data availability statement

The original data used in this article and an earlier publication is available for inspection upon request. An anonymized dataset which can be used to run the provided syntax on will be made available in a repository upon publication. Anonymization was performed by random permutation.[20] Note: the original data structure is not completely retained following anonymization, but more advanced anonymization methods that can retain the original data structure have not yet been developed for survival analysis.[21]

Supplementary materials

The Supplementary materials contain the tutorial with syntax for use in R (*Supplementary materials*). Additional tables with results for the provided, anonymized dataset available from the repository are reported in Table S2.

REFERENCES

1. Carson JL, Stanworth SJ, Dennis JA, et al. Transfusion thresholds for guiding red blood cell transfusion. *Cochrane Database of Systematic Reviews*. 2021;(12) doi:10.1002/14651858.CD002042.pub5
2. Shah A, Brunskill SJ, Desborough MJ, Doree C, Trivella M, Stanworth SJ. Transfusion of red blood cells stored for shorter versus longer duration for all conditions. *Cochrane Database Syst Rev*. Dec 22 2018;12:Cd010801. doi:10.1002/14651858.CD010801.pub3
3. 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. *Transfusion Medicine Reviews*. 2018/04/01/ 2018;32(2):77-88. doi:https://doi.org/10.1016/j.tmrv.2018.02.002
4. Hernán MA. The C-Word: Scientific Euphemisms Do Not Improve Causal Inference From Observational Data. *American Journal of Public Health*. 2018;108(5):616-619. doi:10.2105/ajph.2018.304337
5. Hernán MA RJ. Causal Inference: What If. Boca Raton: Chapman & Hall/CRC; 2020:chap Chapter 20.
6. 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.
7. Zhao J, Sjölander A, Edgren G. Mortality Among Patients Undergoing Blood Transfusion in Relation to Donor Sex and Parity: A Natural Experiment. *JAMA Internal Medicine*. 2022;182(7):747-756. doi:10.1001/jamainternmed.2022.2115
8. Middelburg RA, Briet E, van der Bom JG. Mortality after transfusions, relation to donor sex. *Vox Sang*. Oct 2011;101(3):221-9. doi:10.1111/j.1423-0410.2011.01487.x
9. Alshalani A, Uhel F, Cremer OL, et al. Donor-recipient sex is associated with transfusion-related outcomes in critically ill patients. *Blood Adv*. Jun 14 2022;6(11):3260-3267. doi:10.1182/bloodadvances.2021006402
10. Caram-Deelder C, Kreuger AL, Evers D, 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*. Oct 17 2017;318(15):1471-1478. doi:10.1001/jama.2017.14825
11. Edgren G, Murphy EL, Brambilla DJ, et al. Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. *Jama*. Jun 11 2019;321(22):2183-2192. doi:10.1001/jama.2019.7084
12. Edgren G, Ullum H, Rostgaard K, et al. Association of Donor Age and Sex With Survival of Patients Receiving Transfusions. *JAMA Intern Med*. Jun 1 2017;177(6):854-860. doi:10.1001/jamainternmed.2017.0890
13. Hern, xe, n MA, et al. A Structural Approach to Selection Bias. *Epidemiology*. 2004;15(5):615-625.
14. Chasse M, Tinmouth A, English SW, et al. Association of Blood Donor Age and Sex With Recipient Survival After Red Blood Cell Transfusion. *JAMA Intern Med*. Sep 1 2016;176(9):1307-14. doi:10.1001/jamainternmed.2016.3324
15. Robins JM, Finkelstein DM. Correcting for noncompliance and dependent censoring in an AIDS Clinical Trial with inverse probability of censoring weighted (IPCW) log-rank tests. *Biometrics*. Sep 2000;56(3):779-88. doi:10.1111/j.0006-341x.2000.00779.x

16. Heddle NM, Cook RJ, Liu Y, et al. The association between blood donor sex and age and transfusion recipient mortality: an exploratory analysis. *Transfusion*. Feb 2019;59(2):482-491. doi:10.1111/trf.15011
17. 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;51doi:10.1016/j.eclinm.2022.101628
18. Hernán MA, Robins JM. Using Big Data to Emulate a Target Trial When a Randomized Trial Is Not Available. *Am J Epidemiol*. 2016;183(8):758-764. doi:10.1093/aje/kwv254
19. Simmons JP, Nelson LD, Simonsohn U. False-Positive Psychology: Undisclosed Flexibility in Data Collection and Analysis Allows Presenting Anything as Significant. *Psychological Science*. 2011;22(11):1359-1366.
20. Ängquist L. Stata tip 92: Manual implementation of permutations and bootstraps. *The Stata Journal*. 2010;10(4):686-688.
21. Kroes SKS, van Leeuwen M, Groenwold RHH, Janssen MP. Generating synthetic mixed discrete-continuous health records with mixed sum-product networks. *Journal of the American Medical Informatics Association*. 2022;30(1):16-25. doi:10.1093/jamia/ocac184

Supplement: Clinical transfusion-outcomes research: A practical guide

Contents:

Supplemental methods

Inverse probability of censoring weighting method (IPW)

The dataset was organized as longitudinal survival data (with *t_begin* representing start of follow-up and *t_end* representing the end of follow-up for each patient row), for use in the *ipw* and *survey* package in R.[1] Initial follow-up is ordered as daily intervals for the first 28 days, followed by 4-week intervals ("blocks"). Weighted Cox proportional hazards models were fitted to correct for censoring and confounding.[1] Analyses were performed in R (version 3.6.3) and R Studio (version 2022.02.0+443) software.

The following variables were included in the multinomial logistic regression to estimate the baseline inverse probability of treatment weights: year of first transfusion exposure (*Transfusion_Year_first,* continuous), patient blood group (*Patient_ABORh*, categorical), hospital (*Hospital*, categorical). The outcome variable for the logistic regression was the categorical variable *Arm* (taking 0 if exposure was to the reference of male donors, 1 if exposure was to ever-pregnant female donors, and 9 if exposure was to other/mixed products).

The cumulative number of transfusions was included as the only covariate in the model for the generation of inverse probability of censoring weights (*Arm_Total_cum*), as a time-varying continuous variable. The outcome for this model was the censoring variable (*Censored*). Because patients could contribute multiple transfusion episodes, robust standard errors were used for the computation of

the confidence limits.[2] Only patients exposed to the reference arm (male, *Arm* taking the value 0) donors or the exposure arm (ever-pregnant female, *Arm* taking the value 1) were included in the estimation of censoring weights. Censoring weights were generated for the dataset weighted by the inverse probability of treatment weights generated earlier. Weights were plotted within strata of follow-up time to determine the distribution of the weights with *ipwplot*.

The resulting weights were multiplied to create the final weights. Truncation, or trimming, of the weights in case of extreme weights (e.g. >10) is optional. The spread of the weights was assessed by calculating the 0.5^{th} and 99.5^{th} percentiles of the weights.

If patients were censored or died in a block, they were interval-censored. The actual end of follow-up, the variable *t_end_new,* was then used to replace the block time *t_end* for use in the Cox proportional hazards model.

The weighted Cox proportional hazards model was specified with the exposure (*Arm*), the outcome (*Death*), the time variables (*t_begin*, *t_end)* and the final weights. Only uncensored lines (*Censored* = 0) were included in the model.

A detailed R code including all steps described above is available at the end of the Supplemental materials.

Time-varying exposure and confounding adjustment method

Cox proportional hazards models were fitted, adjusted for: cumulative number of transfusions (restricted cubic spline with three knots); hospital (categorical); blood group (categorical); calendar year (categorical); age of the donor (cumulative number of transfusions from donors aged >50 years, continuous); interaction term for cumulative number of transfusions and hospital.(see Chapter 5) Exposure is included as a binary, categorical variable.

This method is expected to be biased if treatment-confounder feedback is present due to limitations of traditional regression analysis. Analyses were performed in Stata, version 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

Restriction method

Similar to method described above, with the distinction that only patients who received transfusions from the same exposure category as the first, are included and Cox PH regression is performed without a time-varying component.

This method conditions on information from the future follow-up of the patients, and is also expected to lead to bias. Analyses were performed in Stata, version 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

Tutorial for use of IPW for transfusion-outcomes research in R

The below provided syntax can be used to perform an inverse probability of treatment- and censoring-weighted analysis(see Chapter 5) for blood product exposures related to hemoglobin increment raising capacity of the product, on a provided, anonymized dataset. Note that this dataset does not retain all original features of the real dataset, and the treatment-confounder feedback structure was lost due to the anonymization process. The provided anonymized dataset is a representative example of a dataset generated with random permutation of the variables *Arm* (exposure, assigned randomly from original distribution), *Hospital* (category for the hospital where the patient received the transfusion, assigned randomly in one of four categories from original distribution of six hospitals), *Patient_ABORh* (category of the blood group ABO and Rhesus type, assigned randomly from original distribution) and *Transfusion_Year_first* (year of the first transfusion of the patient, i.e. year of patient's start follow up, assigned randomly from original distribution). All other variables were kept identical to the original dataset.

Tutorial syntax in R:

The tutorial is organized as follows:

Step 0. Specify working directory and prepare files

Step 1. Inverse probability of treatment weights (IPTW) estimation with multinomial logistic regression

Step 2. Inverse probability of censoring weights (IPCW) estimation with weighted Cox regression

Step 3. Multiplication of weights (IPTW*IPCW) to create final weights

Step 4. IPW-corrected Cox model

Step 0

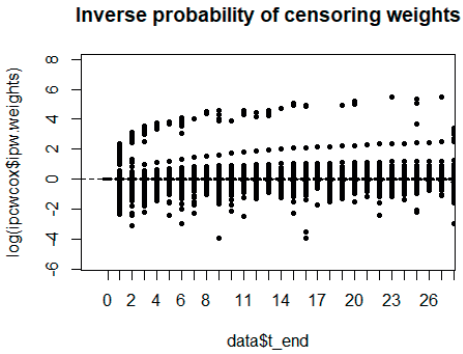
```
#Tutorial Clinical transfusion-outcomes research: A practical guide #required: file
= "Datafile-clinicaltransfusion.Rdata" (available upon request)
##############################################
#Tutorial
#Male patients only
#Comparison: Male (0) vs Ever-pregnant female (1)
#Variables in the dataset are:
```

```
#PIN: unique patient identifier.
#Arm: 0: control, patients whose first transfusion was donated by a male donor; 1: exposure, patients whose first transfusion was donated by a female donor who had been pregnant; 9: patients whose first transfusion was donated by blood donated by any other than exposure and control, i.e. female without history of pregnancy or sex of the donor unknown, and/or mixed exposure on day 1).
#Transfusion_Year_first:  year of the first transfusion of the patient, i.e. year of patient's start follow up).
#Patient_ABORh: patient blood group, category.
#Hospital: hospital name, category.
#Censored: censoring indicator (0 if patient received all transfusions from the same Arm group, 1 if patient no longer adhered to initial group assignment).
#Arm_Total_cum: cumulative number of transfusions, continuous.
#t_begin and t_end: time variables, each line refers to a single time period (t_begin refers to the start of the follow up, as required by the ipw package; all t_begin lines are rescheduled having -1 as reference; the first 28 days of follow up are included as one line per day and after day 28 the lines refer to blocks of 28 days).
#t_end_new: time variable, adjusted from block size (only blocks of 28 days are allowed) to real end of follow-up (individual days are allowed, e.g. if the patient has died at day 30, t_end_new would be '30', while t_end would be '56').
#Death: indicator for event at time t_end.
################################################
#step 0. specify working directory and prepare files
#install packages
#install.packages("ipw")
#install.packages("survival")
#install.packages("survey")
#install.packages("dplyr")
#load packages
library(ipw)
library(survival)
library(survey)
library(dplyr)
#set working directory: complete the path with the local where the datafile "Datafile-clinicaltransfusion.Rdata") is located
setwd("C:\\dir")
#clear workspace
rm(list=ls())
#load files
load(file= "Datafile-clinicaltransfusion.Rdata")
```

Step 1-4

```
################################################
#step 1. inverse probability of treatment weights (IPTW) estimation with multinomial
logistic regression
confounder_weight <- ipwpoint(exposure = Arm, family = "multinomial", numerator =
~1, denominator =~Transfusion_Year_first + Patient_ABORh + Hospital, data = data)
#OUTPUT
# weights: 6 (2 variable)
## initial value 925365.525208
## iter 10 value 345710.424406
## iter 10 value 345710.424400
## iter 10 value 345710.424397
## final value 345710.424397
## converged
## # weights: 42 (26 variable)
## initial value 925365.525208
## iter 10 value 420905.812229
## iter 20 value 388504.584486
## iter 30 value 361899.264984
## iter 40 value 349123.738592
## iter 50 value 345305.718723
## iter 60 value 345284.712165
## iter 70 value 345284.435350
## final value 345284.422337
## converged
data$iptwlogweights <- confounder_weight$ipw.weights
summary(data$iptwlogweights)
#OUTPUT
## Min. 1st Qu. Median Mean 3rd Qu. Max.
## 0.6927 0.9952 1.0009 1.0000 1.0066 1.3027
#selection of subset of exposed (ever-pregnant, F1: coded as 1) and reference (male,
M: coded as 0); excluding Unknown, F0 and mixed (coded as 9) after estimation of
iptwlogweights
data<-subset(data, Arm!=9)
################################################
#step 2. inverse probability of censoring weights (IPCW) estimation with weighted
Cox regression
#IPTCW is estimated in the population weighted by IPTW
ipcwcox <- ipwtm(
```

```
 exposure = Censored,
 family = "survival",
 numerator = ~ 1,
 denominator = ~ Arm_Total_cum ,
 id = PIN,
 tstart = t_begin,
 timevar = t_end,
 type = "first",
 data = data,
 weight = data$iptwlogweights)
data$ipcwcoxweights <- ipcwcox$ipw.weights
summary(data$ipcwcoxweights)
#OUTPUT
## Min. 1st Qu. Median Mean 3rd Qu. Max.
## 0.0039 0.8961 0.9788 1.0004 1.0004 2384.5971
#plot IPCW weights
ipwplot(weights = ipcwcox$ipw.weights, timevar = data$t_end,
 binwidth = 1, main = "Inverse probability of censoring weights" , xlim = c(0, 28))
#OUTPUT
```




```
#interpretation:
#weights are depicted for the first 28 days; the distribution of the weights is
balanced with the exception of some large weights. Weights are selected for only the
uncensored lines in step 3., leading to less extreme weights.
#preparation of data for IPW-corrected model
#selection of non-censored observations only to limit the model to follow-up time
eligible for analysis (Arm=0 or Arm=1)
data2<-subset(data, Censored!=1)
################################################
#step 3. multiplication of weights (IPTW*IPCW) to create final weights
```

```
data2$weights <- (data2$ipcwcoxweights*data2$iptwlogweights)
summary(data2$weights)
#OUTPUT
## Min. 1st Qu. Median Mean 3rd Qu. Max.
## 0.6753 0.8999 0.9664 0.9792 0.9995 60.2151
#store ranges of weights for assessment of extreme weights and weights distribution
min <- min(data2$weights)
max <- max(data2$weights)
pct005 <- quantile(data2$weights, c(.005))
pct995 <- quantile(data2$weights, c(.995))
#store extreme weights
extreme <- subset(data2, weights>10)
#truncate weights (optional: large weights lead to instability of the IPW estimator; truncation can reduce variance, but increase bias)
#data2["weights"][data2["weights"] >10] <- 10
#change t_end (to no longer be the 'block t_end', but the 'real t_end' from patient final follow-up date)
data2$t_end <- data2$t_end_new
################################################
#step 4. IPW-corrected Cox model
surveydesign1<-svydesign(id = ~ PIN, strata = ~ Arm, weights = ~ data2$weights, data = data2)
summary(svycoxph(Surv(t_begin, t_end, Death) ~ as.factor(Arm), design = survey-design1))
#OUTPUT
## Stratified 1 - level Cluster Sampling design (with replacement)
## With (12395) clusters.
## svydesign(id = ~PIN, strata = ~Arm, weights = ~data2$weights,
## data = data2)
## Call:
## svycoxph(formula = Surv(t_begin, t_end, Death) ~ as.factor(Arm),
## design = surveydesign1)
##
## n= 830334, number of events= 2297
##
## coef exp(coef) se(coef) robust se z Pr(>|z|)
## as.factor(Arm)1 0.01564 1.01576 0.06244 0.07283 0.215 0.83
##
## exp(coef) exp(-coef) lower .95 upper .95
```

```
## as.factor(Arm)1 1.016 0.9845 0.8806 1.172
##
## Concordance= 0.503 (se = 0.004 )
## Likelihood ratio test= NA on 1 df, p=NA
## Wald test = 0.05 on 1 df, p=0.8
## Score (logrank) test = NA on 1 df, p=NA
##
## (Note: the likelihood ratio and score tests assume independence of
## observations within a cluster, the Wald and robust score tests do not).
msm <- svycoxph(Surv(t_begin, t_end, Death) ~ as.factor(Arm), design = survey-
design1)
a <- exp(coef(msm))
b <- exp(confint(msm))
#store counts for Deaths/Recipients, by exposure (0/1)
n_distinct(data$PIN)
## [1] 12395
data00 <- subset(data2, Arm==0)
c <- n_distinct(data00$PIN) #Recipients 0, total
data01 <- subset(data2, Arm==0 & Death==1)
d <- n_distinct(data01$PIN) #Recipients 0, died
data10 <- subset(data2, Arm==1)
e <- n_distinct(data10$PIN) #Recipients 1, total
data11 <- subset(data2, Arm==1 & Death==1)
f <- n_distinct(data11$PIN) #Recipients 1, died
#create output table
Tutorialclinicaltransfusion <- data.frame(expcoef = a,
 confint = b,
 total0 = c,
 deaths0 = d,
 total1 = e,
 deaths1 = f,
 min = min,
 max = max,
 pct005 = pct005,
 pct995 = pct995,
 name = "Tutorialclinicaltransfusion")
#view output
View(Tutorialclinicaltransfusion)
```

Output

Supplemental results

The characteristics of the anonymized dataset are presented in Table S1.

Table S1. Patient and product characteristics for the anonymized dataset

Characteristics	Complete population	No-mixture subset*	Restriction subset†
Number of patients	N=18,206	N=13,361	N=7,659
Number of deaths, (%)	7,092 (39%)	2,234 (17%)	2,234 (29%)
Follow-up, median (IQR), days‡	1,819 (389-2,744)	341 (7-2,253)	2,051 (679-2,977)
Person-time, sum in years	87,382	42,999	41,107
Age of patients, median (IQR), years	65 (49-75)	65 (44-75)	64 (27-74)
0 to 17	2,754 (15%)	2,589 (19%)	1,796 (23%)
18 to 50	1,947 (11%)	1,287 (10%)	660 (9%)
51 to 70	6,825 (37%)	4,737 (35%)	2,568 (34%)
≥71	6,680 (37%)	4,748 (36%)	2,635 (34%)
Transfusions of red blood cell units per patient, median (IQR)	3 (2-6)	2 (1-2)	2 (1-2)
Units of red blood cells transfused, Number (%)§	103,016	25,600	14,172
male donor	65,239 (63%)	22,454 (88%)	12,617 (89%)
female donor, ever-pregnant	22,931 (22%)	1,939 (8%)	982 (7%)
female donor, never-pregnant	14,474 (14%)	1,207 (5%)	573 (4%)

* Consists of all the follow-up time during which patients either received all their red blood cell transfusions exclusively from one exposure category: female donors with a history of pregnancy (ever-pregnant donors), never-pregnant female donors, or male donors. The IPW analysis and Time-varying analysis use this definition. Follow-up time was censored at the time this inclusion criterion was violated.
† Consists of patients who received only one type of exposure (ever-pregnant, never-pregnant or male donor only) during the period in which they were followed up. Complete follow-up from these patients was included in the Restriction analysis.
‡ Median follow-up time is defined as the longest time any patient is in one of the comparisons. Exposure categories are: ever-pregnant donors and male donors.
§ Includes 372 (0.4%) transfusions with unknown donor sex and pregnancy history in the Complete population.

Below, the results for the anonymized dataset are presented (Table S2).

Table S2. Results for the different methods applied to the anonymized dataset

Analysis	No. of Deaths	No. of Recipients	HR (95% CI)
Restriction method			
Male (reference)	1,860	6,316	1 (reference)
Ever-pregnant female	263	884	1.00 (0.88-1.14)
Time-varying exposure and confounding adjustment method			
Male (reference)	1,860	10,901	1 (reference)
Ever-pregnant female	263	1,494	1.01 (0.89-1.15)
Inverse probability of censoring weighting method			
Male (reference)	1,860	10,901	1 (reference)
Ever-pregnant female	263	1,494	1.02 (0.88-1.17)

Here, due to the random permutation of the different variables, the original structure of the data was not maintained. Thus, the treatment-confounder feedback necessitating the use of the here described Inverse probability of censoring weighting method is not present, and all methods perform similarly. This, as opposed to the performance of these methods on the original data, can be seen in Table 2 of the main article.

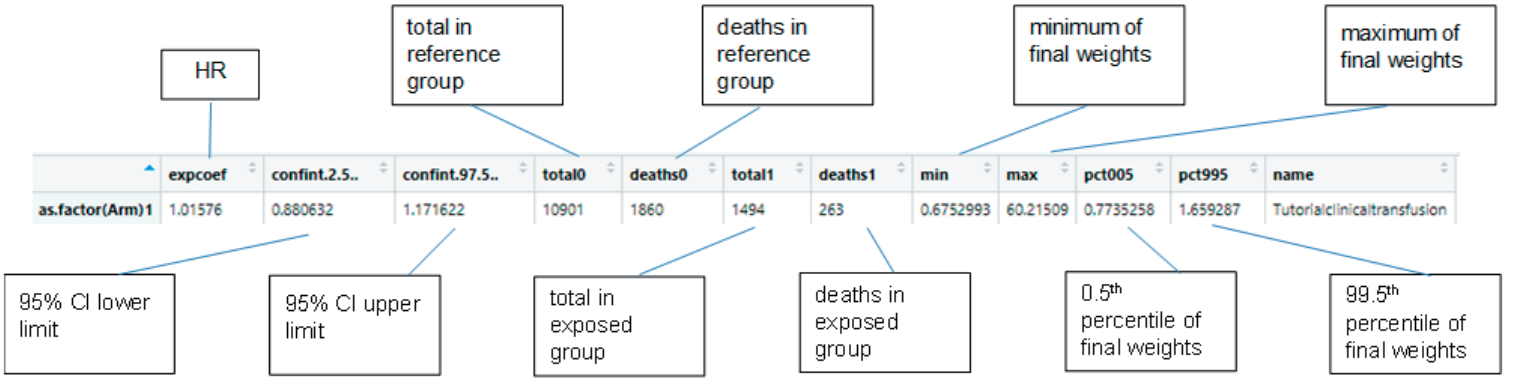


Figure S1. Output of tutorial syntax in R

7

Chapter 7

General discussion and summary

General discussion and summary

Blood products, by many measures, have evolved to be an extremely safe and fundamental part of hospital care. Moreover, they are a valuable resource that should be respected and safeguarded. In this thesis, we studied the relation of donor and product characteristics with patient outcomes in detail. By using thorough epidemiological methods, we found that there are still causes for concern pertaining to donor characteristics and transfusion recipient outcomes. In all chapters, in addition to describing the results and the most relevant aspects for clinical transfusion practice, we extensively described study limitations, and in a number of chapters we acknowledge that methodological limitations preclude causal claims. It should be noted that the goal of the research included in this thesis is not to criticize the use of blood products as a whole. Rather, the continuous improvement of a therapy's safety and effectivity for those in need of it is always justified, and is the ultimate target of the research described here.

Donor parity and storage of blood products: where do we stand?

In **Chapter 2,** the different potential biological mechanisms that could underlie the association between donor sex and donor pregnancy history with mortality are discussed. We propose the most likely mechanism is the unintentional transfer of donor-derived white blood cells of ever-pregnant female donors, potentially specific to male-targeted minor histocompatibility antigens (HY-antigens), and provide the biological rationale to study this in a later chapter. In brief, exposure to alloantigens during pregnancy, transfusion, or transplantation can induce human neutrophil antigen or human leukocyte antigen (HLA)-specific leukocytes, which we theorize are transferred to recipients by means of a transfusion. We further elaborate on this potential biological mechanism in **Chapter 3**, where we hypothesize that blood product storage, and with it, the decay of residual donor leukocytes in the blood product, might explain the observations pertaining to storage of blood products. In this chapter, we investigate the role of storage in the association between exposure to ever-pregnant donor red blood cell units with mortality. Although we are unable to conclusively show that storage modifies the association between donor characteristics and patient mortality, the most likely direction of the association seems to be harm from fresh products. Therefore, storage remains a factor of interest in the study of blood product safety and effectiveness.

Storage of blood products and its association with patient outcomes indeed have been frequently studied, and researchers remain divided on its relevance.[1] Observational studies were conducted, and an association between prolonged storage and mortality was found, with storage hypothesized to affect red blood

cell transfusion efficacy and safety due to the so-called "storage lesion". However, the more early studies on this matter were conducted while not taking into account the decreasing probability of patients to receive all their transfusions from a single storage category, and are therefore not estimating a causal effect of storage. Moreover, we now understand that even when carefully designing these studies and incorporating the time-varying aspects of transfusion exposures, their conclusions can still be subject to biases. This is due to the possibility of treatment-confounder feedback by smaller hemoglobin-increments observed with stored products, as described in this thesis and in the publication by Zhao et al.[2]. Products that are stored, indeed have a lower capacity to increase the patient's hemoglobin concentration, with a dose-response relationship between storage and hemoglobin increment.[3] Randomized controlled trials have also not given a decisive answer, with the results from a large meta-analysis showing potential for 1-2% benefit and up to 9% harm (HR 1.04, 95% confidence interval 0.98-1.09).[4] The difficulty with these randomized controlled trials is that they investigated overlapping categories of storage and did not restrict themselves to the very fresh and very old groups, which led to a lack of power to determine the effect of the very furthest ends of the storage spectrum. It is understandable that physicians might not want to subject their patients to perceived inferior blood products, being very fresh or very old, respectively. However, as the evidence base for the impact of storage on mortality is not yet fully established, we do consider such trials to be justified, in particular, to find out if a minimum storage threshold might be an easy to implement measure to decrease transfusion-associated risks.

In relation to the aforementioned treatment-confounder feedback by differing levels of product hemoglobin, we would like to draw attention to a relatively recent development in clinical transfusion: patient blood management (PBM). PBM is a strategy that aims to reduce the amount of blood products and thereby make the restrictive transfusion policy more common, while also reducing the patient's own blood loss and, if possible, salvaging the blood that a patient loses and returning it to them. Together, these actions have been shown to lead to a safer and more cost-effective blood supply.[5] We propose that a better understanding of the effects of product characteristics (i.e. storage or donor sex) could contribute to the evolution of PBM. As transfusion researchers, we need to consider the ultimate target of transfusion: to improve tissue oxygenation by providing oxygen-carrying capacity in the form of hemoglobin on the red blood cell. So, why then are donor and product characteristics associated with hemoglobin increment not taken into account here? It would be interesting to see whether patient outcomes might be improved by using information known

to be associated with lower hemoglobin-raising capacity of products (again, as can be caused by donor sex and storage, but also by novel concepts like 'poor storing donors'[6]) to tailor transfusions to patients with certain characteristics (e.g. different strategies for transfusion indications and/or relevant patient characteristics such as sex). The undertaking of a pragmatic randomized controlled trial comparing such a tailored transfusion strategy to a standard one could help further understanding of associations in observational research, and subsequently improve both patient outcomes and cost-effectiveness.

Convalescent plasma for COVID-19

The objective of **Chapter 4** was to quantify the available evidence on the efficacy and safety of convalescent plasma or hyperimmune immunoglobulin transfusion as a treatment for individuals with COVID-19. The results of the review indicated that the evidence on the effectiveness of convalescent plasma therapy for individuals hospitalized with COVID-19 is highly uncertain due to inconsistent reporting of results, which made it difficult to draw definitive conclusions. We found very low-certainty evidence on the effectiveness and safety of convalescent plasma therapy for individuals with COVID-19, as all studies included in the review had a high risk of bias and low reporting quality. Furthermore, at the time of publication there were no completed randomized controlled trials or controlled non-randomized studies that evaluated the benefits and harms of convalescent plasma therapy. Since then, a plethora of studies of varying quality have become public and it has become clear that convalescent plasma does not reduce mortality and has little to no impact on clinical improvement for hospitalized patients with moderate to severe disease (*Figure 1*).[7] For outpatient with mild disease, evidence from five randomized trials suggests that early and high-titer convalescent plasma is safe and effective against hospitalization.[8]

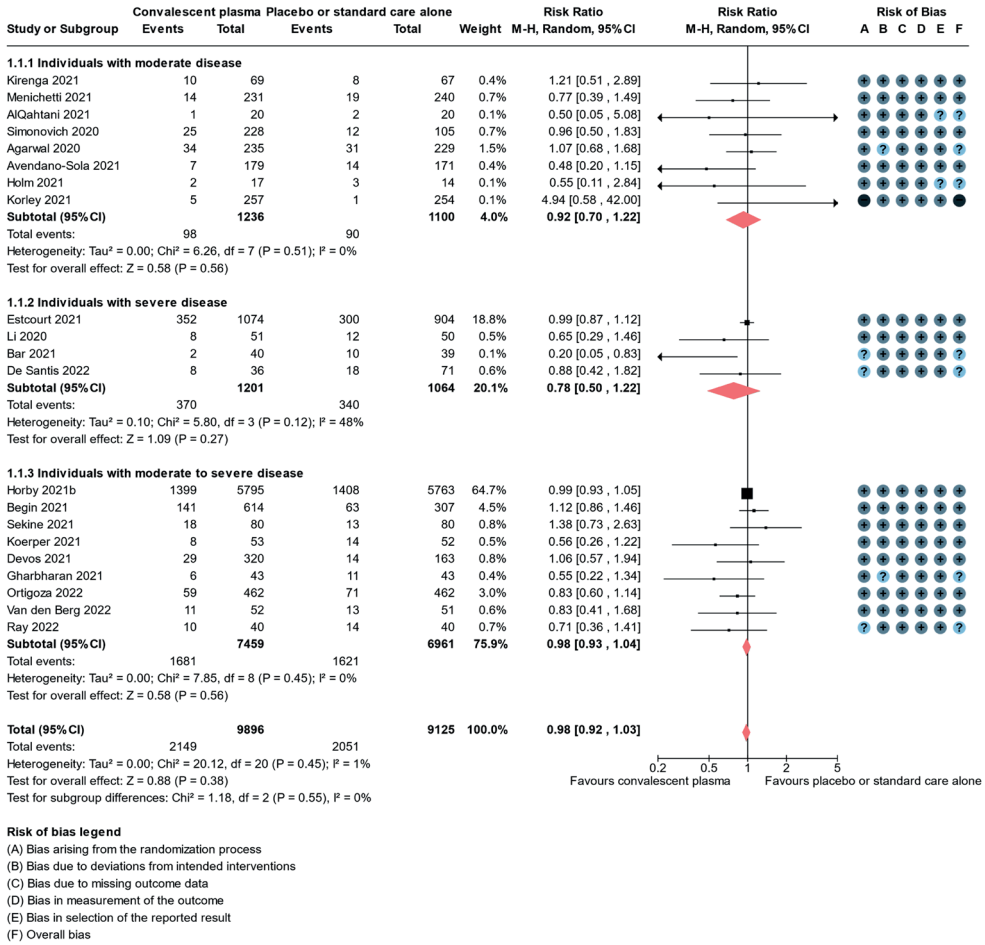


Figure 1

The most recent update of the systematic review investigating the safety and effectiveness of convalescent plasma transfusion as a treatment for individuals with COVID-19 showed there was no benefit in the primary outcome of 28-day mortality when transfusing convalescent plasma compared to placebo or standard care in hospitalized patients with moderate to severe disease (HR 0.98, 95% CI 0.92-1.03, adapted from Iannizzi et al.[7]).

The continued threat of the emergence of variants, like the Omicron variant of concern of SARS-CoV-2, has posed a significant challenge to the treatment of COVID-19 with plasma or antibodies. Omicron indeed has shown increased resistance to the anti-Spike monoclonal antibodies (mAbs) that have been authorized for emergency use.[9] Although plasma obtained following vaccination designed against earlier strains might also be less effective against new variants, cross-reactivity is more likely, while plasma of patients having cleared the new variant might also be available rapidly. As a result, there is ongoing interest in the use of COVID-19 convalescent plasma, particularly for immunocompromised patients.[10]

In the context of the COVID-19 pandemic, notwithstanding effectivity given early and with high titers for also immunocompetent patients, immunosuppressed patients could especially benefit. They are more vulnerable to severe illness and death, due to their decreased ability to mount an effective immune response to the virus, and an estimated 2% of patients without a functional B-cell response present with persistent COVID-19 and progressive respiratory failure.[11] On top of this, immunosuppressed patients are viewed as a potential public health threat because of the development of new variants under these conditions.[12] There are indications that a proportion of patients without antibody responses to SARS-CoV-2 vaccines are able develop T cell responses, but evidence is inconsistent.[13] Better treatment and prevention strategies are needed to protect this vulnerable patient population, both now and in the future.

We recognize there are ample grounds to consider the use of convalescent plasma in immunocompromised patients as a treatment option.[10] First, immunocompetent individuals have responded well to early antibody-based therapy of sufficient dosage, with some studies indicating benefit in patients without antibodies at baseline.[7, 14] Second, convalescent plasma contains a broader spectrum of also non-IgG type immunoglobulins than other formulations, such as hyperimmune immunoglobulin[15] and monoclonal antibodies[9]. Third, preliminary evidence suggests that even late administration of convalescent plasma may have potential benefits in immunocompromised patients.[10] While the definitive benefits of convalescent plasma in immunocompromised patients remain to be demonstrated, the available evidence supports its efficacy as a treatment option and the therapy is available on compassionate need basis in the Netherlands. A trial is in preparation on this subject.

The role of donor sex and pregnancy history of the donor

In 2017, our research group was the first to show that exposure to ever-pregnant donor red blood cell products was associated with mortality, with a hazard ratio (HR) of 1.43 (95% confidence interval (CI) 1.13-1.82) in male patients between 18 and 50 years of age.[16] This research was rooted in multiple studies consistently indicating an association between female donor sex and mortality of male patients.[16-26] However, the association between donor parity and mortality was not confirmed in a large observational cohort study in Sweden, Denmark, and the USA[27], while another study applying different statistical methodology also was unable to detect an association[2]. What's more, the recent publication of the iTADS trial showed that there was no difference in mortality rates at 30 days, 3 months, 6 months, 1 year, and 2 years when comparing assignment to male donor units to assignment to female donor units in a large pragmatic random-

ized controlled trial in Canada.[28] There was, however, a noteworthy association between exposure to female donor units and mortality in the combined group of male and female patients aged between 20-29 years, which was not explained. The question remains: why did these studies not find the same effect? The answer could lie in differences in country-specific production methods, population differences in both donors and patients, and differences in the applied statistical analyses. This last point pertaining to methodology, as we are able to show in **Chapter 5**, does not alter our initial observation on the potential for harm from exposure to ever-pregnant female donors for male patients.

We investigated whether offspring sex explains the association between donor pregnancy history and mortality using newly collected data from the municipality registration in the Netherlands on over 130,000 female donors, and we relate this to patient mortality using marginal structural models. Offspring sex was not shown to play a role in the earlier observed association, and thereby the hypothesis that blood products from female donors with sons was driving the previously observed associations in clinical transfusion research could not be confirmed. One noteworthy aspect that could have influenced the interpretation of the study's findings, is that female donors who have been pregnant are on average older compared to never-pregnant women, meaning the association could also (partly) be driven by this factor. Notwithstanding any donor age-mediated effects, the methodology used here was able to account for treatment-confounder feedback due to donor hemoglobin concentration differences, and thereby this research question has been thoroughly investigated.

Interestingly, the study was able to independently replicate that exposure to ever-pregnant donors is associated with male mortality in the age group between 18 and 50 years. Residual leukocytes from blood products persisting in the patient, with some products containing up to $5x10^6$ residual white blood cells following leukodepletion, might cause this consistent observation. We can only speculate, however, why male patients of the age group between 18 and 50 could be more sensitive to these female products. First of all, concerning the higher sensitivity of males between 18 and 50, we hypothesize that this is not because of their Y-chromosome, but might be due to the different transfusion indications and medical needs in that male age group. Young male patients indeed more often receive their transfusions for trauma indications[29], in which the induced raise in Hb (or lack of it, e.g. by female donor units) could be more critical. Additionally, trauma is known to lead to a rapid and, at times, sustained depression in cellular and humoral immunity (*Figure 2*).[30] It is important to point out the exclusion of a proportion of the trauma patients from the study by design, because these

patients often receive multiple transfusions, leading to limited generalizability to this subgroup. Still, these patients are present in the study population, albeit in lower numbers

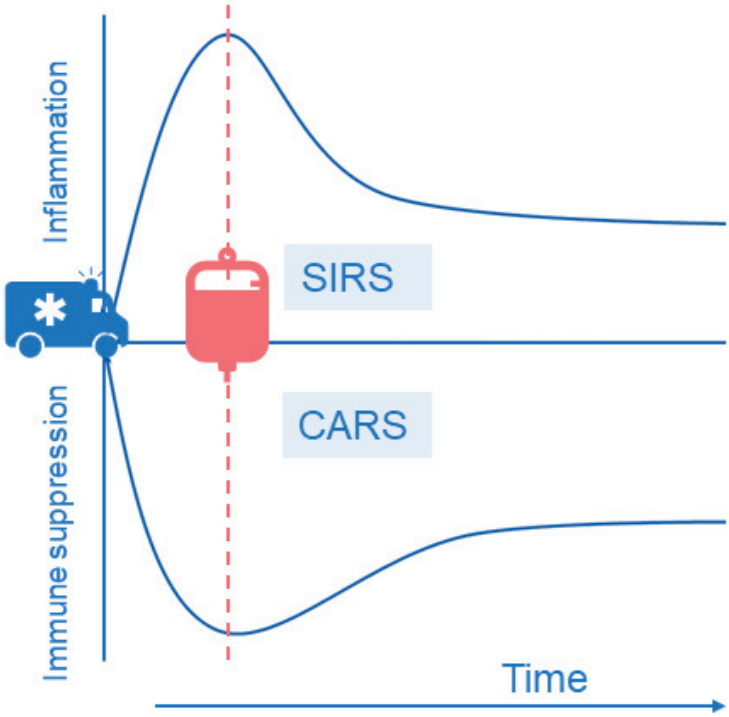


Figure 2

The immune response to trauma forms a complex interplay of both pro-inflammatory compounds and mechanisms (i.e. cytokines, fever, capillary leak, and early organ dysfunction) and anti-inflammatory responses (i.e. anti-inflammatory mediators, suppressed cytokine production, antigen presentation capacity, and cellular immunity) known as the systemic inflammatory response syndrome (SIRS) and the compensatory anti-inflammatory response syndrome (CARS). Transfusions are given at a time when these two responses are active, and the delayed return to homeostasis over time is hypothesized to be a facilitator of microchimerism development. (adapted from Shah et al.[31])

Acting in tandem with the latter, is that HLA alloimmunization of women is more common after pregnancy, and this immunization also having been shown to be a strong predictor of Graft-vs-Host disease after stem cell transplantation.[32] While it would be interesting to study the association between exposure to multiparity of the donor, and patient mortality, this is complicated by the small sample size of patients exposed only to women with multiple children, and a dose-response relationship will be difficult to establish. Nevertheless, there is still a need to further clarify 1. whether male patients of a certain age group are really at risk, 2. if the underlying transfusion indication further drives the epidemiological associations, and 3. what the causative agent(s) in the transfusion product might

be, inducing that risk. Moreover, these speculations should include storage time if the interest lies in studying its combined effects, with two different potential risk modulating mechanisms: leukocytes are less present in older products with hence less expected immunomodulating effects, and older products induce a lower Hb increase and hence less efficiency. Because of the many expected opposing risk modulations of blood product characteristics, the combined effect of these mechanisms is hard to predict. Data on cause-specific mortality have an important role here and should have a high priority, as these could give valuable insight into the potential biological mechanisms underlying the observed epidemiological associations.

Methodological implications for the field of transfusion medicine

Following up on the previous chapter, in Chapter 6 the importance of a thorough understanding of clinical epidemiology in the clinical transfusion research field is further emphasized. We present a structured approach to investigate any exposure that is associated with both the subsequent probability of receiving additional transfusions, and the outcome. This approach can be widely adopted by researchers studying transfusion exposures, which are generally sustained over time (e.g. trauma patients, receiving multiple transfusions in a short time frame, or chronically transfused cancer patients receiving multiple transfusions divided over a long period). Before this can be attempted, however, we recommend the use of directed acyclic graphs (DAGs) to visualize the researcher's assumptions about the data before embarking on the analytical task.

DAGs are a visual tool for causal inference, and can be used in conjunction with the target trial framework, aiding variable selection in statistical models and thereby avoiding self-inflicted bias by the researcher.[33] By definition, DAGs are acyclic, meaning variables cannot have effects on their own parent variables occurring before them in time. While this has led to the misconception that DAGs cannot display bidirectional effects, bidirectional effects can, in fact, be incorporated into DAGs by specifying how causal relationships evolve over time.[34] Incorporating bidirectional effects can improve the validity of statistical models and provide a more accurate representation of complex biological relationships. Not properly doing so can lead to mistakes that make a causal interpretation of estimates from studies inappropriate. Therefore, clinical transfusion researchers are encouraged to embrace incorporating bidirectional effects into DAGs, as we demonstrated in this thesis.

When to stop? An epidemiological perspective on innovation vs. replication

In the academic world, there is a lack of decisiveness regarding the need for pursuing the same research topic, or on the other hand, replicating and validating previous findings. This is reflected in the overrepresentation of new prediction models and the difficulty in publishing validation studies.[35] The sentiment can be summarized in the following quote, which was studied in psychological science but can be extrapolated to science in general: 'Innovation points out paths that are possible; replication points out paths that are likely; progress relies on both.'[36] Therefore, it is crucial for clinical transfusion researchers to strike a balance between innovation and replication to ensure that progress is made towards improving transfusion practices and patient outcomes. Both can coexist even within the same paper, as we demonstrated, but the decision to study either one of them or both should be made after careful consideration.

The goal of clinical transfusion research is to further the knowledge and understanding about transfusion products and their effects, and thereby help improve health. It is sometimes not clear when this goal is reached, and what a new study would add to the available body of evidence on an intervention. There are always additional research questions, study limitations and new research ideas, often indicated by the phrase 'more research is needed' at the end of a manuscript. Rather than paving the road for promising new research questions, this phrase has become an empty one without meaning. There comes a time when the quality of evidence is sufficient to change direction; we think this time has arrived for the research on donor pregnancy and patient mortality. The next section on future perspectives therefore lacks further validation studies of this research question, but instead focuses on novel study designs investigating personalized transfusion strategies over population effects.

Future perspectives

Future perspectives for causal inference in clinical transfusion research should focus on defining research questions with high clinical relevance and investigating important knowledge gaps. In this regard, we conclude that:

- The association between donor parity and subgroups of causes of death in young men is of interest, and the causal mechanisms could be investigated in a matched cohort study. This, however, would require the collaboration of blood banks and hospitals to be successful, and the formation of a consortium with representatives from hematology, blood banks and epidemiology. The size of such a study should involve a large national or international collaboration of research groups. Probing the interest and building the foundation for such a study would be an excellent first step.

- The incorporation into clinical studies, of assays quantifying potential pregnancy induced micro-chimerism and leukocyte transfer from female donors to patients, is required for complete understanding of the biological mechanisms underlying the observed epidemiological associations.
- The role of storage of blood products and its association with patient outcomes – as it is still incompletely understood – should be studied using g-methods (for which we refer to Chapter 6), or alternatively with well-designed randomized controlled trials investigating different storage thresholds.
- The effect of convalescent plasma for the treatment and prevention of respiratory viral infections in immunosuppressed people[10] or for prophylaxis[37] is still not clear and should be investigated to aid pandemic preparedness.

We expect the use of thorough epidemiological methods can help elucidate these research questions and are confident to have contributed to their future development with the research described in this thesis.

Reference list

1. Dilday JC, Martin MJ. Storage Wars: Is It Time to Retire the Myth of the "Storage Lesion" in Red Cell Transfusion?*. *Critical Care Medicine*. 2023;51(3):427-430. doi:10.1097/ccm.0000000000005786
2. Zhao J, Sjölander A, Edgren G. Mortality Among Patients Undergoing Blood Transfusion in Relation to Donor Sex and Parity: A Natural Experiment. *JAMA Internal Medicine*. 2022;182(7):747-756. doi:10.1001/jamainternmed.2022.2115
3. Rydén J, Clements M, Hellström-Lindberg E, Höglund P, Edgren G. A longer duration of red blood cell storage is associated with a lower hemoglobin increase after blood transfusion: a cohort study. https://doi.org/10.1111/trf.15215. *Transfusion*. 2019/06/01 2019;59(6):1945-1952. doi:https://doi.org/10.1111/trf.15215
4. 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. *Transfusion Medicine Reviews*. 2018/04/01/ 2018;32(2):77-88. doi:https://doi.org/10.1016/j.tmrv.2018.02.002
5. Goodnough Lawrence T, Shander A, Riou B. Patient Blood Management. *Anesthesiology*. 2012;116(6):1367-1376. doi:10.1097/ALN.0b013e318254d1a3
6. Hadjesfandiari N, Khorshidfar M, Devine DV. Current understanding of the relationship between blood donor variability and blood component quality. *International journal of molecular sciences*. 2021;22(8):3943.
7. Iannizzi C, Chai KL, Piechotta V, et al. Convalescent plasma for people with COVID-19: a living systematic review. *Cochrane Database of Systematic Reviews*. 2023;(2)
8. Levine AC, Fukuta Y, Huaman MA, et al. COVID-19 Convalescent Plasma Outpatient Therapy to Prevent Outpatient Hospitalization: A Meta-analysis of Individual Participant Data From 5 Randomized Trials. *Clinical Infectious Diseases*. 2023;doi:10.1093/cid/ciad088
9. Cox M, Peacock TP, Harvey WT, et al. SARS-CoV-2 variant evasion of monoclonal antibodies based on in vitro studies. *Nature Reviews Microbiology*. 2023/02/01 2023;21(2):112-124. doi:10.1038/s41579-022-00809-7
10. Senefeld JW, Franchini M, Mengoli C, et al. COVID-19 convalescent plasma for the treatment of immunocompromised patients: A systematic review and meta-analysis. *JAMA Network Open*. 2023;6(1):e2250647-e2250647.
11. Yetmar ZA, Khodadadi RB, Seville MT, et al. Outcomes of B-Cell-Depleted Patients With Coronavirus Disease 2019 Treated With Antispike Monoclonal Antibodies. *Open Forum Infectious Diseases*. 2022;9(7)doi:10.1093/ofid/ofac204
12. Clark SA, Clark LE, Pan J, et al. SARS-CoV-2 evolution in an immunocompromised host reveals shared neutralization escape mechanisms. *Cell*. 2021/05/13/ 2021;184(10):2605-2617.e18. doi:https://doi.org/10.1016/j.cell.2021.03.027
13. Shree T. Can B cell-deficient patients rely on COVID-19 vaccine-induced T-cell immunity? *Br J Haematol*. Jun 2022;197(6):659-661. doi:10.1111/bjh.18210
14. Mair-Jenkins J, Saavedra-Campos M, Baillie JK, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. *J Infect Dis*. Jan 1 2015;211(1):80-90. doi:10.1093/infdis/jiu396

15. Daniele F, Marco T, Guido A, Fabrizio M. What is the optimal usage of Covid-19 convalescent plasma donations. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2020;
16. Caram-Deelder C, Kreuger AL, Evers D, 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*. Oct 17 2017;318(15):1471-1478. doi:10.1001/jama.2017.14825
17. Valk SJ, Caram-Deelder C, Zwaginga JJ, van der Bom JG, Middelburg RA. Donor sex and recipient outcomes. *ISBT Science Series*. 2019;doi:10.1111/voxs.12528
18. Middelburg RA, Briet E, van der Bom JG. Mortality after transfusions, relation to donor sex. *Vox Sang*. Oct 2011;101(3):221-9. doi:10.1111/j.1423-0410.2011.01487.x
19. Barty RL, Cook RJ, Liu Y, Acker J, Eikelboom JW, Heddle NM. Exploratory analysis of the association between donor sex and in hospital mortality in transfusion recipients. *Transfusion*. 2015 2015;Conference: AABB Annual Meeting 2015 Anaheim:September 2015.Not in File.
20. Bjursten H, Dardashti A, Bjork J, Wierup P, Algotsson L, Ederoth P. Transfusion of sex-mismatched and non-leukocyte-depleted red blood cells in cardiac surgery increases mortality. *J Thorac Cardiovasc Surg*. Jul 2016;152(1):223-232.e1. doi:10.1016/j.jtcvs.2015.12.022
21. Chasse M, Tinmouth A, English SW, et al. Association of Blood Donor Age and Sex With Recipient Survival After Red Blood Cell Transfusion. *JAMA Intern Med*. Sep 1 2016;176(9):1307-14. doi:10.1001/jamainternmed.2016.3324
22. 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;51doi:10.1016/j.eclinm.2022.101628
23. Alshalani A, Uhel F, Cremer OL, et al. Donor-recipient sex is associated with transfusion-related outcomes in critically ill patients. *Blood Adv*. Jun 14 2022;6(11):3260-3267. doi:10.1182/bloodadvances.2021006402
24. Murphy T, Chawla A, Tucker R, Vohr B. Impact of Blood Donor Sex on Transfusion-Related Outcomes in Preterm Infants. *J Pediatr*. Oct 2018;201:215-220. doi:10.1016/j.jpeds.2018.04.023
25. Heddle NM, Cook RJ, Liu Y, et al. The association between blood donor sex and age and transfusion recipient mortality: an exploratory analysis. *Transfusion*. 2018;0(0) doi:doi:10.1111/trf.15011
26. Gupta V, Patidar GK, Hote M, Mehar R, Dhiman Y, Hazarika A. Association of blood donor's biological characteristics on outcomes of cardiac surgery patients receiving red blood cells transfusion. *Transfusion Clinique et Biologique*. 2023/02/01/2023;30(1):130-136. doi:https://doi.org/10.1016/j.tracli.2022.09.068
27. Edgren G, Murphy EL, Brambilla DJ, et al. Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. *Jama*. Jun 11 2019;321(22):2183-2192. doi:10.1001/jama.2019.7084
28. Chassé M, Fergusson DA, Tinmouth A, et al. Effect of Donor Sex on Recipient Mortality in Transfusion. *New England Journal of Medicine*. 2023;388(15):1386-1395. doi:10.1056/NEJMoa2211523
29. Borkent-Raven BA, Janssen MP, Van Der Poel CL, Schaasberg WP, Bonsel GJ, Van Hout BA. The PROTON study: profiles of blood product transfusion recipients in the

Netherlands. *Vox Sanguinis*. 2010;99(1):54-64. doi:https://doi.org/10.1111/j.1423-0410.2010.01312.x
30. Shah S, Spinella PC, Muszynski JA. Immunologic effects of trauma and transfusion. *Journal of Trauma and Acute Care Surgery*. 2017;82(6S):S50-S56. doi:10.1097/ta.0000000000001434
31. Shah A, Brunskill SJ, Desborough MJ, Doree C, Trivella M, Stanworth SJ. Transfusion of red blood cells stored for shorter versus longer duration for all conditions. *Cochrane Database Syst Rev*. Dec 22 2018;12:Cd010801. doi:10.1002/14651858.CD010801.pub3
32. Loren AW, Bunin GR, Boudreau C, et al. Impact of donor and recipient sex and parity on outcomes of HLA-identical sibling allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. Jul 2006;12(7):758-69. doi:10.1016/j.bbmt.2006.03.015
33. Hernán MA, Sauer BC, Hernández-Díaz S, Platt R, Shrier I. Specifying a target trial prevents immortal time bias and other self-inflicted injuries in observational analyses. *J Clin Epidemiol*. Nov 2016;79:70-75. doi:10.1016/j.jclinepi.2016.04.014
34. Murray EJ, & Kunicki, Z. . As the Wheel Turns: Causal Inference for Feedback Loops and Bidirectional Effects. *OSF Preprints*. 2022;doi:10.31219/osf.io/9em5q
35. Collins GS, de Groot JA, Dutton S, et al. External validation of multivariable prediction models: a systematic review of methodological conduct and reporting. *BMC medical research methodology*. 2014;14(1):1-11.
36. Collaboration OS. Estimating the reproducibility of psychological science. *Science*. 2015/08/28 2015;349(6251):aac4716. doi:10.1126/science.aac4716
37. Valk SJ, Piechotta V, Kimber C, et al. Convalescent plasma and hyperimmune immunoglobulin to prevent infection with SARS-CoV-2. *Cochrane Database of Systematic Reviews*. 2021;2020(12)

Appendices

Summary

Blood product characteristics have intrigued researchers since, in 1667, the transfusion of docile lambs' blood was thought to cure psychiatric illness.[1] More recently, the transfusion of 'young blood' was made commercially available to invigorate those seeking to return to their youthful self.[2] Clearly, these therapies are far-fetched, not evidence-based, and should not be investigated further. However, a number of clinically relevant, unresolved questions pertaining to characteristics of blood products still puzzle epidemiologists. The research in this thesis aims to answer several research questions related to blood product characteristics, by using thorough epidemiological methods. Throughout this thesis, clinical transfusion research in different shapes and forms is the central theme. Clinical epidemiology and observational study design is an important aspect of clinical transfusion research, and new developments in this field that are applicable to future studies are covered extensively, including a tutorial to apply inverse-probability weighted marginal structural models to longitudinal transfusion data.

In Chapter 1, the different chapters are introduced and the background for the research is described. The chapter consists of two parts: a general introduction and an aim and outline of the thesis.

Chapter 2 contains a narrative review on the association between red blood cell donor sex and patient mortality. In 2017, our research group was the first to show that exposure to ever-pregnant donor red blood cell products was associated with mortality, with a hazard ratio (HR) of 1.43 (95% confidence interval (CI) 1.13-1.82) in male patients between 18 and 50 years of age.[3] This research was rooted in multiple studies consistently showing an association between female donor sex and mortality of male patients, which puzzled transfusion science.[4] However, the association between donor parity and mortality was not seen in a large observational cohort study in Sweden, Denmark, and the USA[5], and it was unclear what the biological mechanism could be that would cause these associations. This chapter brings together the available evidence on donor sex and patient mortality up to the date of publication, evaluates the different biological mechanisms that could underlie the epidemiological associations, and states donor microchimeric cell-mediated immune modulation is the most likely explanation for these associations in clinical transfusion research.

In Chapter 3, we investigated the importance of storage duration of red blood cell units in relation to donor characteristics, in a large cohort of transfusion

recipients in the Netherlands. Blood product storage has been indicated as a potential modifier of transfusion efficacy and safety, but evidence has been conflicting, with studies showing both positive and negative associations between storage duration of red blood cells and clinical outcomes. However, a small potential for harm from fresh red blood cell transfusions has not been ruled out and can be considered more plausible than benefit, based on evidence from randomized trials.[6] In this chapter, we investigated whether storage plays a role in modifying the effect of donor characteristics, namely sex and pregnancy history of the donor. Although a large initial cohort was used, due to blood product distribution practices relying on a 'first in, first out' mechanism, subgroups were small. This meant that the methods described in the later chapters of this thesis (i.e. g-methods) could not be applied, and we were not able to definitively prove storage plays a role in the effect of donor sex and pregnancy history on mortality. This chapter does, however, give a first indication that the direction of the effect is more likely to be towards harm from ever-pregnant donor units that are shortly stored.

Chapter 4 contains the first instalment of the systematic review series on convalescent plasma and hyperimmune immunoglobulin for people with COVID-19. The review was performed during the early stages of the COVID-19 pandemic in 2020, and followed Cochrane rapid review methodology. Evidence sources in this review were of insufficient quality to allow strong conclusions. More recent versions of this review were able to show convalescent plasma is not effective for patients hospitalized with COVID-19[7], with continuously updated reviews expected in the future to inform evidence-based healthcare decision making.

In Chapter 5, we present the results of a large observational cohort of first-ever transfusion recipients in the Netherlands: the MATER study ("Mortality After Transfusion of Ever-pregnant donor Red blood cells"). As we have carefully described, the marginal structural models used for this analysis capture treatment-confounder feedback previously prohibiting a causal interpretation of the association between donor pregnancy history, and mortality.[8] The results confirm that male patients between 18 and 50 years are at risk of increased mortality following transfusion from ever-pregnant donors, with a HR of 1.81 (95% CI 1.31-2.51). Importantly, this was not explained by offspring sex, which we hypothesized could play a role through the unintentional transfer of donor helper T-cells or cytotoxic T-cells targeting HY-antigens, after exposure to female donors with sons.[4] To conclude, we have extensively investigated this research question and found the risk of mortality after exposure to ever-pregnant donors continues to be present in a new cohort, which is intriguing.

Chapter 6 covers the development of a methodology to study the association between blood product characteristics and patient outcomes, when the product characteristics are associated with hemoglobin-raising capacity of the product. In addition to an extensive appraisal of the methods used in previous studies, the application of said methods to real transfusion data, and a visual representation of the epidemiological concepts applicable to this research, a detailed tutorial including R code and an example dataset are included in the chapter.

Chapter 7 contains a summary of the evidence described in this thesis, how these findings tie into the most recent literature, and a discussion of the implications for future research on this topic.

In this thesis, we have covered a range of topics in the clinical transfusion research field, located at the intersection of clinical and fundamental research. The research was performed using thorough epidemiological methods, large cohorts of patient and transfusion data, and critical appraisal of study assumptions.

References

1. King E. An account of the experiment of transfusion, practised upon a man in London. *Philosophical Transactions of the Royal Society of London*. 1667;2(30):557-559.
2. Corbyn Z. Could 'young' blood stop us getting old? Guardian News and Media. Accessed April 6, 2023. https://www.theguardian.com/society/2020/feb/02/could-young-blood-stop-us-getting-old-transfusions-experiments-mice-plasma
3. Caram-Deelder C, Kreuger AL, Evers D, 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*. Oct 17 2017;318(15):1471-1478. doi:10.1001/jama.2017.14825
4. Valk SJ, Caram-Deelder C, Zwaginga JJ, van der Bom JG, Middelburg RA. Donor sex and recipient outcomes. *ISBT Science Series*. 2019;doi:10.1111/voxs.12528
5. Edgren G, Murphy EL, Brambilla DJ, et al. Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. *Jama*. Jun 11 2019;321(22):2183-2192. doi:10.1001/jama.2019.7084
6. 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. *Transfusion Medicine Reviews*. 2018/04/01/ 2018;32(2):77-88. doi:https://doi.org/10.1016/j.tmrv.2018.02.002
7. Iannizzi C, Chai KL, Piechotta V, et al. Convalescent plasma for people with COVID□19: a living systematic review. *Cochrane Database of Systematic Reviews*. 2023;(2)
8. Zhao J, Sjölander A, Edgren G. Mortality Among Patients Undergoing Blood Transfusion in Relation to Donor Sex and Parity: A Natural Experiment. *JAMA Internal Medicine*. 2022;182(7):747-756. doi:10.1001/jamainternmed.2022.2115

Nederlandse samenvatting

Al sinds 1667, zijn kenmerken van bloedproducten en hun relatie met klinische uitkomsten een onderwerp van discussie. Men was bijvoorbeeld in de veronderstelling dat de transfusie van bloed afkomstig van “makke” lammetjes psychiatrische aandoeningen kon genezen.[1] Recent is het transfunderen van 'jong bloed' voor commercieel gebruik in de media besproken, met als doel de ontvanger te verjongen.[2] Het is duidelijk dat deze therapieën niet op betrouwbaar bewijs gebaseerd zijn, oftewel, deze therapieën zijn niet ‘*evidence-based’*. Gedegen klinisch transfusieonderzoek houdt zich bezig met de beantwoording van klinisch relevante vragen over bloedproducten met behulp van solide onderzoeksmethoden, waardoor een oorzaak-gevolg relatie kan worden aangetoond.

Het onderzoek in dit proefschrift heeft als doel verschillende vraagstukken over de kenmerken van bloedproducten op te helderen, door middel van gedegen epidemiologische methoden. Klinisch transfusieonderzoek in verschillende vormen en studie designs vormt het centrale thema van dit proefschrift. Observationeel onderzoek, dat wil zeggen, onderzoek met gegevens die verzameld zijn uit de klinische praktijk en niet uit een gerandomiseerd onderzoek met controlegroep (Engels: *randomized controlled trial,* RCT), is een belangrijk onderdeel van klinisch transfusieonderzoek en vormt het hart van dit proefschrift. Nieuwe ontwikkelingen op het gebied van observationeel studie-design die relevant zijn voor toekomstige studies worden daarnaast uitgebreid behandeld, inclusief een praktische handleiding voor het toepassen van zogenaamde g-methoden voor gebruik met observationele transfusiegegevens.

In Hoofdstuk 1 worden de verschillende hoofdstukken geïntroduceerd en wordt de achtergrond van het onderzoek beschreven. Het hoofdstuk bestaat uit twee delen: een algemene inleiding, en de doelstelling en het overzicht van het proefschrift.

Hoofdstuk 2 bevat een beschrijvend overzicht van het onderzoek naar de associatie tussen het geslacht van de bloeddonor en de mortaliteit van de patiënt. In 2017 toonde onze onderzoeksgroep als eerste aan dat blootstelling aan bloedproducten van vrouwelijke donoren die ooit zwanger waren geweest, geassocieerd was met mortaliteit bij mannelijke patiënten tussen 18 en 50 jaar, met een hazard ratio (HR) van 1.43 (95% betrouwbaarheidsinterval (BI) 1.13-1.82).[3] Dit onderzoek was gebaseerd op meerdere studies die een associatie lieten zien tussen het vrouwelijke geslacht van de donor en mortaliteit bij mannelijke patiënten, een bevinding die niet kon worden verklaard en tot vragen leidde in de transfu-

siewetenschap.[4] Echter, de associatie tussen zwangerschappen van de donor en mortaliteit werd niet waargenomen in een grote observationele cohortstudie in Zweden, Denemarken en de Verenigde Staten[5], en het was onduidelijk wat het biologische mechanisme zou kunnen zijn dat deze associaties veroorzaakte. Dit hoofdstuk brengt het beschikbare bewijsmateriaal tot aan de datum van publicatie over het geslacht van de donor en mortaliteit van de patiënt samen, evalueert de verschillende biologische mechanismen die ten grondslag kunnen liggen aan de epidemiologische associaties, en stelt dat de overdracht van donorcellen specifiek voor mannelijk DNA (d.w.z. *HY-antigenen*) de meest waarschijnlijke verklaring is voor deze associaties in klinisch transfusieonderzoek.

In Hoofdstuk 3 hebben we onderzocht hoe de bewaarduur van transfusies samenhangt met kenmerken van de donor, in een groot cohort van ontvangers van bloedtransfusies in Nederland. Bewaarduur van bloedproducten is een potentiële factor die de effectiviteit en veiligheid van transfusies kan beïnvloeden, maar het bewijs is tegenstrijdig, waarbij studies zowel positieve als negatieve associaties laten zien tussen de bewaarduur van rode bloedcellen en klinische uitkomsten. Niettemin kan een klein potentieel schadelijk effect van 'verse' bloedtransfusies niet worden uitgesloten en lijkt dit waarschijnlijker dan een positief effect, op basis van bewijs uit RCTs.[6] In dit hoofdstuk hebben we onderzocht of de bewaarduur een rol speelt bij het modificeren van het effect van het geslacht en de zwangerschapsgeschiedenis van de donor. Hoewel er aanvankelijk gebruik werd gemaakt van een groot cohort, waren de subgroepen klein vanwege de praktijk van verdeling van bloedproducten op basis van het "first in, first out"-mechanisme. Dit betekende dat de in latere hoofdstukken van dit proefschrift beschreven methoden (d.w.z. g-methoden) niet konden worden toegepast, en de vraag of bewaarduur een rol speelt bij het effect van donorgeslacht en zwangerschapsgeschiedenis op mortaliteit blijft onvoldoende beantwoord. Dit hoofdstuk geeft echter wel een eerste voorzichtige aanwijzing dat een negatief effect waarschijnlijker is bij bloedtransfusies van ooit-zwangere donoren, die kort werden bewaard.

Hoofdstuk 4 bevat het eerste deel van de serie systematische reviews over convalescent plasma en hyperimmuun immunoglobuline voor mensen met COVID-19. Het onderzoek werd uitgevoerd tijdens de vroege stadia van de COVID-19-pandemie in 2020 en volgde de methodologie van Cochrane Rapid Reviews. De kwaliteit van de geïncludeerde studies in deze review was onvoldoende om sterke conclusies te kunnen trekken. Meer recente versies van deze review hebben kunnen aantonen dat convalescent plasma niet effectief is voor mensen die in het ziekenhuis zijn opgenomen met COVID-19[7]. Snel bijgewerkte reviews worden gepubliceerd om besluitvorming in de gezondheidszorg te ondersteunen.

In Hoofdstuk 5 presenteren we de resultaten van een grote observationele cohortstudie van ontvangers van een eerste bloedtransfusie in Nederland: de MATER-studie ("Mortality After Transfusion of Ever-pregnant donor Red blood cells"). Zoals we zorgvuldig hebben beschreven, zijn g-methoden die voor deze analyse zijn gebruikt in staat te corrigeren voor factoren die anders een causale interpretatie van de associatie tussen zwangerschappen van de donor en mortaliteit belemmeren.[8] De resultaten bevestigen dat mannelijke patiënten van 18 tot 50 jaar een verhoogd risico op mortaliteit hebben na transfusie van bloed van ooit-zwangere donoren, met een hazard ratio van 1.81 (95% BI 1.31-2.51). We zagen dat dit niet werd verklaard door het geslacht van het nageslacht, waarvan we vermoedden dat dit een rol zou kunnen spelen door de onbedoelde overdracht van donorcellen die gericht zijn tegen HY-antigenen, na blootstelling aan vrouwelijke donoren met zonen.[4] In conclusie: we hebben deze onderzoeksvraag uitgebreid onderzocht en gevonden dat het risico op mortaliteit na blootstelling aan ooit-zwangere donoren nog steeds aanwezig is in een nieuw cohort van transfusie-ontvangers.

Hoofdstuk 6 behandelt de ontwikkeling van een methode om de associatie tussen kenmerken van bloedproducten en patiëntuitkomsten te bestuderen, wanneer de productkenmerken geassocieerd zijn met de toename van het hemoglobinegehalte na transfusies. Deze methodologie, *inverse-probability weighted* marginale structurele modellen, valt onder de groep g-methoden. Naast een uitgebreide beoordeling van de gebruikte methoden in eerdere onderzoeken, de toepassing van een drietal methoden op een grote transfusiedataset en een visuele toelichting van de epidemiologische concepten die van toepassing zijn op dit onderzoek, bevat het hoofdstuk een gedetailleerde handleiding inclusief R-code en toegang tot een voorbeelddataset.

Hoofdstuk 7 bevat een samenvatting van het beschreven bewijs in dit proefschrift, hoe deze bevindingen aansluiten bij de meest recente literatuur en een bespreking van de implicaties voor toekomstig onderzoek naar deze onderwerpen.

In dit proefschrift hebben we een breed scala aan onderwerpen behandeld op het gebied van klinisch transfusieonderzoek, op het snijvlak van klinisch en fundamenteel onderzoek. Het onderzoek is uitgevoerd met behulp van geavanceerde epidemiologische methoden, grote cohorten van patiënt- en transfusiegegevens en kritische beoordeling van aannames die ten grondslag liggen aan het beschreven onderzoek.

Referenties

1. King E. An account of the experiment of transfusion, practised upon a man in London. *Philosophical Transactions of the Royal Society of London*. 1667;2(30):557-559.
2. Corbyn Z. Could 'young' blood stop us getting old? Guardian News and Media. Accessed April 6, 2023. https://www.theguardian.com/society/2020/feb/02/could-young-blood-stop-us-getting-old-transfusions-experiments-mice-plasma
3. Caram-Deelder C, Kreuger AL, Evers D, 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*. Oct 17 2017;318(15):1471-1478. doi:10.1001/jama.2017.14825
4. Valk SJ, Caram-Deelder C, Zwaginga JJ, van der Bom JG, Middelburg RA. Donor sex and recipient outcomes. *ISBT Science Series*. 2019;doi:10.1111/voxs.12528
5. Edgren G, Murphy EL, Brambilla DJ, et al. Association of Blood Donor Sex and Prior Pregnancy With Mortality Among Red Blood Cell Transfusion Recipients. *Jama*. Jun 11 2019;321(22):2183-2192. doi:10.1001/jama.2019.7084
6. 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. *Transfusion Medicine Reviews*. 2018/04/01/ 2018;32(2):77-88. doi:https://doi.org/10.1016/j.tmrv.2018.02.002
7. Iannizzi C, Chai KL, Piechotta V, et al. Convalescent plasma for people with COVID-19: a living systematic review. *Cochrane Database of Systematic Reviews*. 2023;(2)
8. Zhao J, Sjölander A, Edgren G. Mortality Among Patients Undergoing Blood Transfusion in Relation to Donor Sex and Parity: A Natural Experiment. *JAMA Internal Medicine*. 2022;182(7):747-756. doi:10.1001/jamainternmed.2022.2115

List of publications

1. Valk SJ, Caram-Deelder C, Zwaginga JJ, van der Bom JG, Middelburg RA (2019) "Donor sex and recipient outcomes." ISBT Science Series.
2. Valk SJ, Piechotta V, Kimber C, Chai KL, Monsef I, Doree C, Wood EM, Lamikanra AA, Roberts DJ, McQuilten Z et al. Convalescent plasma and hyperimmune immunoglobulin to prevent infection with SARSCoV-2. Cochrane Database Syst Rev 2021, Issue 1. Art. No.: CD013802.
3. Valk SJ, Caram-Deelder C, Evers D, de Vooght KMK, van de Kerkhof D, Wondergem MJ, Péuéiaux NCV, Hudig F, Zwaginga JJ, de Korte D, van de Watering LMG, Middelburg RA, van der Bom JG (2023) "Donor pregnancies and transfusion recipient mortality: a role for red blood cell storage?" (under review)
4. Valk SJ, Caram-Deelder C, Groenwold RHH, Evers D, de Vooght KMK, van de Kerkhof D, Wondergem MJ, Péuéiaux NCV, Hudig F, Zwaginga JJ, Middelburg RA, van der Bom JG (2023) "Transfusion of ever-pregnant donor red blood cells and mortality of male patients" (under review)
5. Valk SJ, Caram-Deelder C, Groenwold RHH, van der Bom JG (2023) "Clinical transfusion-outcomes research: A practical guide" (in preparation)
6. So-Osman C & Valk SJ (2022). "High-dose immunoglobulins from convalescent donors for patients hospitalised with COVID-19." Lancet 399(10324): 497-499.
7. So-Osman C, Bruggeman CY, Valk SJ, te Boekhorst PAW (2021) "Antistofgerelateerde behandelingen voor covid-19 bij hematologiepatiënten: een update" NTVH 4, June 2021.
8. Kimber C, Valk SJ, Chai KL, Piechotta V, Iannizzi C, Monsef I, Wood EM, Lamikanra AA, Roberts DJ, McQuilten Z et al. (2023). "Hyperimmune immunoglobulin for people with COVID-19." Cochrane Database Syst Rev 1(1): Cd015167.
9. Chai KL, Valk SJ, Piechotta V, Kimber C, Monsef I, Doree C, Wood EM, Lamikanra AA, Roberts DJ, McQuilten Z et al. (2020). "Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review." Cochrane Database Syst Rev 10: Cd013600.
10. Iannizzi C, Chai KL, Piechotta V, Valk SJ, Kimber C, Monsef I, Wood EM, Lamikanra AA, Roberts DJ, McQuilten Z et al. Convalescent plasma for people with COVID-19: a living systematic review. Cochrane Database Syst Rev 2023, Issue 2. Art. No.: CD013600. DOI: 10.1002/14651858.CD013600.pub5. Accessed 17 July 2023.
11. Kimber C, Lamikanra AA, Geneen LJ, Sandercock J, Doré C, Valk SJ, Estcourt LJ. (2023). "A systematic review of the safety and efficacy of convalescent plasma or immunoglobulin treatment for people with severe respiratory viral infections due to coronaviruses or influenza." Transfus Med 33(1): 26-38.
12. Kreuzberger N, Hirsch C, Chai KL, Tomlinson E, Khosravi Z, Popp M, Neidhardt M, Piechotta V, Salomon S, Valk SJ et al. (2021). "SARS-CoV-2-neutralising monoclonal antibodies for treatment of COVID-19." Cochrane Database Syst Rev 9(9): Cd013825.
13. Piechotta V, Chai KL, Valk SJ, Doree C, Monsef I, Wood EM, Lamikanra A, Kimber C, McQuilten Z, So-Osman C et al. (2020). "Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review." Cochrane Database Syst Rev 7(7): Cd013600.
14. Piechotta V, Iannizzi C, Chai KL, Valk SJ, Kimber C, Dorando E, Monsef I, Wood EM, Lamikanra AA, Roberts DJ et al. (2021). "Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: a living systematic review." Cochrane Database Syst Rev 5(5): Cd013600.

Curriculum vitae

Sarah Valk was born 21 August 1994 in Woerden, the Netherlands. She is a clinical epidemiologist whose research efforts are concentrated on epidemiology and blood transfusion safety and efficacy, particularly the association between donor characteristics and patient outcomes. She has obtained a Bachelor of Science degree in Biology in 2015 and a Master of Science degree in Biomedical Science in 2018 from Leiden University in the Netherlands. Sarah has published a number of research articles in the field of transfusion medicine while working as a PhD student at Sanquin Research. In addition, she contributed to the development and continued updating of Cochrane systematic reviews on antibody-based therapies for COVID-19. Sarah followed a number of courses during her PhD trajectory to become certified as Epidemiologist B. Currently, Sarah is working on the prevention and control of healthcare-associated infections in the Netherlands, at the Dutch National Institute for Public Health and the Environment. In this capacity, she remains dedicated to strengthening the field of epidemiology.

Acknowledgements

I would like to thank everyone who has helped me over the past 5 years of performing this research. It has been a joy to work with a group of professional and dedicated researchers studying many aspects of transfusion at the Center for Clinical Transfusion Research, first at the Pelican building, then at the Poortgebouw and at the LUMC, and at the Plesmanlaan in Amsterdam. I first want to wholeheartedly acknowledge my thesis advisors. Anske, I appreciate all you have done for me, I will strive to continue working in clinical epidemiology and take your lessons with me. Jaap Jan, your suggestions and comments have always been extremely valuable and I appreciate you as a team member. Camila, without your steadfast presence and regular conversations about epidemiology and so much more, this thesis could not have been written.

Rutger Middelburg, I want to thank you for setting me on this path and allowing me to continue this important work. Aad Pors, Yavanna van Oostveen, and Dorien Heijting: thank you for all your help with the data management. I thank all the investigators of the R-FACT for supporting this research and allowing me to continue to build on this work: Dorothea Evers, Karen de Vooght, Daan van de Kerkhof, Marielle Wondergem, Nathalie Péquériaux, and Francisca Hudig. Eugenie Gemen, I want to thank you for your help and your valuable contributions. Josine Oud, thank you for the work you did for me and for sharing this PhD experience with me. All of your help is much appreciated.

During my time at the LUMC, at the Department of Clinical Epidemiology, I learned so much, both during courses and while taking in the research from other groups. I especially want to thank prof. dr. Rolf Groenwold for the valuable discussions about study design of transfusion exposure studies. Prof. dr. Frits Roosendaal, thank you for allowing me to be a part of this stimulating environment, also during the last phase of my PhD project. Suzanne Cannegieter and Masja de Haas, thank you for being in my guidance committee. Yvonne Souverein and Tamara Wienen-Groen, thank you for making me feel welcome at the department. To all my fellow PhD students: thank you for allowing me to learn, to grow and to have fun! Hilde, Lisanne, and Nina, from book club to feedback on yet another presentation, you were there. Heather, thanks for being a great roommate and my epi-buddy even before starting this PhD.

At the Rijksdienst voor Identiteitsgegevens, we tremendous support from Khadija el Hamdaoui, Diana van den Brink, and Marie-Claire Breet, which made challenging projects possible. Nicole Skoetz, Vanessa Piechotta, Khaili Chai and

Catherine Kimber, we worked a lot together and I always enjoyed it. I also want to thank Cynthia So-Osman for sharing this project with me and being available to discuss any open issues in a timely and pleasant manner.

At Sanquin Research and Blood Supply Foundation, I have felt supported and welcome. Sander Meijer, thank you for arranging anything I needed. Leo van de Watering, thank you for sharing your views and providing feedback. Dirk de Korte, you have given me guidance and insight on blood products and their contents: thank you for being available Anne-Marie van Walraven, Marieke von Lindern, and Lela Saou: thank you for meticulously going over the privacy aspects of the MATER study. Florine van Milligen and Inge Klanker, thank you for supporting my PhD trajectory over these years. Ed Slot, thank you for guiding me through the process of performing this study at Sanquin. Shannon, sharing the 'puppykamer' was a joy, and although I did not perform a simulation study in the end, I did learn a lot. Katja van den Hurk and Femmeke Prinsze, thank you for your time commitment in supporting the data collection in a safe and secure manner. Thank you all for these contributions. I know there are still many people who helped me along the way who I did not thank, or did not thank enough, so please accept my appreciation in making this PhD project a personal success.

I could not have performed these research projects without my own personal support team: Martijn, thank you for being there with a listening ear, a supportive word and a comforting snack; Daniël and Safira, thanks for the hugs, for the jokes and laughter, and for all the board games. I also want to thank my mom, Ries, and Joey for supporting us through this journey. I want to thank my dad, and Ellis, Ruud, Anneloes and Liselotte. Thank you all for your support.

As a last remark: I want to acknowledge the incredibly useful contributions of large language model GPT3.5 in the following chapters for text generation and text correction: chapter 1, chapter 7, Biography and Dutch summary; and for translation of the Dutch summary.