Short and long-term clinical effects of blood donor characteristics in transfusion recipients

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Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
in partial fulfillment of the requirements
for the Doctorate in Philosophy degree in Epidemiology

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Contents

ACKNOWLEDGEMENTS

This research could not have been conducted without the invaluable support of many colleagues and friends.

I am indebted to Dr. Dean Fergusson for expertly guiding me through this project. His world-class scientific expertise, combined with his flawless mentorship and personal generosity made possible the accomplishment of this endeavor. His contagious enthusiasm and optimism kept me constantly engaged with my research and I am convinced that his mentorship and friendship helped me become a better person.

I am deeply grateful to Dr. Lauralyn McIntyre for her scientific input. Her commitment to me in terms of time and scientific and moral support was invaluable. I wish to thank Dr. Alan Forster for his mentorship, friendship and dedication in supporting me in every step of this project. I would also like to express my appreciation to my Thesis Advisory Committee members, Dr. Greg Knoll, Dr. Alan Tinmouth and Dr. Tim Ramsay for their assistance throughout this project. I extend my appreciation to the Department of Epidemiology and Community Medicine at the University of Ottawa for providing an excellent learning environment. I would also like to acknowledge the time, expertise and support of all the investigators mentioned in this thesis as each of them were essential for this project to succeed.

This thesis could not have been possible without the collaboration of Canadian Blood Services (Dr. Jason Acker), and the Institute of Clinical Evaluative Sciences (Dr. Carl van Walraven). By the collaboration between the University, The Ottawa Research Institute,

Canadian Blood Services and the Institute of Clinical Evaluative Sciences, we conducted a research program that could not have been possible without their constant involvement. For their research assistance and administrative assistance, I am grateful to Jodi Peters, Nancy Cleary, Diana Wolfe, Pauline Quach and Robin Ducharme.

To my family, thank you for being supportive at all time. Mum, Dad, you have always been unconditionally there for me, days and nights, for my entire life. Thanks to my brothers and to my sister for being such good friends. A special word to my brother Pascal who is fighting for his life. You are my brother, my friend and despite all the life hurdles you are going through, you remain positive. You are an example for me.

To my wife Audrey, my son Édouard, my daughters

Victoria and Charlie-Rose, this work is for you.

ABSTRACT

Introduction: Transfusion of blood products, especially red blood cells (RBC) is the most common medical intervention administered in North-American hospitals. The indications for transfusion are diverse but they largely aim at increasing oxygen delivery to tissues to improve patient clinical outcomes. Transfusion can also have deleterious effects. In fact, there is evidence that RBC transfusion may be ineffective, or even harmful in some populations where its use should in theory be beneficial. Seeking explanations for the beneficial and deleterious effects of red blood cell transfusions is necessary. The primary objective of this research is to investigate the associations between donor characteristics and RBC transfusion recipient outcomes.

Methods: My thesis consists of a systematic review and meta-analysis of the literature regarding the association between blood donor characteristics and outcomes of the recipient, and the development of a framework linking the donor-recipient continuum using data collected from blood donors by Canadian Blood Services and clinical outcome data from large hospital and provincial clinical-administrative databases. Based on the framework, an epidemiological analysis was conducted to assess the effect of donor sex, age and ABO-Rh mismatch on RBC recipient outcomes.

Results: Our systematic review found 58 studies evaluating 17 different donor characteristics. Five studies evaluated donor age as a risk factor for RBC transfusion outcome and 17 studies evaluated donor sex. We successfully developed an analytical framework allowing for a robust analysis of the impact of donor characteristics on RBC recipient outcomes that included 30,503 RBC recipients, 80,755 blood donors and a total of

187,960 transfusion episodes. We found that young age and female sex are donor characteristics significantly associated with adverse outcomes after RBC transfusion. Our newly developed framework, as well as our epidemiological findings, have the potential to influence future research in transfusion medicine and transfusion practices.

CHAPTER 1: INTRODUCTION

In many clinical situations when the red blood cell (RBC) counts are low (anemia), one common intervention is the transfusion of RBC. RBCs are administered to increase oxygen delivery to tissue. Approximately 1.1 million RBC units are collected and transfused each year in Canada[1,2], accounting for the most frequent procedure performed in hospitalized patients[3]. They are used across a variety of medical and surgical situations, including in more than 30% of critical care patients and 50% of cardiac surgery patients during their hospital stay[4,5]. Unfortunately, even if anemia is clearly associated with adverse outcomes, it is unclear if transfusion of RBC will improve outcomes in all patients. In fact, several large and robust clinical trials in critical care[6], cardiac surgery[7] or orthopedic surgery[8] suggest that a liberal transfusion strategy is not helpful and may even be harmful. Additionally, a recent systematic review has suggested that RBC transfusions may be associated with increased morbidity and mortality[9]. Seeking explanations for the beneficial and deleterious effects of red blood cell transfusions is necessary to ensure the safe and optimal use of a precious biological resource.

1.1 BLOOD DONOR SELECTION TO REDUCE RISK

The risks associated with blood transfusion are well documented and described. Critical improvements in blood compatibility testing and red cell preservation at the beginning of the 20th century allowed the large-scale use of blood during the wars, and, thereafter, for the general population[10]. The transfusion community is keenly aware of the infectious and immunological risks associated with transfusion and a variety of measures, including donor questionnaires that assess risk, improved infectious disease screening, and other quality

measures, were implemented to improve the safety of transfusions[11,12]. Those strategies are aiming mainly at insuring safety for the blood donor, as well as to potentially improve short-term outcome in the recipient. Such is the case for example with the exclusion of donors with a history of cancer. Although this policy is implemented widely, it is based at best on anecdotal evidence. In fact, a large cohort study evaluating the risk of cancer transmission through blood transfusion found no association between development of cancer in donors following blood donation and the risk of cancer in the recipient[13]. The evidence is extremely limited regarding the impact of donor characteristics on the outcome of transfusion recipients.

"Standard" blood products have important biologic variability due to the fact that each unit is collected from an individual volunteer donor and administered randomly to a recipient. This variability of interactions between donor and recipient may affect the clinical efficacy and safety of a blood transfusion. In situations where the benefits of an intervention are uncertain, and known risks are present, it is of paramount importance to minimize the risks and maximize the potential for clinical benefit. Thus, we need to examine all factors (especially donor factors) that could contribute to the effect of RBC transfusions on morbidity and mortality. Interestingly, donor characteristics have not been comprehensively assessed as a potential modifier of risk or benefit.

1.2 Risks of RBC transfusions

To appropriately study the association between blood donor characteristics and transfusion outcomes, it is important to understand what is currently known about non-infectious

transfusion related adverse outcomes in RBC recipients (transfusion reactions, volume overload, transfusion related immunomodulation and transfusion related acute lung injury) and how they are related to the donor. We will then discuss how the donor characteristics affect the blood and how this may translate in different outcomes for the recipient.

RBC transfusion reactions

Transfusion reactions can be classified as immune and non-immune. Immune reactions are caused by either cytokines such as interleukins and tumor necrosis factor coming from the donor, or from antibodies contained in residual plasma, also from the donor[4,11]. Their effect may range from benign fever to very severe reactions such as anaphylaxis, shock or death. Various interventions have been proposed to reduce their risk such as leukoreduction, washing of red cells and platelets, duplicate ID identification checks to avoid administration errors (transfusion of mismatched blood), etc.[14]. Some of these interventions are effective (e.g. reduction of clerical errors[15]) but the clinical benefit of other interventions such as leukoreduction (beyond patients with hematologic malignancies), RBC washing and other interventions aiming to reduce the "immunogenicity of the product" are still a matter of much debate[11]. Interestingly, a recent cohort study of allergic transfusion reactions to platelets by Ness and colleagues reported that this particular adverse event was associated "more with donor and recipient factors than with product attributes"[16]. Donor factors can affect the transfused product quality and characteristics to the extent that a peanut anaphylactic reaction has been documented on a transfused patient where the donor had consumed peanuts in the hours preceding blood donation[17]. Non-immune reactions, such as iron overload, hyperkalemia, citrate toxicity or mechanical problems associated with the

transfusion such as air embolism are not hypothesized to be associated with donor characteristics and will not be further addressed.

The volume of RBC we transfuse matters

Transfusion Associated Circulatory Overload (TACO) is a common adverse complication wherein congestive heart failure, pulmonary edema secondary to volume overload occur following a blood transfusion. TACO results in increased mortality and length of stay [18].The incidence of this complication is underestimated but is thought to be the second leading cause of transfusion-related mortality, after Transfusion Related Acute Lung Injury (TRALI)[19,20]. The influence of other mechanisms beyond simple fluid overload is unclear in that 20% of reported cases of TACO involved the transfusion of only 1 unit of RBCs, or that fever is often associated with TACO and suggest that there are characteristics of the blood that are not accounted for that are likely donor related[21].

RBC transfusion related immunomodulation (TRIM)

A major postulated mechanism for adverse outcomes after transfusion is transfusion related immunomodulation (TRIM). Such immunomodulation has been associated with adverse clinical outcomes such as infection, accelerated cancer growth, multiple organ dysfunction and mortality after transfusion[4,22]. As of 1999, all RBC units in Canada undergo pre-storage leukoreduction. These units contain red blood cells, residual amounts of leucocytes and plasma from the donor. Leucocytes are often incriminated for many transfusion related adverse events such as allo-immunization, febrile non-immune transfusion reactions, immunosuppression, infection, and transfusion associated graft

versus host disease. Allogeneic transfusions may also induce immune suppression in recipients[23]. This immunomodulation may also be responsible for an increased risk of cancer in the recipients. A large number of studies have suggested that allogeneic transfusions accelerate cancer growth[23–28] perhaps due to altered immune surveillance. These altered immune responses following allogeneic red cell transfusions may also predispose critically ill transfusion recipients to nosocomial infections[29–34] and increased rate of multiple system organ failure[35] which may ultimately result in higher mortality rates. Interventions such as leukoreduction, irradiation and pathogen inactivation reduce leucocytes in RBCs and have been hypothesized as a solution to reduce transfusion related adverse events[36,37]. The fact that, despite these interventions, the immune response still seems to be affected by transfusions suggests that there may be other donor-related aspects associated with TRIM.

Transfusion Related Acute Lung Injury (TRALI)

Transfusion related acute lung injury (TRALI), an acute lung injury occurring within 6 hours after the transfusion of a blood product, is the leading cause of transfusion related mortality[38]. Recent epidemiological studies associated the presence of Human Leucocytes Antibodies and Human Neutrophil Antibodies in the donors' plasma, and a history of pregnancy with the risk of TRALI (female gender, OR 4.5 95% CI 1.08, 3.04) [38]. This led to a policy change in 2007 to use predominately male plasma for transfusion and a subsequent reduction in TRALI (OR 2.57 95% CI 1.92, 3.86 for reduction of TRALI) in Canada[39]. Similar results using TRALI reduction strategies have been observed in the UK[40] and the USA[38].

1.3 TRANSFUSIONS HAVE ORGANIZATIONAL IMPACTS

In addition to the growing list of ways that RBC transfusion may adversely affect transfusion recipients, the characteristics of blood products (and RBCs particularly) are worth considering. Much effort has been undertaken over the last 30 years to mitigate these risks and to promote appropriate practice regarding the screening, collection, storage, distribution and transfusion of blood products. Due to their common clinical use and the necessary precautions to ensure their safety, the use of blood products is associated with significant costs to the Canadian health care system. In 2012, the total Canadian Blood Services' budget for transfusable products was $473 million dollars[1] and close to $366 million dollars for Héma-Québec[2]. These totals do not account for the cost of administering the blood products or the additional societal costs associated with transfusion. For RBCs, the societal cost associated with a transfusion has been estimated to be greater than $400 per unit[41]. With more than 1.1 million RBC units delivered to Canadian hospitals and clinics in 2012, one can only begin to grasp the clinical and economic importance and impact of RBC transfusions on our health care system[1,2]. Given such a precious resource, we need to ensure that we maximize benefit and minimize risks in transfusion recipients.

In summary, transfusion of RBCs are associated with many adverse events including transfusion reactions, circulatory overload, acute lung injury, cancer, infections and mortality. Many of these adverse reactions have been shown to be related to donor factors (TRALI, allergies) whereas other adverse outcomes have been hypothetically related to donor factors (mortality, cancer, infections). Through our study, we will evaluate and identify factors that affect outcomes related to blood transfusion.

1.4 DONOR CHARACTERISTICS: THE SCIENCE

Age affects blood

Increased age affects erythropoiesis. Evidence obtained from in vitro, animal and human studies suggest that with increased age, there is a decreased number of erythrocytes precursors, leading to an increased prevalence of anemia[42]. Moreover, an increased risk of DNA damage at the stem cell level due to aging itself has been shown to negatively impact cellular function, and possibly increase the oncogenic risk[43]. In addition to the effect on RBC precursors, circulating RBCs are subject to changes in cell membrane compositions (decreased phospholipid and cholesterol content, appearance of senescence surface antigen) that have been associated with altered immunology and circulation[44]. Clinically, these changes have been associated with premature RBC destruction and an increased risk of anemia. The activity of cytokines circulating in plasma is also increased in the aging human. Despite advanced blood collection systems, a significant amount of plasma is transfused with RBCs. These cytokines (TNF-a is an example) have been suggested to increase the oncogenic risk, as well as altering response to pathogens, increasing the risk of infections[45]. In summary, aging of the hematopoietic system is associated with many physiological perturbations such as decreased precursors and increased oncogenic risk, changes in RBC membrane properties, increased RBC destruction and premature cellular death, immune modulations and increased inflammation. These changes have clinical impact on the donor and support that “old” blood may not be equivalent to “young” blood.

Evidence that age of donor may impact outcome of transfusion recipients may also be found in stem cell transplantation, where age is a significant determinant of transplantation outcome. In the stem cell transplantation population, age (>45 years old) has been consistently associated with adverse events such as mortality (RR 1.10, 95% CI 1.06-1.14 per decade), a higher rate of graft versus host disease (GVHD), graft failure and post transplantation obstructive lung disease[46,47]. This increased GVHD risk may suggest that the immune system of older donors have been exposed to a wider variety of antigens, decreasing tolerance to foreign antigens[46]. This hypothesis is supported by a pre-clinical study showing a decreased concentration of naïve T-cells towards more memory T-cells[46]. One may argue that the worse outcomes in bone marrow transplantation were due to decreased cellularity in the bone marrow of older donors. However, the results are unchanged when adjusting for the dose of cells transplanted, suggesting other responsible mechanisms. The strong associations found with donors' age (45 years-old being a particularly important cut-off in most studies) in stem cells transplantation supports the hypothesis that age could be a significant determinant of outcomes in a RBC transfusion.

Sex affects blood

In normal physiologic conditions, male and female RBC are comparable regarding their O_2 delivery capacity, their deformability and composition[48,49]. Sex differences however exist when erythrocytes are exposed to adrenaline. It has been shown that the enzymatic activity (acetylcholinesterase) decreases in females but not in males, there is an increased RBC membrane rigidity and a decreased affinity to oxygen[48]. These perturbations at the cellular level could contribute to explain the different tissue responses to stress between sexes[48].

Blood composition is also affected by sex. Blood transfusion obtained from women affects transfusion outcome in at least one clinically well-defined situation, TRALI. Because the transfusion of RBC implies the transfusion of a certain amount of plasma, immunologic products are inevitably transfused at the same time as the RBCs. TRALI is an excellent example of how a donor characteristic (sex) affects transfusion outcome. It supports the hypothesis that there may be other relevant donor characteristics that can affect transfusion outcomes, as well as provides some mechanistic explanation for this association.

Donor-recipient compatibility

In cardiac surgery[50] and acute leukemia[51,52], the transfusion of non-identical ABO group platelet products has been associated with increased morbidity and mortality. These finding have been attributed to the formation of ABO immune complexes, which promote inflammatory responses, increase the risk of multiorgan dysfunction and down-regulate immune responses. While RBCs have a relatively small amount of plasma, non ABO identical RBC transfusions will lead to the formation of immune complexes with the potential to affect short and longer term outcomes in transfusion recipients[22]. On the other hand, transfusion of blood with at least 1 shared HLA haplotype between donor and recipients has been associated with microchimerism (long-term persistence of donor cells in blood transfusion recipients)[53,54]. The clinical effects of donor-recipient compatibility (ABO group or microchimerism) is unknown and needs to be investigated[22,53,54]. Regarding donor-recipient sex mismatch, there is only one study looking at this association and the risk of mortality after transfusion of RBC[55]. In this interesting study, gender mismatched

transfusions were associated with an increased risk of mortality, and the risk was higher for recipients aged > 55 years (1.8; 95% CI. 1.2, 2.1). The authors proposed that the minor sex-specific histocompatibility antigens may be the cause and they suggested repeating their study in a different population, studying other characteristics and a potential dose-response relationship[55].

Analogy between blood transfusion and solid organ transplantation

Organ transplantation involves the retrieval of an organ (for example, a kidney) and its transplantation in another patient. Such intervention requires the careful selection of the correct donor with adequate characteristics, extensive compatibility testing at the organ level, and careful monitoring and immunomodulation of the organ recipient to ensure the most optimal outcomes. The transfusion of a blood component also involves the retrieval of an organ (blood) from a donor, post-donation processing and storage, and transplantation" (transfusion) in recipients. In both cases, the donor undergoes detailed screening, the organ transplanted (or blood) undergoes testing for transfusable diseases, and a suitable recipient receives the organ. For solid organ transplantation, the majority of screening is performed based on physiological and immunological characteristics of the donor. Post organ retrieval analyses are seldom performed on the organ itself, except for biopsies. In the solid organ transplantation community and for bone marrow transplantation, several donor characteristics have been associated with adverse outcomes for the recipients.

In heart transplantation, several donor characteristics are associated with short and long-term survival. Increasing *age* is consistently associated with increased mortality. Female

sex, anthropometric markers such as body surface area, body mass index, height and weight, and cardiac related characteristics are also associated with survival[56]. For lung transplantation, donor smoking status, female sex and *age* have been associated with increased mortality[57,58]. Although the sex association was not seen in all studied cohorts, advanced donor *age* was always a significant predictor of adverse outcomes after transplantation. Similarly, younger *age* of liver donors have been consistently associated with an increased survival in liver recipients and a reduction of graft loss[59]. Other outcomes such as the reactivation of hepatitis C infection or histological changes such as fibrosis on the liver recipient were also associated with an increased *age* in the donor[60]. Several studies also observed such associations with gender, race and height. Predictive survival models (such as the Liver Donor Risk Index) are now published to better select donors and predict outcomes in liver recipients[61]. Kidney donors' characteristics have been extensively associated with outcomes for the recipient. In addition to *age*, race and height, comorbidities such as hypertension, diabetes, creatinine level and weight have all been associated with kidney graft survival[61]. The American Organ Procurement and Transplantation Network is even considering the use of scores derived from donors' characteristics to change the current kidney allocation system to optimize allocation of organs[61]. In pancreas transplantation, one of the main determinants of outcome is donor selection. The young *age* of the donor (between 10 and 40 years-old), body mass index below 27.5 kg/m^2 and brain death (other than a cerebrovascular accident) as a cause of death have all been associated with better transplantation outcomes[62].

As stated, in solid organ transplantation, physicians make important clinical decisions on the selection of the organ recipient based on the clinical characteristics of the organ donor. Interestingly, for blood transfusion, in contrast with solid organ transplantation, physiological characteristics of the donors have not traditionally been a focus. Instead, blood operators and regulators have focused more on donor activities or risk factors that may increase risk of transfusion transmissible diseases. Some donor characteristics, such as a history of cancer[63] or *age* greater than 70 years old for first blood donation will render a particular donor ineligible for donation, albeit limited scientific evidence exists for this policy. We suggest that donor characteristics need to be explored as potential factors that affect clinical outcomes in transfusion recipients**.**

Clinical and biological rationale

Although donor characteristics affect RBC and plasma properties, there is a paucity of data regarding the impact of donor characteristics on recipient outcome. This is especially true for the potential effects of donor age. In Canada, the upper age limit for existing blood donors was recently removed. While the previous age limit criterion was primarily for donor safety, there has been no evaluation the impact of donor age on transfusion outcome. We suggest the following evidence to support our rationale to examine the effect of donor age on transfusion outcomes: 1) RBC themselves are altered by aging; 2) aging has impacts on blood composition (immune tolerance, increased inflammation, oncogenicity, premature cellular turnover); 3) there are proven immunological phenomena related to donors such as the anti-leukocyte antibodies (anti HLA or anti-neutrophil antibodies) that occur after pregnancies (e.g. gender effect on TRALI) and these phenomena can affect clinical outcome;

4) there is extensive evidence in stem cell transplantation and solid organ transplantation demonstrating the effect of age (and also gender) on recipient short-term and long-term outcomes; and 5) successful interventions can be implemented to reduce the occurrence of adverse events and improve the safety of blood transfusion(for example, collection of male only plasma).

1.5 Proposed Methods and Research Question

The primary objective of my thesis to investigate the association of donor characteristics on RBC transfusion recipient outcomes.

To achieve our primary objective, we designed a PhD thesis program consisting of: 1) a systematic review and meta-analysis of the literature regarding the association between blood donor characteristics and outcomes of the recipient; 2) the development of a novel donor-to recipient continuum framework using administrative and clinical databases; and 3) a multi-site, retrospective, longitudinal cohort study using data collected from blood donors by Canadian Blood Services, and clinical outcome data from large hospital and provincial clinical-administrative databases. My thesis includes the thorough analysis of the available literature, the development of a framework and analytical methods to allow the study of the donor-recipient continuum, an in-depth epidemiological analysis to answer our research questions, and a careful interpretation of the results to assist the decision-making process of clinicians, researchers and policy makers in transfusion medicine.

PICOTS for the systematic review

We used the PICOTS format to define our research question:

The target **P**opulation: All patient populations that received RBCs

1) The studied exposure (**I**ntervention): Any donor characteristic
2) **C**ondition: Any indication for RBC transfusion
3) **O**utcomes: Any outcome
4) **T**ime: No time limit
5) **S**etting: Inpatients and outpatients

PICOTS of our research question

We use the PICOTS format to define our research question:

1) The target **P**opulation: All patients that received RBC
2) The studied exposure (**I**ntervention):
    - Main exposure: Donor Age
        i. Secondary exposure: Donor Sex, donor-recipient ABO-Rh mismatch.
3) **C**ondition: Any indication for transfusion
4) **O**utcomes:
    - Primary: Survival in post-transfusion recipients
    - Secondary: New cancer rate, infection rates (*Clostridium difficile* and Methicillin-Resistant *Staphylococcus aureus*) and occurrence of new myocardial infarction.

5) **T**ime: 25 October 2006 to 31 December 2013

6) **S**etting: The Ottawa Hospital – General Campus, The Ottawa Hospital – Civic Campus, The University of Ottawa Heart Institute, and The Ottawa Hospital – Riverside Campus

1.6 RATIONALE OF METHODS

There is currently no "real time" way in Canada to study the donor-recipient impact for RBC transfusion. To perform such a study, one has to be able to track blood from "vein to vein". Unfortunately, the blood systems in most parts of the world, including Canada, is not structured to directly allow such analysis. Blood is usually collected and processed with all information pertaining to the donor stored in a central database of blood service organizations. The blood is then delivered to hospitals where it is managed by their respective blood banks. Mechanisms exist to monitor transfusion-related reactions and a "per-case" analysis can be performed on blood units that may be associated with such reactions. If needed, the original donor may be tracked (for example, in cases of novel infections), from a unique identifier on the blood unit. However, this is done manually. With the event of HIV, hepatitis B and C, and other blood transmissible pathogens, hemovigilance has improved. Prospective registries of blood donors, tests performed on blood, and the ability to trace blood has been implemented in many countries. Example of such databases are The Serious Hazards of Transfusion (SHOT) database from UK[14], and the Retrovirus Epidemiology Donor Study (REDS) from US[64]. The SHOT database, which depends on outcome reporting by transfusing hospitals, records only adverse reactions from blood transfusions; donor data and data for other transfused patients where no event has been

reported is not collected. The third phase of the REDS study (REDS-III) will allow the ability to link donor and recipients of blood yet the assessment of outcomes in recipients is still approximately 7 to 10 years away[65]. Internationally, very few countries and/or organizations can follow blood from the donor to the recipients. Potentially the most advanced database of this kind is the Scandinavian Donations and Transfusions (SCANDAT) database, a joint project from Sweden and Denmark providing complete follow-up on the outcome of transfused patients. It consists of a database linking the different blood donation organizations and transfused patients administrative databases with a central data harmonization and cleaning[66]. This group has published a few papers using this database in the last 5 years, which shows the feasibility of conducting such studies. They first studied the association of donor cancer occurring in the 5 years following blood donation, and the cancer rate on transfused patients, finding no association[63]. Subsequently, they conducted epidemiological studies examining the effect of age of stored RBCs on mortality (weak association between increased mortality and longest storage of RBC)[67] and the effect of blood group on gastric cancer and gastric ulcer (A blood group associated with gastric cancer)[68]. Unfortunately, the SCANDAT database does not contain important patient information such as comorbidities, biochemistry results, and other important factors necessary for robust analyses.

The Danish Transfusion Database (DTDB) has the ability to track blood from donors to recipients. However, this database is less focused on outcomes, and more focused on blood use. Finland created a similar database in 2002. To date, no studies have been published, but it may have the ability to track blood use from vein to vein[69]. Finally, to study blood

utilization in their country, the Netherlands recently performed this type of linkage between donors and recipients, and published a limited study of transfusion practices[70].

No existing database or study has investigated the association between donor age and other characteristics and the risk of adverse transfusion outcomes. To conduct such a study prospectively would be impractical and expensive. Using our proposed methodology, we will have the capacity to conduct a thorough analysis of the association between donors' characteristics and transfusion recipients. The data necessary to perform such analyses are available in different high-quality datasets. After the required data collection, linkages and epidemiological analyses are performed, we will be able to obtain precise results to support (or not) our hypothesis.

This thesis has the potential to confirm the current age policy in blood donation. On the other hand, different outcomes according to age would support further study. One will want to consider positive and negative impacts to the recipient, health provider, blood supplier, and blood donor as well as both health and economic impact from the government (funders), blood supplier and societal perspectives. For example, adverse outcomes associated with blood donated by older donors may only result in adverse outcomes for specific recipient populations, such as immunosuppressed patients. Another possibility would be to use blood from young donors for the sickest transfusion recipients. This may lead health providers to consider selecting the blood product based on a given donor characteristics. Such changes in behavior could have impacts on the blood suppliers (responsible to maintain an appropriate supply), blood donors (potential for increased burden on donors with favorable characteristics), as well as economic impacts (depending

on the increased burden in the last factors) and finally on a societal perspective (given the limited availability of the blood product, choices will have to be made on whom should receive that resource, similarly to the solid organ transplantation field). We believe that this thesis will shed light on this important topic; the association of blood donor age, sex, and potential other characteristics in transfusion recipients, and will allow to support (or not) current policies, and potentially suggest new research hypotheses to improve the safety of our blood system. There is precedence. Many of these donor selection considerations, albeit on a smaller scale, have been present in the field solid organ transplant.

1.7 ETHICS AND PRIVACY

By the nature of the data used for this project, individual consent from patients has been waived, in accordance with the Ontario Personal Health Information Protection Act. We ensured approval from Research Ethics board of all involved institutions, as well as from privacy offices from CBS, ICES and The Ottawa Hospital Data Warehouse. All data collection and management was performed in accordance with the *Personal Health Information Protection Act of Ontario, Regulation 329/04.* All patients were identified by a unique anonymous number and no patient identifiers was kept with clinical data. The resulting database was encrypted and stored centrally. The access was restricted and only designated members of the team had access to the dataset.

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CHAPTER 2 - MANUSCRIPT 1 - EFFECT OF BLOOD DONOR CHARACTERISTICS ON TRANSFUSION OUTCOMES: A PROTOCOL FOR SYSTEMATIC REVIEW AND META-ANALYSIS

2.1 PREFACE TO MANUSCRIPT 1

To better evaluate the donor characteristics that may be of interest, as well as the available evidence regarding the subject of this thesis, we performed a systematic review of the literature. The first manuscript describes the rationale and methods used to perform the systematic review.

2.2 MANUSCRIPT 1

Chassé M, English SW, McIntyre L, Knoll G, Shehata N, Forster A, Wilson K, van Walraven C, Tinmouth A, Fergusson DA. Effect of blood donor characteristics on transfusion outcomes: a protocol for systematic review and meta-analysis. Syst Rev 2014;3:28.

PubMed ID: 24650633

Effect of blood donor characteristics on transfusion outcomes: a protocol for systematic review and meta-analysis

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ABSTRACT

Background: Optimal selection of blood donors is of paramount importance in ensuring the safety of blood products. The current selection process is concerned principally with the safety of the blood donor and the safety of the patient that receives the blood. Recent evidence suggests that the characteristics of the donor may affect transfusion outcomes for the recipient.

Methods: We will conduct a systematic review of the association between major blood donor characteristic and RBC transfusion outcomes. The primary objective is to assess the association of blood donor characteristics and the risk of adverse short-term and long-term clinical outcomes after red blood cell (RBC) transfusion. We will search MEDLINE, EMBASE, Cochrane Central databases, as well as perform manual searches of top transfusion medical journals for prospective and retrospective studies. Study characteristics will be reported and the methodological quality of studies will be assessed. When appropriate, we will provide pooled odds ratio with 95% confidence intervals of the effect estimates, study clinical heterogeneity using pre-defined sensitivity and subgroup analyses, and study statistical heterogeneity using the I^2 test.

Discussion: The results of this systematic review will provide an evidence-base regarding the potential clinical effects of donor characteristics on transfusion recipients to better guide policy and clinical practice. The evidence gathered from this review will also identify strengths and weaknesses of published studies regarding donor characteristics and transfusion outcome and will identify knowledge gaps to inform future research in this field of transfusion medicine. PROSPERO Registration Number: CRD42013006726

BACKGROUND

Transfusion of blood products is a necessary, lifesaving intervention. More than 85 million units of red blood cells (RBCs) are collected and transfused every year worldwide [1]. RBCs are given to increase oxygen delivery to tissues and used across a variety of medical and surgical situations. For instance, approximately 30% of critical care patients, and more than 50% of cardiac surgery patients will receive a transfusion during their hospital stay [2,3].

Importantly, the use of RBC transfusion is not without risks, including infectious and immunologic. These risks can be reduced by a variety of measures, including donor questionnaires that assess risk, infectious disease blood screening, and other quality measures already implemented by blood agencies [4,5]. An important aim of current measures in reducing risks for patients is to better select blood donors. These measures have been shown to be efficient in improving transfusion outcome. For example, better screening of donors and microbiological testing of the blood products have led to the reduction of transfusion-related infections as low as 1 in 8 million for HIV, 1 in 6.7 million for Hepatitis C virus and 1 in 1.7 million for Hepatitis B virus [6]. More recently, female sex, a history of pregnancy and the presence of anti-leukocytes antibodies in blood products have been associated with the risk of transfusion related acute lung injury which is the current leading cause of mortality after transfusion. These findings have led directly to successful interventions (collection of male only plasma for example), with a subsequent decrease in the occurrence of transfusion related acute lung injury in Canada [7,8]. Finally, red blood cell units have important biologic variability due to the fact that each unit is

collected from an individual volunteer donor. This variability and the variability of interactions between donor and recipient may affect the clinical efficacy and safety of a blood transfusion. Although many measures are undertaken to select blood donors based on their clinical characteristics, there are no systematic reviews evaluating the impact of donor characteristics on transfusion recipient morbidity and mortality.

LIMITATIONS OF EVIDENCE

Notably, the medical literature is extensive regarding the impact of donor characteristics on outcome of solid organ and bone marrow transplanted patients. These characteristics are used routinely to determine if a specific organ may be suitable for transplantation. Patient characteristics are also used to select the best recipient for a particular organ and to optimize follow-up of transplanted patients when some characteristics from the donor are not optimal. In contrast, when it comes to blood transfusion (which is similar to the transplantation of a different organ) there is a paucity of data regarding the impact of donor characteristics on outcome. We suggest the following evidence to support our rationale to examine the effect of donor characteristics on transfusion outcomes: 1) literature for solid organ transplantation demonstrates the effect of age and gender on recipient outcomes[9–13]; 2) there are immunological modifications that are associated with age such as the increase in memory T cells from naïve T cells[13]; 3) decreased tolerance of the immune system with age[13]; 4) in platelet transfusion, allergic transfusion reactions seem to be associated more with recipient and donor factors than with product attributes[14]; and 5) immunological phenomena in the donor such as the anti-leukocyte antibodies (anti-human-leukocyte antibodies or anti-neutrophil antibodies) that occur after

pregnancies (e.g. gender effect on transfusion related acute lung injury) and the successful interventions (collection of male only plasma) to reduce the occurrence of adverse events and improve the safety of RBC transfusion[7,8]. The biological plausibility, clinical evidence in solid organ transplant, and current policies that select donors based on their characteristics mandates a systematic review to provide a rigorous evidence base for the potential effects of donor characteristics and their impact on outcome.

RESEARCH OBJECTIVES

The primary objective of this systematic review is to evaluate the association of blood donor characteristics (e.g. age, gender, ABO blood type) and the risk of adverse short-term and long-term clinical outcomes after RBC transfusion in recipients. Our secondary objectives are 1) to assess the risk of bias of studies informing current policies for selection of blood donors on their characteristics; and 2) to identify knowledge gaps and potential future research directions in the selection of blood donors based on their characteristics.

METHODS/DESIGN

The methods to be used for this systematic review will follow strict methodological standards based on the Cochrane Collaboration recommendations [15]. Our approach will include, in addition to our research goals and objectives, a thorough process for study identification and selection, a descriptive quality assessment of included studies, and a data-analysis, and interpretation.

SEARCH STRATEGY

We will develop a comprehensive and systematic search strategy with an information specialist trained in the conduct of systematic reviews. We will use MeSH (or EMTREE equivalent) terms in addition with free text terms representing the included population, exposure to RBC and donor characteristics and outcomes, to be sensitive and inclusive (Appendix 1). We will search MEDLINE database, EMBASE database and Cochrane Central databases (that include the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials, the Cochrane Methodology Register, the Database of Abstracts of Reviews of Effects, the Health Technology Assessment Database and the NHS Economic Evaluation Database) from inception and updated until authors are ready for final abstraction of selected references. There will be no limitation of the search strategy based on language. Reference lists of published narrative reviews, systematic reviews, and eligible studies, will be searched for additional references. We will finally perform manual screening of published articles since the year 2000 of five journals in the field of transfusion medicine, according to the 2012 Thomson Reuters' impact factor and from expert opinion of the most clinically relevant journals in the field (Blood, Transfusion Medicine Review, British Journal of Hematology, Vox Sanguinis and Transfusion).

STUDY SCREENING AND INCLUSION

Using the results of our comprehensive search strategy, we will obtain title and abstract from all references. If an abstract is not available, full text will be obtained unless the title is clearly irrelevant. From abstracts and titles, a screening process will be performed by two

independent reviewers for each reference using the inclusion and exclusion criteria below. Full text copies of relevant reports will then be obtained for independent analysis by two reviewers for final inclusion decision. Two independent reviewers we then will collect the required data from selected studies using a piloted electronic form. Disagreements will be resolved by consensus and by consultation with a third independent reviewer when needed.

INCLUSION CRITERIA FOR REVIEW

Study Type:

We will include both observational studies (including cross-sectional and cohort studies) and interventional studies. Our aim in synthesizing all available evidence is to provide not only estimates of association but also the clinical and methodological quality of the evidence and identify important gaps in the evidence.

Population:

The aim of RBC transfusions is to improve oxygen delivery to tissues. Such is the case for patients with active bleeding of any cause, or for patients with subacute or chronic anemia, including patients with cancer, patients hospitalized in intensive care units or patients with terminal renal failure. For this systematic review, the population of interest will be patients (in-hospital or outpatient) with any medical condition requiring at least one RBC unit. There will be no age restriction for this systematic review.

Intervention (exposure):

RBC transfusion accounts for the major part of Blood Supply Organizations blood product manufacture activities [16–18]. A RBC unit comes from a unique donor, whereas plasma and platelets may come from several different donors and pooled into a single unit before transfusion. Since RBC products are the most commonly used blood product, have the most important societal and clinical outreach, and a single unit comes from a single donor, our review will focus on RBC transfusions only. We will include all studies evaluating donor characteristics, focusing on age, sex and blood group that assess at least one pre-defined donor characteristic and its effect on recipients

Outcomes, setting and timeframe:

The primary outcome for this review is mortality. However, to better appraise the impact of donor characteristics on outcome, we will not restrict outcomes in the search strategy. Rather, the search strategy will capture any clinical or surrogate outcomes related to donor characteristics. We will use no limit for year of publication in this review.

EXCLUSION CRITERIA

- Studies in which the study population includes non-transfused patients and from which it is impossible to obtain results for transfused patients.
- Studies with no measure of association between donor characteristics and transfusion outcome.
- Case reports (≤ 2 cases) and case series.
- Duplicates or “sub cohorts” of already published cohorts.

ANALYSIS PLAN

A descriptive analysis of all included studies will be first performed in tables and text form. Clinical, demographic, and methodological quality characteristics and results within study will be reported and discussed. An in depth discussion of heterogeneity between studies will be provided where applicable. We will perform meta-analyses of suitable studies and provide a discussion of the results.

STUDY CHARACTERISTICS

Each study will be categorized as observational or interventional. Interventional studies will be categorized as randomized or non-randomized, and observational studies will be further categorized as cohort (retrospective or prospective) and case-control studies. Further major characteristics of the study design will be reported. We will describe the population studied including the total number of patients, clinical characteristics, the number of transfused patients, the blood products transfused with the proportion for each blood product, the characteristics of included patients (age, gender and reasons for transfusion), and inclusion and exclusion criteria.

RISK OF BIAS

We expect that most of included studies will be observational. Meta-analyses of observational studies may be more prone to bias [16]. Particular care will be taken to adequately investigate the methodological and clinical risk of bias before proceeding with the meta-analysis.

Methodological quality assessment

The methodological quality of the included studies in this systematic review will be evaluated by two independent reviewers using the Downs and Black tool for assessing risk of bias [15]. The Downs and Black tool is the updated evidence based quality tool for use in assessing risk of bias when including non-randomized studies in systematic reviews of interventions recommended by the Cochrane Collaboration [16]. Specific coding instructions adapted for this review will be included for reviewers and will be piloted prior to implementation on all selected references. Overall and study specific appraisal of methodological strengths and weaknesses will be reported.

Clinical risk of bias and effect modification

There is a high risk of clinical heterogeneity introduced by the many potential indications of transfusions of RBCs. The studied populations will most likely be very different between studies. We will take appropriate care to adequately define the clinical characteristics of the transfused patients and the indication for transfusion and if possible contact authors for further clarification. Sensitivity analyses will be performed to evaluate impact of these differences.

Changes in manufacture and storage policies, as well as donor inclusion and exclusion criteria changed significantly over the years. For example, age limits for blood donation have increased in many jurisdictions [17,18], preservative solutions used for RBCs have changed and the manufacture process has been modified by performing universal leukoreduction on RBC products [1,4]. In cases where the exact donor inclusion criteria and

manufacture characteristics are not reported, we will attempt to contact authors for further details.

PRIMARY ANALYSIS

For our primary outcome of mortality, we will provide for each of the donor characteristics the reported number of alive and dead patients for exposed and non-exposed patients. We will also report the number of patients lost to follow-up to assess the completeness of the reported events. We will collect adjusted mortality risk as odds ratios or relative risks if reported. When appropriate, we will provide pooled effect estimates as pooled log-odds ratios with 95% confidence intervals using random effect modeling approach. The results will be presented in tables and Forest plots. Continuous endpoints will be described and pooled using weighted means ratios with random effect modeling. Statistical heterogeneity will be reported using the I^2 test with 95% confidence intervals. If the number of studies included is sufficient (≥10), we will investigate the presence of publication bias using funnel plot techniques.

PLANNED SUBGROUP ANALYSES TO EXPLAIN CLINICAL HETEROGENEITY

Given that our study has the potential, by design, to include different populations that have received RBC transfusions, clinical heterogeneity is expected. To explore clinical and statistical heterogeneity, assuming that pooling of data is possible and appropriate, we will report pooled log-odds ratio for the following subgroups: 1) Adults ≥ 18 years versus mixed children/adults, only children vs only adult and we will consider subgroups among children (< 1 month and neonates versus other children < 18 years; 2) Hospitalized patients versus

outpatient transfused patients; 3) Patients transfused in the intensive care units versus hospitalized but not in the intensive care units; 4) Significant changes in donor inclusion criteria or manufacture strategy; 5) Surgical versus medical population (≥75% of included patients); 6) Patients with acute versus chronic anemia; and 7) Continent where the studies were conducted (North America, South America, Europe, Asia, Australia and Oceania).

PLANNED SENSITIVITY ANALYSES TO INVESTIGATE IMPACT OF STUDY DESIGN

We will conduct subgroup analyses to explore and explain heterogeneity in effects sizes and investigate robustness of our results. It is impossible to identify all potential sensitivity analyses, but we can pre-specify important sensitivity analyses based on the *Downs and Black* for the quality assessment of randomized and non-randomized studies that will have to be performed: selection bias, comparability of groups, and appropriateness of outcome assessment (blinded, length of follow-up, complete follow-up)[15].

QUALITY OF EVIDENCE

The quality of the evidence obtained from this systematic review will be influenced by the different designs of included studies. Two reviewers will evaluate the quality of evidence for our primary outcome (mortality) according to five domains: study limitations, inconsistency of results, indirectness of evidence, imprecision and reporting bias, using the GRADE methodology [19]. We will report the quality as very low, low, moderate or high.

DISCUSSION

The rigorous systematic review methodology used for this project will ensure the best available knowledge synthesis on this topic. The internal peer-review and a priori submission of the protocol to PROSPERO will reduce the risk of bias of this review and ensure a transparent process throughout the project. The internal validation and piloting undertaken at every step of the review will decrease the risk of selection bias and systematic extraction errors. We will pilot and then use an evidence based tool for the evaluation of methodological quality and will use the results for pre-specified subgroup analysis to explore potential methodological cause for heterogeneity.

The results from a systematic review are dependent on the quality of the underlying primary studies conducted. For the current review, we are aware that most, if not all studies will be cohort or case control studies, and many will be retrospective. As such, we decided purposively to be very inclusive in our eligibility criteria so we can better appraise the extent of the available literature as we hypothesize that most of the evidence regarding the association between donor characteristics and transfusion outcomes may be of low methodological quality. Such a comprehensive approach will allow us to assess the potential impact of low quality versus high quality studies on the estimation of the observed associations.

The results of this systematic review will provide the best evidence regarding the potential clinical effects of donor characteristics on transfusion recipients to better guide policy and clinical practice. Our systematic review will synthesize and update current knowledge

regarding evidence pertaining to blood donor selection. In addition, the results of our systematic review will identify clinical and methodological strengths and weaknesses of existing evidence regarding donor characteristics and recipient outcome. It will serve as a foundation for further research in the field and for the development of studies evaluating present or establishing novel policies regarding outcome improvement in transfusion medicine. The results of our work will allow blood collection and supply organizations to ensure that their current policies are evidence-based and could result in updating national policies regarding donor selection for blood transfusion. The potential optimal identification of donor characteristics that are associated with better RBC transfusion outcome may ultimately lead to an improvement in health outcomes in the transfused patient population.

COMPETING INTERESTS

None to declare

SOURCE OF FUNDING

No specific funding has been received for this systematic review.

AUTHORS' CONTRIBUTIONS

MC and DF designed the review, conducted the scoping searches, drafted and revised the manuscript. SE, LM, GK, NS, AF, KW, CvW, AT provided content expertise, feedback on the design of the review, the protocol and on the manuscript. MC, DF, SE will be the reviewers for the systematic review. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We would like to thank Risa Shorr, information specialist, for her help and support constructing the search strategy.

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APPENDIX 1: SEARCH STRATEGY

Database: Embase Classic+Embase <1947 to 2013 December 11>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>
Search Strategy:

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1 blood transfusion/ or erythrocyte transfusion/ (168687)
2 ((rbc or red blood$ cell$ or red cell$ or erythrocyte$) adj5 transfusion$).tw. (18530)
3 1 or 2 (175580)
4 Blood Donors/ (46008)
5 ((rbc or blood or red cell$ or erythrocyte$) adj1 (donor$ or donation$)).tw. (53965)
6 4 or 5 (72574)
7 3 and 6 (16580)
8 (risk or mortality).mp. or cohort.tw. (5021473)
9 prognosis/ or treatment outcome/ (2048249)
10 predictor$.tw. (498103)
11 diagnosed.tw. (854089)
12 exp models, statistical/ (397700)
13 death/ (185240)
14 or/8-13 (7507718)
15 7 and 14 (5724)
16 15 use prmz (1893)

17 *blood transfusion/ (70716)
18 *erythrocyte transfusion/ (6471)
19 ((rbc or red blood$ cell$ or red cell$ or erythrocyte$) adj5 transfusion$).tw. (18530)
20 17 or 18 or 19 (88179)
21 *Blood Donors/ (20782)
22 ((rbc or blood or red cell$ or erythrocyte$) adj1 (donor$ or donation$)).tw. (53965)
23 21 or 22 (59904)
24 20 and 23 (8332)
25 (risk or mortalit$ or cohort).tw. (3978080)
26 follow-up.mp. or prognos$.tw. or ep.fs. (4675345)
27 25 or 26 (7304408)
28 24 and 27 (3007)
29 28 use emczd (1814)
30 16 or 29 (3707)
31 remove duplicates from 30 (2993)

Cochrane Library
Description:

ID Search
#1 MeSH descriptor: [Blood Transfusion] this term only
#2 MeSH descriptor: [Erythrocyte Transfusion] this term only
#3 rbc near/1 transfusion:ti,ab,kw or "red blood cell* NEAR1 transfusion":ti,ab,kw or red cell near/1 transfusion:ti,ab,kw or erythrocyte near/1 transfusion:ti,ab,kw (Word variations have been searched)
#4 platelet* near/1 transfusion:ti,ab,kw (Word variations have been searched)
#5 #1 or #3 or #4
#6 MeSH descriptor: [Blood Donors] explode all trees
#7 rbc near/1 (donor* or donation*):ti,ab,kw or "blood" near/1 (donor* or donation*):ti,ab,kw or "red cell" near/1 (donor* or donation*):ti,ab,kw or "erythrocyte" near/1 (donor* or donation*):ti,ab,kw (Word variations have been searched)
#8 #6 or #7
#9 #5 and #8

Cochrane Reviews (9)
Trials (130)
Technology Assessments (3)
Economic Evaluations (22)

CHAPTER 3 - MANUSCRIPT 2 - EFFECT OF BLOOD DONOR CHARACTERISTICS ON TRANSFUSION OUTCOMES: A SYSTEMATIC REVIEW AND META-ANALYSIS

3.1 PREFACE TO MANUSCRIPT 2

In this paper, we present the results of our systematic review designed in Chapter 2. Fifty-eight studies discussing 17 different donor characteristics met our inclusion criteria. Of the 58 eligible studies, 5 studies evaluated donor age as a risk factor for RBC transfusion outcome and 17 studies for donor sex. Only one study for donor age reported mortality as an outcome of interest and the results were not reported in the primary publication but rather obtained from communications with the main author of the paper. Detailed description of the reporting quality, risk of bias, methodological quality and of the results are provided. Exploratory meta-analyses are performed for some donor characteristics where such analyses were possible.

3.2 MANUSCRIPT 2

Chassé M, Tinmouth A, English SW, McIntyre L, McIntyre L, Knoll G, Wolfe D, Wilson K, Shehata N, Forster A, van Walraven C, Fergusson DA. Effect of blood donor characteristics on transfusion outcomes: a systematic review and meta-analysis.

This manuscript has been submitted to Transfusion Medicine Reviews

Effect of blood donor characteristics on transfusion outcomes: a systematic review and meta-analysis

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ABSTRACT

Background: Optimal selection of blood donors is critical for ensuring the safety of blood products. The current selection process is concerned principally with the safety of the blood donor at the time of donation and of the recipient at the time of transfusion. Recent evidence suggests that the characteristics of the donor may affect short and long-term transfusion outcomes for the transfused recipient.

Methods: We conducted a systematic review with primary objective to assess the association between blood donor characteristics and red blood cell (RBC) transfusion outcomes. We searched MEDLINE, EMBASE, Cochrane Central databases, and performed manual searches of top transfusion journals for all available prospective and retrospective studies. We described study characteristics, methodological quality and risk of bias, provided study-level effect estimates, and when appropriate, pooled estimates with 95% confidence intervals using the Mantel-Haenszel or inverse variance approach. The overall quality of the evidence was graded using GRADE methodology.

Results: From 6120 citations identified by our literature search, 58 studies met our eligibility criteria (49 observational, 9 interventional). We identified the evaluation of association of 17 donor characteristics on RBC transfusion outcome. The risk of bias and confounding of the included studies were high. The quality of evidence was graded as very low to low for all 17 donor characteristics. Potential associations were observed for donor sex with reduced survival at 90 days and 6 months in male recipients that receive donated blood from females (HR 2.60 [1.09, 6.20] and HR 2.40 [1.10, 5.24] respectively, n=1), HLA-DR matched transfusions (OR 0.39 [0.15, 0.99] for the risk of transplant allo-immunization n=9),

presence of anti-leukocyte antibodies (OR 5.84 [1.66, 20.59] for risk of transfusion related acute lung injury, n=4) and donor RBC antigens matching (OR 0.19 [0.03, 1.02] for risk of allo-immunization, n=3).

Conclusion: The available evidence and its quality are insufficient to draw definitive conclusions regarding the association of donor characteristics and transfusion recipients. Albeit based on poor quality evidence, positive anti-leukocyte antibodies, female donor to male recipients, mismatched HLA-DR RBC transfusion or donor RBC antigen may affect RBC transfusion outcome. Well-designed studies are needed to robustly assess the potential clinical impact of blood donor characteristics on recipients.

PROSPERO Registration Number: CRD42013006726

BACKGROUND

Transfusion of red blood cells (RBCs) is one of the most commonly used medical interventions in hospital, accounting for more than 108 million yearly units worldwide[1]. The supply of RBCs in North America relies on voluntary whole blood donation. The quality of RBC products can be greatly affected by characteristics of these donors (health status, phenotypes) and an important aim of current measures in reducing risks for recipients is to better select blood donors. Many infectious pathogens can be transmitted by blood transfusion and can lead to recipient contamination potentially affecting their long-term outcome. Such is the case of human immunodeficiency virus (HIV) and other viruses. These risks can be reduced by a variety of measures, including donor questionnaires that assess infectious risk, infectious disease blood screening, and other quality measures already implemented by blood supply agencies[2,3]. For example, better screening of donors and microbiological testing of the blood products have led to the reduction of transfusion-related infections[4]. Other characteristics have been suggested to affect outcome of transfusion recipients, notably in plasma transfusion. For example, female sex, a history of pregnancy and the presence of anti-leukocytes antibodies in blood products have been associated with the risk of transfusion related acute lung injury (TRALI) which is the current leading cause of mortality after transfusion[5]. These findings have led to potentially successful interventions (transfusion of male-only plasma), with a subsequent decrease in the occurrence of TRALI in Canada[5,6].

Although one could easily draw comparisons between RBC transfusions (“transplanting” blood from a donor to a suitable recipient) and solid organ or bone marrow transplantation,

there is a paucity of data regarding the impact of donor characteristics on transfusion outcome. In contrast, the medical literature is extensive regarding the impact of donor characteristics on outcome of solid organ and bone marrow transplanted patients[7–11]. Donor characteristics (age, sex, blood groups, comorbities, etc.) are used routinely to determine if a specific organ may be suitable for transplantation, to select the best recipient for a particular organ, and to optimize follow-up of transplanted patients when some characteristics from the donor are not optimal. Given the variability in the donor population, such characteristics may also affect RBC transfusion outcome.

We hypothesize that donor characteristics, other than the screening measures undertaken aiming mostly to reduce the risk of transfusion transmissible infection, may be associated with RBC transfusion outcome and should be evaluated. To inform future transfusion policy making and research, and in light of the absence of summative evaluations of the impact of donor characteristics on RBC transfusion recipient outcome, we conducted a systematic review of the evidence.

RESEARCH OBJECTIVES

The primary objective of this systematic review is to evaluate the association of blood donor characteristics (e.g. age, gender, RBC antibodies) and the risk of short-term and long-term clinical outcomes after RBC transfusion in recipients. Our secondary objectives are 1) to assess the methodological quality and the risk of bias of eligible studies; and 2) to identify knowledge gaps and potential future research directions in the selection of blood donors based on their characteristics.

METHODS/DESIGN

We conducted a systematic review of studies evaluating the association between whole blood donor characteristics and RBC transfusion outcome. The protocol of this systematic review has been published previously and registered in PROSPERO (www.crd.york.ac.uk/prospero) CRD42013006726[12].

SEARCH STRATEGY

We developed a comprehensive, systematic search strategy with an information specialist trained in the conduct of systematic reviews. We searched MEDLINE, EMBASE and Cochrane Central databases (that include the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials, the Cochrane Methodology Register, the Database of Abstracts of Reviews of Effects, the Health Technology Assessment Database and the NHS Economic Evaluation Database) from inception to December 31, 2013, irrespective of language. We used MeSH (or EMTREE equivalent) terms in addition with free text terms representing the included population, exposure to blood products and donor characteristics and outcomes, to be sensitive and inclusive (Appendix 1). At the stage of the literature search, we applied no restriction on the type of blood product to ensure the most sensitive search strategy. Reference lists of published narrative reviews, systematic reviews, and eligible studies, were searched for additional references up to January 2015. We performed manual screening of published articles since the year 2000 of five journals in the field of transfusion medicine, according to the 2012 Thomson Reuters' impact factor and from expert opinion of the most clinically relevant journals in the field (Blood, Transfusion Medicine Reviews, British Journal of Hematology, Vox Sanguinis and Transfusion).

STUDY SCREENING AND INCLUSION

We obtained title and abstracts of citations identified by our search strategy. When an abstract was not available, full text was obtained unless the title was clearly irrelevant. All abstracts and titles were screened by two independent reviewers using pre-specified inclusion and exclusion criteria. Full text copies of relevant reports were then obtained for independent analysis by two reviewers for final inclusion decision. Eligible studies were then abstracted by two independent reviewers using a piloted, standardized electronic form. Disagreements were resolved by consensus and by consultation with a third independent reviewer when needed.

INCLUSION CRITERIA FOR REVIEW

Study Type:

We included observational studies and interventional studies.

Population:

The population of interest was patients (in-hospital or outpatient) with any medical condition requiring at least one RBC unit. We included neonatal, pediatric and adult patient populations.

Intervention (exposure):

For this review, we were interested in the impact of whole blood donor characteristics in relation to transfusion of RBC products only. We included all studies evaluating at least one donor characteristic and its clinical effect on recipients. When a study included recipients of blood products other than RBCs, the study was eligible only if we could extract the

proportion of patients receiving RBC transfusions, either from the manuscript or after contacting the corresponding author. When the intervention was labelled as “whole blood transfusion” specifically, the study was excluded as these products contain a significant proportion of plasma. Studies reporting “blood products”, without any further description regarding the type of blood products transfused were also excluded after contacting the corresponding author of the study if the proportion of RBC transfused patients and outcome data were not available.

Outcomes, setting and timeframe:

Our primary outcome was mortality. However, we did not restrict outcomes in the search strategy or for inclusion. We included any clinical or surrogate outcomes related to donor characteristics.

EXCLUSION CRITERIA

We excluded studies in which the study population included non-transfused patients and from which it was impossible to obtain results for transfused patients. To be eligible, a study also had to report a measure of association between a donor characteristic and a transfusion outcome. We excluded studies reporting expected associations between donor and recipients (for example, using mathematical modeling). Case series were excluded unless an interrupted time-series design was used. We excluded case reports (≤ 2 cases) and duplicates or “sub cohorts” of already published studies.

ANALYSIS PLAN

Study synthesis

For each eligible study, we described the study origin (country, date) and design characteristics (retrospective, prospective, interventional, etc.). We provided a rich description of the population studied including the total number of patients, clinical characteristics, the number of transfused patients, the blood products transfused with the proportion for each type of blood product, the characteristics of included patients (age, sex and reasons for transfusion), and inclusion and exclusion criteria.

Methodological quality assessment

The methodological quality of the included studies in this systematic review was evaluated by two independent reviewers using the Downs and Black tool for assessing risk of bias[13]. Specific coding instructions were provided to the reviewers and were piloted prior to implementation.

Primary analysis

For each of the donor characteristics, we abstracted the number of exposed and non-exposed patients with the studied outcome. We also abstracted unadjusted and adjusted odds ratios, relative risks, risk ratios or hazard ratios. When appropriate, we provided pooled effect estimates as pooled log-odds ratios or risk ratios with 95% confidence intervals using a random effects modeling approach. For dichotomous variables, we used the Mantel-Haenszel approach and computed odds ratios and 95% CIs when the number of

exposed and non-exposed patients was available. For continuous outcomes, we computed mean differences and 95% CIs using the inverse variance approach. Statistical heterogeneity was reported using the I^2 test with 95% confidence intervals. To investigate publication bias, the funnel plot techniques will be used. All analyses were performed using RevMan version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

Planned subgroup or sensitivity analysis

To explore clinical and statistical heterogeneity, assuming that pooling of data is possible and appropriate, we reported pooled log-odds ratio for the following subgroups: 1) adults ≥18 years versus mixed children/adults, only children versus only adult, and we considered subgroups among children (<1 month and neonates versus other children <18 years; 2) hospitalized patients versus outpatient transfused patients; 3) patients transfused in an intensive care unit versus hospitalized but not in an intensive care unit; 4) significant changes in donor inclusion criteria or manufacture strategy; 5) surgical versus medical populations (≥75% of included patients); 6) patients with acute versus chronic anemia; and 7) continent where the studies were conducted.

Quality of evidence

Two reviewers evaluated the quality of the evidence according to five domains: study limitations, inconsistency of results, indirectness of evidence, imprecision and reporting bias using the GRADE methodology[14]. We reported the quality as very low, low, moderate or high.

RESULTS

RESULT OF THE SEARCH

Our search strategy identified 6120 citations of which 6044 citations were obtained from our electronic search and 76 citations were found by hand search (Figure 1). Following the screening of titles and abstracts to exclude non-eligible studies by two independent reviewers, 104 reports remained for full text screening and abstraction. A further 46 reports were excluded (Table S2 for description of excluded reports), and thus 58 studies remained for the review.

STUDY CHARACTERISTICS

Overall, 9 studies (n=5,143 patients) were interventional (5 randomized) and 49 were observational (n=not always reported). Of the observational studies, 38 were retrospective and 11 prospective. Observational designs included time-series (n=18), cohort studies (n=18), lookback (n=3), trace-back (n=5) and case-control (n=5). All but 5 studies (1 Uganda, 1 Jamaica, 1 Columbia, and 2 Japan) were conducted in North-America (n=26) or Europe (n=27). Publication years ranged from 1976 to 2013, with 34 (58.6%) published since 2000. We included 2 studies where the exact proportion of RBC transfusions were not reported but the authors reported an adjusted analysis by blood component[15,16]. One study reported RBC-related TRALI events without denominator data[17]. For 24 studies, we were able to extract data for patients that received RBCs only (i.e. no other blood products). Four of those studies were for the RBC antigens antibody blood donor characteristic, 4 for donor sex, 12 for the HLA mismatch blood donor characteristic in the transplant population and 2 for donor age.

DONOR CHARACTERISTICS

We identified 17 different donor characteristics studied (Figure 1). Detailed results of our analyses are presented in Table 1 to Table 5 and in Figure S2. We summarized the results by patients either receiving RBC products only and those receiving RBC and other blood products.

Donor age

We identified 5 studies that provided clinical outcome estimates for donor age (Table 1 and Figure S2). The outcomes of interests were mortality (n=1), TRALI (n=3), change in HTLV infection status (n=1) and HIV infection (n=1). All were retrospective and included a total of 586 recipients.

RBC only

Two studies[18,19] presented data for patients receiving RBC transfusions only. One study reported the incidence of acute lung injury[18], whereas the other study reported the rate of immune mediated versus non-immune related TRALI[19]. Both studies reported no significant association between donor age and the risk of TRALI (age difference -0.18 [-6.67, 6.31] for maximum donor age) or immune-related TRALI (age difference 7.0 [-13.64, 27.64] for maximum donor age).

RBC and other blood components

In three studies reporting donor age as a potential risk factor for adverse RBC transfusion outcome, recipients were also transfused other blood products in addition to RBCs (Table 1). From data provided by the author of one study[15], maximum donor age was in average 6.24 years lower in patients who did not survive hospitalization (age difference -6.24 [-12.43, -0.05]). Despite design limitations (retrospective case-control study), and the fact that all patients also received plasma transfusion, this study was of good reporting quality with low risk of bias. Two additional studies tested the association between donor age and HIV[20] or HTLV[21] transmission. No numerical values were provided by either study but the association was reported as not significant. Those studies had poor reporting quality and were at high risk of bias and confounding (Figure S1).

Donor sex

Seventeen studies (1 prospective, 16 retrospective) were eligible for review for the donor sex characteristic (Table 2). Seven studies were time series studying "before and after" an organizational policy change for blood donation. The other 10 studies were retrospective case-control (n=4), cohorts (n=4) or trace-back studies (n=2). 3 studies reported data for RBC transfusions separately. Two types of donor sex comparisons were available from the included studies: donor sex alone and donor-recipient sex mismatch. The reported outcomes were mortality (n=2), TRALI (n=13), change in HTLV infection status (n=1), mosaicism (n=1) and the risk of necrotizing enterocolitis (n=1).

RBC only

Three studies[19,22,23] reported a measure of association between donor sex and transfusion outcome and one study for donor sex mismatch[24]. In a retrospective cohort of 122 patients, Paul & colleagues[23] found that in neonates developing necrotizing enterocolitis within 48h after transfusion 84% of RBC units transfused were from male donors compared to 62% in neonates that developed necrotizing enterocolitis greater than 48h after transfusion (p=0.03). A study including 10 patients by Porretti[19] did not find an association between donor sex and immune-mediated TRALI. Finally, a study of 10 neonates[22] receiving RBC transfusions found no evidence of karyotype mosaicism according to donor sex. The risk of bias was high for all studies.

For donor sex mismatch, Middelburg & al. [24] found an improved survival at 90 days and 6 months among male recipients under 55 years of age who received only red cell transfusion from male donors (HR 2.60 [1.09, 6.20] and (HR 2.40 [1.10, 5.24], respectively). Statistical significance was no longer observed at 5 years (HR 1.40 [0.94, 2.08]). There was no such association in female recipients. Reporting quality was good and the risk of bias low.

RBC and other blood components

For donor sex, 7 studies reported the rate of RBC-related TRALI before and after policy changes that included implementation of predominantly-male plasma donors and in some reports, the exclusion of previously allo-exposed donors[5,17,25–29]. In those studies, the interventions were aiming to reduce exposure of female blood in plasma products, but we extracted RBC-related TRALI. A meta-analysis of those 7 included reports showed a reduced

risk of TRALI in favour of the policy change of reduced exposure to female blood donations (RR 0.50 [0.35, 0.72], I^2 0%). Patients included in those studies also received other blood products in a proportion ranging between 14.6% and 100% for plasma. Given the design (before-and-after studies) of those reports[5,17,25–29], the risk of bias and confounding was high for all included studies. We pooled 4 studies (3 case-control and 1 trace-back study) directly measuring the risk of TRALI in RBC recipients and found no such association (OR 1.03 [0.64, 1.68], I^2 0%)[5,15,19,30]. One before-and-after study reported cases of RBC-associated TRALI, and the total number of units transfused during the study period and found no evidence of association[31]. One recipient trace-back study reported a significantly increased risk of TRALI-related death for recipients of blood from antibody-positive female donors compared to male donors or antibody-negative donors (RR 9.00 [1.92, 42.24]) [32]. This study was of low reporting quality, included 18 recipients, was at risk of confounding and 59% of the blood products transfused were plasma. One study looking at the risk of HTLV transmission found no association with donor sex[21].

One report estimated the risk of mortality and of acute lung injury for donor sex mismatch[15]. In this study, for the subgroup of patients that received RBCs, there was no association between donor sex mismatch and the risk mortality or acute lung injury (OR 1.04 [0.50, 2.73] for mortality, 1.05 [0.40, 2.73] for acute lung injury). All patients also received plasma. Reporting quality was good and the risk of bias low.

White Blood Cell antibodies

Seven studies assessed at least one donor antibody directed to any type of WBC antigen (Table 3). One study found no association between donor WBC antigen and mortality. Four observational studies suggested an increased risk of TRALI (OR 5.84 [1.66, 20.59], I^2 62%). The risk of bias and the statistical heterogeneity for the 4 studies were high. One study measured the risk of TRALI in relation to the anti-WBC antibodies titers and reported a significant association, favoring low antibody titers (OR 3.20 [1.52, 6.74]). All studies included patients that received RBC and other blood products.

RBC antigens

4 studies (3 observational, 1 non-randomized intervention study) reported the association between donor-recipient RBC surface antigen matching (Table 3) [33–36]. One study found no association between a donor-recipient cross-match (vs no crossmatch) with the risk of clinical hemolysis (OR 0.74 [0.03, 15.83]). Three studies reported the risk of allo-immunization after transfusion of mismatched vs matched blood. Overall, transfusion of matched RBC products was associated with a reduction in the risk of allo-immunization (OR 0.19 [0.03, 1.02], I^2 65%). The heterogeneity and risk of bias were high for all studies. One study was not included due to incomplete reporting[34].

HLA-DR mismatch

We identified 12 studies (2 RCTs, 3 non-randomized intervention studies, 5 prospective cohorts and 2 retrospective cohorts), reporting the association between HLA-DR mismatch between donor and recipients (Table 3). All recipients were transplant candidates (renal in 11 studies and cardiac in 1 study). All patients received only RBC transfusions. There was no evidence of association between HLA-DR mismatch and mortality (n = 1 study) patient survival (n = 2 studies), graft survival (n = 4 studies) and microchimerism (n = 1 study). Pre-transplantation HLA-DR matched transfusion (as opposed to mismatched) reduced the rate of renal rejection < 6 months after transplantation in 2 studies (OR 0.50 [0.29, 0.88], I^2 0%), at any time after transplantation in 1 study (OR 0.47 [0.16, 1.35]), and the number of rejection episodes post-transplant in 2 studies (OR 0.50 [0.29, 0.88], I^2 0%). In 9 studies (1 RCT, 3 non-randomized intervention trial and 5 observation studies), the rate of allo-immunization post-transplantation was reported. The overall risk of allo-immunization was reduced by the transfusion of HLA-DR matched RBC products (OR 0.39 [0.15, 0.99], I^2 66%). All studies were of different design, at high risk of bias and confounding, and the statistical heterogeneity was high.

Other non-infectious donor characteristics

An additional 4 non-infectious donor characteristics were identified (Table 4).

RBC only

One study published in 1976 including 123 patients assessed CMV and HBV infection risks in pediatric recipients of RBCs from "walking donors" versus "usual" donor and found no association (OR 1.67 [0.18, 15.02] for CMV, not estimable for HBV). One RCT in 40 neonates found no association between maternal donors, paternal donors or unrelated donors and the risk of transfusion reaction, change in hematocrit, abnormal post-transfusion creatinine and bilirubin level. Both studies were at high risk of bias.

RBC and other blood components

Four studies reported previous allo-exposures (transfusion or pregnancy) as the exposure of interest, with no regard to the antibody status, and found no association with mortality or TRALI[5,15,37,38]. One large population study of good methodological quality and at low risk of bias found no association between donor cancer and the risk of cancer in transfusion recipients (RR 1.00 [0.94, 1.06])[16]. The proportion of RBC transfusions was not reported but the authors performed statistical adjustments by blood products showing no association with the type of product transfused.

Infectious donor characteristics

We identified 18 studies reporting infectious donor characteristics (Table 5). The studied pathogens were babesiosis (n = 2), CMV (n = 3), HBV (n = 3), hepatitis C (n = 5), HHV8 (n = 1), HTLV (n = 4) and Parvovirus B19 (n = 1). The included studies were in general at high risk of bias, used various designs and had imprecise estimates. A total of 3 studies included patients that received RBC transfusions only (Babesiosis n=1, CMV n=1, Parvovirus B19 n=1). Donor infectious testing was associated with a statistically significant reduced infection risk for HCV (OR 0.12 [0.02, 0.84], I^2 91% for policy change), HHV8 (OR 0.52 [0.27, 0.98]), HTLV (OR 0.02 [0.00, 0.09]) and parvovirus B19 (OR 0.03 [0.00, 0.58] for DNA positive) but non-significantly reduced risk for babesiosis (RR 0.16 [0.02, 1.31], I^2 0%) and HBV (OR 0.61 [0.32, 1.17]).

SUBGROUP OR SENSITIVITY ANALYSIS

Given the low number of eligible studies for each donor characteristics and outcome, no meaningful pre-specified subgroup or sensitivity analysis stated in our protocol could be performed.

Risk of bias

The overall risk of bias and study-specific risk of bias analyses are reported in Figure 2 and Figure S1. Overall, the quality of reporting was variable. 80% of studies clearly described objectives, exposures, outcomes and study population, but less than 50% clearly described

the distribution of confounders, measures of random variability, lost to follow-up and measure of probability values. More than 50% of studies presented at least one serious risk of bias, especially concerning blinding of the subjects or outcome assessments, as well as the selection of the appropriate analysis for different lengths of follow-up. The risk of confounding was high in general, as more than 65% of studies did not appropriately consider confounding or losses of patients to follow-up. Five studies were randomized but we were unable to determine if allocation concealment was appropriate. Given the low number of included studies for each donor characteristics and outcome, no funnel plots were produced to appropriately estimate the risk of publication bias.

QUALITY OF EVIDENCE

We applied the GRADE methodology to assess the quality of the evidence obtained from this review (Table S4). It was estimated to be very low for all outcomes except for donor cancer and risk of cancer in the recipient (low), the risk of allo-immunization after transfusion of HLA-DR mismatched blood in renal transplant recipients (low) and the change in hematocrit after transfusion of maternal versus paternal RBCs or random versus parental RBCs (low).

DISCUSSION

In our systematic review, we identified 17 unique donor characteristics and their potential impact on RBC transfusion recipient outcomes from 58 studies. The quality of the evidence regarding the association between non-infectious donor characteristics and recipient outcomes was low to very low, suggesting that any estimate of effect is either uncertain or that additional research will likely have an important impact and affect our estimates[14]. Because the common design choice was observational (and retrospective), the risk of bias and confounding was high.

A very low number of studies reported donor age as a potential effect modifier of outcome in RBC transfusion recipients and was a stated outcome in only one small study[15]. No study reported any evidence of association between donor age and transfusion outcome. From personal communications with the authors of one study, we found that the maximum donor age was lower in patients who died in hospital versus patients who survived[15]. This analysis was performed on the subset of patients who received RBC transfusions, but all patients also received plasma. All studies were small and at high risk of confounding and no conclusion can be drawn from the gathered evidence.

Whether the risk of TRALI is increased after RBC transfusion from female donors is unclear. From this review, we found very low quality evidence suggesting that a policy change limiting female plasma donors or plasma donors positive for anti-leukocytes antibodies reduces the risk of RBC-related TRALI. Such findings are surprising since female donors were not restricted in any included studies for RBC transfusions. Given the study design of those studies (before-and-after), the findings are potentially confounded by transfusion of other

plasma-rich products to the recipients. This hypothesis is supported by a null association between female or mixed RBC donors and the risk of TRALI from an analysis including 4 observational studies (OR 1.03 [0.64, 1.68]), although most patients in those studies were also co-transfused with plasma-rich products. The association between female donors and TRALI has been associated with allo-immunization and the presence of WBC antibodies. When studying specifically the presence of WBC antibodies in the blood donor, our meta-analysis of 4 studies suggested that the presence of antibodies in the donor was associated with an increased risk of TRALI in RBC transfusion recipients (OR 5.84 [1.66, 20.59]). However, any result from such analyses should be hypothesis generating only as the obtained estimate is likely unreliable due to poor study design and high risk of bias.

One study suggested that donor sex may be associated with survival when donor and recipient sex was mismatched. In this study by Middelburg and colleagues, the subgroup below 55 years of age that received only female donor RBCs transfused in male recipients had a significantly decreased survival at 6 months (HR 2.40 [1.10, 5.24]). This association was also seen when not restricting to only RBC transfusions (HR 3.1 [1.5, 6.3])[39]. If verified, the mechanism of such an association remains to be explained and will need further study. One could argue that this sex mismatch association may be due again to the presence of WBC antibodies. However, given the lack of association in female recipients who received blood from female donors, and a trend for a decreased survival in females who receive blood from males, the presence of antibodies is unlikely to be the only explanation. One group proposed that microchimerism may be present in donor-recipient mismatch transfusion but this was not observed in their study[40].

Other antibodies or antigens have been studied in RBC transfusions and assessed recipients' clinical outcomes. Compatibility of the pre-transplantation histocompatibility complex (HLA-DR) has been studied in 8 studies in kidney transplantation and 1 in cardiac transplantation. We found no association with survival. However, transfusion of HLA-DR matched blood was reported to be associated with a reduced rate of graft rejection and allo-immunization. Such interventions are no longer used clinically to prevent graft rejection. It however supports that donor mismatch may affect recipient outcome by altering the recipient's immune response. In the general population, similar observations were obtained from three studies studying RBC antigen compatibility and the risk of clinical allo-immunization. Although routine testing for blood compatibility is performed prior to transfusion, for time and availability considerations, patients often receive unmatched blood for minor and sometimes major RBC antigens. This associated increased risk of allo-immunization is however of uncertain long-term clinical significance and robust evaluations are still needed.

Three additional non-infectious characteristics were evaluated for their effect on RBC transfusion outcomes. One large bi-national study analyzed the association between diagnosis of cancer after blood donation and the risk of cancer in the transfusion recipient and showed no association. The estimate was not provided for RBC transfused patients only but the authors reported a multivariable analysis by blood product that did not modify the risk estimate. This unique study provides weak evidence that pre-cancerous patients may not increase the risk of cancer in the recipient. In pediatric populations, one study reported no difference in risk of transmission of CMV when using random donors or donors

specifically asked to provide blood for pediatric care ("walking donors"). Another study comparing blood donated by the mother, the father or random donors also showed no difference in clinical outcome. Given the observational methods used and the small sample size of those two last characteristics, whether such characteristics can affect transfusion outcome remains unclear.

Transfusion has long been a recognized risk of transmission for certain infections such as hepatitis and HIV. In our review, we identified studies that assessed the risk of transfusion transmitted diseases. Our inclusion criteria required that we can measure an outcome associated with RBC transfusions, and that an effect estimate can be measured (rather than estimated), as such several studies were excluded from this review (Table S2). It is therefore impossible to use the observed estimates to compare the risks of transmission of blood transmissible pathogens to other blood products or to the overall risk of transmission when using whole blood. We however found 18 studies that reported a direct measure of association between donor infectious and transfusion outcome. As expected, despite the low number of studies meeting our inclusion criteria, we found a significant association between a positive infectious status for CMV (low viral titer), hepatitis C, HHV8, HTLV and parvovirus B19. For hepatitis B and babesiosis, the included studies did not reach statistical significance, although the trend for infectious transmission was toward a protective effect of negative donors. No studies looking at HIV positive donors and RBC transfusion met our inclusion criteria. It may seem at first surprising to find so few studies that assessed directly the risk of transmissible pathogens. Many studies published in the field tested for the infectious status of the donor, excluded such donors from the transfusion pool, and

performed probabilistic estimations of the risk of having an infected unit in the released units for transfusion. Many more studies did not report the risk of transmission for RBC units, but only for the global transfused population, or for whole blood transfusion, rendering estimation of risk for RBC impossible.

We believe that our rigorous methodology allowed us to provide the most extensive systematic review of donor characteristics that may affect RBC transfusion outcome. We feel confident that our comprehensive search strategy, detailed risk of bias assessment and review methodology allowed us to provide a comprehensive and complete review of the evidence regarding the associations of interest. The quality of the evidence gathered by this review is limited by the quality and design of the included studies. The pooled estimates provided are highly hypothesis generating in nature given the high statistical and clinical heterogeneity, the different study designs, and the high risk of bias and confounding of most included studies. Moreover, a significant amount of studies were excluded because it was impossible to extract estimates for RBC transfusions. This affects mainly the effect estimates for infectious characteristics as only 3 studies were excluded for the donor sex characteristic. Additionally, we excluded only 5 studies that looked at non-infectious donor characteristics (prison donors (n=1), drug users (n=1), parental donors (n=1) and paid donors (n=2)), all observational in design. Those exclusions are very unlikely to influence the conclusions of our study for non-infectious donor characteristics.

In summary, based on very low to low quality evidence, some donor characteristics may affect RBC transfusion outcome. Female donor sex, positive white blood cells antibodies, mismatched HLA-DR donor-recipient status and donor infectious status may be associated with RBC transfusion outcomes. However, the designs and methodologies are at a high risk of bias and confounding, and the number of studies that support these findings are limited. The chosen clinical outcomes of interest are most commonly TRALI and change in infectious status. Survival outcomes are rarely reported. Importantly, the evidence is insufficient to draw definitive conclusions for any donor characteristics. Given the potential outcome benefits or risks observed in some studies, further well designed studies are needed to better evaluate if an improved selection of donors by their characteristics (age, sex, etc.) improves RBC transfusion outcome.

COMPETING INTERESTS

None to declare

SOURCE OF FUNDING

No specific funding has been received for this systematic review.

AUTHORS' CONTRIBUTIONS

MC and DF designed the review, conducted the scoping searches, drafted and revised the manuscript. SE, LM, GK, DW, NS, AF, KW, CvW, AT provided content expertise, feedback on the design of the review, the protocol and on the manuscript. MC, DW designed the

abstraction forms, performed study selection and review. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We would like to thank Risa Shorr, information specialist, for her help and support constructing the search strategy. We are also grateful to Pauline Quach who helped perform screening and double abstraction of studies.

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TABLE 1: ESTIMATES OF ASSOCIATION BETWEEN DONOR AGE AND RBC TRANSFUSION OUTCOMES

Outcome or subgroup	Studies	Effect estimate (with 95% CI)	Interpretation
Donor age			
Hospital mortality			
Mean donor age	1	MD -4.62 [-9.92, 0.64]	Null, favours older donors
Maximum donor age	1	MD -6.24 [-12.43, -0.05]	Favours older donor
ALI ≤ 6h after transfusion			
Mean donor age	2	MD 3.40 [-4.97, 11.78]	Null
Maximum donor age	2	MD 0.47 [-5.73, 6.66]	Null, favours younger donors
ALI ≤ 72 hours after transfusion	1	OR 1.14 [0.52, 2.49]	Null, favours no donor > 63
ALI 72 hours to 28 days after transfusion	1	OR 0.86 [0.38, 1.96]	Null, favours at least one donor > 63
ALI ≤ 28 days after transfusion	1	OR 0.98 [0.51, 1.88]	Null
HTLV transmission	1	Multivariate model including donor age as a risk factor for HTLV-I conversion. 54 patients analyzed. Numerical value not provided. Reported as not significant.	Null
HIV transmission	1	Maximum likelihood estimation of HIV transmission adjusted for covariates. Reported as: "The HIV-1 transmission rate did not vary by the donor's age[…]"	Null

TABLE 2: ESTIMATES OF ASSOCIATION BETWEEN DONOR SEX AND RBC TRANSFUSION OUTCOMES

Outcome or subgroup	Studies	Effect estimate (with 95% CI)	Interpretation
Donor sex			
RBC associated TRALI risk after policy change	7	RR 0.50 [0.35, 0.72], I^2 0%	Favours after policy change
RBC associated TRALI from male vs mixed donors	4	OR 1.03 [0.64, 1.68], I^2 0%	Null
Non-fatal TRALI risk / unit distributed	1	OR 0.87 [0.40, 1.88]	Null, favours male donors only
Fatal TRALI risk / unit distributed	1	OR 0.37 [0.07, 1.93]	Null, favours male donors only
TRALI-related death vs non-TRALI-related death	1	RR 9.00 [1.92, 42.24]	Favours male donors and antibody negative female donors
Transfusion reactions	1	OR 1.06 [0.94, 1.19]	Null
TRALI / number of units			
Male donors	1	OR 0.96 [0.73, 1.26]	Null
Female donors	1	OR 1.03 [0.75, 1.41]	Null
Antibody-mediated TRALI vs non-antibody mediated	1	OR 4.20 [0.12, 151.96]	Null, favours male donors
Genetic mosaicism	1	Male vs usual donor populations. Statistical method unclear. Authors report: "Given the male to female donor ratio in our blood bank of approximately 1:1, the probability of any patient being transfused with blood from a donor of opposite sex is 50%. The observation of no instances of even mosaic discordant karyotypic sex in any of our 10 patients is highly significant, with a likelihood of , 0.001."	Null
Necrotizing enterocolitis	1	84% of RBC units were from males in the NEC within 48h of transfusion group, 62% of RBC units were from males in the NEC > 48h group. Authors report a p-value of 0.03.	Favours female donors
HTLV infection	1	Multivariate model including donor sex as a risk factor for HTLV-I conversion. 54 patients analyzed. Numerical value not provided. Reported as not significant.	Null
Donor sex mismatch			
Hospital mortality			
Female recipients	1	OR 1.05 [0.22, 5.13]	Null
Male recipients	1	OR 0.70 [0.18, 2.75]	Null, favours sex mismatch
All recipients	1	OR 1.05 [0.40, 2.73]	Null
Survival 1 month post-transfusion			
Female recipients	1	HR 1.10 [0.43, 2.84]	Null, favours no sex mismatch
Male recipients	1	HR 2.20 [0.86, 5.64]	Null, favours no sex mismatch
All recipients	1	HR 1.40 [0.73, 2.68]	Null, favours no sex mismatch

Survival 90 days post-transfusion	1		
Female recipients	1	HR 1.50 [0.59, 3.79]	Null, favours no sex mismatch
Male recipients	1	HR 2.60 [1.09, 6.20]	Favours no sex mismatch
All recipients	1	HR 1.80 [1.00, 3.24]	Favours no sex mismatch
Survival 6 months post-transfusion	1		
Female recipients	1	HR 1.20 [0.56, 2.59]	Null, favours no sex mismatch
Male recipients	1	HR 2.40 [1.10, 5.24]	Favours no sex mismatch
All recipients	1	HR 1.60 [0.96, 2.67]	Null, favours no sex mismatch
Survival 5 years post-transfusion	1		
Female recipients	1	HR1.20 [0.69, 2.09]	Null, favours no sex mismatch
Male recipients	1	HR 1.70 [0.92, 3.14]	Null, favours no sex mismatch
All recipients	1	HR 1.40 [0.94, 2.08]	Null, favours no sex mismatch
Acute Lung Injury	1		
Female recipients	1	OR 0.78 [0.20, 3.01]	Null, favours sex mismatch
Male recipients	1	OR 1.38 [0.35, 5.44]	Null, favours no sex mismatch
All recipients	1	OR 1.05 [0.40, 2.73]	Null

TABLE 3: ESTIMATES OF ASSOCIATION BETWEEN DONOR ANTIBODIES OR ANTIGEN AND RBC TRANSFUSION OUTCOMES

Outcome or subgroup	Studies	Effect estimate (with 95% CI)	Interpretation
WBC antibody			
Mortality	1	OR 0.45 [0.14, 1.48]	Null, favours presence of antibody
TRALI	4	OR 5.84 [1.66, 20.59], I^2 62%	Favours absence of antibody
TRALI – after implementation of a no WBC antibody policy	1	RR 0.64 [0.07, 5.85]	Null, favours after policy change
Fatal vs non-fatal TRALI	1	OR 0.71 [0.11, 4.65]	Null, favours presence of antibody
Increase of TRALI risk by antigen titer increase	1	OR 3.20 [1.52, 6.74]	Favours low antibody titer
RBC antigen			
Haemolysis	1	OR 0.74 [0.03, 15.83]	Null, Favours positive cross-match
Allo-immunization	3	OR 0.19 [0.03, 1.02], I^2 65%	Null, favours matched blood
HLA-DR mismatch			
Mortality	1	OR 0.45 [0.16, 1.29]	Null, favours HLA-DR matched
One-year graft survival	3	OR 1.27 [0.37, 4.36], I^2 49%	Null, favours HLA-DR mismatched
5-year graft survival	2	OR 1.10 [0.56, 2.18], I^2 0%	Null, favours HLA-DR mismatched
1-year patient survival post-transplant	2	OR 0.45 [0.10, 2.02], I^2 0%	Null, favours HLA-DR matched
5-year patient survival post-transplant	2	OR 1.02 [0.11, 9.79], I^2 68%	Null
Acute renal rejection < 6 months post-transplant	2	OR 0.36 [0.14, 0.91], I^2 0%	Favours HLA-DR matched
Acute renal rejection at any time after transplant	1	OR 0.47 [0.16, 1.35]	Favours HLA-DR matched
> 1 acute renal rejection episode over follow-up	2	OR 0.50 [0.29, 0.88], I^2 0%	Favours HLA-DR matched
Allo-immunization	9	OR 0.39 [0.15, 0.99], I^2 66%	Favours HLA-DR matched
Microchimaerism			
1-4 days post transfusion	1	Not estimable	Not estimable
5-7 days post transfusion	1	OR 17.89 [0.76, 420,49]	Null, favours HLA-DR mismatched
2-4 weeks post transfusion	1	OR 11.67 [0.92, 147.56]	Null, favours HLA-DR mismatched
5-8 weeks post transfusion	1	OR 1.33 [0.10, 17.10]	Null

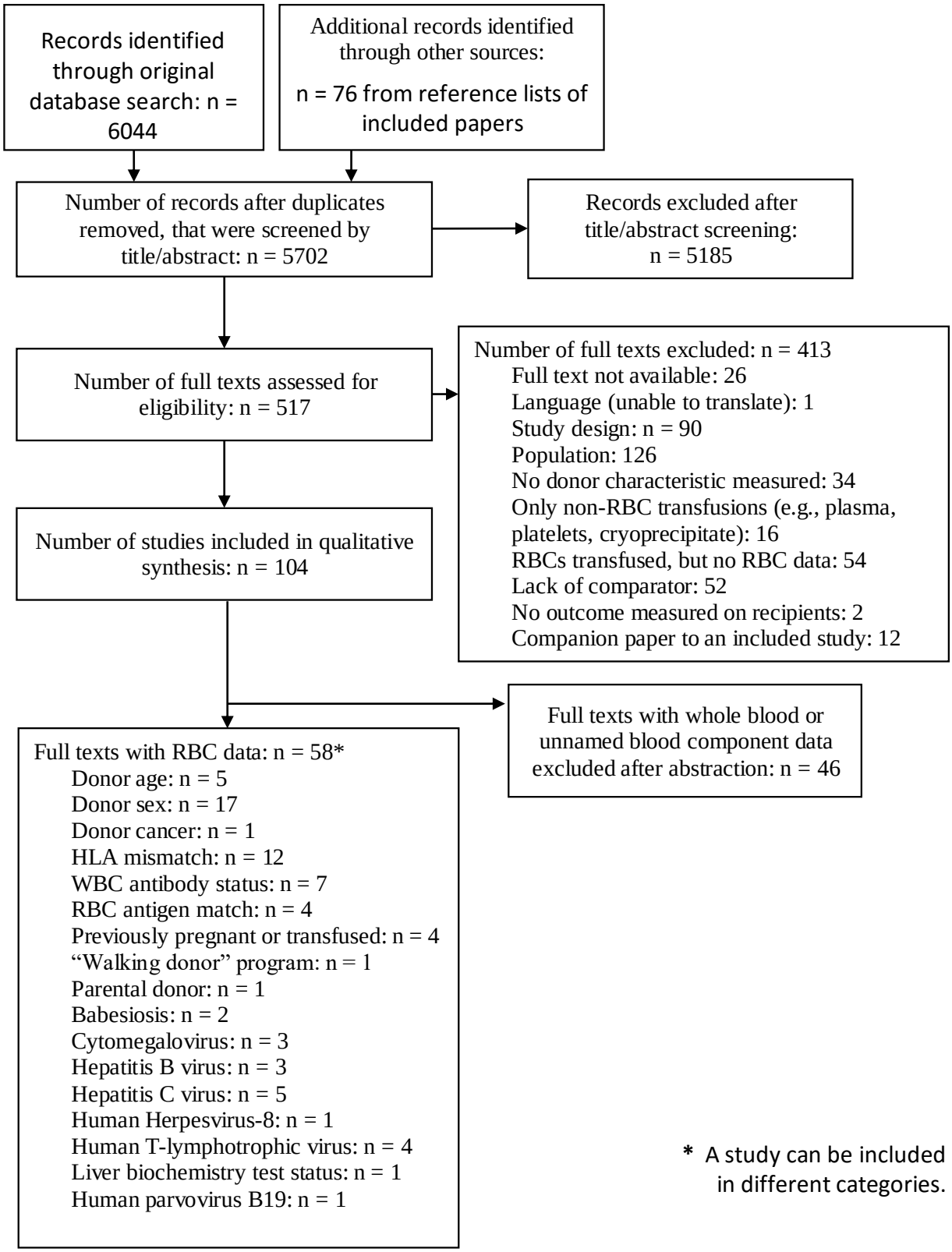
TABLE 4: ESTIMATES OF ASSOCIATION BETWEEN OTHER NON-INFECTIOUS DONOR CHARACTERISTICS AND RBC TRANSFUSION OUTCOMES

Outcome or subgroup	Studies	Effect estimate (with 95% CI)	Interpretation
Donor cancer			
Recipient cancer	1	RR 1.00 [0.94, 1.06]	Null
Previous allo-exposure			
Mortality	1	OR 1.48 [0.48, 4.51]	Null, favours allo-exposure
TRALI	2	OR 0.86 [0.59, 1.27]	Null, favours no allo-exposure
TRALI risk after policy change	2	OR 0.78 [0.45, 1.35], I^2 0%	Null, favours after policy change
Walking donors vs random donors			
CMV infection	1	OR 1.67 [0.18, 15.02]	Null, favours no walking donor
HBV infection	1	Not estimable	Not estimable
Parental donors vs unrelated donors			
Transfusion reaction			
Maternal	1	Not estimable	Not estimable
Paternal	1	OR 3.62 [0.14, 95.78]	Null, favors random donor
Maternal or Maternal	1	OR 2.33 [0.09, 60.85]	Null, favors random donor
Change in hematocrit			
Maternal	1	MD 0.0 [-3.45, 3.45]	Null
Paternal	1	MD -0.20 [-3.36, 2.96]	Null
Abnormal creatinine			
Maternal	1	OR 0.65 [0.02, 17.65]	Null, favours parental donor
Paternal	1	OR 1.14 [0.07, 20.02]	Null, favours random donor
Maternal or Maternal	1	OR 0.73 [0.04, 12.52]	Null, favours random donor
Abnormal bilirubin	1	Not estimable	Not estimable
Maternal donors vs paternal donors			
Transfusion reaction	1	OR 0.57 (0.02, 15.58]	Null, favours maternal donor
Change in hematocrit	1	MD 0.20 [-3.37, 3.77]	Null
Abnormal creatinine	1	OR 0.57 [0.02, 15.58]	Null, favours maternal donor
Abnormal bilirubin	1	Not estimable	Not estimable

TABLE 5: ESTIMATES OF ASSOCIATION BETWEEN INFECTIOUS DONOR CHARACTERISTICS AND RBC TRANSFUSION OUTCOMES

Outcome or subgroup	Studies	Effect estimate (with 95% CI)	Interpretation
Babesiosis screening			
Babesiosis infection	2	RR 0.16 [0.02, 1.31], I^2 0%	Null, favours after screening
CMV positive donors			
Seroconversion to CMV			
Screened vs not screened	2	OR 1.13 [0.86, 1.48], I^2 85%	Null, favours screened
High titer vs low titer	1	OR 7.04 [1.44, 34.49]	Favours low titer
HBV positive donors			
Anti-HBc seroconversion	1	OR 0.33 [0.09, 1.27]	Null, favours anti-HBs positive
Any hepatitis	1	OR 0.61 [0.32, 1.17]	Null, favours screening
Non-A non-B hepatitis	1	OR 0.17 [0.01, 3.21]	Null, favours anti-HBc positive
HCV positive donors			
HCV infection (before and after policy change)	3	OR 0.12 [0.02, 0.84], I^2 91%	Favours after policy change
HCV infection (positive vs negative blood)	2	OR 0.65 [0.44, 0.95], I^2 0%	Favours negative donor
HHV8 positive donors			
HHV8 seroconversion	1	OR 0.52 [0.27, 0.98]	Favours HHB8 negative donor
HTLV positive donors			
HTLV seroconversion	4	OR 54.87 [11.49, 262.01], I^2 75%	Favours HTLV negative donor
Parvovirus B19 positive donors			
Seroconversion			
DNA positive	1	OR 0.03 [0.00, 0.58]	Favours low donor viral load
IgM positive	1	OR 0.53 [0.04, 6.51]	Null, favours low donor viral load
IgG positive	1	OR 0.04 [0.00, 0.34]	Favours low donor viral load
Donor abnormal liver function test			
Post-transfusion hepatitis	1	OR 1.63 [0.85, 3.12]	Null, favours normal donor liver function test
Post-transfusion HCV infection	1	OR 3.39 [0.93, 12.35]	Null, favours normal donor liver function test

FIGURE 1: PRISMA FLOW CHART



* A study can be included in different categories.

FIGURE 2: RISK OF BIAS

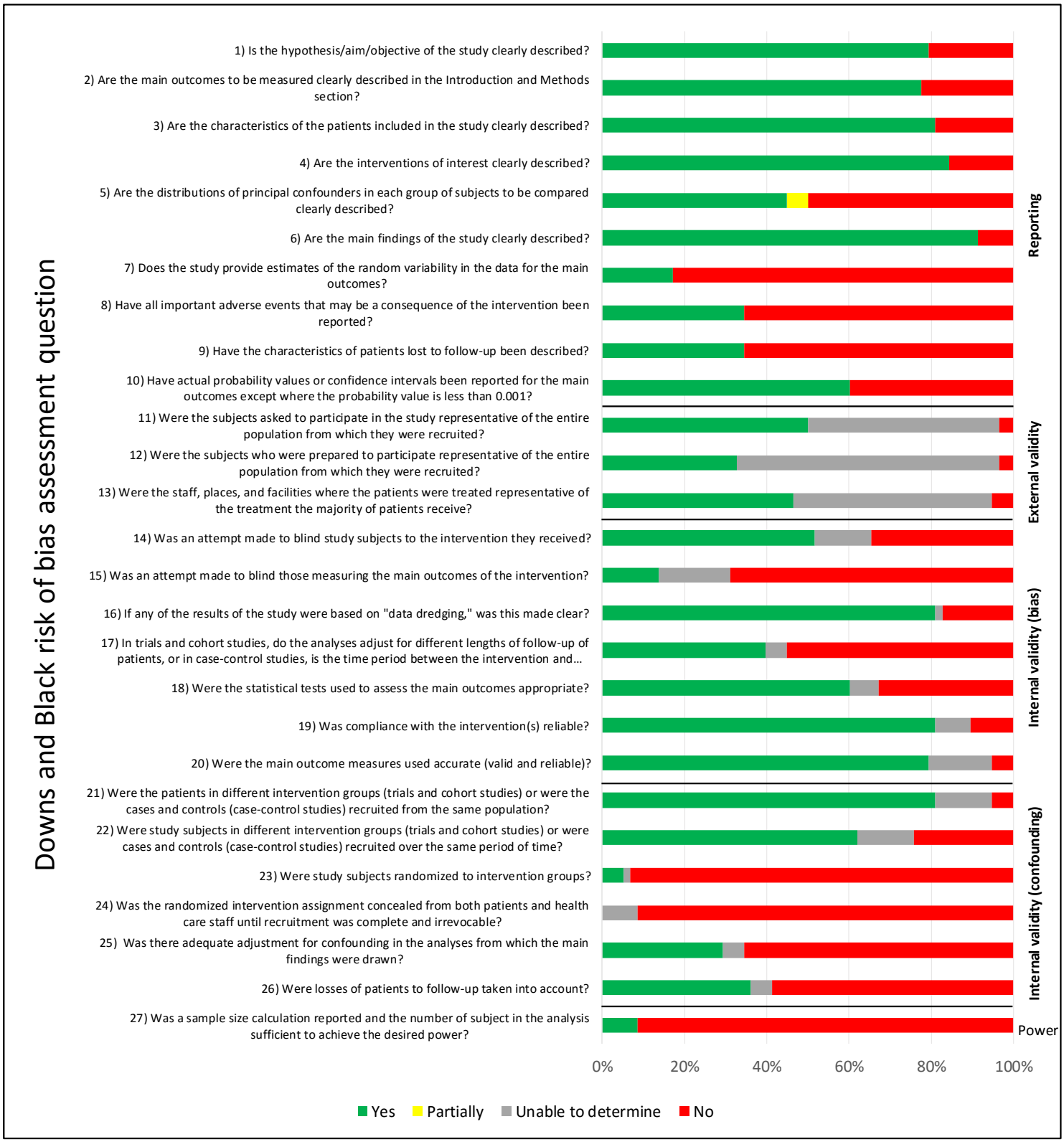


TABLE S1: DESCRIPTION OF INCLUDED STUDIES

Study	Timeframe	Recipients included	Design	Donor population	Donor characteristics	Recipient population	Products transfused	Recipient outcome
Donor age								
Gajic[15] 2007 USA	NR (2 years)	75 * From personal communications with the author	Case-control	Not reported	Donor sex; Pregnancy; donor antibody status, donor age	RBC transfused critically ill adult patients.	All patients included in the review received plasma and RBC transfusions.	ALI, Mortality
Manns[21] 1992 Jamaica	1987-1988	54	Prospective cohort	Not reported Conducted in Kingston, Jamaica. Collaboration with the National Cancer Institute (NCI), USA with the National Transfusion Service, Jamaican Ministry of Health and the University of the West Indies.	HTLV-I positive vs. HTLV-I negative, donor age, donor sex	Recipients of HTLV-positive blood that was not removed from the system before it was transfused	RBCs: 32.1% Plasma: 35.3% Platelets: 6.0% Whole blood: 26.6%	Change in HTLV status
Nowlis[18] 2012 USA	NR	268	Retrospective analysis of a clinical trial (Abstract)	Not reported	Donor age > 63 years (median)	RBC transfused patients after trauma	RBCs: 100%	TRALI
Petersen[20] 1994 USA	1985-1991	179	Recipient trace-back study	HIV positive adult donors with at least one prior HIV negative donation	Donor age and sex	Adult recipients of blood from HIV seroconverters	Component types not explicitly reported, but adjusted by blood component	Change in HIV status

Porretti[19] 2012 Italy	2008-2010	10	Recipient trace-back study	Blood donors whose recipients developed TRALI	Donor age and sex; previous allo-exposure	Adult and pediatric RBC transfusion recipients	RBCs: 100%	TRALI (Immune related versus non-immune related)
Donor sex								
Arinsburg[25] 2012 USA	2006-2008	461,598 blood components	Before-and-after study	Not reported	Sex (male plasma policy); pregnancy status;	RBC transfused patients after implementation of a male plasma donor policy	RBCs: 56.6% Plasma: 21.7% Platelets: 21.7%	TRALI
Chapman[26] 2009 UK	1996-2006	193	Before-and-after study	Not reported	Sex (male plasma policy)	Adult and pediatric TRALI cases from RBC transfused patients captured in the Serious Hazards of Tranfusion scheme (SHOT), after implementation of a male plasma donor policy	RBCs: 78.6% Plasma: 13.7% Platelets: 7.7%	TRALI
Eder[32] 2007 USA	2003-2005	18	Recipient trace-back study	American Red Cross	High risk donors vs low risk donors (sex; antibodies, pregnancy)	Adult TRALI cases	RBCs: 29.5% Plasma: 59.0% Platelets: 11.5%	TRALI
Eder[31] 2010 USA	2006-2008	41	Before-and-after study	American Red Cross	Sex (male plasma policy), anti-HLA, anti-HNA	Adult and pediatric TRALI cases in patients receiving RBCs after implementation of a male plasma donor policy	RBCs: 40.8% Plasma: 56.3% Platelets: 2.8%	TRALI
Eder[27] 2012 USA	2006-2010	Population	Before-and-after study (Abstract)	American Red Cross	Donor sex (male plasma policy)	Adult TRALI cases in patients receiving RBCs after implementation	Percentage transfused not explicitly stated, but results	TRALI

						of a male plasma donor policy	reported by component.	
Gajic[15] 2007 USA	NR (2 years)	75	Case-control	Not reported	Donor age and sex; previous allo-immunization, antibody status.	Adult RBC transfused critically ill patients.	All patients included in the review received plasma and RBC transfusions.	ALI, Mortality
Héma-Québec[28] 2010 Canada	2005-2009	Population	Before-and-after study	Quebec hemovigilance system, which received reports of transfusion reactions from 88 centers in 2009 (19 designated hospitals, 56 associated hospitals, and 13 affiliated centers). These centers accounted for 96.3% of all "transfusion activity" in Quebec in 2009.	TRALI reduction program	Adult and pediatric recipients of blood products who presented TRALI.	RBCs: 63.5% Plasma: 15.9% Platelets: 8.2% Other: 12.4%	TRALI
Insunza[29] 2004 Spain	2000-2002	Population	Before-and-after study	University Hospital Blood Bank of Cantabria. Provides blood components to the hospitals of the Cantabria region, with a population of 600,000 people.	TRALI reduction program	Adult and pediatric RBC transfused patients who present TRALI	RBCs: 62.1% Plasma: 14.6% Platelets: 23.2%	TRALI
Kulharya[22] 2001 USA	1995-1998	10	Retrospective cohort	Not reported	Donor sex	Infants in the NICU who received RBC	RBCs: 100%	Karyotype mosaicism/micro-chimaerism

						transfusions before chromosomes analysis		
Lin[17] 2012 Canada	2001-2009	Population	Before-and-after study	Regions supplied by Canadian Blood Services (Canada wide, except Quebec).	Sex (male plasma policy)	Any blood transfusion recipient	RBC-TRALI cases reported, no denominator data	TRALI
Manns[21] 1992 Jamaica	1987-1988	54	Prospective cohort	Not reported Conducted in Kingston, Jamaica. Collaboration with the National Cancer Institute (NCI), USA with the National Transfusion Service, Jamaican Ministry of Health and the University of the West Indies.	HTLV-I positive vs. HTLV-I negative, donor age, donor sex	Recipients of HTLV-positive blood that was not removed from the system before it was transfused	RBCs: 32.1% Plasma: 35.3% Platelets: 6.0% Whole blood: 26.6%	Change in HTLV status
Middelburg[30] 2010 USA, The Netherlands, Poland, Spain, Finland, UK	1991-2007	67	Case-control	An international collaborative project was conducted because no individual country would likely have enough cases of TRALI with unisex donation to provide valid inferences. Groups that had previously	Donor sex	Patients presenting with ALI who received RBC from sex-matched or mismatched donors.	RBCs: 84.3% Plasma: 12.0% Platelets: 7.2%	TRALI

				published TRALI cases based on clinical criteria alone (independent of donor sex or antibody status) were contacted to join the International TRALI Unisex Working Group.				
Middelburg[39] 2011 The Netherlands	2004-2009	3806 (805 patients in the RBC-only subgroup)	Retrospective cohort	The Sanquin Blood Bank supplies the center with donor blood and provided donor data on the products transfused during the study period.	Sex-mismatch	Adult and pediatric sex matched or mismatched RBC recipients	Overall: RBCs: 76.3% Plasma: 18.2% Platelets: 4.6% Other: 0.9% Analysis on RBC only subgroup	Death/survival or time to death
Paul[23] 2011 USA	1993-2007	122	Retrospective cohort	Not reported	Donor Sex	Infants who had necrotizing enterocolitis who had RBC transfusion before the event	RBCs: 100%	Necrotizing enterocolitis
Porretti[19] 2012 Italy	2008-2010	10	Recipient trace-back study	Blood donors whose recipients developed TRALI	Donor age and sex; previous alloexposure;	Adult and pediatric RBC transfusion recipients	RBCs: 100%	TRALI
Sanchez[41] 2007 USA	2002-2005	26	Case-Control	Not reported	Donor sex	Adult and pediatric transfusion recipients	RBCs: 31.7% Plasma: 33.3% Platelets: 21.7% Whole blood: 13.3%	TRALI
Toy[5] 2012 USA	2006-2009	47,783	A case-control and a before-and-after	Not reported	Antibodies; cytokines, bioreactive substances;	Adult and pediatric transfusion	RBCs: 36.3% Plasma: 55.7% Platelets: 8.0%	TRALI

			study were both done		Donor Sex, Pregnancy, TRALI reduction program	recipients > 6 months of age		
Donor cancer								
Edgren[16] 2007 Sweden, Denmark	1968-2002	354,094	Retrospective cohort	Blood donors from the SCANDAT database with 5 years follow-up with no prior history of cancer	Cancer	Adult and pediatric Recipients with no prior history of cancer who received blood from eligible donors	Percentage transfused not explicitly stated, but adjusted by blood component.	Incident cancer cases
HLA mismatch								
Albert[42] 1981 Germany	Not reported	47	Intervention, non randomized	Not reported		All patients on kidney transplant list who had never been pregnant or transfused	RBCs: 100%	New allo-antibody, graft rejection
Baudouin[43] 1997 France	Not reported	24	Intervention (randomized)	Volunteer donors	Match for 1 HLA-DR antigen vs. mismatch for all HLA-DR antigens	Adult and pediatric potential kidney transplant recipients who had never had a transfusion, been transplanted or been pregnant	RBCs: 100%	New allo-antibody production
Christiaans[44] 1999 Netherlands	Not reported	247	Prospective cohort	Not reported	HLA-DR match (1 or 2 matches) vs. HLA-DR mismatched	Adult potential kidney recipients on the waiting list with no previous pregnancy, transfusions or transplant.	RBCs: 100%	New allo-antibody production, graft rejection
Hiesse[45] 2001 France	1992-1998	70	Intervention (randomized)	Volunteer donors	HLA-DR match vs. mismatch	Randomization between HLA-DR matched vs mismatched adult or pediatric	RBCs: 100%	Survival, graft function

						recipients prior to kidney transplantation		
Magee[46] 2012 Northern Ireland/UK	2004-2009	68	Intervention (non randomized)	Volunteer donors from the British Bone Marrow Registry	HLA-DR matched units vs random donor units	All patients awaiting renal transplant who had pretransfusion and posttransfusion clotted blood samples available for HLA-specific antibody screening and identification	RBCs: 100%	New allo-antibody production
Mariat[47] 2000 France	1992-1997	156	Intervention (non randomized)	Not reported	HLA-matched protocol vs. HLA-unmatched protocol	Adult first cadaveric kidney graft recipients with no history of transfusion or pregnancy.	RBCs: 100%	New allo-antibody production, graft rejection
Middleton[48] 1994 Ireland	1987-1989	65	Prospective cohort	Volunteer donors	HLA-DR matched vs random donors	Patients awaiting kidney transplantation with no history of pregnancy, transfusion, transplant and no anti-HLA antibody.	RBCs: 100%	New allo-antibody production, graft survival
Scornik[49] 1989 USA	Not reported	24	Prospective cohort	Not reported	HLA mismatch	Adult RBC transfusion recipients.	RBCs: 100%	New allo-antibody production
Van der Mast[50] 1997 Netherlands	1984-1993	100	Retrospective cohort	Not reported	Random donor vs. HLA-DR matched donors	Adult and pediatric recipients of cardiac transplantation	RBCs: 100%	Patient survival, graft rejections, infectious episodes, transplant coronary disease
Van Hooff[51] 1992 Netherlands	Not reported	147	Prospective cohort	Not reported	HLA DR-antigen mismatch (0, 1,	Potential kidney transplant recipients who	RBCs: 100%	New allo-antibody production, graft rejection

					or 2 mismatches)	had never had a transfusion or been pregnant		
Van Twuyver[52] 1991 Netherlands	Not reported	23	Prospective cohort	Not reported	HLA A, B, and DR matching	Adults awaiting first kidney transplantation	RBCs: 100%	Alloimmunization
Vervoordeldonk[40] 1998 Netherlands	1988-1993	21	Retrospective cohort	Not reported	Partially matched (at least one HLA-B and one HLA-DR antigen shared) vs. mismatched	Patients awaiting kidney transplantation	RBCs: 100%	Karyotype mosaicism/microchimaerism
Donor White Blood Cells antigens								
Eder[31] 2010 USA	2006-2008	41	Before-and-after study	American Red Cross	Sex (male plasma policy), anti-HLA, anti-HNA	Adult and pediatric TRALI cases in patients receiving RBCs after implementation of a male plasma donor policy	RBCs: 40.8% Plasma: 56.3% Platelets: 2.8%	TRALI
Eder[32] 2007 USA	2003-2005	18	Recipient trace-back study	American Red Cross	High risk donors vs low risk donors (sex; antibodies, pregnancy)	Adult TRALI cases	RBCs: 29.5% Plasma: 59.0% Platelets: 11.5%	TRALI
Funk[38] 2012 Germany	2006-2010	Population	Before-and-after study	RBC donors in Germany	Implementation of a policy (female donors with history of pregnancy and positive for WBC antibodies for FFP)	Adult TRALI cases. Study restricted to plasma recipients but provides data for RBC transfusions.	RBCs: 13% Plasma: 77% Platelets: 10%	TRALI
Gajic[15] 2007 USA	NR (2 years)	75	Case-control	Not reported	Donor age and sex; previous allo-immunization,	RBC transfused critically ill adult patients.	All patients included in the review received plasma and RBC transfusions.	ALI, Mortality

					antibody status.			
Toy[5] 2012 USA	2006-2009	47,783	A case-control and a before-and-after study were both done	Not reported	Antibodies; cytokines, bioreactive substances; Donor Sex, Pregnancy, TRALI reduction program	Transfusion recipients > 6 months of age	RBCs: 36.3% Plasma: 55.7% Platelets: 8.0%	TRALI
Vlaar[53] 2011 Netherlands	2006-2019	48	Case-control	Not reported	Antibodies Donor sex (male plasma)	Consecutive adults in cardiac surgery who received transfusions.	RBCs: 61.2% Plasma: 28.4% Platelets: 10.4%	TRALI
Zupanska[54] 2007 Poland	5 years, early 2000	44	Recipient trace-back study	Not reported	Donor antibodies	Adult and pediatric patients who presented TRALI and were transfused	RBCs: 88.6% of patients Plasma: 4.5% of patients Platelets: 6.8% of patients	TRALI
RBC antigens								
Ambruso[33] 1987 USA	1974-1984	12	Before-and-after study	Volunteer donors	Random donors vs donor selected for phenotype compatibility	Adult and Children with sickle cell/hemoglobin C/beta-thalessemia	RBCs: 100%	New allo-antibody production
Heddle[34] 1992 Canada	2 years	130 (transfusion events)	Prospective cohort	Not reported	Donor-recipient antiglobulin cross-match test (vs. not using the test)	Patients > 16 years who received transfusions	RBCs: 100%	New allo-antibody production, Laboratory evidence of hemolysis (e.g., HCT, Hgb, bilirubin),
Michailmeriano u[35] 1987 Greece	1980-1985	120	Intervention study (randomizatio n unclear)	Volunteer donors	Random vs. selected compatible donors (phenotyped for ABO and 17	Infants and children starting transfusion treatment	RBCs: 100%	New allo-antibody production

					other blood groups)			
Tahhan[36] 1994 USA	1980-1993	86	Retrospective cohort	Not reported	Antigen-matched RBCs vs. antigen-matched and unmatched RBCs (beyond ABO and D)	Children and adolescent with high likelihood to be put on a high transfusion regiment.	RBCs: 100%	New allo-antibody production
Previous allo-exposure								
Funk[38] 2012 Germany	2006-2010	Population	Before-and-after study	RBC donors in Germany	Implementation of a policy (female donors with history of pregnancy; (2) female donors with history of pregnancy and positive for WBC antibodies for FFP)	Adult and pediatric TRALI cases. Study restricted to plasma recipients but provides data for RBC transfusions.	RBCs: 13% Plasma: 77% Platelets: 10%	TRALI
Gajic[15] 2007 USA	NR (2 years)	75	Case-control	Not reported	Donor age and sex; previous allo-immunization, antibody status.	RBC transfused critically ill adult patients.	All patients included in the review received plasma and RBC transfusions.	ALI, Mortality
Middelburg[37] 2011 Netherlands	2004-2008	19	Case-control study	Netherland national blood supply	Donor alloexposure status (transfusion and/or pregnancy history)	Recipients of plasma-poor products (RBCs and platelets)	RBCs: 72.4% Plasma: 18.9% Platelets: 4.4% Other: 4.2%	TRALI
Toy[5] 2012 USA	2006-2009	47,783	A case-control and a before-and-after study were both done	Not reported	Antibodies	Transfusion recipients > 6 months of age	RBCs: 36.3% Plasma: 55.7% Platelets: 8.0%	TRALI

Walking donors								
Pass[55] 1976 USA	1972-1974	123	Prospective cohort	Volunteer donors	CMV status,"Walking donor" blood vs. blood bank blood	Pediatric RBC recipients	RBCs: 100%	Change in infection status or presence of antibody/antigen related to an infectious agent post-transfusion,New allo-antibody production
Parental donors								
Strauss[56] 2000 USA	NR (27 months)	40	Intervention study, randomized	Not reported	Maternal vs. paternal vs. unrelated donors	Infants in the NICU	RBCs: 100%	Transfusion reaction, hematrocrit, creatinine, bilirubin,
Babesiosis								
Johnson[57] 2012 USA	1999-2005	63	Before-and-after study	American Red Cross study in a Babesia endemic area of Conneticut.	Before exclusion of IFA-positive donors and after exclusion of IFA-positive donors	Adult and pediatric blood recipients who developed babesiosis	Cellular components (RBCs, whole-blood derived platelets, whole blood): 100%	Change in infection status
Young[58] 2012 USA	2005-2011	7287 units	Before-and-after donor screening study	Not reported	Before and after implementation of a babesiosis screening program	Neonates with sickle cell, or thalassemia who received RBC units	RBCs: 100%	Change in infection status
CMV								
Nichols[59] 2003 USA	1994-2000	807	Population-level study or before-and-after donor screening study at the patient	Not reported	CMV status	CMV-seronegative patients undergoing autologous SC transplantation or receiving allografts from CMV-	RBCs: 42.4% Platelets: 57.6%	Change in infection status

						seronegative donors		
Pass[55] 1976 USA	1972-1974	123	Prospective cohort	Volunteer donors	CMV status,"Walking donor" blood vs. blood bank blood	Neonate RBC recipients	RBCs: 100%	Change in infection status or presence of antibody/antigen related to an infectious agent post-transfusion,New allo-antibody production
Preiksaitis[60] 1997 Canada	1989-1994	30	Intervention study, randomized	Volunteer donors	CMV status	Children < 16 years of age, CMV negative oncology patients requiring chemotherapy and transfusion.	RBCs: 36.6% Plasma: 0.5% Platelets: 63.0%	Change in infection status
Hepatitis B								
Allain[61] 2013 Croatia, Denmark, Germany, Poland, Spain, UK	2005-2010	74	Recipient trace-back study	Not reported	Anti-HBc status	Adult recipients from donors who developed HBV, or donors of acute HBV infection in a transfused patient	RBCs: 70.5% Plasma: 18.1% Platelets: 11.4%	Change in infection status
Aymard[62] 1986 France	NR	64	Prospective cohort	Volunteer donors	Anti-HBc status	Adult cardiac surgery patients	RBCs: 56.9% Plasma: 43.1%	Post-transfusion hepatitis
Blajchman[63] 1995 Canada	1988-1993	4588	Intervention, randomized	Canadian Red Cross	HBcAg, HCV or abnormal ALT	Adults requiring in-hospital transfusions	RBCs: 83.5% RBCs + Plasma or Plasma only: 11.2% RBCs + Other or Other only: 5.1%	Post-transfusion hepatitis
Hepatitis C								
Beltran[64] 2005 Colombia	1988-1992	490	Cross-sectional	Volunteer donors, Commercial/paid donors	Donor screening for HCV	Recipients with hemophilia, sickle cell disease, thalassemia, acute bleeding,	Of Recipients: RBCs: 87.8% Plasma: 26.8% Platelets: 37.4%	HCV infections

						oncological illnesses or renal failure requiring haemodialysis, receiving > 10 units of allogeneic blood or blood components.	Whole blood: 2.6%	
Blajchman[63] 1995 Canada	1988-1993	4588	Intervention, randomized	Canadian Red Cross	HBcAg, HCV or abnormal ALT	Adults requiring in-hospital transfusions	RBCs: 83.5% RBCs + Plasma or Plasma only: 11.2% RBCs + Other or Other only: 5.1%	Post-transfusion hepatitis
O'Riordan[65] 1998 Ireland	1991-1995	20	Look-back study	Not reported	HCV RNA positive vs negative	Neonatal recipients of RBC from anti-HCV positive donors	RBCs: 95% Platelets: 5%	HCV RNA positive vs negative
English National Blood Service[66] 2002 UK	1991-1997	964	Donor look-back study	UK National blood service	HCV RNA PCR status	Adult and pediatric recipients of a unit of blood from a HCV PCR-positive or HCV-indeterminate donor	RBCs: 56.3% Plasma: 6.4% Platelets: 11.5% Unknown: 1.1%	Change in infection status
Vogt[67] 2004 Germany	1991-1999	669	Before-and-after donor screening study	Volunteer donors	Before and after donor screening for anti-HCV	Children undergoing cardiac surgery	RBCs: 13.2% Plasma: 52.4% Platelets: 0.6% Whole blood: 33.8%	Post-transfusion anti-HCV positive
HHV8								
Hladik[68] 2006 Uganda	2000-2001	991	Prospective cohort	Volunteer donors	Any HHV-8 seropositive blood vs. all HHV-8 seronegative blood	Adult and pediatric recipients with pretransfusion specimen seronegative for HHV-8 completing	RBCs: 79.2% of patients Plasma: 0.2% of patients Whole blood: 14.6% of patients	Change in infection status

						at least 2 months of follow up	Unknown: 6.0% of patients	
HTLV								
Herr[69] 1993 USA	1988-1990	12	Donor look-back study	Not reported	HTLV-I vs HTLV-II	Adult RBC recipients of positive HTLV donors	RBCs: 66.7% Plasma: 5.6% Platelets: 5.6% Whole blood: 22.2%	Change in infection status
Manns[21] 1992 Jamaica	1987-1988	118	Prospective cohort	Not reported Conducted in Kingston, Jamaica. Collaboration with the National Cancer Institute (NCI), USA with the National Transfusion Service, Jamaican Ministry of Health and the University of the West Indies.	HTLV-I positive vs. HTLV-I negative, donor age, donor sex	Recipients of HTLV-positive blood that was not removed from the system before it was transfused	RBCs: 32.1% Plasma: 35.3% Platelets: 6.0% Whole blood: 26.6%	Change in HTLV status
Okochi[70] 1986 Japan	1981-1986	1151	Before-and-after study	Red Cross blood centers in Kyushu	Implementation of HTLV screening	Transfused patients.	RBCs: 59.7% Plasma: 28.7% Platelets: 7.8% Whole blood: 3.9%	Change in HTLV status
Sato[71] 1992 Japan	1981-1991	4001	Before-and-after study	Red Cross blood centers in Kyushu	Implementation of HTLV screening	Transfused patients.	RBCs: 42.1% Plasma: 30.1% Platelets: 23.4% Whole blood: 4.5%	Change in HTLV status
Parvovirus B19								
Hourfar[72] 2011 Germany	2005-2007	34	Prospective Cohort	German Red Cross	B19 virus load: <10^5 IU/ml vs. >10^5 IU/ml	Adults blood recipients receiving parvoB19 positive blood	RBCs: 100%	Change in infection status

Abnormal liver function tests								
Blajchman[63] 1995 Canada	1988-1993	4588	Intervention, randomized	Canadian Red Cross	HBcAg, HCV or abnormal ALT	Adults requiring in-hospital transfusions	RBCs: 83.5% RBCs + Plasma or Plasma only: 11.2% RBCs + Other or Other only: 5.1%	Post-transfusion hepatitis

TABLE S2: DESCRIPTION OF EXCLUDED STUDIES AT FULL-TEXT SCREENING

Study	Design	Donor characteristic	Recipient outcome
Excluded because no RBC estimate was reported			
Adler 1986 USA	Intervention study	CMV status	CMV
Adler 1983 USA	Observational study	CMV status	CMV
Aggarwal 1997 India	Observational study	HIV status, Change in donor screening (before-and-after population-based study)	HBV, HCV, CMV, Malaria, Herpes simplex virus-2, HDV
Allen 1966 USA	Observational study	Prison donors vs. Volunteer donors	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Arguin 1999 USA	Observational study	Tick-borne infection status (e.g., Rocky Mountain Spotted Fever, ehrlichiosis, babsesiosis)	Tick-borne infection (e.g., Rocky Mountain Spotted Fever, ehrlichiosis, babesiosis)
Arndt 1971 Germany	Observational study	Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis, Australia antigen (Au) status	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Ashton 1994 Australia	Observational study	AIDS developed within 10 years of HIV infection	Death/survival or time to death
Bhumbra 1988 USA	Observational study	CMV status	CMV
Busch 1996 USA	Observational study	HIV status	HIV
Byrne 2011 England and Wales	Observational study	HIV status	HIV
Chambers 1991 USA	Observational study	HBV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis, Change in donor screening (before-and-after population-based study)	HBV, NANB hepatitis, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Cohen 1968 USA	Observational study	Drug addicts vs. Not drug addicts	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Donahue 1992 USA	Observational study	HBV, HCV, Liver biochem tests, Change in donor screening (before-and-after population-based study)	HCV
Dunbar 2010 USA	Observational study	Maternal/parental vs. Not maternal/parental donors	TRALI or TRALI-like event
Edgren 2013 Sweden	Observational study	Alzheimer's disease (future development)	Development of Alzheimer's disease

Fowlkes 2009 USA	Observational study	HHV-8 status	HHV-8
Freimanis 2013 Ghana	Observational study	Malaria status	Malaria
Gjesdal 1987 Norway	Intervention study	CMV status	CMV
Goncales 1993 Brazil	Observational study	HBV status, HCV status	HBV, HCV
Grady 1972 USA	Observational study	HBV status, Paid (commercial) vs. volunteer donors	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Hollinger 1974 USA	Observational study	HBV status	HBV, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc., Liver biochemistry abnormalities (e.g., ALT)
Huang 1994 Taiwan	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	HCV, CMV, Liver biochemistry abnormalities (e.g., ALT)
Imoto 2007 Japan	Observational study	Sex, HLA/HNA/WBC/granulocyte antibody status	TRALI or TRALI-like event, Clinical but non-TRALI transfusion reaction (e.g., pyrexia, chills, hypotension, dyspnea, TACO)
Ismay 1995 Australia	Observational study	HBV status, HCV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	HCV, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc., Liver biochemistry abnormalities (e.g., ALT)
Jullien 1993	Observational study	HBV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis, Change in donor screening (before-and-after population-based study)	HCV
Keller-Stanislawski 2010 Germany	Observational study	HLA/HNA/WBC/granulocyte antibody status, "Blood mismatch" for the purpose of our study	Death/survival or time to death, TRALI or TRALI-like event
Koretz 1973 USA	Observational study	HBV status	HBV, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Koretz 1976 USA	Observational study	HBV status	HBV, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Lopez-Jimenez 1995 Spain	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.

Mathiesen 1992 Sweden	Observational study	HCV status	NANB hepatitis
Matsuoku 1994 Japan	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	HCV
Moore 2001 Kenya	Observational study	HIV status	HIV
Mosley 1996 USA	Observational study	HBV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	HCV, NBNC hepatitis
Mosley 1995 USA	Observational study	HBV status	HBV
Nabavizadeh 2007 Iran	Observational study	G6PD deficiency status	Laboratory evidence of hemolysis (e.g., HCT, Hgb, bilirubin), Clinical but non-TRALI transfusion reaction (e.g., pyrexia, chills, hypotension, dyspnea, TACO)
Nakahara 1986 Japan	Observational study	Guanase activity	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Olowe 1981 Nigeria	Observational study	G6PD deficiency status, Hgb-AS status (sickle cell trait)	Laboratory evidence of hemolysis (e.g., HCT, Hgb, bilirubin)
Perham 1971 England	Observational study	CMV status	CMV
Pintera 1986 Czech Republic	Observational study	Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis, Change in donor screening (before-and-after population-based study)	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Robitaille 2010 Canada	Observational study	IgA deficiency, with or without anti-IgA	Clinical but non-TRALI transfusion reaction (e.g., pyrexia, chills, hypotension, dyspnea, TACO)
Satake 2007 Japan	Observational study	HBV status	HBV
Steinbrecher 1983 Canada	Observational study	Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Stevens 1984 USA	Observational study	HBV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	NANB hepatitis, Liver biochemistry abnormalities (e.g., ALT)
Stute 1979 West Germany	Observational study	HBV status, Change in donor screening (before-and-after population-based study)	HBV, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc., Liver biochemistry abnormalities (e.g., ALT)
Sugg 1988 Germany	Not discernible	HBV status	NANB hepatitis

Takano 1993 Japan	Observational study	HBV status, HCV status, Change in donor screening (before-and-after population-based study)	NANB hepatitis
Takano 1992 Japan	Observational study	Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Ward 1989 USA	Observational study	HIV status	Death/survival or time to death
Hollinger 1992 USA	Observational study	HBV status	HBV, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Hashimoto 1991 Japan	Observational study	Sex, HLA/HNA/WBC/granulocyte antibody status, Donor age	TRALI or TRALI-like event
Japanese Red Cross Non-A, Non-B Hepatitis Research Group 1991 Japan	Observational study	HBV status, HCV status, Change in donor screening (before-and-after population-based study)	NANB hepatitis
Lazda 1990 USA	Observational study	HLA/HNA/WBC/granulocyte antibody status, "Blood mismatch" for the purpose of our study	New allo-antibody production, Tissue graft/transplant rejection or loss
van der Poel 1989 The Netherlands	Observational study	HBV status, HCV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	NANB hepatitis
Koziol 1986 USA	Observational study	HBV status	HBV, CMV, NANB hepatitis, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Excluded because whole blood or unnamed blood components			
Aach 1980 USA	Observational study	Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis, Paid (commercial) vs. volunteer donors	NANB hepatitis, Liver biochemistry abnormalities (e.g., ALT)
Aach 1991 USA	Observational study	HCV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	HCV
Aach 1981 USA	Observational study	Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis, Paid (commercial) vs. volunteer donors	NANB hepatitis
Akbari 2011 Iran	Observational study	HCV status	HCV
Allain 1999 UK (England)	Observational study	HBV status	HBV, Liver biochemistry abnormalities (e.g., ALT)
Alter 1972 USA	Observational study	HBV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Alter 1981 USA	Observational study	Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	General post-transfusional hepatitis not related to a specific organism or

			identified as NANB - defined or not defined by ALT levels, etc.
Azar 1993 France	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	HCV, NANB hepatitis
Barrera 1991 Spain	Observational study	HBV status, HCV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	HCV, NANB hepatitis
Bedarida 1978 Italy	Observational study	HBV status, Change in donor screening (before-and-after population-based study)	HBV
Bernardin 2010 USA	Observational study	TTV, TTMDV, or TTMV (anelloviridae) status, GBV-C, HGV status	TTV, TTMDV, TTMV, GBV-C, HGV
Cardoso 1996 Germany	Observational study	HCV status	HCV
Conrad 1977 USA	Observational study	HBV status	HBV, NANB hepatitis
Esteban 1990 Spain	Intervention study	HBV status, HCV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	HCV, NANB hepatitis
Foberg 1996 Sweden	Observational study	HCV status	HCV, Liver biochemistry abnormalities (e.g., ALT)
Gocke 1972 USA	Observational study	Australia antigen (Au) status	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc., Liver biochemistry abnormalities (e.g., ALT), Australia (Au) antigen/antibody
Goldfield 1974 USA	Observational study	HBV status, Change in donor screening (before-and-after population-based study), Paid (commercial) vs. volunteer donors	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Goldman 2006 Canada	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	HCV
Gonzalez 1995 Spain	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	HCV, NANB hepatitis
Grindon 1988 USA	Observational study	HIV status	HIV
Hau 1993 Hong Kong (China)	Observational study	HBV status, HCV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	HBV, HCV, NANB hepatitis
Hoofnagle 1978 USA	Observational study	HBV status	HBV, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Ito 1988 Japan	Observational study	Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis, Change in donor screening (before-and-after population-based study)	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc., Liver

			biochemistry abnormalities (e.g., ALT)
John 1974 India	Observational study	HBV status, Change in donor screening (before-and-after population-based study)	Death/survival or time to death, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Katchaki 1981 The Netherlands	Observational study	HBV status	HBV
Mirmomen 2006 Iran	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	HCV
Moore 1995 USA	Observational study	HCV status	HCV
Nishioka 1996 Japan	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	HCV, NANB hepatitis
Norda 1995 Sweden	Observational study	HCV status	HCV
Sanwald 1970 Germany	Observational study	Australia antigen (Au) status	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc., Australia (Au) antigen/antibody
Saraswat 1996 India	Observational study	HBV status, HCV status, Liver biochemistry tests (e.g., ALT) or surrogate markers for any hepatitis	HBV, HCV
Seeff 1975 USA	Observational study	HBV status, Change in donor screening (before-and-after population-based study), Paid (commercial) vs. volunteer donors	HBV, General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
van der Poel 1990 The Netherlands	Observational study	HCV status, Liver biochem tests (e.g., ALT) or surrogate markers for any hepatitis	HCV, NANB hepatitis
Volkow 2004 Mexio	Observational study	Paid (commercial) vs. volunteer donors	Time to diagnosis of AIDS in HIV-infected recipients
Vrielink 1997 The Netherlands	Observational study	HCV status	HCV
Walsh 1970 USA	Intervention study	Paid (commercial) vs. volunteer donors	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Wang 1995 Taiwan	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.
Wang 1994 Taiwan	Observational study	HCV status, Change in donor screening (before-and-after population-based study)	General post-transfusional hepatitis not related to a specific organism or identified as NANB - defined or not defined by ALT levels, etc.

Contreras 1991 United Kingdom	Observational study	HCV status	HCV
Kumar 1980 USA	Observational study	CMV status	CMV
Lee 1992 China (Taiwan)	Observational study	CMV status	CMV
Saw 2013 Canada	Observational study	Sex, HLA/HNA/WBC/granulocyte antibody status, Donor age	TRALI or TRALI-like event
Busson 1997 France	Intervention study	HLA/HNA/WBC/granulocyte antibody status	New allo-antibody production, Tissue graft/transplant rejection or loss
Bayle 1995 France	Observational study	Change in donor screening (before-and-after population-based study), HLA/HNA/WBC/granulocyte antibody status	Tissue graft/transplant rejection or loss
Lagaaij 1989 The Netherlands	Observational study	HLA/HNA/WBC/granulocyte antibody status	New allo-antibody production, Tissue graft/transplant rejection or loss
Burlingham 1989 USA	Observational study	HLA/HNA/WBC/granulocyte antibody status	New allo-antibody production

TABLE S3: THE DOWNS AND BLACK TOOL

Questions	Possible answers
Reporting	
1) Is the hypothesis/aim/objective of the study clearly described?	Yes/No
2) Are the main outcomes to be measured clearly described in the Introduction and Methods section?	Yes/No
3) Are the characteristics of the patients included in the study clearly described? (This question refers specifically to inclusion/exclusion criteria.)	Yes/No
4) Are the interventions of interest clearly described?	Yes/No
5) Are the distributions of principal confounders in each group of subjects to be compared clearly described?	Yes/No/Partially
6) Are the main findings of the study clearly described?	Yes/No
7) Does the study provide estimates of the random variability in the data for the main outcomes?	Yes/No
8) Have all important adverse events that may be a consequence of the intervention been reported?	Yes/No
9) Have the characteristics of patients lost to follow-up been described?	Yes/No
10) Have actual probability values (e.g., 0.035 rather than <0.05) or confidence intervals been reported for the main outcomes except where the probability value is less than 0.001?	Yes/No
External validity	
11) Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	Yes/No/Unable to determine
12) Were the subjects who were prepared to participate representative of the entire population from which they were recruited?	Yes/No/Unable to determine
13) Were the staff, places, and facilities where the patients were treated representative of the treatment the majority of patients receive?	Yes/No/Unable to determine

Internal validity (bias)	
14) Was an attempt made to blind study subjects to the intervention they received?	Yes/No/Unable to determine
15) Was an attempt made to blind those measuring the main outcomes of the intervention?	Yes/No/Unable to determine
16) If any of the results of the study were based on "data dredging," was this made clear?	Yes/No/Unable to determine
17) In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	Yes/No/Unable to determine
18) Were the statistical tests used to assess the main outcomes appropriate?	Yes/No/Unable to determine
19) Was compliance with the intervention(s) reliable?	Yes/No/Unable to determine
20) Were the main outcome measures used accurate (valid and reliable)?	Yes/No/Unable to determine
Internal validity (confounding)	
21) Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	Yes/No/Unable to determine
22) Were study subjects in different intervention groups (trials and cohort studies) or were cases and controls (case-control studies) recruited over the same period of time?	Yes/No/Unable to determine
23) Were study subjects randomized to intervention groups?	Yes/No/Unable to determine
24) Was the randomized intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	Yes/No/Unable to determine
25) Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	Yes/No/Unable to determine
26) Were losses of patients to follow-up taken into account?	Yes/No/Unable to determine
Power	
27) Was a sample size calculation reported and the number of subject in the analysis sufficient to achieve the desired power?	Yes/No/Unable to determine

TABLE S4: RATING OF THE EVIDENCE QUALITY

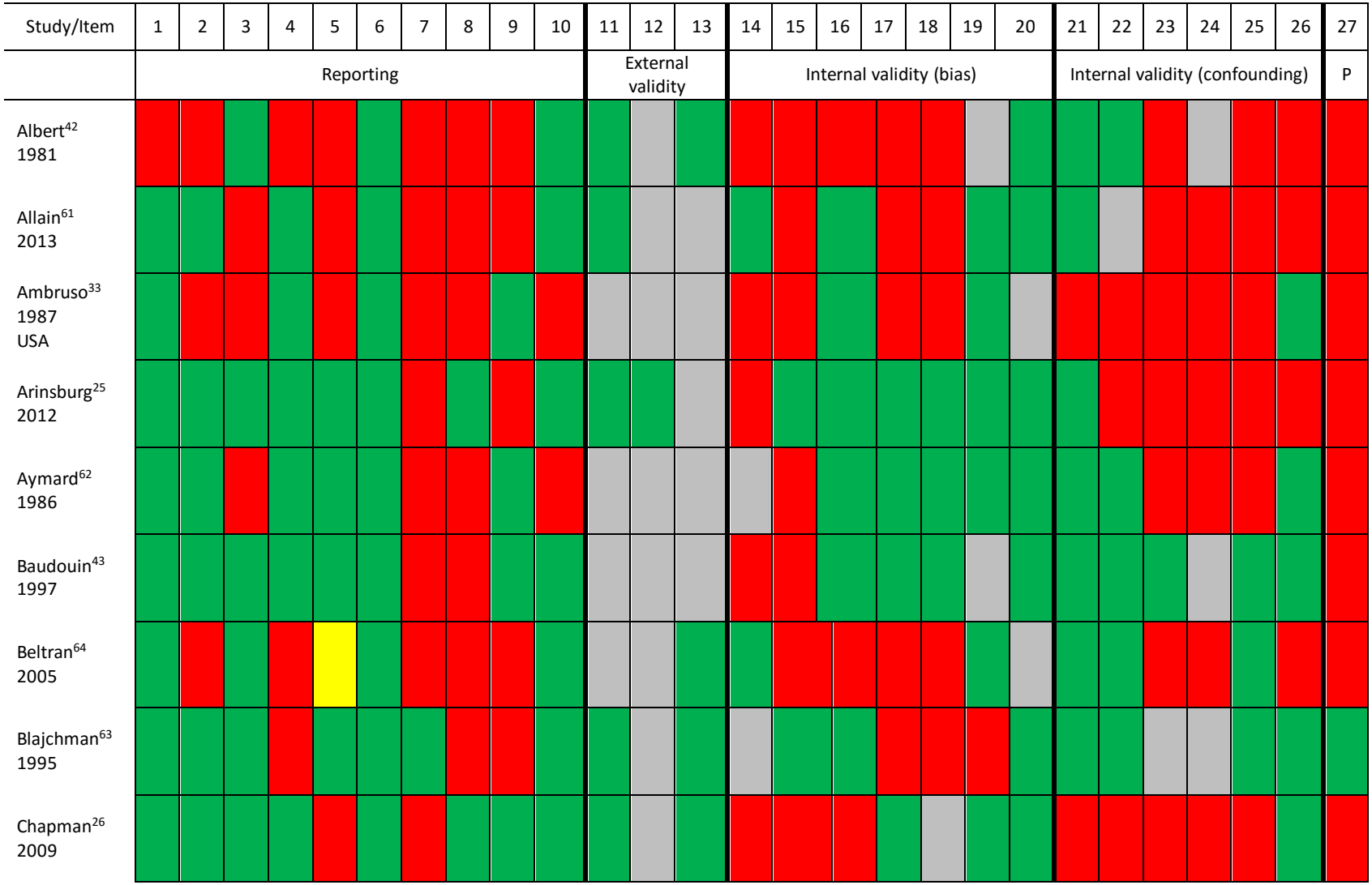
Outcome	Studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Quality of the evidence (GRADE)
Donor age							
ALI ≤ 6h after transfusion	2 (Obs)	Serious limitations	Serious inconsistency	Serious indirectness[π]	Serious imprecision	Likely present	+
ALI ≤ 72 hours after transfusion	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
ALI 72 hours to 28 days after transfusion	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
ALI ≤ 28 days after transfusion	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
HTLV transmission Manns 1992	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
HIV transmission Peterson 1994	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Donor sex							
Hospital mortality	1 (Obs)	No serious limitation	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
RBC associated TRALI risk after policy change	7 (Obs)	Serious limitations	No serious inconsistency	Serious indirectness	No serious imprecision	Likely present	+
RBC associated TRALI from male vs mixed RBC donors	4 (Obs)	Serious limitations	Serious inconsistency	Serious indirectness	Serious imprecision	Likely present	+
Non-fatal TRALI risk / unit distributed	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Fatal TRALI risk / unit distributed	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
TRALI-related death vs non-TRALI-related death	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Transfusion reactions	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	No serious imprecision	Likely present	+
TRALI / number of units	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	No serious imprecision	Likely present	+
Antibody-mediated TRALI vs non-antibody mediated	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Genetic mosaicism	1 (Obs)	Very Serious limitations	Unable to determine	Very serious indirectness	Serious imprecision	Likely present	+

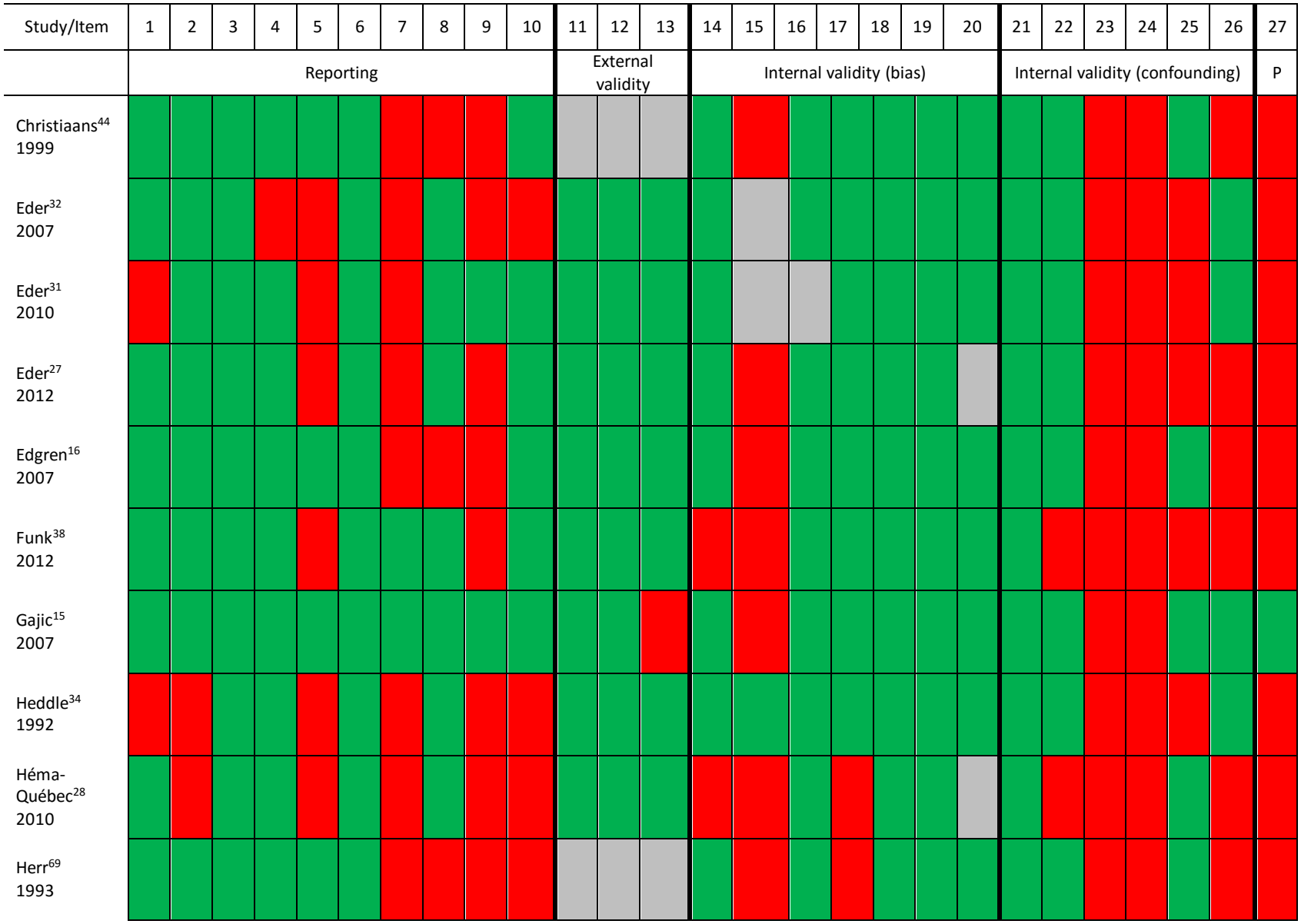
Necrotizing enterocolitis	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
HTLV infection	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Donor sex mismatch							
Hospital mortality	1 (Obs)	No serious limitation	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Survival 1 month post-transfusion	1 (Obs)	No serious limitation	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Survival 90 days post-transfusion	1 (Obs)	No serious limitation	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Survival 6 months post-transfusion	1 (Obs)	No serious limitation	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Survival 5 years post-transfusion	1 (Obs)	No serious limitation	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Acute Lung Injury	1 (Obs)	No serious limitation	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Donor cancer							
Recipient cancer	1 (Obs)	No serious limitation	Unable to determine	No serious indirectness	No serious imprecision	Likely present	++
HLA-DR mismatch							
Mortality	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
One-year graft survival	1 (RCT) 2 (NCT)	Serious limitations	Serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
5-year graft survival	1 (RCT) 1 (Obs)	Serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
1-year patient survival post-transplant	1 (RCT) 1 (Obs)	Serious limitations	Serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
5-year patient survival post-transplant	1 (RCT) 1 (Obs)	Serious limitations	Serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
Acute renal rejection < 6 months post-transplant	1 (RCT) 1 (Obs)	Very serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
Acute renal rejection at any time after transplant	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
> 1 acute renal rejection episode over follow-up	1 (NCT) 1 (Obs)	Serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
Allo-immunization	1 (RCT) 3 (NCT) 5 (Obs)	Serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	Likely present	++

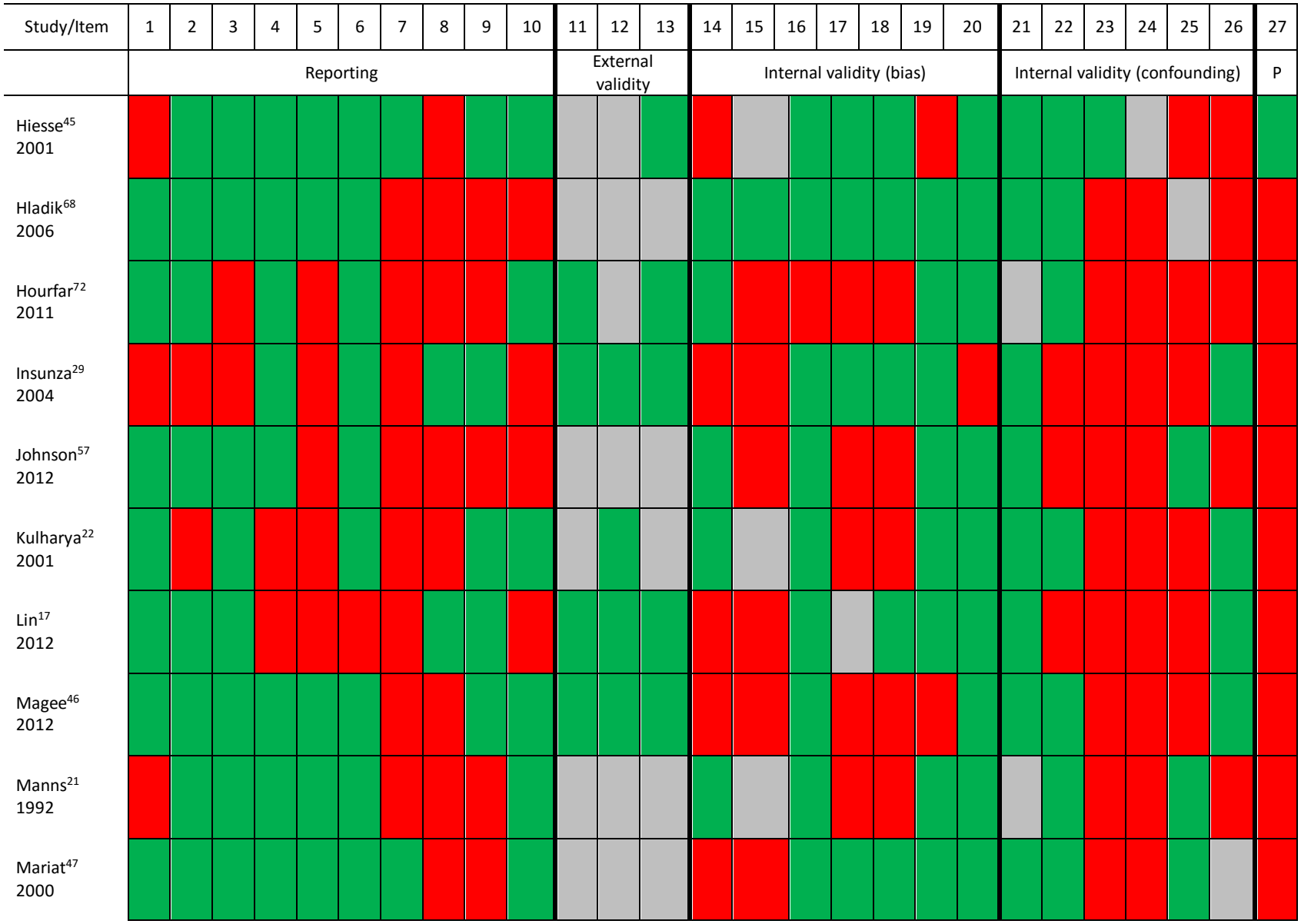
Microchimaerism	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
WBC antigen							
Mortality	1 (Obs)	No serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
TRALI	4 (Obs)	Serious limitations	Serious inconsistency	Serious indirectness	Serious imprecision	Likely present	+
TRALI – after implementation of a no WBC antibody policy	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Fatal vs non-fatal TRALI	1 (Obs)	Serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Increase of TRALI risk by antigen titer increase	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious inconsistency	Likely present	+
RBC antigen							
Haemolysis	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Allo-immunization	1 (NCT) 2 (Obs)	Serious limitations	Serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
Previous allo-exposure							
Mortality	1 (Obs)	No serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
TRALI	2 (Obs)	No serious limitations	No serious inconsistency	Serious indirectness	Serious imprecision	Likely present	+
TRALI risk after policy change	2 (Obs)	Serious limitations	Serious inconsistency	Serious indirectness	Serious imprecision	Likely present	+
Walking donors vs random donors							
CMV infection	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
HBV infection	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Parental donors vs unrelated donors							
Transfusion reaction	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Change in hematocrit	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	No serious imprecision	Likely present	++
Abnormal creatinine	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Abnormal bilirubin	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Maternal donors vs paternal donors							

Transfusion reaction	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Change in hematocrit	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	No serious imprecision	Likely present	++
Abnormal creatinine	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Abnormal bilirubin	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Babesiosis screening							
Babesiosis infection	2 (Obs)	Serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
CMV positive donors							
Seroconversion to CMV	1 (RCT) 2 (Obs)	Serious limitations	Serious inconsistency	Serious indirectness	Serious imprecision	Likely present	+
HBV positive donors							
Anti-HBc seroconversion	1 (Obs)	Very serious limitations	Unable to determine	Serious indirectness	Serious imprecision	Likely present	+
Any hepatitis	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Non-A non-B hepatitis	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
HCV positive donors							
HCV infection (before and after policy change)	1 (RCT) 2 (Obs)	Very serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
HCV infection (positive vs negative donor)	2 (Obs)	Serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
HHV8 positive donors							
HHV8 seroconversion	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	No serious imprecision	Likely present	+
HTLV positive donors							
HTLV seroconversion	4 (Obs)	Serious limitations	No serious inconsistency	No serious indirectness	Serious imprecision	Likely present	+
Parvovirus B19 positive donors							
Seroconversion	1 (Obs)	Serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Donor abnormal liver function test							
Post-transfusion hepatitis	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+
Post-transfusion HCV infection	1 (RCT)	Very serious limitations	Unable to determine	No serious indirectness	Serious imprecision	Likely present	+

FIGURE S1: RISK OF BIAS SUMMARY




Study/Item
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Reporting
External validity
Internal validity (bias)
Internal validity (confounding)
P
Christiaans[44] 1999
Eder[32] 2007
Eder[31] 2010
Eder[27] 2012
Edgren[16] 2007
Funk[38] 2012
Gajic[15] 2007
Heddle[34] 1992
Héma-Québec[28] 2010
Herr[69] 1993


Study/Item
1
2
3
4
5
6
7
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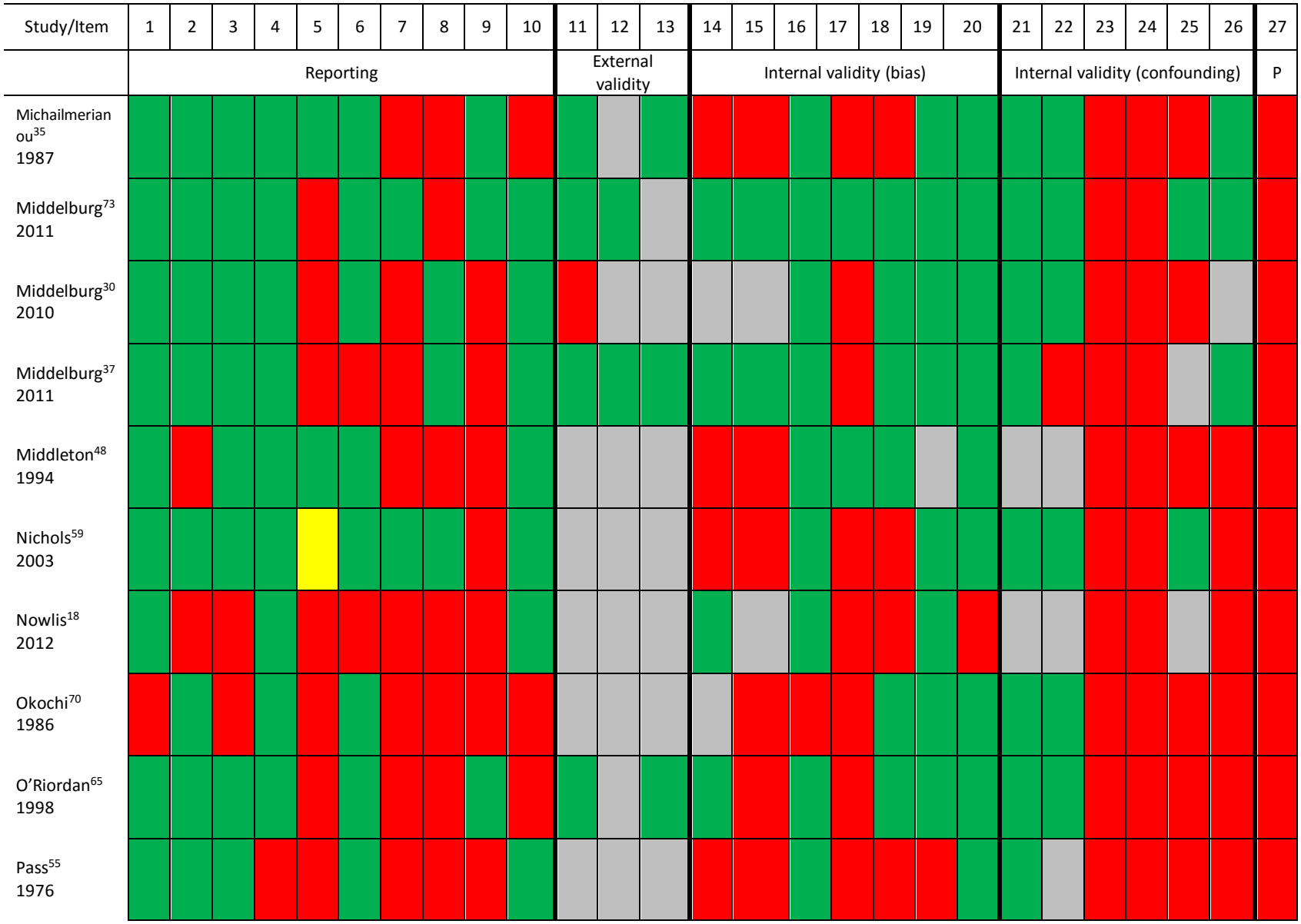
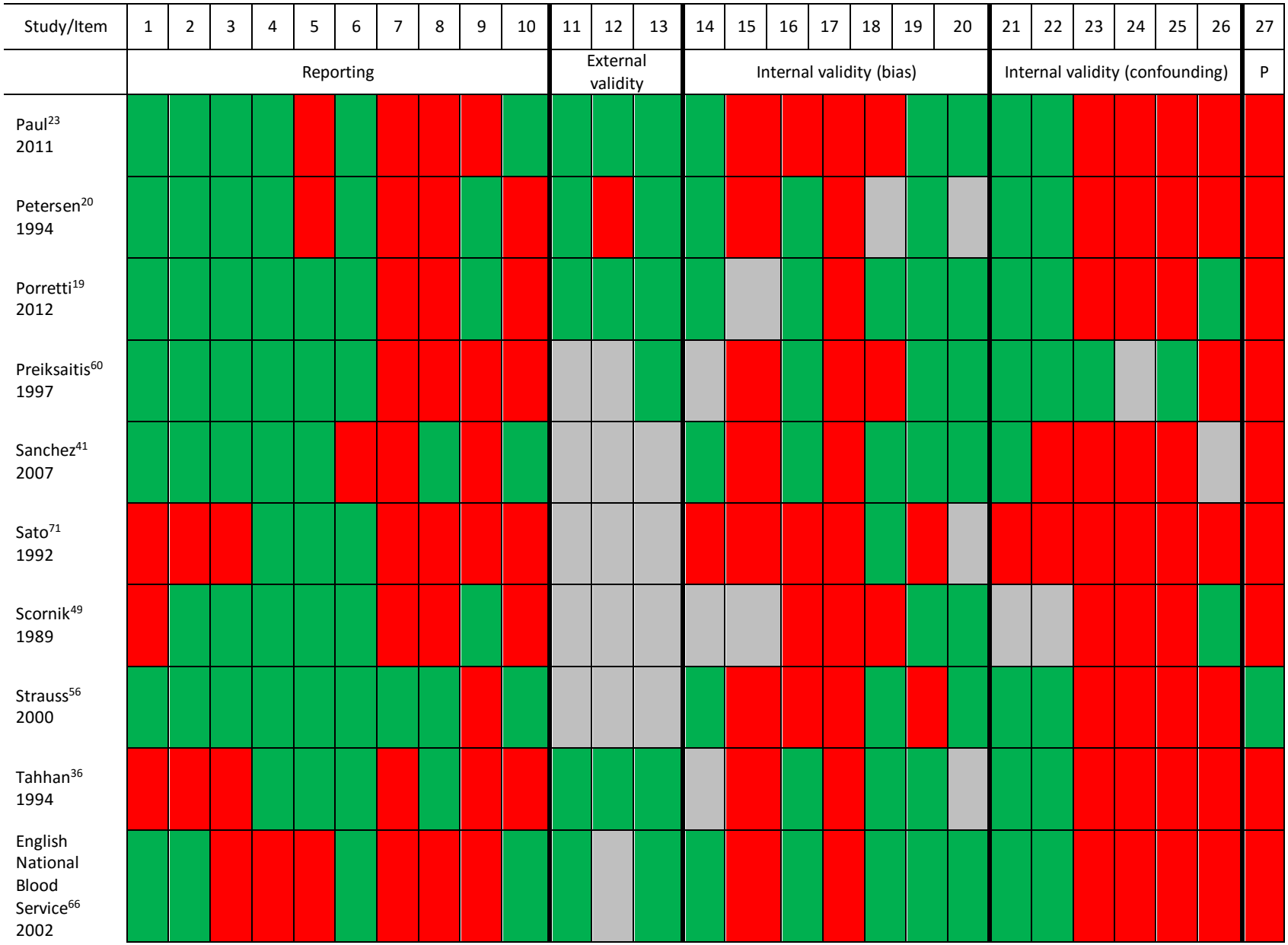
26
27
Reporting
External validity
Internal validity (bias)
Internal validity (confounding)
P
Hiesse[45] 2001
Hladik[68] 2006
Hourfar[72] 2011
Insunza[29] 2004
Johnson[57] 2012
Kulharya[22] 2001
Lin[17] 2012
Magee[46] 2012
Manns[21] 1992
Mariat[47] 2000


Study/Item
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Reporting
External validity
Internal validity (bias)
Internal validity (confounding)
P
Michailmerianou[35] 1987
Middelburg[73] 2011
Middelburg[30] 2010
Middelburg[37] 2011
Middleton[48] 1994
Nichols[59] 2003
Nowlis[18] 2012
Okochi[70] 1986
O'Riordan[65] 1998
Pass[55] 1976

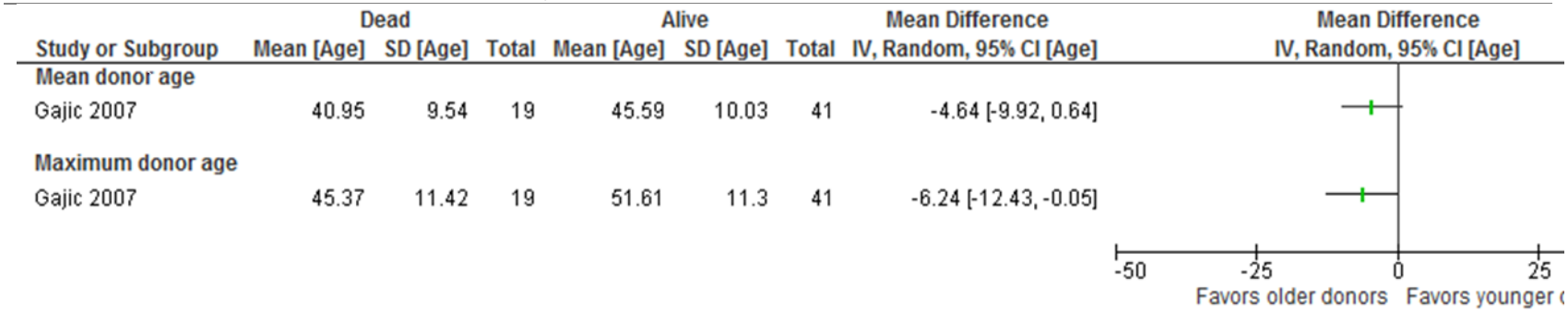

Study/Item
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Reporting
External validity
Internal validity (bias)
Internal validity (confounding)
P
Paul[23] 2011
Petersen[20] 1994
Porretti[19] 2012
Preiksaitis[60] 1997
Sanchez[41] 2007
Sato[71] 1992
Scornik[49] 1989
Strauss[56] 2000
Tahhan[36] 1994
English National Blood Service[66] 2002

Study/Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	Reporting										External validity			Internal validity (bias)							Internal validity (confounding)						P
Toy[5] 2012																											
Van der Mast[50] 1997																											
Van Hooff[51] 1992																											
Van Twuyver[52] 1991																											
Vervoordeldonk[40] 1998																											
Vlaar[53] 2011																											
Vogt[67] 2004																											
Young[58] 2012																											
Zupanska[54] 2007																											

Legend: Green: Yes; Red: No; Yellow: Partially

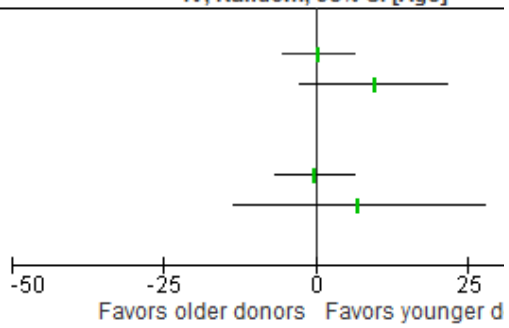
FIGURE S2: FOREST PLOTS

Outcome or subgroup	Studies	Statistical Method	Effect estimate	Interpretation
Donor age				
Hospital mortality				
Mean donor age	1	Mean Difference (IV, Random, 95% CI)	-4.62 [-9.92, 0.64]	Null, favours older donors
Maximum donor age	1	Mean Difference (IV, Random, 95% CI)	-6.24 [-12.43, -0.05]	Favours older donor



ALI ≤ 6h after transfusion				
Mean donor age	2	Mean Difference (IV, Random, 95% CI)	3.40 [-4.97, 11.78]	Null
Maximum donor age	2	Mean Difference (IV, Random, 95% CI)	0.47 [-5.73, 6.66]	Null, favours younger donors

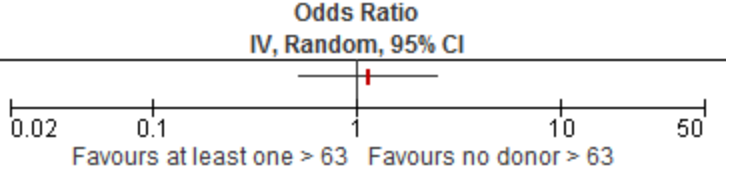
Study or Subgroup	ALI Mean [Age]	ALI SD [Age]	ALI Total	No ALI Mean [Age]	No ALI SD [Age]	No ALI Total	Mean Difference IV, Random, 95% CI [Age]
1.1.1 Mean donor age							
Gajic 2007	44.4	12.66	23	43.95	8.19	37	0.45 [-5.36, 6.26]
Porretti 2012 (1)	47.17	1.61	3	37.58	10.6	3	9.59 [-2.54, 21.72]
1.1.2 Maximum donor age							
Gajic 2007	49.52	13.6	23	49.7	10.4	37	-0.18 [-6.67, 6.31]
Porretti 2012 (2)	49.67	4.04	3	42.67	17.79	3	7.00 [-13.64, 27.64]



Footnotes
(1) Compares immune mediated TRALI cases versus non immune TRALI mediated cases
(2) Compares immune mediated TRALI cases versus non immune TRALI mediated cases

ALI ≤ 72 hours after transfusion	1	Odds Ratio (IV, Random, 95% CI)	1.14 [0.52, 2.49]	Null, favours no donor > 63

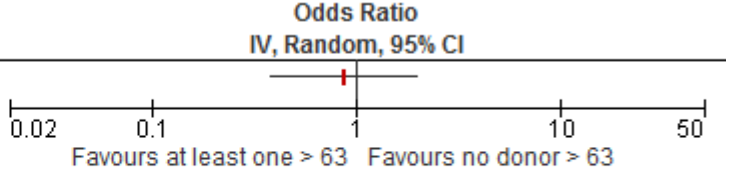
Study or Subgroup	log[Odds Ratio]	SE	Odds Ratio IV, Random, 95% CI
Nowlis 2012 (1)	0.1337	0.397	1.14 [0.52, 2.49]



Footnotes
(1) abstract, 212 patients included, not reported by group.

ALI 72 hours to 28 days after transfusion	1	Odds Ratio (IV, Random, 95% CI)	0.86 [0.38, 1.96]	Null, favours at least one donor > 63

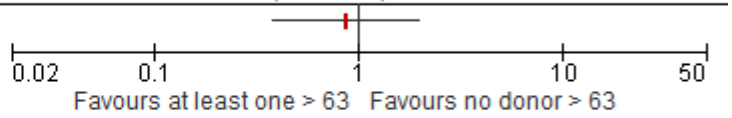
Study or Subgroup	log[Odds Ratio]	SE	Odds Ratio IV, Random, 95% CI
Nowlis 2012 (1)	-0.1508	0.4203	0.86 [0.38, 1.96]



Footnotes
(1) abstract, 212 patients included, not reported by group.

ALI ≤ 28 days after transfusion	1	Odds Ratio (IV, Random, 95% CI)	0.98 [0.51, 1.88]	Null

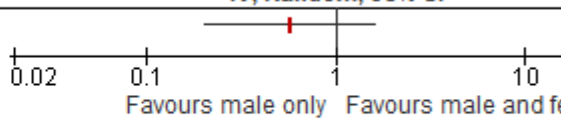
Study or Subgroup	log[Odds Ratio]	SE	Odds Ratio IV, Random, 95% CI	Odds Ratio IV, Random, 95% CI
Nowlis 2012 (1)	-0.1508	0.4203	0.86 [0.38, 1.96]	



Footnotes
(1) abstract, 212 patients included, not reported by group.

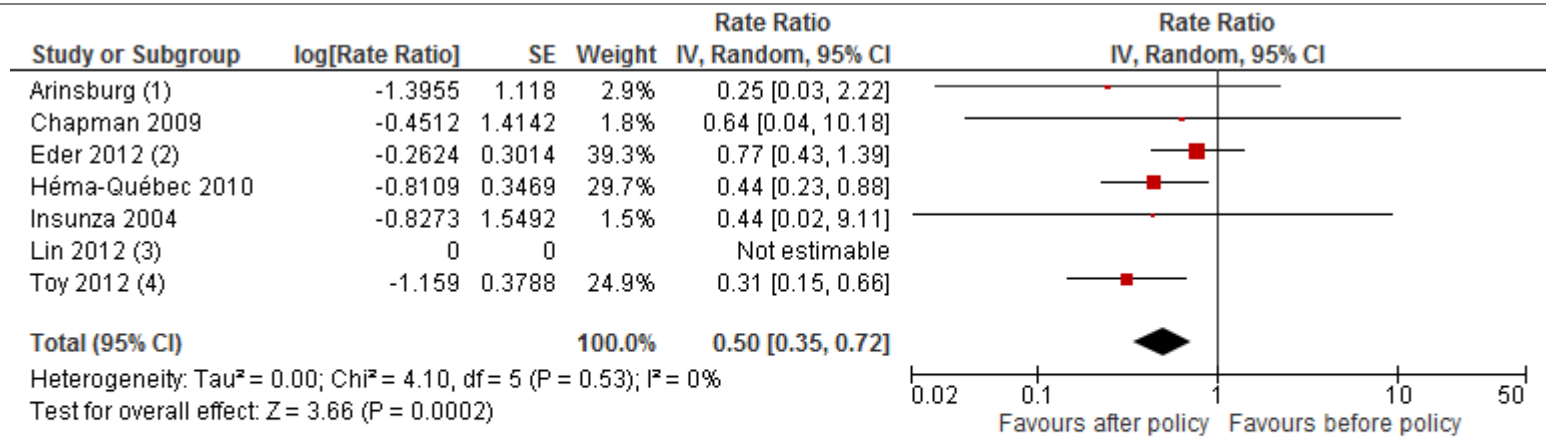
HTLV transmission	1	Multivariate model including donor age as a risk factor for HTLV-I conversion.	54 patients analyzed. Numerical value not provided. Reported as not significant.	Null
HIV transmission	1	Maximum likelihood estimation of HIV transmission adjusted for covariates.	Reported as: "The HIV-1 transmission rate did not vary by the donor's age[...]"	Null
Donor sex				
Hospital mortality	1	Odds Ratio (IV, Random, 95% CI)	0.57 [0.20, 1.61]	Null, favours male donors only

Study or Subgroup	log[Odds Ratio]	SE	Male and female Total	Male only Total	Odds Ratio IV, Random, 95% CI	Odds Ratio IV, Random, 95% CI
Gajic 2007 (1)	-0.5618	0.5301	51	24	0.57 [0.20, 1.61]	



Footnotes
(1) From data sent by the author.

RBC associated TRALI risk after policy change	7	Rate Ratio (IV, Random, 95% CI)	0.50 [0.35, 0.72], I^2 0%	Favours after policy change

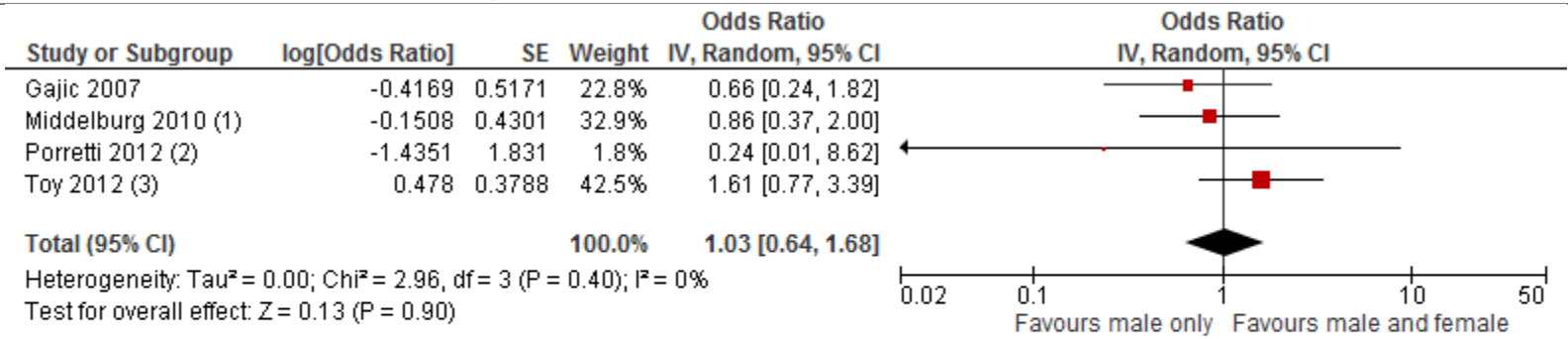


Study or Subgroup	log[Rate Ratio]	SE	Weight	Rate Ratio IV, Random, 95% CI
Arinsburg (1)	-1.3955	1.118	2.9%	0.25 [0.03, 2.22]
Chapman 2009	-0.4512	1.4142	1.8%	0.64 [0.04, 10.18]
Eder 2012 (2)	-0.2624	0.3014	39.3%	0.77 [0.43, 1.39]
Héma-Québec 2010	-0.8109	0.3469	29.7%	0.44 [0.23, 0.88]
Insunza 2004	-0.8273	1.5492	1.5%	0.44 [0.02, 9.11]
Lin 2012 (3)	0	0		Not estimable
Toy 2012 (4)	-1.159	0.3788	24.9%	0.31 [0.15, 0.66]
Total (95% CI)			**100.0%**	**0.50 [0.35, 0.72]**

Heterogeneity: Tau² = 0.00; Chi² = 4.10, df = 5 (P = 0.53); I² = 0%
Test for overall effect: Z = 3.66 (P = 0.0002)

Footnotes
(1) Per unit transfused before and after implementation of plasma policy, but for RBC-associated TRALI.
(2) Per unit distributed, for RBC-associated TRALI.
(3) The authors report the number of TRALI cases per year, no denominator data.
(4) The policy was only for plasma and platelet donor

RBC associated TRALI from male vs mixed donors	4	Odds Ratio (IV, Random, 95% CI)	1.03 [0.64, 1.68], I^2 0%	Null



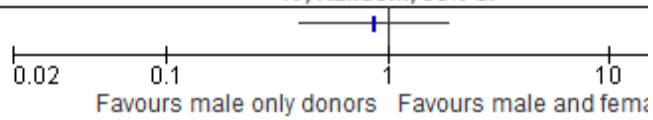
Study or Subgroup	log[Odds Ratio]	SE	Weight	Odds Ratio IV, Random, 95% CI
Gajic 2007	-0.4169	0.5171	22.8%	0.66 [0.24, 1.82]
Middelburg 2010 (1)	-0.1508	0.4301	32.9%	0.86 [0.37, 2.00]
Porretti 2012 (2)	-1.4351	1.831	1.8%	0.24 [0.01, 8.62]
Toy 2012 (3)	0.478	0.3788	42.5%	1.61 [0.77, 3.39]
Total (95% CI)			**100.0%**	**1.03 [0.64, 1.68]**

Heterogeneity: Tau² = 0.00; Chi² = 2.96, df = 3 (P = 0.40); I² = 0%
Test for overall effect: Z = 0.13 (P = 0.90)

Footnotes
(1) Cases of TRALI, compared to the population attribuable risk.
(2) Compares immune mediated TRALI cases versus non immune TRALI mediated cases
(3) Any RBC from female donor versus no female donor RBC

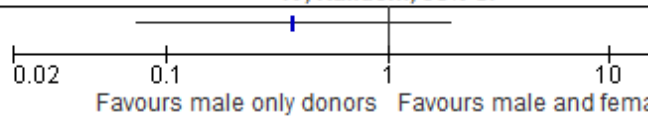
Non-fatal TRALI risk / unit distributed	1	Odds Ratio (IV, Random, 95% CI)	0.87 [0.40, 1.88]	Null, favours male donors only

Study or Subgroup	Male only donors Events	Total	Male and female donors Events	Total	Odds Ratio IV, Random, 95% CI
Eder 2010	12	6000000	14	6086956	0.87 [0.40, 1.88]



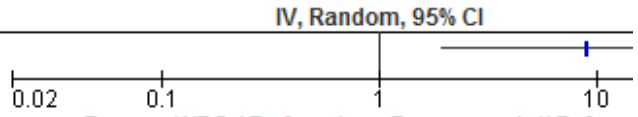
Fatal TRALI risk / unit distributed	1	Odds Ratio (IV, Random, 95% CI)	0.37 [0.07, 1.93]	Null, favours male donors only

Study or Subgroup	Male only donors Events	Total	Male and female donors Events	Total	Odds Ratio IV, Random, 95% CI
Eder 2010	2	6666666	5	6250000	0.37 [0.07, 1.93]



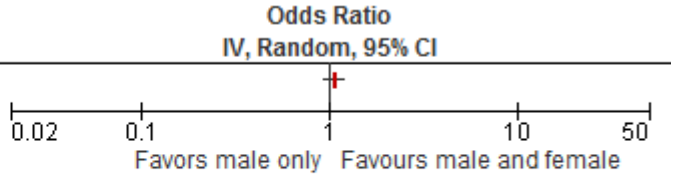
TRALI-related death vs non-TRALI-related death	1	Risk Ratio (IV, Random, 95% CI)	9.00 [1.92, 42.24]	Favours male donors and antibody negative female donors

Study or Subgroup	WBC+ Female donor Events	Total	WBC AB- female or male Events	Total	Risk Ratio IV, Random, 95% CI
Eder 2007	4	4	1	14	9.00 [1.92, 42.24]



Transfusion reactions	1	Odds Ratio (IV, Random, 95% CI)	1.06 [0.94, 1.19]	Null

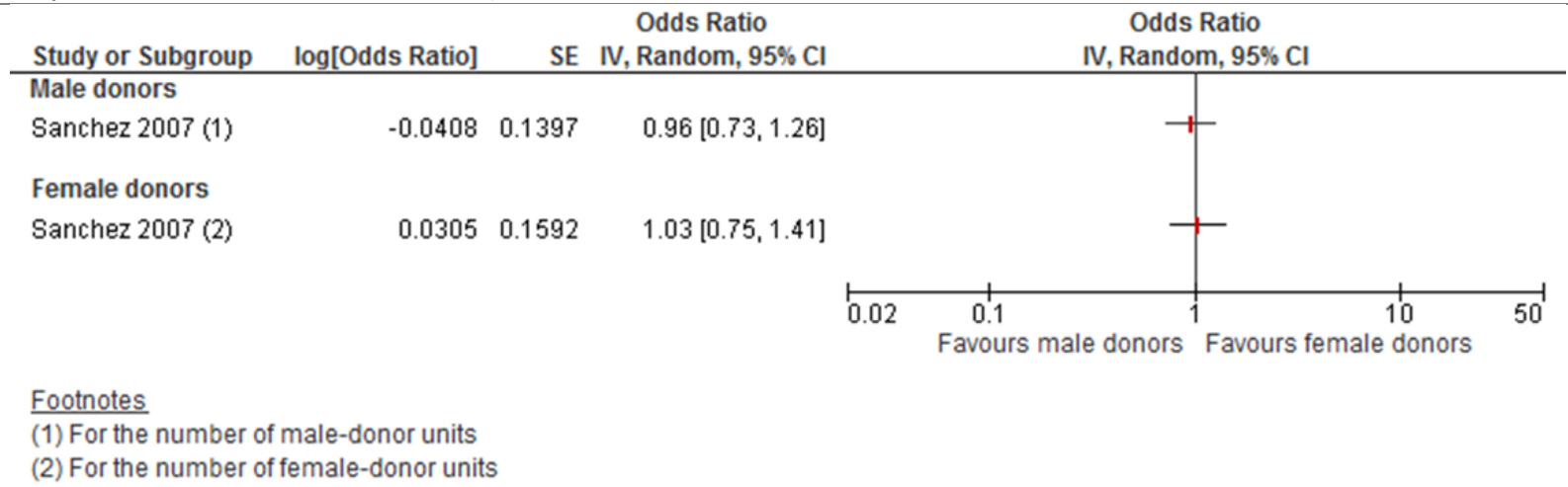
Study or Subgroup	log[Odds Ratio]	SE	Odds Ratio IV, Random, 95% CI
Eder 2012 (1)	0.0556	0.0619	1.06 [0.94, 1.19]



Footnotes
(1) Per unit distributed

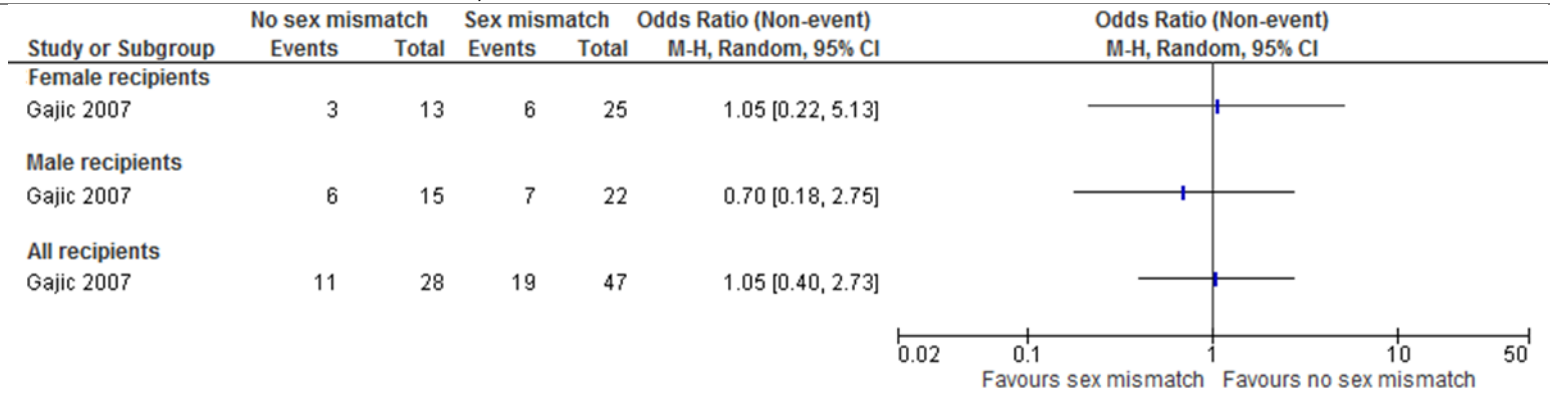
TRALI / number of units				
Male donors	1	Odds Ratio (IV, Random, 95% CI)	0.96 [0.73, 1.26]	Null

Female donors	1	Odds Ratio (IV, Random, 95% CI)	1.03 [0.75, 1.41]	Null
Antibody-mediated TRALI vs non-antibody mediated	1	Odds Ratio (IV, Random, 95% CI)	4.20 [0.12, 151.96]	Null, favours male donors

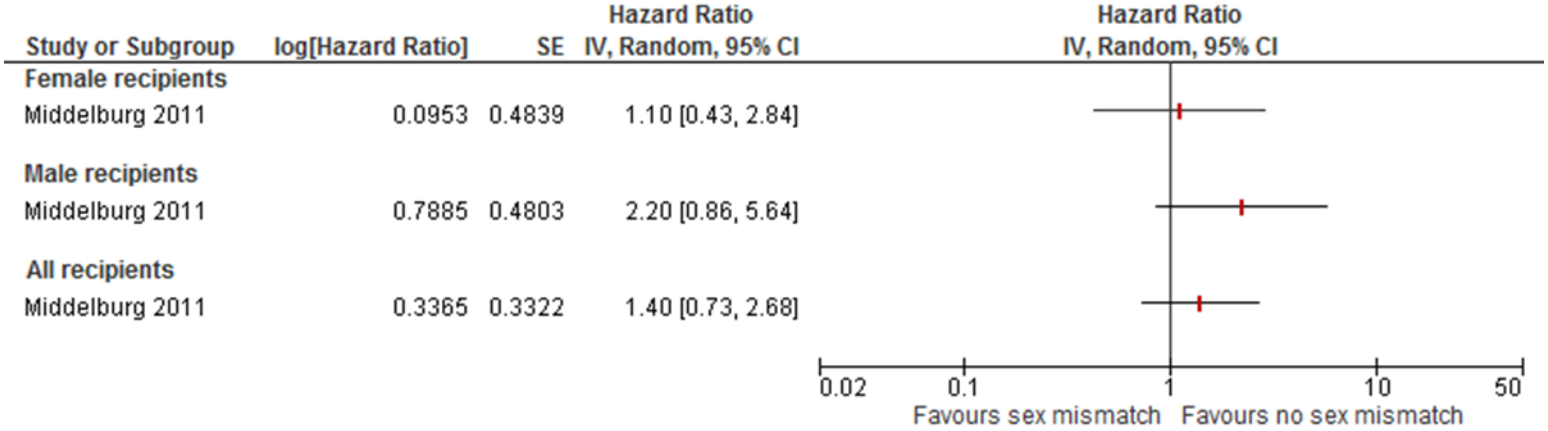


Genetic mosaicism	1	Male RBC donors vs usual donor populations. Statistical method unclear.	Authors report: "Given the male to female donor ratio in our blood bank of approximately 1:1, the probability of any patient being transfused with blood from a donor of opposite sex is 50%. The observation of no instances of even mosaic discordant karyotypic sex in any of our 10 patients is highly significant, with a likelihood of , 0.001."	Null
Necrotizing enterocolitis	1	Proportion of all RBC units transfused to outcome group that were from donors of male sex.	84% of RBC units were from males in the NEC within 48h of transfusion group, 62% of RBC units were from males in the NEC > 48h group. Authors report a p-value of 0.03.	Favours female donors
HTLV infection	1	Multivariate model including donor sex as a risk factor for HTLV-I conversion.	54 patients analyzed. Numerical value not provided. Reported as not significant.	Null
Donor sex mismatch				
Hospital mortality				
Female recipients	1	Odds Ratio (IV, Random, 95% CI)	1.05 [0.22, 5.13]	Null

Male recipients	1	Odds Ratio (IV, Random, 95% CI)	0.70 [0.18, 2.75]	Null, favours sex mismatch
All recipients	1	Odds Ratio (IV, Random, 95% CI)	1.05 [0.40, 2.73]	Null

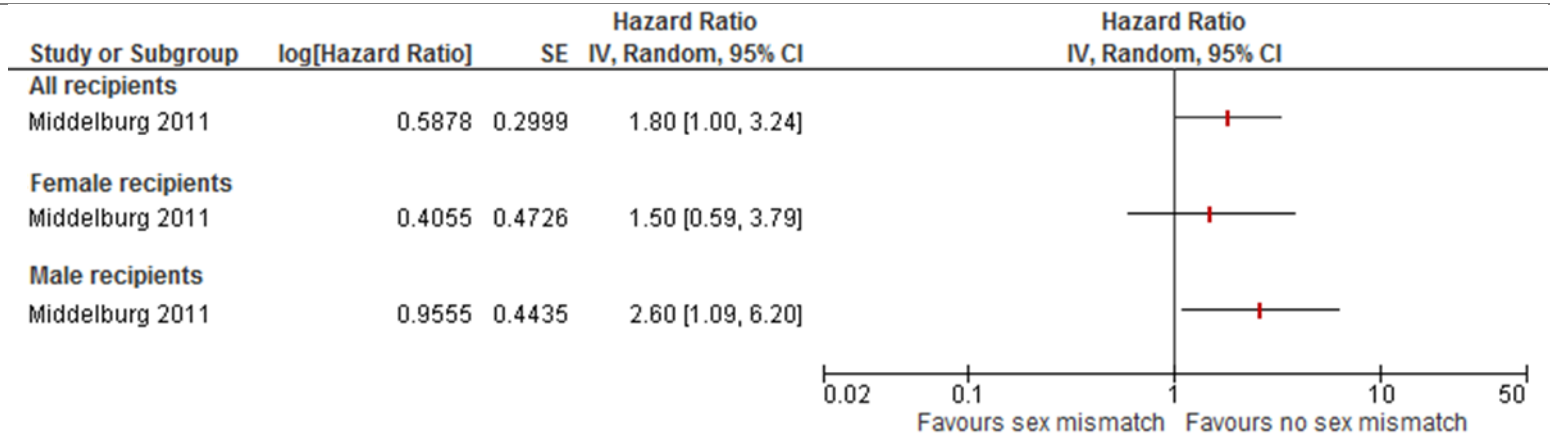


Survival 1 month post-transfusion				
Female recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.10 [0.43, 2.84]	Null, favours no sex mismatch
Male recipients	1	Hazard Ratio (IV, Random, 95% CI)	2.20 [0.86, 5.64]	Null, favours no sex mismatch
All recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.40 [0.73, 2.68]	Null, favours no sex mismatch

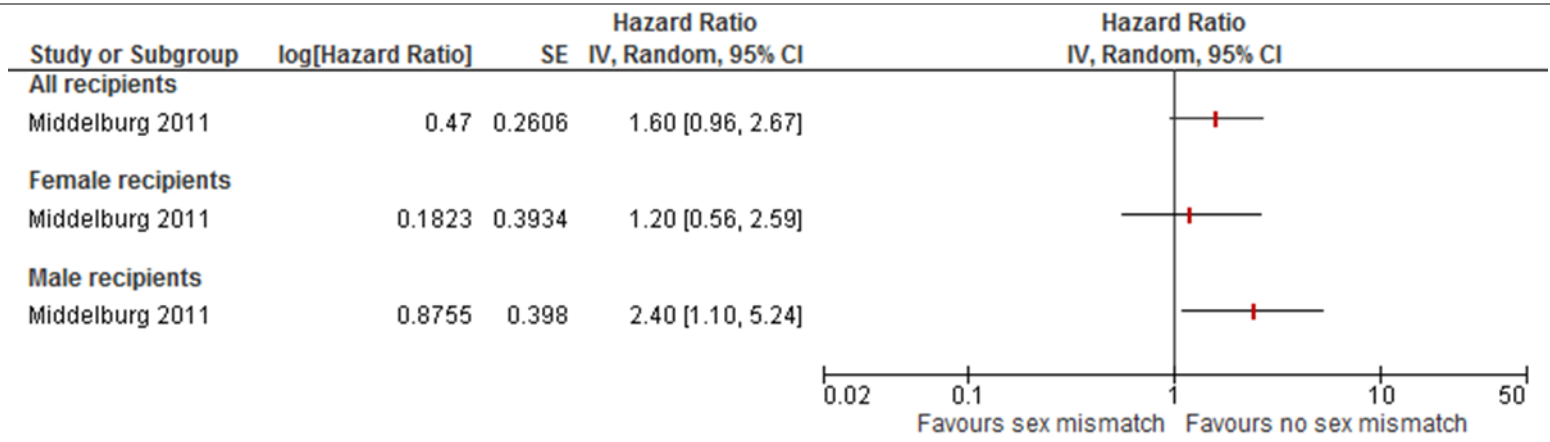


Survival 90 days post-transfusion	1

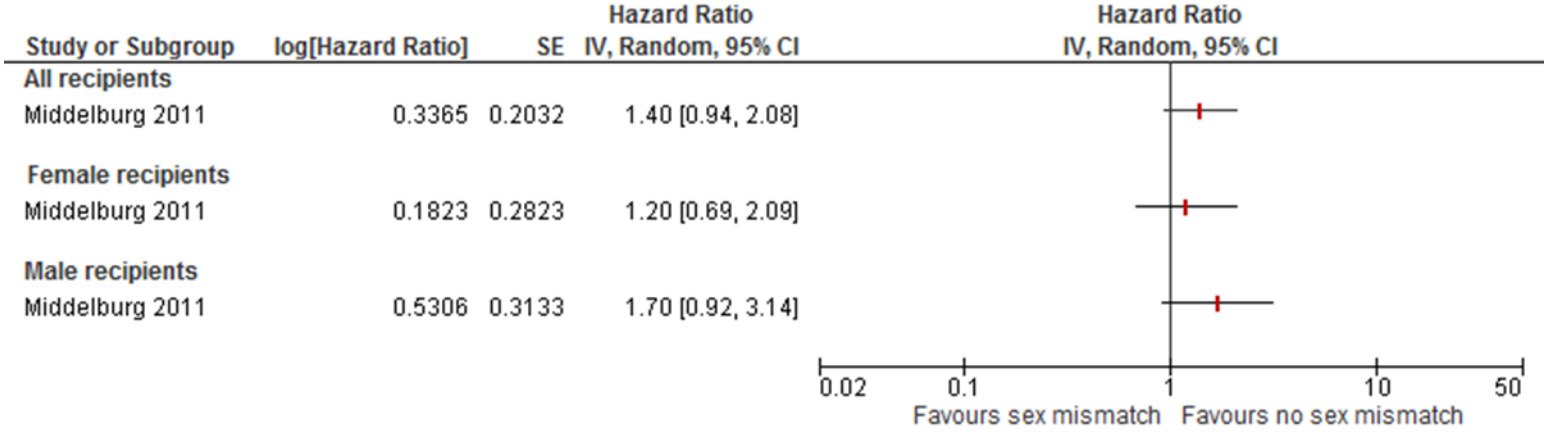
Female recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.50 [0.59, 3.79]	Null, favours no sex mismatch
Male recipients	1	Hazard Ratio (IV, Random, 95% CI)	2.60 [1.09, 6.20]	Favours no sex mismatch
All recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.80 [1.00, 3.24]	Favours no sex mismatch



Survival 6 months post-transfusion	1			
Female recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.20 [0.56, 2.59]	Null, favours no sex mismatch
Male recipients	1	Hazard Ratio (IV, Random, 95% CI)	2.40 [1.10, 5.24]	Favours no sex mismatch
All recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.60 [0.96, 2.67]	Null, favours no sex mismatch

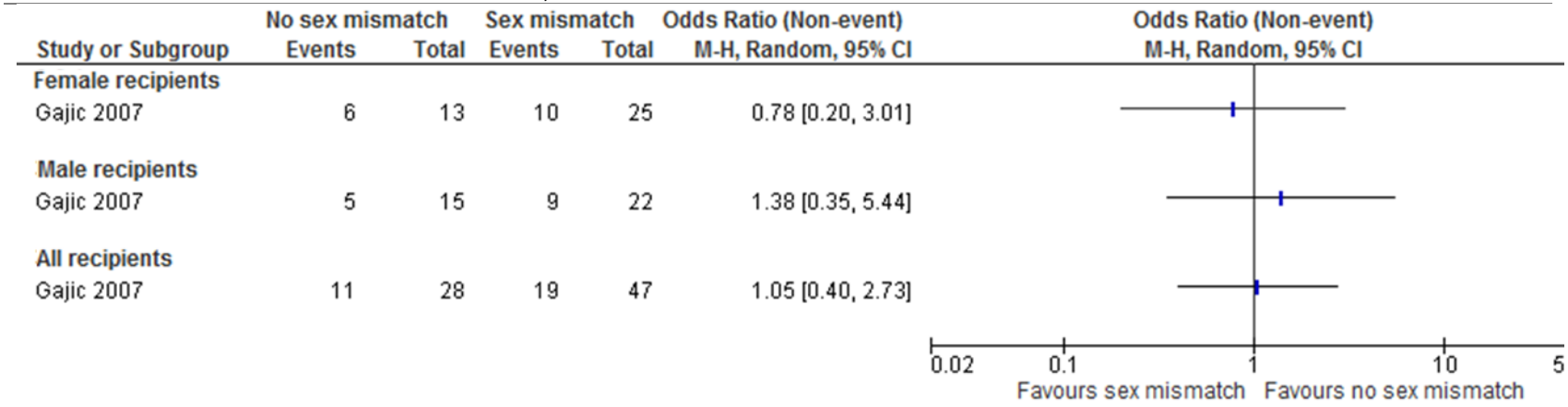


Survival 5 years post-transfusion	1			
Female recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.20 [0.69, 2.09]	Null, favours no sex mismatch
Male recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.70 [0.92, 3.14]	Null, favours no sex mismatch
All recipients	1	Hazard Ratio (IV, Random, 95% CI)	1.40 [0.94, 2.08]	Null, favours no sex mismatch



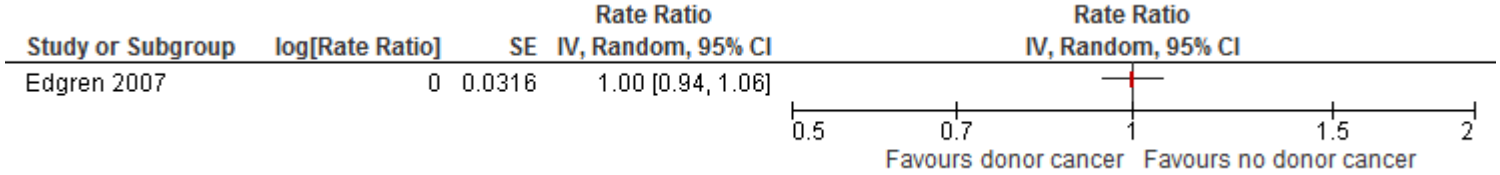
Acute Lung Injury	1			
Female recipients	1	Odds Ratio (M-H, Random, 95% CI)	0.78 [0.20, 3.01]	Null, favours sex mismatch

Male recipients	1	Odds Ratio (M-H, Random, 95% CI)	1.38 [0.35, 5.44]	Null, favours no sex mismatch
All recipients	1	Odds Ratio (M-H, Random, 95% CI)	1.05 [0.40, 2.73]	Null



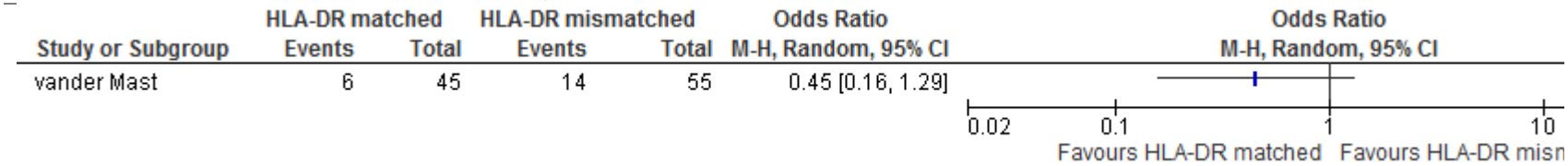
Donor cancer

Recipient cancer	1	Rate Ratio (IV, Random, 95% CI)	1.00 [0.94, 1.06]	Null



HLA-DR mismatch

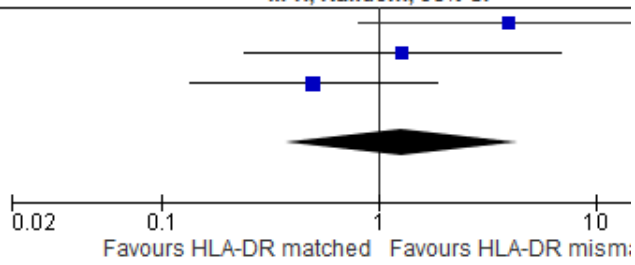
Mortality	1	Odds Ratio (M-H, Random, 95% CI)	0.45 [0.16, 1.29]	Null, favours HLA-DR matched



One-year graft survival	3	Odds Ratio (M-H, Random, 95% CI)	1.27 [0.37, 4.36], I^2 49%	Null, favours HLA-DR mismatched

	HLA-DR matched		HLA-DR mismatched			Odds Ratio	Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	M-H, Random, 95% CI
Albert 1981	10	14	5	13	31.5%	4.00 [0.80, 20.02]	
Hiesse 2001	3	31	3	39	30.1%	1.29 [0.24, 6.86]	
Mariat 2000	3	49	12	103	38.4%	0.49 [0.13, 1.84]	
Total (95% CI)		**94**		**155**	**100.0%**	**1.27 [0.37, 4.36]**	
Total events	16		20				

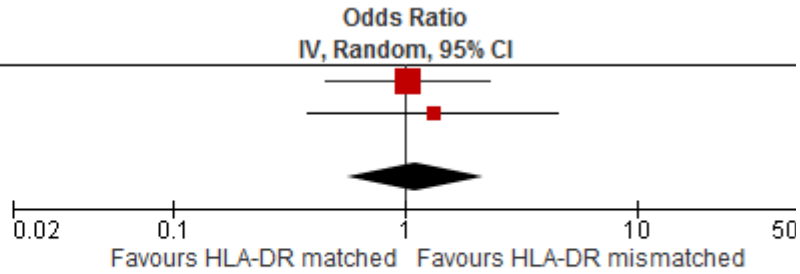
Heterogeneity: Tau² = 0.58; Chi² = 3.91, df = 2 (P = 0.14); I² = 49%
Test for overall effect: Z = 0.39 (P = 0.70)



5-year graft survival	2	Odds Ratio (IV, Random, 95% CI)	1.10 [0.56, 2.18], I^2 0%	Null, favours HLA-DR mismatched

				Odds Ratio	Odds Ratio
Study or Subgroup	log[Odds Ratio]	SE	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Christiaans 1999	0.0198	0.4175	69.8%	1.02 [0.45, 2.31]	
Hiesse 2001 (1)	0.2776	0.6353	30.2%	1.32 [0.38, 4.58]	
Total (95% CI)			**100.0%**	**1.10 [0.56, 2.18]**	

Heterogeneity: Tau² = 0.00; Chi² = 0.12, df = 1 (P = 0.73); I² = 0%
Test for overall effect: Z = 0.28 (P = 0.78)

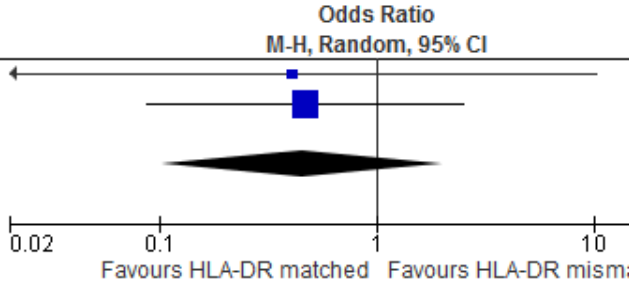


Footnotes
(1) Odds ratio estimated from survival table.

1-year patient survival post-transplant	2	Odds Ratio (M-H, Random, 95% CI)	0.45 [0.10, 2.02], I^2 0%	Null, favours HLA-DR matched

Study or Subgroup	HLA-DR matched Events	Total	HLA-DR mismatched Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Hiesse 2001 (1)	0	31	1	39	21.4%	0.41 [0.02, 10.35]
vander Mast (2)	2	45	5	55	78.6%	0.47 [0.09, 2.52]
Total (95% CI)		**76**		**94**	**100.0%**	**0.45 [0.10, 2.02]**
Total events	2		6			

Heterogeneity: Tau² = 0.00; Chi² = 0.01, df = 1 (P = 0.94); I² = 0%
Test for overall effect: Z = 1.04 (P = 0.30)

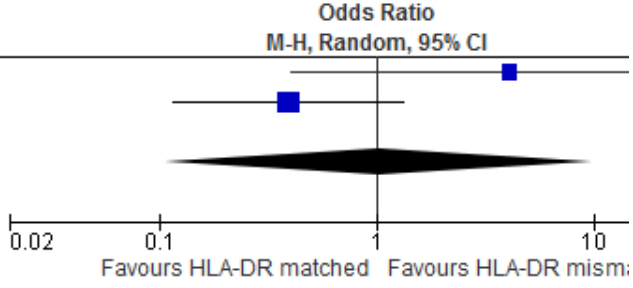


Footnotes
(1) Kidney transplant
(2) Cardiac transplant

5-year patient survival post-transplant	2	Odds Ratio (M-H, Random, 95% CI)	1.02 [0.11, 9.79], I^2 68%	Null

Study or Subgroup	HLA-DR matched Events	Total	HLA-DR mismatched Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Hiesse 2001 (1)	3	31	1	39	40.9%	4.07 [0.40, 41.23]
vander Mast (2)	4	45	11	55	59.1%	0.39 [0.12, 1.32]
Total (95% CI)		**76**		**94**	**100.0%**	**1.02 [0.11, 9.79]**
Total events	7		12			

Heterogeneity: Tau² = 1.87; Chi² = 3.10, df = 1 (P = 0.08); I² = 68%
Test for overall effect: Z = 0.02 (P = 0.99)

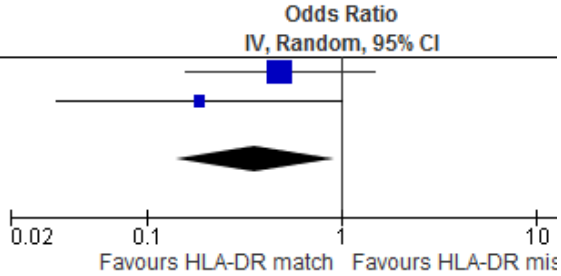


Footnotes
(1) Renal transplant
(2) Cardiac transplant

Acute renal rejection < 6 months post-transplant	2	Odds Ratio (IV, Random, 95% CI)	0.36 [0.14, 0.91], I^2 0%	Favours HLA-DR matched

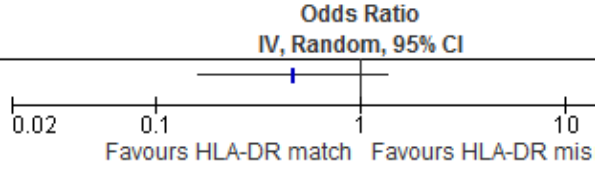
Study or Subgroup	HLA-DR matched blood Events	Total	HLA-DR mismatched blood Events	Total	Weight	Odds Ratio IV, Random, 95% CI
Hiesse 2001	6	31	13	39	69.6%	0.48 [0.16, 1.46]
Middleton 1994	14	29	10	12	30.4%	0.19 [0.03, 1.01]
Total (95% CI)		**60**		**51**	**100.0%**	**0.36 [0.14, 0.91]**
Total events	20		23			

Heterogeneity: Tau² = 0.00; Chi² = 0.84, df = 1 (P = 0.36); I² = 0%
Test for overall effect: Z = 2.16 (P = 0.03)



Acute renal rejection at any time after transplant	1	Odds Ratio (IV, Random, 95% CI)	0.47 [0.16, 1.35]	Favours HLA-DR matched

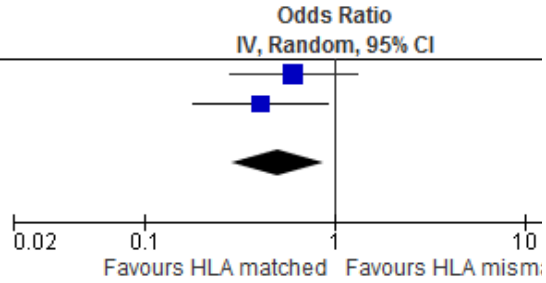
Study or Subgroup	HLA-DR matched blood Events	Total	HLA-DR mismatched blood Events	Total	Odds Ratio IV, Random, 95% CI
Hiesse 2001	7	31	15	39	0.47 [0.16, 1.35]



> 1 acute renal rejection episode over follow-up	2	Odds Ratio (IV, Random, 95% CI)	0.50 [0.29, 0.88], I^2 0%	Favours HLA-DR matched

Study or Subgroup	HLA-DR matched blood Events	Total	HLA-DR mismatched blood Events	Total	Weight	Odds Ratio IV, Random, 95% CI
Mariat 2000	12	49	36	103	53.6%	0.60 [0.28, 1.30]
vander Mast	14	45	29	55	46.4%	0.40 [0.18, 0.92]
Total (95% CI)		**94**		**158**	**100.0%**	**0.50 [0.29, 0.88]**
Total events	26		65			

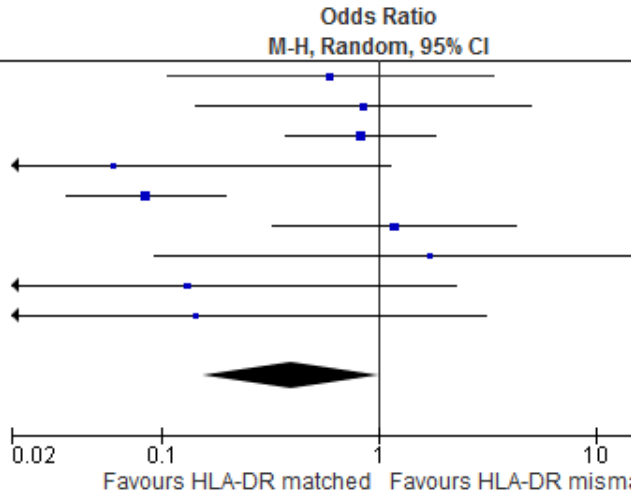
Heterogeneity: Tau² = 0.00; Chi² = 0.48, df = 1 (P = 0.49); I² = 0%
Test for overall effect: Z = 2.41 (P = 0.02)



Allo-immunization	9	Odds Ratio (M-H, Random, 95% CI)	0.39 [0.15, 0.99], I^2 66%	Favours HLA-DR matched

Study or Subgroup	HLA-DR matched Events	Total	HLA-DR mismatched Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Albert 1981 (1)	2	16	6	31	11.9%	0.60 [0.11, 3.35]
Baudouin 1997	9	13	8	11	11.7%	0.84 [0.14, 4.97]
Christiaans 1999	12	76	18	97	17.6%	0.82 [0.37, 1.83]
Magee 2012	0	31	5	25	6.7%	0.06 [0.00, 1.13]
Mariat 2000	9	49	78	107	17.4%	0.08 [0.04, 0.19]
Middleton 1994	11	46	4	19	14.6%	1.18 [0.32, 4.30]
Scornik 1989	1	8	1	13	6.8%	1.71 [0.09, 31.92]
van Hooff 1992	0	17	23	130	7.0%	0.13 [0.01, 2.25]
Vantwuyver	10	20	3	3	6.3%	0.14 [0.01, 3.12]
Total (95% CI)		**276**		**436**	**100.0%**	**0.39 [0.15, 0.99]**
Total events	54		146			

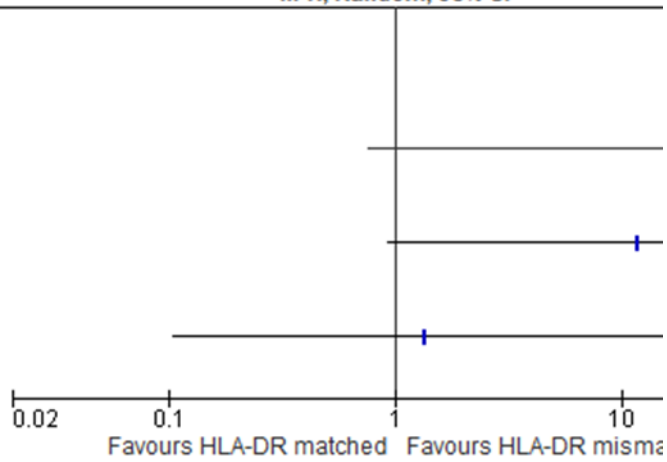
Heterogeneity: Tau² = 1.13; Chi² = 23.67, df = 8 (P = 0.003); I² = 66%
Test for overall effect: Z = 1.97 (P = 0.05)



Footnotes
(1) Greater than 10% of a panel of 90 antibodies was considered a positive outcome.

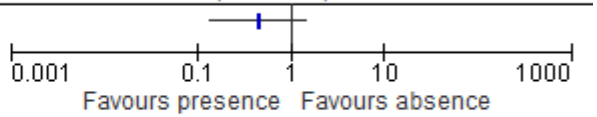
Microchimaerism				
1-4 days post transfusion	1	Odds Ratio (M-H, Random, 95% CI)	Not estimable	Null
5-7 days post transfusion	1	Odds Ratio (M-H, Random, 95% CI)	17.89 [0.76, 420,49]	Null, favours HLA-DR mismatched
2-4 weeks post transfusion	1	Odds Ratio (M-H, Random, 95% CI)	11.67 [0.92, 147.56]	Null, favours HLA-DR mismatched
5-8 weeks post transfusion	1	Odds Ratio (M-H, Random, 95% CI)	1.33 [0.10, 17.10]	Null

Study or Subgroup	HLA-DR matched Events	Total	HLA-DR mismatched Events	Total	Odds Ratio M-H, Random, 95% CI
1-4 days post transfusion					
Vervoordeldonk 1998	4	4	1	1	Not estimable
5-7 days post transfusion					
Vervoordeldonk 1998	11	11	4	7	17.89 [0.76, 420.49]
2-4 weeks post transfusion					
Vervoordeldonk 1998	7	8	3	8	11.67 [0.92, 147.56]
5-8 weeks post transfusion					
Vervoordeldonk 1998	3	12	1	5	1.33 [0.10, 17.10]



WBC antibody				
Mortality	1	Odds Ratio (M-H, Random, 95% CI)	0.45 [0.14, 1.48]	Null, favours presence of antibody

Study or Subgroup	Presence of antibody Events	Total	Absence of antibody Events	Total	Odds Ratio M-H, Random, 95% CI
Gajic 2007 (1)	5	21	16	39	0.45 [0.14, 1.48]



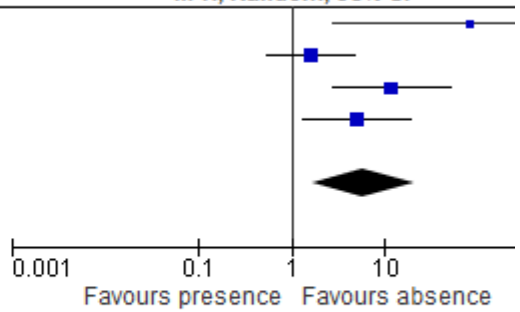
Footnotes

(1) All patients also received plasma

TRALI	4	Odds Ratio (M-H, Random, 95% CI)	5.84 [1.66, 20.59], I^2 62%	Favours absence of antibody

Study or Subgroup	Presence of antibody Events	Total	Absence of antibody Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Eder 2007	4	4	1	14	10.5%	81.00 [2.78, 2364.18]
Gajic 2007 (1)	10	21	14	39	32.8%	1.62 [0.55, 4.77]
Vlaar 2011	10	14	6	34	27.4%	11.67 [2.72, 50.07]
Zupanska 2007 (2)	10	17	6	27	29.2%	5.00 [1.33, 18.81]
Total (95% CI)		**56**		**114**	**100.0%**	**5.84 [1.66, 20.59]**
Total events	34		27			

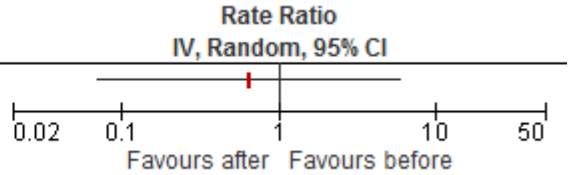
Heterogeneity: Tau² = 0.95; Chi² = 7.91, df = 3 (P = 0.05); I² = 62%
Test for overall effect: Z = 2.75 (P = 0.006)



Footnotes
(1) All patients also received plasma, outcome was ALI
(2) TRALI vs ALI that are probably not transfusion related

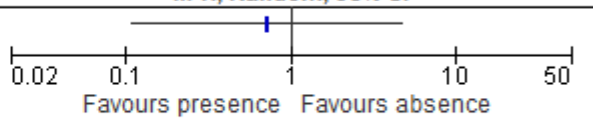
TRALI – after implementation of a no WBC antibody policy	1	Rate Ratio (IV, Random, 95% CI)	0.64 [0.07, 5.85]	Null, favours after policy change

Study or Subgroup	log[Rate Ratio]	SE	Rate Ratio IV, Random, 95% CI
Funk 2012	-0.4463	1.129	0.64 [0.07, 5.85]



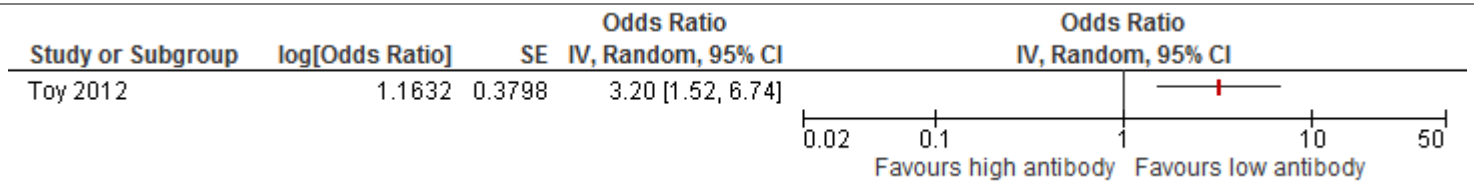
Fatal vs non-fatal TRALI	1	Odds Ratio (M-H, Random, 95% CI)	0.71 [0.11, 4.65]	Null, favours presence of antibody

Study or Subgroup	Presence of antibody Events	Total	Absence of antibody Events	Total	Odds Ratio M-H, Random, 95% CI
Eder 2010 (1)	6	27	2	7	0.71 [0.11, 4.65]

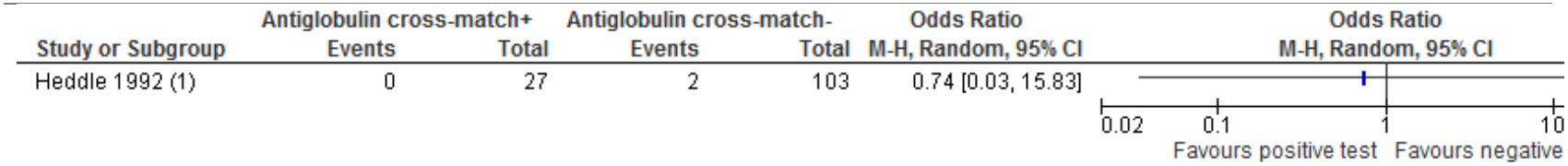


Footnotes
(1) HLA, HNA, cognate or cross-reactive match

Increase of TRALI risk by antigen titer increase	1	Odds Ratio (IV, Random, 95% CI)	3.20 [1.52, 6.74]	Favours low antibody titer

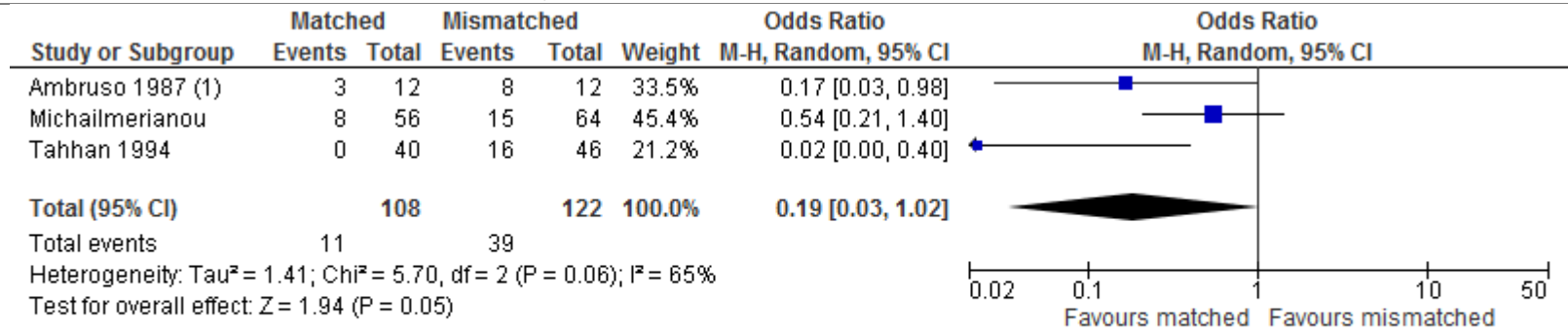


RBC antigen				
Haemolysis	1	Odds Ratio (M-H, Random, 95% CI)	0.74 [0.03, 15.83]	Null, Favours positive cross-match



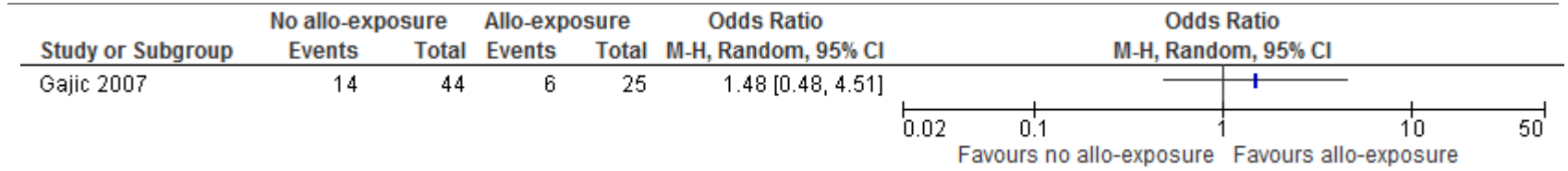
Footnotes
(1) Transfusion episodes

Allo-immunization	3	Odds Ratio (M-H, Random, 95% CI)	0.19 [0.03, 1.02], I^2 65%	Null, favours matched blood

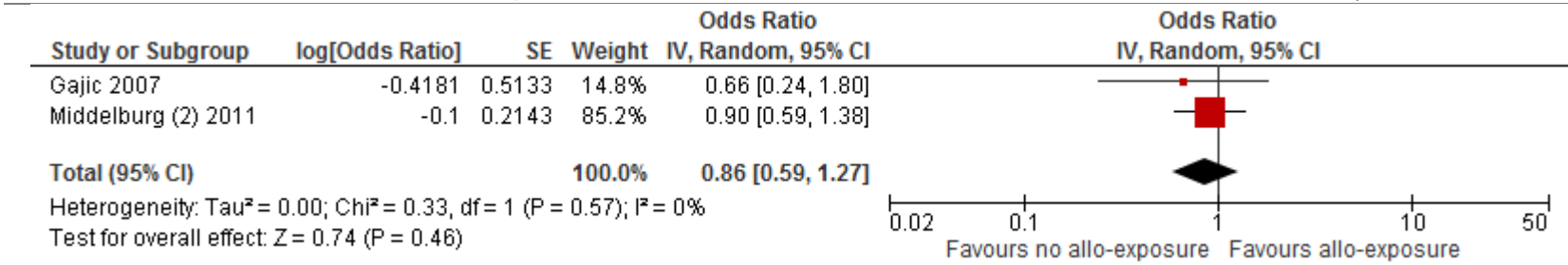


Footnotes
(1) 12 patients acted as their own control.

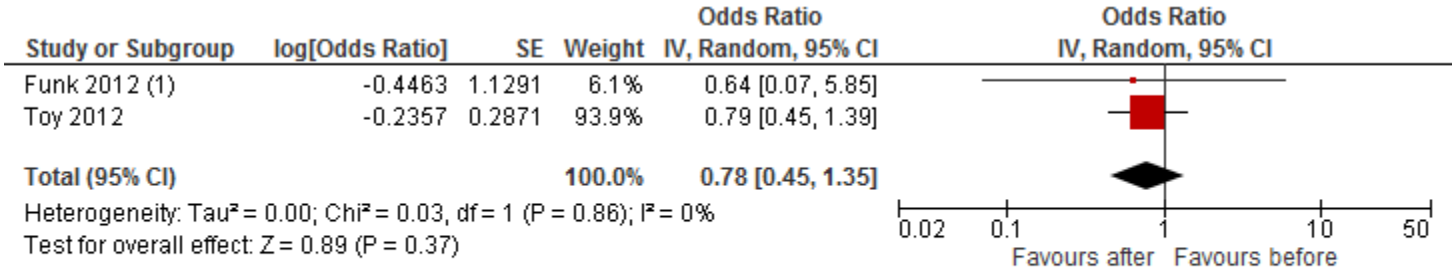
Previous allo-exposure				
Mortality	1	Odds Ratio (M-H, Random, 95% CI)	1.48 [0.48, 4.51]	Null, favours allo-exposure



TRALI	2	Odds Ratio (IV, Random, 95% CI)	0.86 [0.59, 1.27]	Null, favours no allo-exposure



TRALI risk after policy change	2	Odds Ratio (IV, Random, 95% CI)	0.78 [0.45, 1.35], I^2 0%	Null, favours after policy change

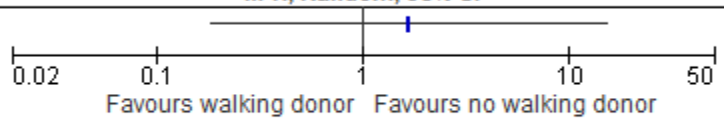


Footnotes

(1) Use of donors without history of pregnancy or WBC antibody, Rate Ratios were approximated to Odds Ratio

Walking donors vs random donors				
CMV infection	1	Odds Ratio (M-H, Random, 95% CI)	1.67 [0.18, 15.02]	Null, favours no walking donor

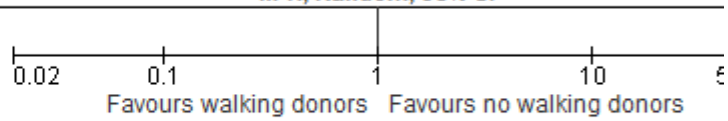
Study or Subgroup	Walking donor Events	Total	No walking donor Events	Total	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% CI
Pass 1976 (1)	23	92	1	6	1.67 [0.18, 15.02]	



Footnotes
(1) Serum or urine CMV positive

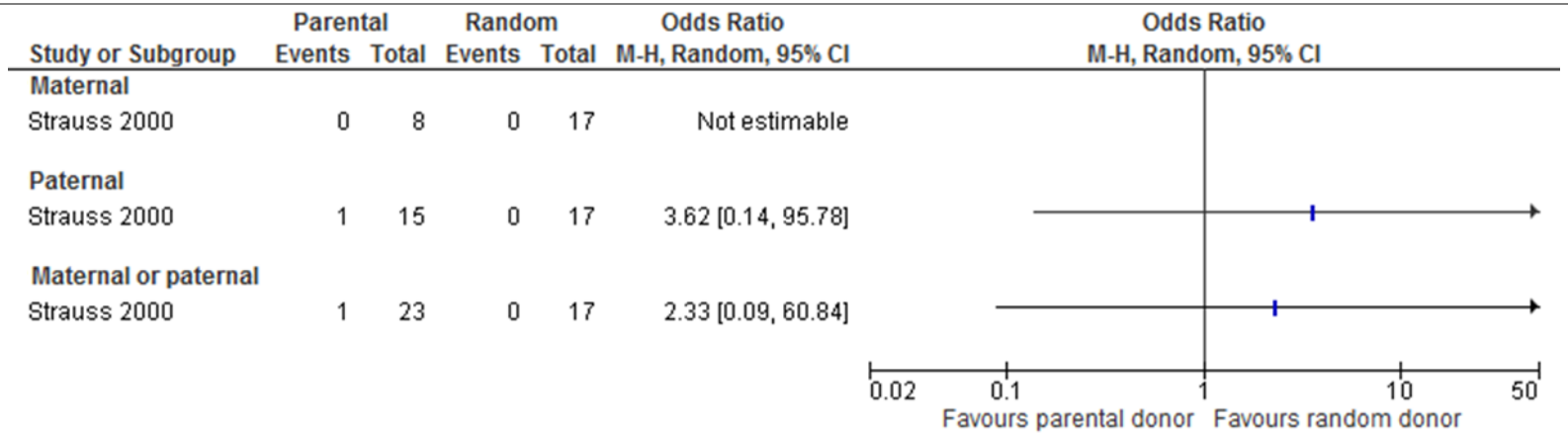
HBV infection	1		Not estimable	

Study or Subgroup	Walking donor Events	Total	No walking donor Events	Total	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% CI
Pass 1976 (1)	0	79	0	2	Not estimable	

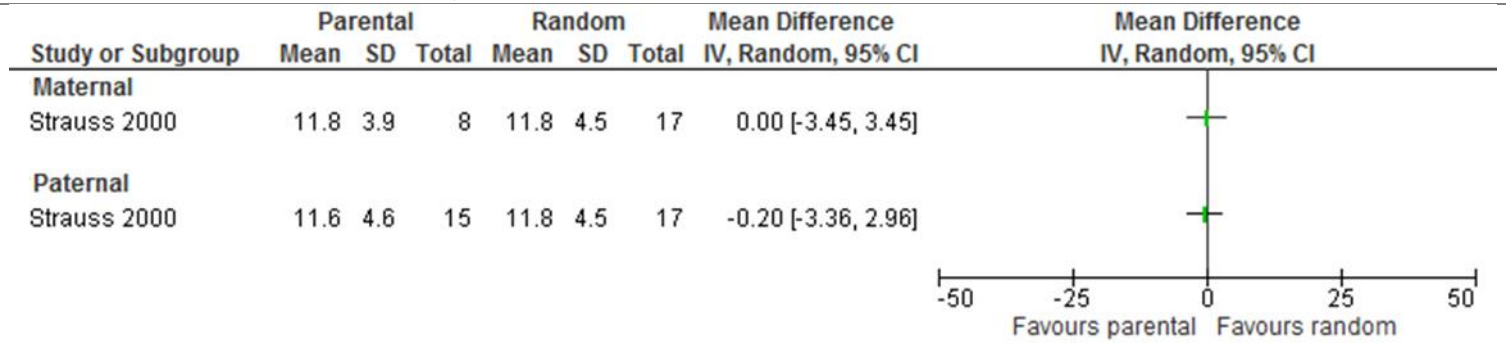


Footnotes
(1) Serum or urine CMV positive

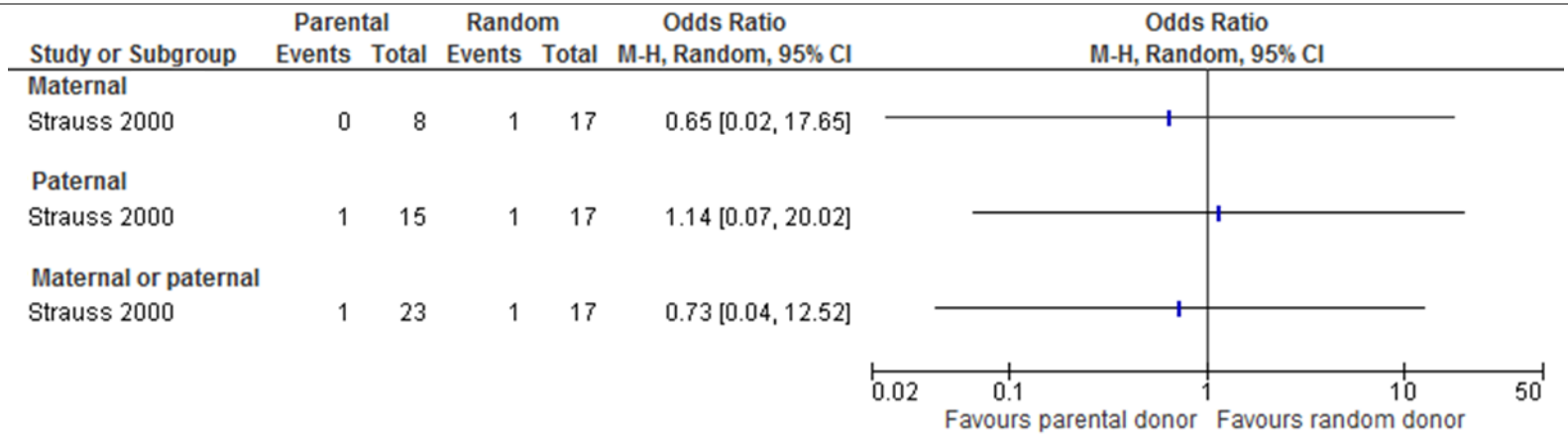
Parental donors vs unrelated donors				
Transfusion reaction				
Maternal	1	Odds Ratio (M-H, Random, 95% CI)	Not estimable	
Paternal	1	Odds Ratio (M-H, Random, 95% CI)	3.62 [0.14, 95.78]	Null, favors random donor
Maternal or Maternal	1	Odds Ratio (M-H, Random, 95% CI)	2.33 [0.09, 60.85]	Null, favors random donor



Change in hematocrit				
Maternal	1	Mean Diff (IV, Random, 95% CI)	0.0 [-3.45, 3.45]	Null
Paternal	1	Mean Diff (IV, Random, 95% CI)	-0.20 [-3.36, 2.96]	Null

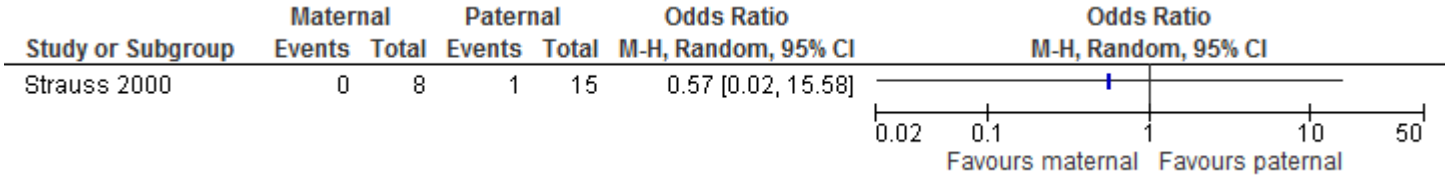


Abnormal creatinine				
Maternal	1	Odds Ratio (M-H, Random, 95% CI)	0.65 [0.02, 17.65]	Null, favours parental donor
Paternal	1	Odds Ratio (M-H, Random, 95% CI)	1.14 [0.07, 20.02]	Null, favours random donor
Maternal or Maternal	1	Odds Ratio (M-H, Random, 95% CI)	0.73 [0.04, 12.52]	Null, favours random donor

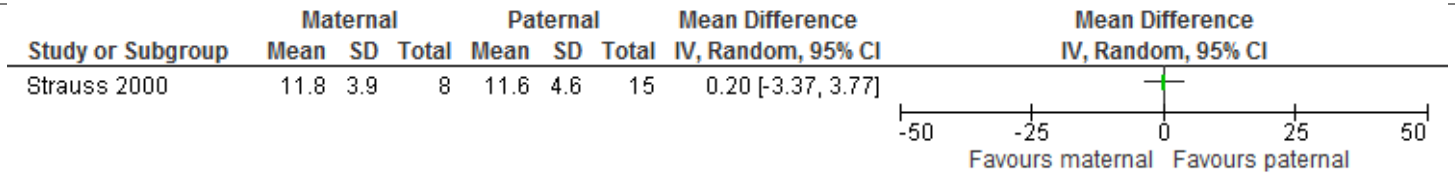


Abnormal bilirubin	1		Not estimable	

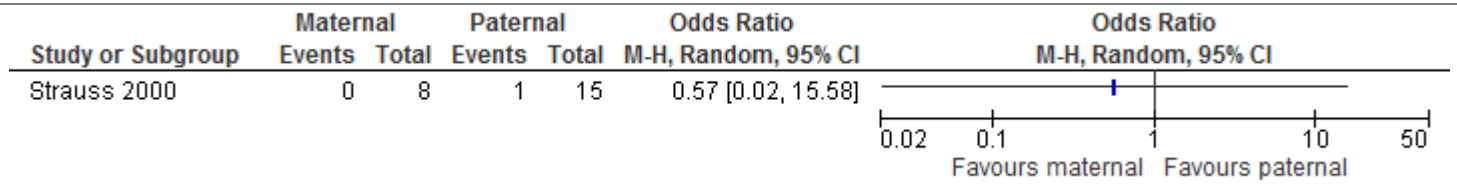
Maternal donors vs paternal donors				
Transfusion reaction	1	Odds Ratio (M-H, Random, 95% CI)	0.57 (0.02, 15.58]	Null, favours maternal donor



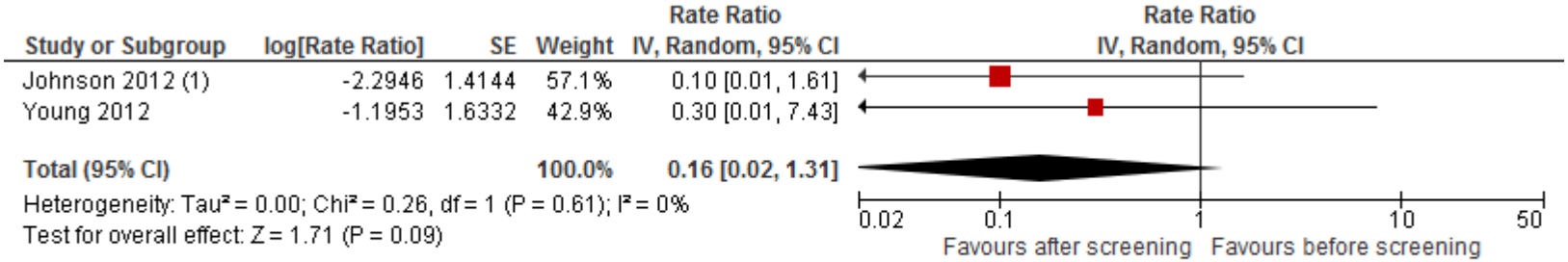
Change in hematocrit	1	Mean Diff (IV, Random, 95% CI)	0.20 [-3.37, 3.77]	Null



Abnormal creatinine	1	Odds Ratio (M-H, Random, 95% CI)	0.57 [0.02, 15.58]	Null, favours maternal donor



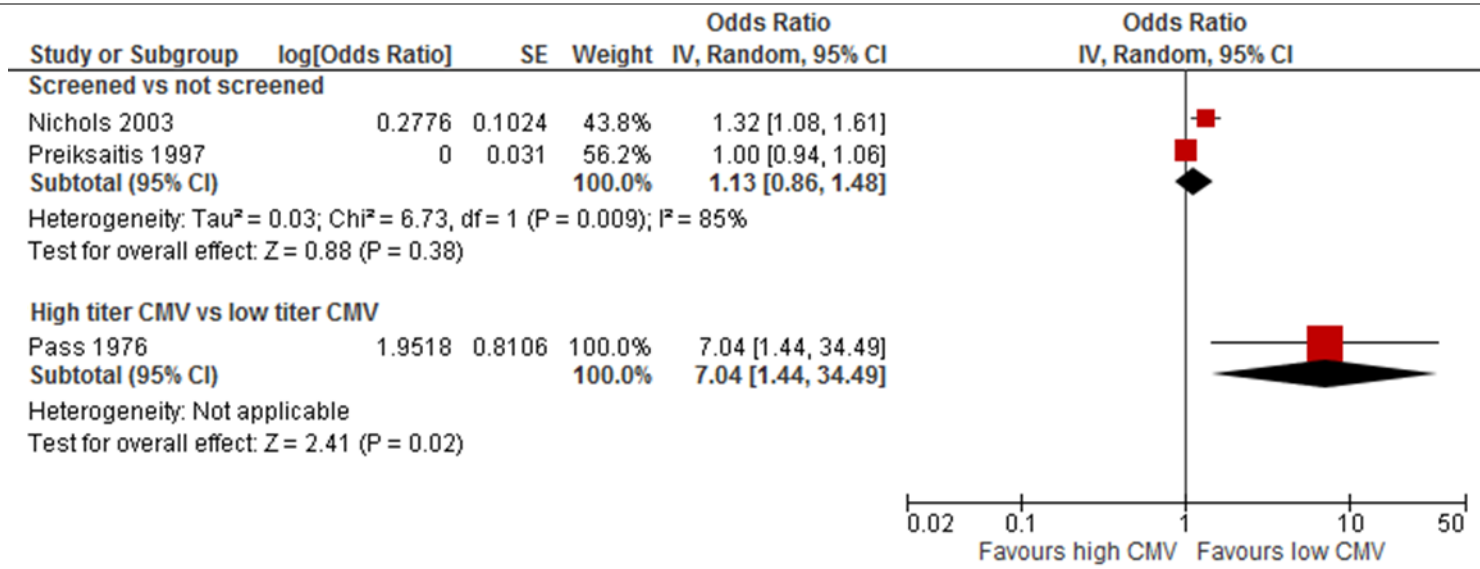
Abnormal bilirubin	1		Not estimable	
Babesiosis screening				
Babesiosis infection	2	Rate Ratio (IV, Random, 95% CI)	0.16 [0.02, 1.31], I^2 0%	Null, favours after screening



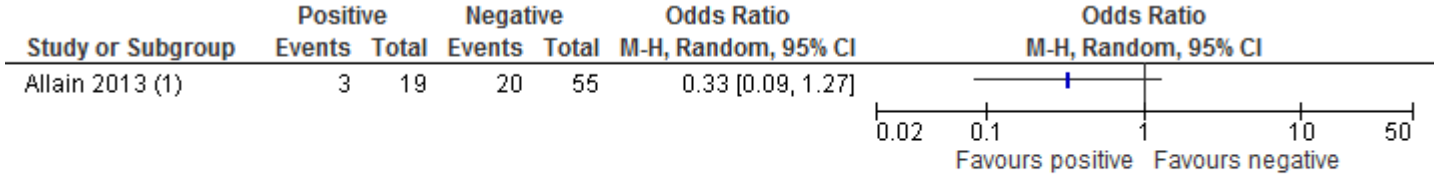
Footnotes

(1) 0.5 was added to the 0-cell after implementation of screening

CMV positive donors				
Seroconversion to CMV				
Screened vs not screened	2	Odds Ratio (IV, Random, 95% CI)	1.13 [0.86, 1.48], I^2 85%	Null, favours screened
High titer vs low titer	1	Odds Ratio (IV, Random, 95% CI)	7.04 [1.44, 34.49]	Favours low titer



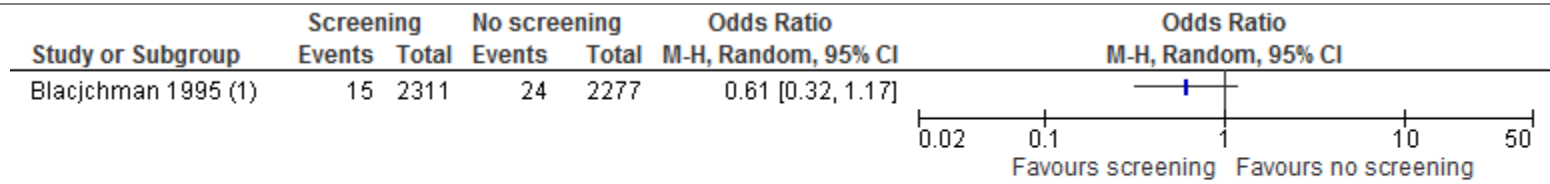
HBV positive donors			
Anti-HBc seroconversion	1	Odds Ratio (M-H, Random, 95% CI)	0.33 [0.09, 1.27]



Footnotes

(1) All had HBV, compares anti-HBs positive vs negative among ocult HBV infected patients

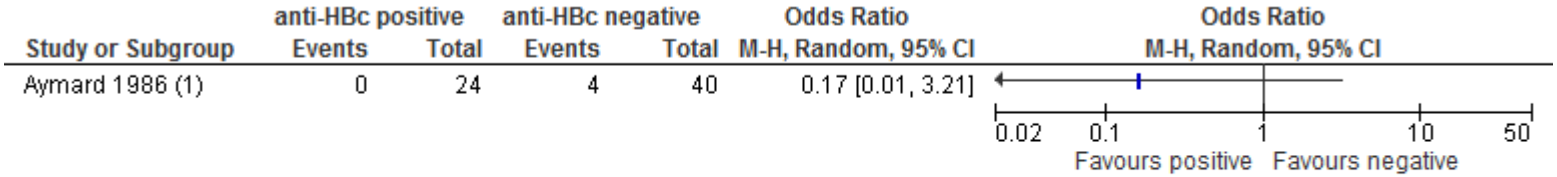
Any hepatitis	1	Odds Ratio (IV, Random, 95% CI)	0.61 [0.32, 1.17]



Footnotes

(1) Any type of transfusion hepatitis

Non-A non-B hepatitis	1	Odds Ratio (IV, Random, 95% CI)	0.17 [0.01, 3.21]	Null, favours anti-HBc positive

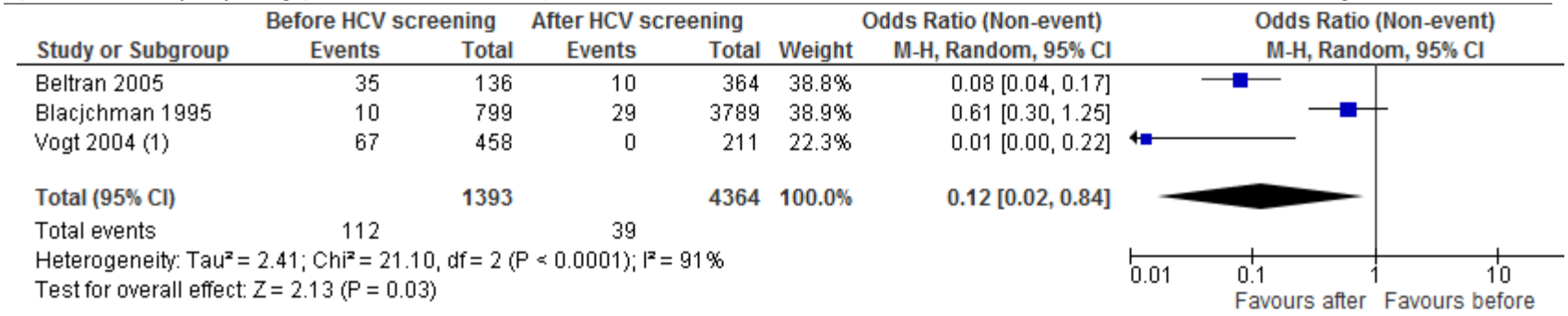


Footnotes

(1) Authors tested wheter anti-HbC positive donors was a surrogate predicter of non-A non-B hepatitis

HCV positive donors

HCV infection (before and after policy change)	3	Odds Ratio (M-H, Random, 95% CI)	0.12 [0.02, 0.84], I^2 91%	Favours after policy change



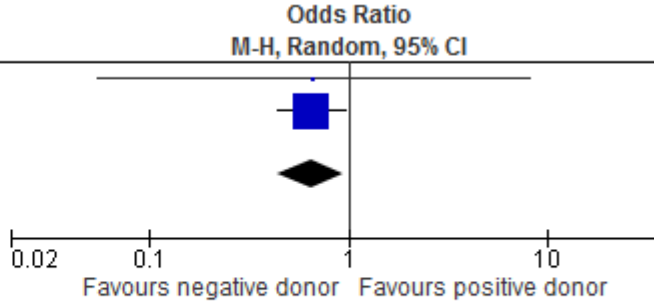
Footnotes

(1) The after policy is no use of fresh blood, warm blood or whole blood. Plasma use was predictor of infection.

HCV infection (positive vs negative blood)	2	Odds Ratio (IV, Random, 95% CI)	0.65 [0.44, 0.95], I^2 0%	Favours negative donor

Study or Subgroup	Negative donor Events	Total	Positive donor Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
O'Riordan 1998 (1)	1	4	5	15	2.3%	0.67 [0.05, 8.16]
UK Lookback 2002	481	831	91	134	97.7%	0.65 [0.44, 0.96]
Total (95% CI)		**835**		**149**	**100.0%**	**0.65 [0.44, 0.95]**
Total events	482		96			

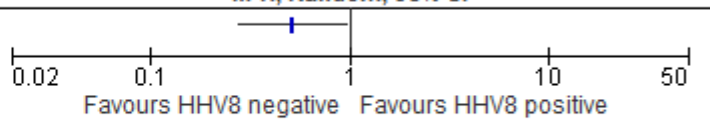
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.98); I² = 0%
Test for overall effect: Z = 2.20 (P = 0.03)



Footnotes
(1) HCV DNA positive vs negative donors

HHV8 positive donors				
HHV8 seroconversion	1	Odds Ratio (IV, Random, 95% CI)	0.52 [0.27, 0.98]	Favours HHB8 negative donor

Study or Subgroup	HHV8 positive Events	Total	HHV8 negative Events	Total	Odds Ratio (Non-event) M-H, Random, 95% CI
Hladik 2006 (1)	24	425	17	566	0.52 [0.27, 0.98]



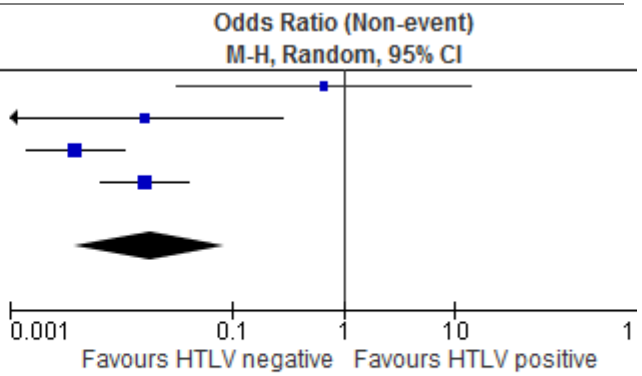
Footnotes
(1) Any type of blood, includes RBC transfusion

HTLV positive donors				
HTLV seroconversion	4	Odds Ratio (IV, Random, 95% CI)	54.87 [11.49, 262.01]. I^2 75%	Favours HTLV negative donor

Study or Subgroup	HTLV Positive Events	Total	HTLV Negative Events	Total	Weight	Odds Ratio (Non-event) M-H, Random, 95% CI
Herr 1993 (1)	6	10	1	2	15.6%	0.67 [0.03, 14.03]
Manns 1992	24	66	0	52	17.0%	0.02 [0.00, 0.28]
Okochi 1984	99	191	4	960	33.1%	0.00 [0.00, 0.01]
Sato 1992 (2)	108	1099	5	2794	34.2%	0.02 [0.01, 0.04]
Total (95% CI)		**1366**		**3808**	**100.0%**	**0.02 [0.00, 0.09]**
Total events	237		10			

Heterogeneity: Tau² = 1.65; Chi² = 11.92, df = 3 (P = 0.008); I² = 75%

Test for overall effect: Z = 5.02 (P < 0.00001)



Footnotes

(1) The exposure group is HTLV-1, the comparator is HTLV-II, the outcome is postive vs negative

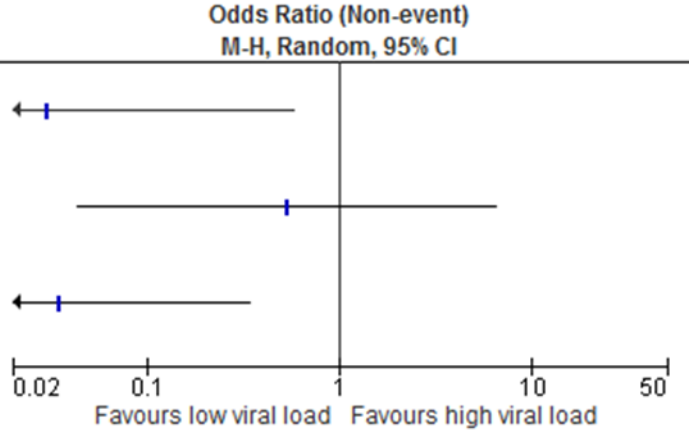
(2) Before and after implementation of a HTLV screening strategy

Parvovirus B19 positive donors

Seroconversion

DNA positive	1	Odds Ratio (M-H, Random, 95% CI)	0.03 [0.00, 0.58]	Favours low donor viral load
IgM positive	1	Odds Ratio (M-H, Random, 95% CI)	0.53 [0.04, 6.51]	Null, favours low donor viral load
IgG positive	1	Odds Ratio (M-H, Random, 95% CI)	0.04 [0.00, 0.34]	Favours low donor viral load

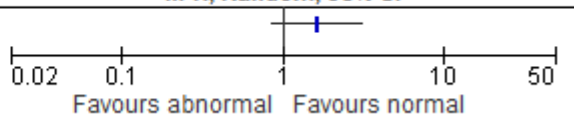
Study or Subgroup	High viral load Events	Total	Low viral load Events	Total	Odds Ratio (Non-event) M-H, Random, 95% CI
DNA positive					
Hourfar 2011	9	18	0	16	0.03 [0.00, 0.58]
IgM positive					
Hourfar 2011	2	18	1	16	0.53 [0.04, 6.51]
IgG positive					
Hourfar 2011	17	18	6	16	0.04 [0.00, 0.34]



Donor abnormal liver function test

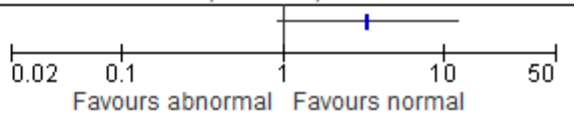
Post-transfusion hepatitis	1	Odds Ratio (M-H, Random, 95% CI)	1.63 [0.85, 3.12]	Null, favours normal donor liver function test

Study or Subgroup	Abnormal liver markers Events	Total	Normal liver markers Events	Total	Odds Ratio M-H, Random, 95% CI
Blacjchman 1995	24	2277	15	2311	1.63 [0.85, 3.12]



Post-transfusion HCV infection	1	Odds Ratio (M-H, Random, 95% CI)	3.39 [0.93, 12.35]	Null, favours normal donor liver function test

Study or Subgroup	Abnormal liver markers Events	Total	Normal liver markers Events	Total	Odds Ratio M-H, Random, 95% CI
Blacjchman 1995	10	2277	3	2311	3.39 [0.93, 12.35]



APPENDIX 1: SEARCH STRATEGY

Database: Embase Classic+Embase <1947 to 2013 December 31>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>
Search Strategy:

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1 blood transfusion/ or erythrocyte transfusion/ (168687)
2 ((rbc or red blood$ cell$ or red cell$ or erythrocyte$) adj5 transfusion$).tw. (18530)
3 1 or 2 (175580)
4 Blood Donors/ (46008)
5 ((rbc or blood or red cell$ or erythrocyte$) adj1 (donor$ or donation$)).tw. (53965)
6 4 or 5 (72574)
7 3 and 6 (16580)
8 (risk or mortality).mp. or cohort.tw. (5021473)
9 prognosis/ or treatment outcome/ (2048249)
10 predictor$.tw. (498103)
11 diagnosed.tw. (854089)
12 exp models, statistical/ (397700)
13 death/ (185240)
14 or/8-13 (7507718)
15 7 and 14 (5724)
16 15 use prmz (1893)

17 *blood transfusion/ (70716)
18 *erythrocyte transfusion/ (6471)
19 ((rbc or red blood$ cell$ or red cell$ or erythrocyte$) adj5 transfusion$).tw. (18530)
20 17 or 18 or 19 (88179)
21 *Blood Donors/ (20782)
22 ((rbc or blood or red cell$ or erythrocyte$) adj1 (donor$ or donation$)).tw. (53965)
23 21 or 22 (59904)
24 20 and 23 (8332)
25 (risk or mortalit$ or cohort).tw. (3978080)
26 follow-up.mp. or prognos$.tw. or ep.fs. (4675345)
27 25 or 26 (7304408)
28 24 and 27 (3007)
29 28 use emczd (1814)
30 16 or 29 (3707)
31 remove duplicates from 30 (2993)

Cochrane Library
Description:

ID Search
#1 MeSH descriptor: [Blood Transfusion] this term only
#2 MeSH descriptor: [Erythrocyte Transfusion] this term only
#3 rbc near/1 transfusion:ti,ab,kw or "red blood cell* NEAR1 transfusion":ti,ab,kw or red cell near/1 transfusion:ti,ab,kw or erythrocyte near/1 transfusion:ti,ab,kw (Word variations have been searched)
#4 platelet* near/1 transfusion:ti,ab,kw (Word variations have been searched)
#5 #1 or #3 or #4
#6 MeSH descriptor: [Blood Donors] explode all trees
#7 rbc near/1 (donor* or donation*):ti,ab,kw or "blood" near/1 (donor* or donation*):ti,ab,kw or "red cell" near/1 (donor* or donation*):ti,ab,kw or "erythrocyte" near/1 (donor* or donation*):ti,ab,kw (Word variations have been searched)
#8 #6 or #7
#9 #5 and #8

Cochrane Reviews (9)
Trials (130)
Technology Assessments (3)
Economic Evaluations (22)

CHAPTER 4 - MANUSCRIPT 3 - CLINICAL EFFECTS OF BLOOD DONOR CHARACTERISTICS IN TRANSFUSION RECIPIENTS: PROTOCOL OF A FRAMEWORK TO STUDY THE BLOOD DONOR-RECIPIENT CONTINUUM

4.1 PREFACE TO MANUSCRIPT 3

Manuscript 3 presents our detailed approach to study the donor-recipient continuum. It includes a brief overview of the rationale behind the study of blood donor characteristics, a proposed methods to collect the required data to study the clinical effects of blood donor characteristics on transfusion recipients, an analytical plan and potential exposures and outcomes. We also discuss how the results of such analyses may help inform current policies in blood donation and transfusion.

4.2 MANUSCRIPT 3

Chassé M, McIntyre L, Tinmouth A, Acker J, English SW, Knoll G, Forster A, Shehata N, Wilson K, van Walraven C, Ducharme R, Fergusson DA. Clinical effects of blood donor characteristics in transfusion recipients: protocol of a framework to study the blood donor-recipient continuum. BMJ Open 2015;5(1):e007412.

Clinical Effects of Blood Donor Characteristics in Transfusion Recipients: protocol of a framework to study the blood donor-recipient continuum

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KEYWORDS

Transfusion, Methodology, Blood Donor, Epidemiological Studies, Population Study, Health Care Services

WORD COUNT

Abstract: 299

Full text: 4719

PROTOCOL VERSION 1: 11 DECEMBER 2014

ABSTRACT

Introduction: When used appropriately, transfusion of red blood cells (RBC) is a necessary lifesaving therapy. However, RBC transfusions have been associated with negative outcomes such as infection and organ damage. Seeking explanations for the beneficial and deleterious effects of RBC transfusions is necessary to ensure the safe and optimal use of this precious resource. This study will create a framework to analyze the influence of blood donor characteristics on recipient outcomes.

Methods and analysis: We will conduct a multi-site, longitudinal cohort study using blood donor data routinely collected by Canadian Blood Services, and recipient data from health administrative databases. Our project will include the thorough validation of primary data, the linkage of the various databases into one large longitudinal database, an in-depth epidemiological analysis and a careful interpretation and dissemination of the results to assist the decision-making process of clinicians, researchers and policy makers in transfusion medicine. Our primary donor characteristic will be age of blood donors and our secondary donor characteristics will be donor-recipient blood group compatibility and blood donor sex. Our primary recipient outcome will be a statistically appropriate survival analysis post-RBC transfusion up to a maximum of 8 years. Our secondary recipient outcomes will include: 1-year, 2-year, and 5-year mortality; hospital and intensive care unit length of stay; re-hospitalization; new cancer and cancer recurrence rate; infection rate; new occurrence of myocardial infarctions and need for hemodialysis.

Ethics and dissemination: Our results will help determine whether we need to tailor transfusion based on donor characteristics and perhaps this will improve patient outcome.

Our results will be customized to target the different stakeholders involved with blood transfusions and will include presentations, peer-reviewed publications and the use of the dissemination network of blood supply organizations. We obtained approval from the Research Ethics boards and privacy offices of all involved institutions.

INTRODUCTION

Transfusion of red blood cells (RBC) is a necessary, lifesaving intervention. RBCs are given to increase oxygen delivery to tissues in clinical situations where the hemoglobin level is low (anemia). Approximately 1.1 million RBC units are collected and transfused each year in Canada[1,2]. They are used across a variety of medical and surgical scenarios. Approximately 30% of critical care patients, and more than 50% of cardiac surgery patients will receive blood products during their hospital stay[3,4]. Due to their common clinical use and the necessary precautions to ensure their safety, the use of blood products is associated with significant costs to the Canadian health care system. In 2012, the total Canadian Blood Services' budget for transfusion products was $473 million dollars[1] and close to $366 million dollars for Héma-Québec[2]. These totals do not account for the cost of administering the blood products or the additional societal costs associated with transfusion. For RBCs, the societal cost associated with a transfusion has been estimated to be greater than $400 per unit[5]. With more than 1.1 million RBC units delivered to Canadian hospitals and clinics in 2012, one can only begin to grasp the clinical and economic importance and impact of RBC transfusions on our health care system[1,2]. Given such a precious resource, we need to ensure that we maximize benefit and minimize risks in transfusion recipients.

Much effort has been undertaken over the last 30 years to mitigate risks and promote appropriate practice regarding the screening, collection, storage, distribution and transfusion of blood products. Indeed, the blood system has never been safer. However, there are potentially other factors that may contribute to transfusion safety that have not

been addressed. Transfusion of RBCs are used extensively in clinical practice and the identification of novel risk factors for adverse transfusion outcomes may generate new research hypotheses to improve the safety of RBC transfusion and potentially improve clinical outcomes.

Even if anemia is clearly associated with adverse outcomes, it is unclear if transfusion of RBCs will improve outcomes in all patients. In fact, several large and robust clinical trials[6–8] suggest that a liberal transfusion strategy (i.e. transfusing blood at a higher hemoglobin concentration) is not helpful and may be harmful as compared to a restrictive transfusion strategy. Additionally, a recent systematic review suggested that RBC transfusions may be associated with increased morbidity and mortality[9]. Seeking explanations for the beneficial and deleterious effects of RBC transfusions is necessary to ensure the safe and optimal use of a precious biological resource.

MITIGATING THE RISKS: CAREFUL SELECTION AND SCREENING OF DONORS

The risks associated with blood transfusion have been well documented and described[9–11]. Critical improvements in blood compatibility testing and red cell preservation at the beginning of the 20th century allowed the large-scale use of blood for both soldiers during times of war and the general population[12]. The discovery of blood-transmissible diseases such as syphilis and hepatitis B, and the tragedies of contaminated blood with hepatitis C and HIV in the 1980s and 1990s, prompted systems changes to markedly increase the safety of blood transfusion and a keen awareness of the infectious and

immunological risks associated with transfusion [10,11]. Because of interventions to decrease the risk of transfusion associated infections, the risk of transfusion-related infections is now very low (approximately 1 in 8 million for HIV, 1 in 6.7 million for Hepatitis C and 1 in 1.7 million for Hepatitis B)[13].

Recent evidence demonstrates that clinical outcomes following transfusion, including important mortality and morbidity, are impacted by many non-infectious transfusion adverse events including acute and delayed hemolytic reactions, Transfusion Related Acute Lung Injury (TRALI), Transfusion Associated Circulatory Overload (TACO), and hypotensive reactions. Other transfusion-related effects such as Transfusion Related Immunomodulation (TRIM) are well documented in terms of their biological effects[10], but their association with clinical outcomes is not clear. "Standard" blood products have important biologic variability that can create interactions between donor and recipient which can impact the clinical efficacy and safety of a blood transfusion. In situations where the benefits of an intervention are uncertain, and known risks are present, it is essential that risks are minimized and potential clinical benefits are maximized. Thus, we need to examine all factors that could contribute to morbidity and mortality from the effect of RBC transfusions.

BLOOD DONOR CHARACTERISTICS MAY AFFECT TRANSFUSION OUTCOME

Transfusion reactions, immune or non-immune mediated, may occur during, immediately following or be delayed in presentation after a transfusion and affect recipient outcomes. Although non-immune reactions such as citrate toxicity, air embolism and others

are unlikely to be related to donor characteristics, immune reactions may be. Immune reactions in the recipient are caused by either donor cytokines (such as interleukins and tumor necrosis factor) or antibodies present in residual plasma[3,10]. Reactions may range from benign fever to very severe outcomes such as anaphylaxis, shock or death[14].

Transfusion outcomes may however be affected by more than transfusion reactions. TRALI, an acute lung injury occurring within 6 hours after the transfusion of a blood product, is the leading cause of transfusion related mortality[15]. Recent epidemiological studies associate the presence of Human Leucocytes Antibodies and Human Neutrophil Antibodies in the donors' plasma, and a history of pregnancy with an increased risk of TRALI (OR 4.5 95% CI 1.08, 3.04) [15]. This led to a policy change to use predominantly-male plasma for transfusion and a subsequent reduction in TRALI (OR 2.57 95% CI 1.92, 3.86 for reduction of TRALI) in Canada[16]. Similar results using TRALI reduction strategies have been observed in the UK[17] and the USA[15].

TACO is a common adverse complication wherein pulmonary edema secondary to volume overload occurs following a blood transfusion. It is associated with increased mortality and hospital length of stay[18]. It is likely that other mechanisms beyond simple fluid overload is involved since 20% of reported cases of TACO followed the transfusion of only one RBC unit. Further, fever is often associated with TACO suggesting that there are characteristics of the blood that can contribute to events that are likely donor related[19].

Another major postulated mechanism for adverse outcomes after transfusion is TRIM. Immunomodulation secondary to transfusion has been associated with adverse

clinical outcomes such as infection, acceleration of cancer growth, multiple organ dysfunction and mortality after transfusion[3,20]. These transfusion-related adverse outcomes have all been suggested to be related to donor characteristics and such association needs further study.

HOW CAN THE DONOR-RECIPIENT CONTINUUM BE INVESTIGATED?

Studying the impact of donor and recipient factors on outcomes following RBC transfusion is difficult due to the independent nature of the blood system (donation and preservation) and health care settings (cross-matching and transfusion of recipients). To perform such a study, one has to be able to track blood from "vein to vein". The blood supply chain in most parts of the world, including Canada, is not structured to facilitate such analyses. Blood is usually collected, processed, and all information pertaining to the donor is confidentially stored in the central database of blood service organizations. No donor information is encoded on the RBC apart for the blood group and limited information regarding the collection and manufacture process. The blood is then delivered to hospitals where it is managed by the hospital's respective blood bank. Mechanisms exist to monitor transfusion-related reactions and a "per-case" analysis can be performed on blood units that may be associated with such reactions. If needed, the original donor may be tracked (for example, in cases of transmitted infections or TRALI), from a unique identifier on the blood unit. However, this is done manually and is labor intensive.

Hemovigilance has improved with the discovery of HIV, hepatitis B and C, and other blood transmissible pathogens. As a result, prospective registries of blood donors, tests

performed on blood, and the ability to trace blood has been implemented in many countries. Examples of such databases include The Serious Hazards of Transfusion (SHOT) database from the UK[21] and the Retrovirus Epidemiology Donor Study (REDS) from the US[22]. The SHOT database, which depends on outcome reporting by transfusing hospitals, records adverse reactions from blood transfusions; donor data and data for other transfused patients having no adverse event reported are not collected. The third phase of the REDS study will link donor and recipients to assess recipient outcomes in approximately 7 to 10 years[23].

Internationally, very few countries and/or organizations can follow blood from the donor to the recipient. Potentially the most advanced database of this kind is the Scandinavian Donations and Transfusions (SCANDAT) database, a joint project from Sweden and Denmark that provides complete follow-up on the outcome of transfused patients. It consists of a database linking the different blood donation organizations and transfused patients in clinical and administrative databases and a central data harmonization and cleaning[24]. This group demonstrated feasibility of "vein to vein" studies using this database[25–27]. The Danish Transfusion Database also has the ability to track blood from donors to recipients. However, this database is less focused on transfusion recipient outcomes, and more focused on blood use. Finally, to study blood utilization in their country, the Netherlands recently performed this type of linkage between donors and recipients, and published a study of transfusion practices[28].

In Canada, the blood supply is managed by Canadian Blood Services (CBS) in all provinces but Quebec and collects all donor-related data. There are also several data

collection strategies leading to large administrative databases within the Canadian health care system. Despite the potential to follow blood from donors to recipients with these vast and robust datasets, there is currently no interoperability between them. With this proposed framework, and given our information system expertise, we have the ability to create a “vein-to-vein” infrastructure, key to improving the current knowledge of the clinical impact of RBC transfusion. With our unique position, we aim to create a framework that will allow the investigation of the effect of blood donor characteristics on outcomes of transfusion recipients.

METHODS AND ANALYSIS

We will conduct a multi-site, retrospective, longitudinal cohort study using data collected from blood donors by Canadian Blood Services , and clinical short and long-term outcome data from hospital and provincial health administrative databases. This study will include the thorough validation of the primary data if needed, the linkage of the various databases into one large longitudinal database, an in-depth epidemiological analysis to answer our questions, and a careful interpretation and dissemination of the results to assist the decision-making process of clinicians, researchers and policy makers in transfusion medicine. Our study will serve as a framework to develop future blood transfusion surveillance programs to allow for the comprehensive monitoring of the blood donor-recipient continuum.

RESEARCH QUESTION

The target population will be all patients who receive a RBC transfusion. The studied exposures include donor age as the main exposure, and donor sex, donor-recipient compatibility and blood type as secondary exposures. Our primary outcome will be a statistically appropriate recipient survival analysis post-RBC transfusion up to a maximum of 8 years. Our secondary outcomes will include: 1-year, 2-year, and 5-year mortality; hospital and intensive care mortality; hospital and intensive care unit length of stay; re-hospitalization; new cancer and cancer recurrence rate; infection rate (Methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* as validated infectious outcomes and surrogates for hospital-acquired infections); new occurrence of myocardial infarctions and

need for hemodialysis (as a surrogate for severe chronic renal failure). These secondary outcomes were selected both based on the quality and accuracy of these outcomes in the source registries, and in order to cover a clinically representative range of adverse short and long-term events after transfusion (mortality, cardiovascular, oncology, mortality, infections, renal). The planned study timeframe will be from October 25, 2006 to December 31st, 2013. At this stage of the program, we will include the following hospitals in the Ottawa region: The Ottawa Hospital – General Campus, The Ottawa Hospital – Civic Campus, The University of Ottawa Heart Institute, and The Ottawa Hospital – Riverside Campus.

SOURCE OF DATA

We will obtain the data for the required analyses from different sources. Recipient data will first be obtained from The Ottawa Hospital Data (TOH) Data Warehouse. TOH Data Warehouse integrates data from several systems used at the hospital including, but not limited to patients, encounters, services, emergency visits, census information, health records abstracts, facility and capacity history and laboratory information services. The data are entered in their respective systems and is then transformed and reformatted to be stored centrally at TOH. Additional outcome data will be obtained from the Institute for Clinical Evaluative Sciences (ICES). ICES houses Ontario's health administrative databases. The most relevant ICES datasets for this study include the Canadian Institute for Health Information's Discharge Abstract Database, the Ontario Cancer Registry, the Ontario Health Insurance Plan database, the Registered Persons Database and the Ontario Drug Benefit

database[29]. Data linkage at ICES will allow us to measure patient survival beyond the initial hospitalization, and to collect information on further hospitalizations, renal and cardiovascular outcomes, as well as cancer-related information.

Donor information will be obtained from the CBS database. CBS database includes demographic information on blood donors, the units of all collected blood and the results of the biological tests performed on the individual blood donations. The objective of this database is to collect basic health information, high-risk activities and blood characteristics on blood donors, such as ABO group and microbiological testing. This information is then used to exclude high-risk donors before or after they give blood for safety of the donor or the recipients, and serve as repository of information for trace-back investigations of any adverse transfusion events[30].

IDENTIFICATION OF TRANSFUSED PATIENTS

We will include any patient, hospitalized or not, who received 1 or more allogeneic RBC units between October 25, 2006 and December 31, 2013. October 25, 2006 was the date where all blood products transfused started to be systematically stored centrally in the different included institutions. We will exclude patients who received autologous, directed or dedicated RBC transfusions.

IDENTIFICATION OF BLOOD DONORS

The donors will be identified from the unique RBC transfusion unit numbers from the units given to the recipient. We will not have any a priori exclusion criteria for the identification of donors.

DATABASE CREATION AND LINKAGE STRATEGY

We will first identify all patients over the 8-year period who received at least one allogeneic RBC unit. Patients who received blood up to December 31st 2013 will be included in the study. For each included patient, we will collect the age, sex, hospital service where the transfusion occurred, primary diagnosis, ABO and Rh blood type, co-morbid illnesses (using the validated Charlson index)[31–33], and pre-transfusion hemoglobin values. Short-term outcomes (hospital mortality, hospital and intensive care length of stays) will also be gathered from the TOH Data Warehouse (Figure 1).

Using the unique RBC unit number of each blood product transfused, we will perform a direct linkage with the CBS database to the corresponding blood donor and perform appropriate linkage and data harmonization for analysis (Figure 2). For long-term outcomes (mortality, re-hospitalization events, terminal renal failure, myocardial infarctions and cancer rates), we will use the patient's Ontario Health Insurance Plan number, to link information from the TOH Data Warehouse to patient data housed at ICES.

SAMPLE SIZE

From preliminary descriptive work performed at the included centers, we estimate that more than 200,000 RBC transfusions occurred during the study period, representing approximatively 35,000 unique blood recipients. The exact number of included patients and RBC units will be known once the linkage and validation process is completed. The number of blood donors associated to these patients cannot be estimated at this time.

DATA QUALITY AND VALIDATION

All blood donors in Canada except for the Province of Québec are required to register with CBS before donating blood. Therefore, the registry of blood donors is complete. Data regarding the blood donor is self-reported. CBS performs validation procedures to make sure that the information reported by the donor is encoded properly in the database. The same is true for the results of the tests performed on the blood units. Therefore, the information needed on the donor (unique identifier, age, sex, ABO and Rh group) is accurate and does not need validation for this project.

Data quality and accuracy for recipients will depend on the studied variable or outcome. Objective information such as age, sex and ABO and Rh group are expected to be valid as they are subject to internal validation. Age and sex are entered automatically upon scanning of the patient's health care number, and the ABO group is transferred directly from the laboratory information system. The TOH Data Warehouse contains clinical data regarding comorbidities using ICD-10CA codes for coding, as well as administrative information. The validity of these codes for exposure and outcome may vary from one

variable to another and incorrectly assuming the validity of these codes may lead to biased results[34,35]. To address possible bias, the validity of each variable will be assessed when needed. For codes and outcomes for which the validity is not known, random manual chart abstraction will be performed to compute the sensitivity and specificity of the code.

PRIMARY ANALYSIS

Our primary analysis will aim to investigate the association between donor age and recipient outcome. The most important analytical issue for our primary analysis is that of modeling multiple transfusions of varying transfusion donor ages[36]. Issues of analysis are difficult and none are ideal (mean donor age, median donor age, oldest donor age unit received, youngest donor age unit received etc.). We have considered the varying age of donors, and we will conduct an adjusted analysis by entering the donor age of each unit received as a continuous variable. In order to evaluate the effect of donor age on mortality in transfused recipients we will use clustered Cox regression analysis with time-dependent stratification, an approach used with success in prior similar studies[35,37,38]. The time dependent strata will be defined by the cumulative number of transfusions received over time. Time-dependent stratification allows us to account for mortality risk at each RBC transfusion until the end of follow-up (maximum 8 years) or death. Observations will be considered as censored for outcomes that did not occur by the end of follow-up (December 31, 2013). We will adjust for important risk factors and potential confounders including age of the recipient, sex, ABO and Rh blood type, donor-recipient ABO match, primary diagnosis, co-morbid illnesses (using the Charlson index), storage and manufacturing

characteristics. Continuous risk factors will be entered into the models as continuous measures rather than categorical to improve statistical efficiency. For time-varying covariates, time will be measured from the first transfusion. Regression diagnostics will be performed on all models.

SENSITIVITY ANALYSIS TO ASSESS THE ROBUSTNESS OF OUR PRIMARY ANALYSIS

While our primary analysis will consider donor age as a continuous variable, we will also assess the cumulative exposure to older donor blood and potential dose effects by categorizing donor age of transfused products using quartiles of the overall donor age distribution as discrete cut points as well as categorizing donor age in discrete categories (<18, <45, 45-60, >61). By treating donor age as categorical variables in the last 3 approaches, it will be possible to investigate dose-response relationships between age of blood donor and risk of death. We will also derive a population of patients that received only 1 blood transfusion over the timeframe of the study, hence obtaining a "pure" cohort of patients exposed to a given characteristic.

PLANNED SECONDARY ANALYSES

As per our primary analysis, we will implement Cox proportional hazard models with time dependent analysis to assess the effects of donor ABO and Rh blood types, donor-recipient compatibility and sex on the risk of mortality. New cancer rates, cancer recurrence rates, *Clostridium difficile* and Methicillin-Resistant *Staphylococcus aureus*, terminal renal

failure and myocardial infarction will be considered as categorical or counts and a similar analytical strategy as our primary outcome will be used. This method has been used before for similar exposures and outcomes[39]. Hospital and intensive care unit length of stay and re-hospitalization rates will be analyzed only for patients who were hospitalized and transfused and measured as "time-to-discharge" or "time-to-event". Therefore, Cox proportional hazard models will also be appropriate.

PLANNED SUBGROUP ANALYSES

Secondary analyses are planned for the following subgroups of patients: 1) 1 vs ≥ 2 RBC transfusion episodes while in hospital; 2) patients who received multiple transfusions from the same donor compared to those who received transfusions from multiple donors with comparable characteristics; 3) patient type (medical, surgical, trauma); 4) severity of illness at baseline, as measured by the Charlson index; 5) interaction between study exposures (age X sex, age X blood group) to investigate the presence of effect modification. The interaction analysis is important because patients of similar age may receive blood from different sexes, blood groups or other characteristics. A significant interaction would suggest a different impact of age depending of the other characteristics of the donor. The analytic approach described above for both primary and secondary outcome measures will be undertaken in all subgroup analyses. These analyses will primarily be hypothesis-generating and hypothesis-supporting in nature.

RISK OF BIAS AND CONFOUNDING

Epidemiological studies are subject to selection and information biases. For this proposal, the risk of selection bias is limited since we will include the entire population of patients transfused during the study timeframe and we will use a complete registry that encompasses all blood donors. Regarding information bias, the studied exposures such as age, sex, blood groups, and blood mismatch are objective and accurately encoded in validated registries.

To obtain valid estimates of association between an exposure and an outcome, the *usual strategy* in cohort studies is to adjust for known confounders. Unknown confounders are then unaccounted for and can be distributed unevenly between exposed and non-exposed groups, confounding the effect estimates. Randomization is used to evenly distribute these unmeasured confounders between groups and reduce their impact on the effect estimation, moving observed effects towards the null hypothesis. By the nature of transfusion exposure, the unmeasured confounders will also tend to be evenly distributed between groups. Donor characteristics are always strictly concealed from any caregiver in the hospital where the blood units are used and blood is distributed in a random manner across hospitals. Therefore, the exposure of interest is randomly distributed among recipients. Because of this concealment of the identity and characteristics of the donors, our study will also have similarities with features usually seen in RCTs which protect from sources of bias, such as allocation concealment (allocation of the donors' characteristics is concealed from any health personnel involved in the transfusion of the blood product) and double-blinding (neither the clinicians nor the patients know donors' characteristics)[40,41].

We will carefully document potential confounders on the donor side (sex, blood group, blood characteristics, preservatives, different manufacture processes) and on the recipient side (age, comorbidities, severity of illness using Charlson index) and adjust for them if appropriate.

Use of inclusive and valid databases from ICES and the TOH Data Warehouse will also limit the number of missing values. Although we expect missing values to be minimal, we will report them and perform appropriate multiple imputation and censoring if we find missing covariates and outcome values.

ADJUSTMENT AND STRATIFICATION FOR MANUFACTURE CHARACTERISTICS

The blood product preparation process following donation of RBCs may affect transfusion outcomes and involves a number of manufacturing steps which may affect transfusion outcomes including the anticoagulant solution, preservative and potentially the duration of storage. At the time of donation, whole blood is collected into a plastic bag with an anticoagulant solution (citrate, phosphate and dextrose). The whole blood is then centrifuged and the RBCs are separated from other blood constituents and filtered to reduce the amount of leukocytes (leukoreduction). In Canada, SAGM (saline, adenine, glucose, and mannitol) is added to optimize red cell storage and survival. Once processed, the RBCs are stored in sterile bags for up to 42 days at a temperature between 1°C and 6°C[42]. Any step of this process has the potential to affect transfusion outcome. RBC storage solutions such as AS-3 (citric acid, phosphate, chlorine, adenine, dextrose, citrate) and SAGM, have been demonstrated to increase the survival of red cells after transfusion. The

AS-3 solution has been shown to increase storage time and to provide better quality of RBCs[43]. Regarding storage time, retrospective studies, prospective non-randomized studies, systematic reviews and meta-analysis reached conflicting results regarding the impact of storage time of red blood cells on recipients' outcome[44–47]. Our randomized clinical trial in a vulnerable and heavily transfused population of neonates found no clinical benefit of fresh RBCs[48]. Prospective randomized trials are underway to try and answer this question[49]. While awaiting the results of these important clinical studies, this project will have to take into consideration the potential impact of these potential confounders by describing their distribution in the transfused population, adjusting or stratifying when appropriate.

ETHICS AND DISSEMINATION

Ethics and privacy

By the nature of the data used for this project, individual patient consent will be waived, in accordance with the Ontario Personal Health Information Protection Act. We obtained approval from the Research Ethics boards of all involved institutions, as well as from privacy offices at CBS, ICES and the TOH Data Warehouse. All data collection and management will be performed in accordance with the *Personal Health Information Protection Act of Ontario, Regulation 329/04.* All patients will be identified by a unique anonymous number and no patient identifiers will be stored with clinical data. The resulting database will be encrypted and stored centrally. Access will be restricted and only designated members of the study team will have access to the dataset.

Knowledge transfer plan

The results of our study will help determine whether we need to tailor blood based on donor characteristics and perhaps this will improve utilization of blood, and more importantly, improve patient outcomes. Our study will have a significant impact on the way we evaluate blood transfusions. Having the capacity to rigorously study blood from "vein to vein" will allow the pursuit of many new hypotheses regarding the use of blood products. An example of application of our study results would be to confirm the current age policy in blood donation. On the other hand, different outcomes according to age would support further study. For example, adverse outcomes associated with blood donated by older donors may only result in adverse outcomes for specific recipient populations, such as patients with significant comorbidities. This new information will inform policy makers and transfusion organizations regarding current policies to either support them, or to use this new information as a foundation for further research in the field of transfusion medicine. It may lead to the development of validation or prospective studies regarding factors that may affect outcomes of RBC transfusion.

From the early development of this research project, we involved stakeholders and experts in a wide range of fields involved with the organization, research and the care of patients receiving transfusions (hematologists, intensivists, transfusion specialists, transplantation specialists, health-care researchers, epidemiologists, blood organization decision makers and senior scientists). This diversity of expertise will ensure that the research questions, objectives, methods and result analysis and interpretation answer pertinent questions for clinicians, but also for stakeholders, patients and the overall

population. Our design will allow us to perform the required analyses to impact on the major blood users. The results will also inform blood supply organizations policies.

Results obtained from this research project will be customized to target the different stakeholders involved with blood transfusions. For clinicians and researchers, traditional dissemination will be used, including publication in relevant peer-reviewed medical journals, presentations at local, national and international conference and meetings. We will also use the dissemination network of CBS, for example, by presenting the results on their website for the general public. We will ensure that publications resulting from this work are open-access. We will work closely with the different stakeholders of CBS and the different clinical specialties to provide reports for the specific needs and realities of their organizations/disciplines. Many additional stakeholders such as other medical specialty organizations involved in transfusion, other blood supply organizations such as the Red Cross in the United-States may benefit from the results of this study. In addition to traditional dissemination strategies we plan to directly reach out to these organizations to present our results, and the team members will be readily available for further discussions, meetings or presentations to answer their specific needs and questions.

Through our study, we will develop an analytical framework that will enable the study of factors that may affect outcomes related to blood transfusion. We believe that our project will shed light on important hypotheses such as the influence of blood donor age and sex, and potential other characteristics in transfusion recipients, and will allow to support (or not) current policies, and potentially suggest new research ideas to improve the

safety of our blood system. Such findings will have implications for the blood and healthcare systems, donors, and clinicians.

CONTRIBUTORSHIP STATEMENT

All authors participated in conceiving this study. MC, DF, AT, JA, SEW, LM, KW, GK, AF, CvW and NS secured its funding. MC, AT, JA, RD and DF obtained ethics and privacy approval. MC and DF developed the analytic plan. MC drafted the manuscript. All authors provided input into the protocol, critical feedback on the manuscript, and approved the final manuscript.

LIST OF ABBREVIATIONS

RBC: Red Blood Cell

TRALI: Transfusion Related Acute Lung Injury

TACO: Transfusion Associated Circulatory Overload

TRIM: Transfusion Related Immunomodulation

SHOT: The Serious Hazards of Transfusion

REDS: Retrovirus Epidemiology Donor Study

SCANDAT: Scandinavian Donations and Transfusions

CBS: Canadian Blood Services

TOH: The Ottawa Hospital

ICES: Institute of Clinical Evaluative Sciences

COMPETING INTERESTS

The authors declare that they have no competing interests.

SOURCE OF FUNDING

This work was supported by a Canadian Blood Services and Canadian Institute of Health Research Partnership on Blood Supply Risk, grant number CIHR-BSR201403-DF-326493. JA is

Scientist at Canadian Blood Services and participated in conceiving this study. The Canadian Institute of Health Research had no role in this study.

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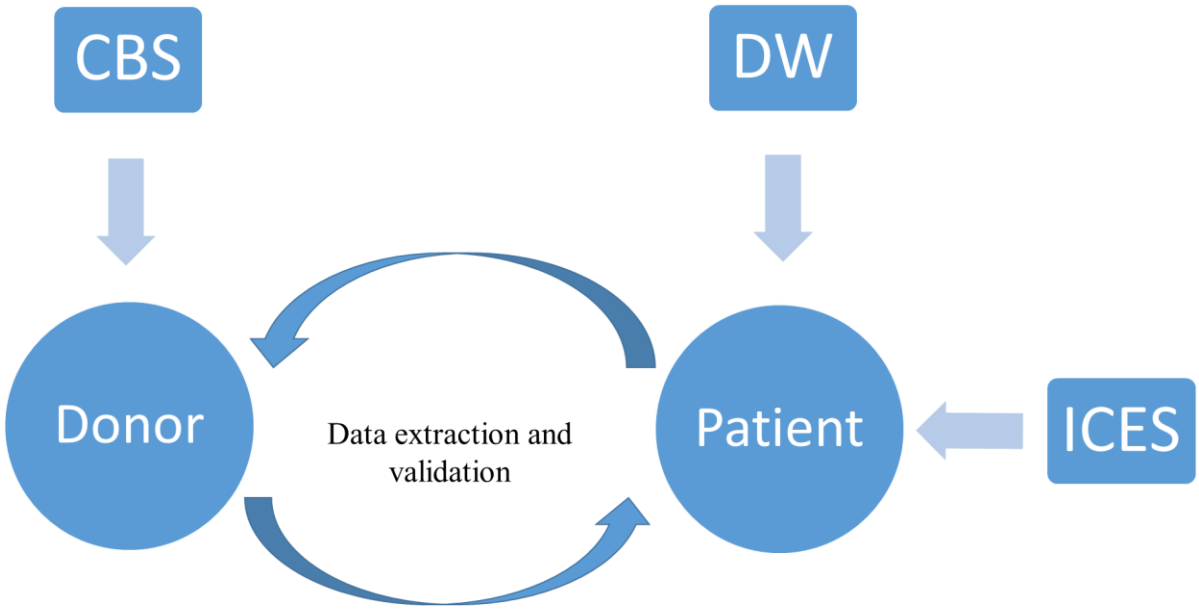
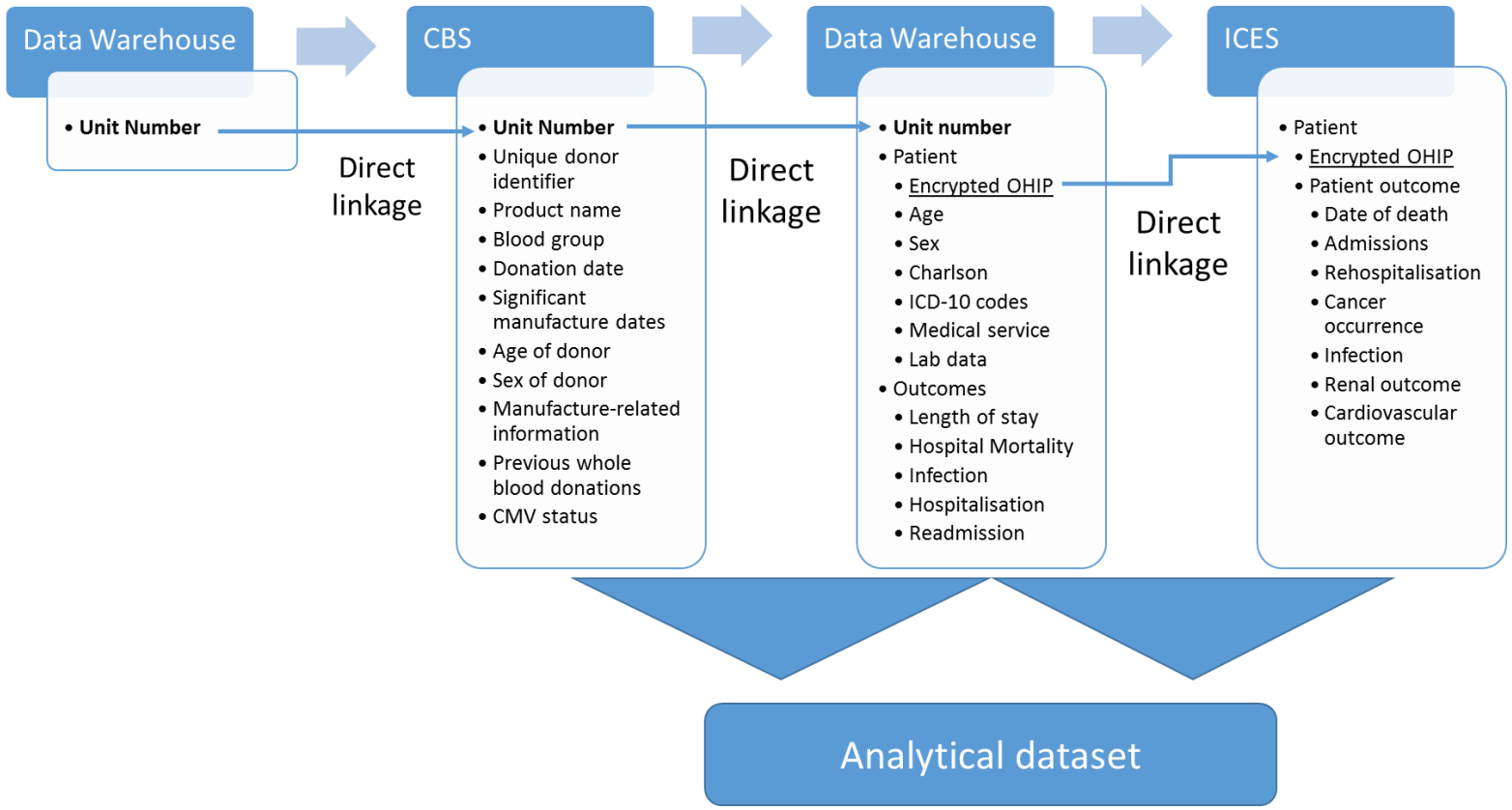
FIGURE 1: SCHEMATIZATION OF THE FRAMEWORK



ICES: Institute for Clinical Evaluative Sciences; CBS: Canadian Blood Services; DW: Data Warehouse

FIGURE 2: FIGURE 2: DATA SOURCES AND LINKAGE STRATEGY



ICES: Institute for Clinical Evaluative Sciences; CBS: Canadian Blood Services; DW: Data Warehouse; OHIP: Ontario Health Insurance Plan

CHAPTER 5 - MANUSCRIPT 4 - ASSOCIATION OF BLOOD DONOR AGE AND SEX WITH SURVIVAL AFTER RED BLOOD CELLS TRANSFUSION

5.1 PREFACE TO MANUSCRIPT 4

After developing the analytical framework for the blood donor-recipient continuum, we conducted the primary analyses of the thesis project. We evaluated the effects of donor age (primary exposure) and donor sex (secondary exposure) on RBC transfusion recipient survival. Adjusted extended Cox regression models were developed to account for the multiple transfusions a patient may receive over time and to consider that each recipient may receive units from different donors, with different donor characteristics. We evaluated the robustness of our analyses by performing pre-specified sensitivity and subgroup analyses. We also provided supportive evidence that donor characteristics are distributed at random among RBC recipients, which is a significant methodological strength.

5.2 MANUSCRIPT 4

Chassé M, Acker J, English S, Forster A, Knoll G, McIntyre L, Ramsay T, Shehata N, Tinmouth A, van Walraven C, Wilson K, Fergusson DA. Association of blood donor age and sex with survival after red blood cells transfusion

This manuscript is in preparation for publication

Association of blood donor age and sex with survival after red blood cells transfusion

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ABSTRACT

Background: Red blood cells transfusion is the most frequent intervention administered in hospitalized patients in North-America. Transfusions are given to improve patient outcomes. However, its use has also been associated with poor outcome. How transfusion of RBC can cause harm is poorly understood. The aim of our study was to investigate the effect of donor age and sex on RBC recipient survival.

Methods: We conducted a longitudinal cohort study involving 4 centers to investigate the association of donor age and sex with survival after RBC transfusion. After linkage of blood procurement organization data with clinical and administrative data, we estimated the chances of survival of RBC transfusion recipients using extended Cox regression models with time-dependent stratification. The primary exposure of our study was donor age and secondary exposure was donor sex. Our primary outcome was recipient survival.

Results: From 25 October 2006 to 31 December 2013 we identified 30,503 RBC transfusion recipients that received a total of 187,960 RBC transfusions from 80,755 blood donors. Younger donor age was associated with higher mortality over the study period (adjusted HR 1.10; 95% CI 1.08 to 1.12 for each additional RBC unit transfused of donors aged 17-20; $p<0.001$; adjusted HR 1.08; 95% CI 1.05 to 1.10 for each additional RBC unit transfused of donors aged 20-30); $p<0.001$). RBCs from female donors were associated with higher mortality (adjusted HR 1.06; 95% CI 1.05 to 1.07 for each additional RBC unit transfused; $p<0.001$).

Conclusions: Cumulative transfusion of young age donors and female donors RBCs was associated with increased mortality in a large cohort of transfused patients. Our findings suggest that the characteristics of the blood donors may affect transfusion outcome.

BACKGROUND

Transfusion of Red Blood Cells (RBC) is the most common medical procedure in contemporary medicine[1]. With the main objective to improve oxygen delivery to tissues[2], transfusion of RBC is used in a variety of medical situations, ranging from the low-grade anemic patients, to the acutely and massively bleeding patient[2–5]. The literature is extensive regarding the best indication for the use of RBC products, but the evidence is limited regarding the type of blood products we should administer. When decision to transfuse has been made, usual practice is to order one or more recipient compatible RBC units from the blood bank. Limited characteristics of the unit can be requested, such as CMV status or particular manufacture characteristics (leukoreduced, irradiation) to improve transfusion outcome[6]. Evidence of such benefit in recipients is scarce. Recently, it has been hypothesized that age of blood may have an effect on RBC recipient outcome. Yet, three large randomized controlled trial examining this effect in critically ill adults, neonates and cardiac surgery patients found no such association[7–9].

The transfusion community is keenly aware of the risks associated with transfusion and a variety of measures, including donor questionnaires assessing risk, improved infectious disease screening, and other quality measures, are implemented to improve the safety of transfusions[10,11]. They are however mainly aimed at reducing donation risks for the donor, and infection transmission in the recipient. Donor age and sex have been considered as potential factors influencing RBC transfusion outcome (chapter 3). We hypothesized that donor age and sex may be associated with survival after transfusion of RBC.

METHODS

STUDY DESIGN AND SETTING

We conducted a 4-center longitudinal cohort study to investigate the association of donor age and sex with recipient survival after RBC transfusion. A detailed version of the study design and methodology has been published[12]. Briefly, we obtained blood donor data collected at time of blood donation from Canadian Blood Services, short-term descriptive and outcome data from hospital clinical and administrative databases and long-term outcome data from the Institute for Clinical Evaluative Sciences of Ontario in Canada. Included centers were the following hospitals in the Ottawa region: The Ottawa Hospital— General Campus, The Ottawa Hospital—Civic Campus, The University of Ottawa Heart Institute and The Ottawa Hospital—Riverside Campus. This study was approved by the Ottawa Hospital Research Ethics Board (protocol #20140111-01H) and the Canadian Blood Services Research Ethics Board (protocol #2014.004). The study was funded by a Canadian Blood Services and Canadian Institute of Health Research – Institute of Circulatory and Respiratory Health partnership on Blood Supply Risk (grant # BSR201403-DF-326493)

RECIPIENT POPULATION

We included all patients of any age that received at least one RBC transfusion between October 25, 2006 to December 31, 2013 and had a valid Ontario Health Insurance Program number (OHIP). The October 25, 2006 date was chosen as it represents the date when all blood products transfused were systematically stored centrally at the 4

included institutions. The patient had to have a valid OHIP number in order for outcome data to be obtained from Provincial databases. We applied no additional eligibility criteria. We excluded patients who received blood units that could not be linked to CBS because they were produced by a different blood collection agency.

DONOR POPULATION

Donor information was obtained from CBS blood donation databases that includes all blood donations in Canada except for the Province of Québec. CBS databases include demographic information on blood donors as well as manufacture characteristics and screening tests results of each unit of blood collected.

DATA COLLECTION

We *a priori* defined the exposures, covariates and outcomes of interest[12]. After REB approval, privacy assessment by the involved organizations (Canadian Blood Services, 4 hospitals, the Institute for Clinical Evaluative Science), and legal assessment for data sharing, we deterministically linked donor information for each RBC unit transfused at the study centers. Blood donor characteristics were provided for each unit transfused. We then linked to hospital-based administrative datasets to determine data about each recipient including recipient age, recipient sex, transfusion history and medical comorbidities. The resulting dataset was then linked deterministically to a population-based registry to determine if and when patients died. The resulting datasets therefore allowed the evaluation of the complete donor-recipient continuum.

STUDIED EXPOSURES

Our main exposure of interest was donor age and our secondary exposure was donor sex. This information was collected at time of donation for each RBC unit transfused. We also collected the date of donation, donor blood group, number of previous whole blood donations, manufacturing characteristics (preservative, bags, separation time, CMV testing, irradiation).

For transfusion recipients, we collected the date and time of each RBC transfusion, the transfusion of any other blood product or derivatives that were transfused for each patient, hospital administrative information (admission and discharge date, discharge disposition), sex of recipient, age of recipient at time of transfusion and laboratory results of blood group testing. For transfusions that occurred in hospital, we also collected the individual elements of the validated Charlson index to obtain the main comorbidities of transfused patients[13–15]. For transfusions that occurred out of hospital, the elements of the Charlson index were coded as not available at time of transfusion (i.e. dummy coded).

STUDY OUTCOME

Our primary study outcome was recipient survival time from first RBC transfusion. To obtain the most accurate measure of our outcome, we identified dates of death using the Registered Persons Database of Ontario. Patients who did not have a death record at the time of completion of this study were assumed alive up to the termination date and censored on December 31, 2013.

DATA ANALYSIS

The principal analysis was an extended Cox regression analysis with time-dependent stratification[16,17]. The overall age and sex of blood donors to which an individual recipient can be exposed changes over time with each additional RBC unit transfused. The time-dependent strata were therefore the cumulative number of RBC transfusions received by any given patient over the study time period. Considering that a patient may receive RBC transfusions from donors of different ages, the donor age at time of transfusion was categorized in groups of 10 years to allow the counting process to occur at time of each transfusion. This strategy allowed us to account for the cumulative risk of receiving RBC units from each donor age group over time (dose-response relationship). We adjusted for important risk factors and potential confounders including age of the recipient, recipient sex, and comorbid illnesses (using the Charlson index). All covariates (except sex) were captured over time and were included as time-varying covariates. We had no missing values for age and sex for both donor and recipient. Missing values for comorbidities were likely for patients who received transfusions as outpatients only. Those patients were still included in the main analysis by using a dummy missing variable in the regression model. We used the same analytical strategy described above for donor sex, but using donor sex as the counting variable (rather than age) to build the model.

To assess the robustness of our findings, we performed two additional a priori planned sensitivity analyses for patients that received RBC transfusions from female donor only

versus male donor only, and from patients that received only blood from young donors versus older donors. The categorization choice of age level for categorization was based on the distribution of effect in our main analysis. One post-hoc sensitivity analysis consisted in the inclusion of the ratio of each age group to the other age groups, and the ratio female to male. This ratio was updated at each transfusion event. Our subgroup analyses compared male recipients to female recipients, recipient age (<1, 1-18, 18-65, >65) and recipient severity of illness (using the Charlson score). All tests of statistical inference reflect a 2-sided α=.05. Analyses were performed using SAS software, version 9.4 (SAS Institute).

RESULTS

From October 25, 2006 to December 31, 2013, 32,798 patients received at least one RBC transfusion. 2,279 patients were excluded because they did not have a valid OHIP number. An additional 16 patients could not be linked to ICES. Finally, 133 RBC units were excluded because the units were obtained from a different blood collection agency. Thus, our cohort for analysis consisted of 30,503 recipients, 80,755 donors, and a total of 187,960 transfusion episodes (Figure 1). Mean patient follow-up was 2.3 (SD 2.1) years and maximum follow-up time was 7.2 years.

Characteristics of the included recipients and donor populations are presented in Tables 1 and 2. The mean age of recipients was 66.2 (18.2) years. Fifty-two percent (n = 15,906) were females. The proportion of patients who had a Charlson Score of 5 or more was 22.8% (n = 6,314). The median number of units received per patient over the course of the study was 3 (2-6). The median age of donors was 42.0 (27.0 to 52.0), 48.7% were female, and they had a history of a median of 5 (1 to 17) whole blood donations.

The characteristics of the products transfused are reported in table 3. All transfused units were leukoreduced. The most commonly used preservative was SAGM (73.6%, n=138,328) and AS-3 (24.7%, n=48,314). A total of 32.1% (n=60,334) of the units were produced using the buffy coat method. The median storage age of the RBC units transfused 17 days (IQR 13, 23). A significant proportion of patients also received other blood products (n = 12,343; 40.5%)(Table 4).

PRIMARY ANALYSIS

Death occurred in 43.0% (n=13,118) of the patients included in our study. The hazard of death was significantly higher for patients receiving RBC transfusions from donors below 20 years (adjusted HR 1.10; 95% CI 1.08 to 1.12 for each additional RBC unit transfused; p<0.001) and 20 to 30 years (adjusted HR 1.08; 95% CI 1.05 to 1.10 for each additional RBC unit transfused; p<0.001). Transfusion of RBC from donors above 70 years was associated with improved survival when compared to patients receiving RBC transfusions from younger donors (adjusted HR 0.91; 95% CI 0.84 to 0.98 for each additional RBC unit transfused, p=0.02) (Figure 2 and Table 5). The transfusion of each additional unit from a donor under 30 years was associated with a reduced survival (adjusted HR 1.09; 95% CI 1.05 to 1.07 for each additional RBC unit transfused; p<0.001) (Figure 3). In a similar analysis (Table 6), increased mortality was observed after transfusion of a 1.0 ratio of RBCs from donors aged below 20 years (adjusted HR 2.25; 95% CI 1.43 to 3.53 compared to a ratio of 1.0 of donors aged ≥ 70 years; p<0.001) and between 20 and 29.9 years (adjusted HR 1.94; 95% CI 1.24 to 3.04 compared to a ratio of 1.0 of donors aged ≥ 70 years; p<0.001).

Donor sex was also associated with survival after RBC transfusion (Figure 2 and Table 5). The transfusion of each additional female blood donor unit was associated with a worse survival (adjusted HR 1.06; 95% CI 1.05 to 1.07 for each additional RBC unit transfused; p<0.001) (Figure 3). The association was the opposite for male donors (adjusted HR 0.98; 95% CI 0.98 to 0.99 for each additional RBC unit transfused, p<0.001). A similar observation was observed for the transfusion of a 1.0 ratio of female donor RBC units (adjusted HR 1.49;

95% CI 1.35 to 1.55 compared to a 1.0 ratio of male donor units at study mean of 6 RBC units; p<0.001).

SENSITIVITY AND SUBGROUP ANALYSES

On subgroup analyses (Table S1), young donor age and female sex was associated with a reduced survival in both male and female recipients. The maximum risk was in male receiving blood from the youngest donor strata (adjusted HR 1.14; 95% CI 1.11 to 1.16 for each additional RBC unit transfused; p<0.001). Receiving blood from older donors was not associated with survival in either recipient category. Male donor transfusions were not associated with survival in female recipients but was associated with marginally improved survival in male recipients (adjusted HR 0.98; 95% CI 0.97 to 0.98 for each additional RBC unit transfused; p<0.001).

Very few mortality events occurred in the age groups < 1 years (n=64) and 1 to 18 years (n=19). Each additional transfusion of blood unit from any age and sex donor group in children increased the risk of death except for the older donor groups and male donors among children between 1 and 18 years. Our subgroup analyses of patients aged between 18 and 64 or 65+ years produced similar results to our main analysis.

When stratifying by the number of comorbidities using the Charlson Index, the association between female donor sex and young donor age was observed among patients with the lowest scores (0 and 1-2). Among the patients with a Charlson index of 3 and 4, young donor age was still associated with reduced survival. However, the increased risk of death per cumulative number of transfusion was comparable for Female and Male donors as well

as for donor age, for recipients with a Charlson Index above 3. Results of our analyses among patients who did not have a Charlson index available also returned comparable results to our main analysis.

We performed two additional pre-specified sensitivity analysis for the subpopulation of patients receiving transfusions from young versus older donors (n=16,133), or from male only versus female only (n=11,176) (Table S2). The age of 30 years was selected to define the young versus older donor cohort because of the findings in our main analysis where the risk was the highest below 30 years. There was a significant interaction between donor age and the number of units transfused ($p < 0.001$). Therefore the results are reported by strata of transfused units. Donor age < 30 years was associated with a reduced survival at each transfusion strata after the first transfusion (adjusted HR 2.80; 95% CI 1.92 to 4.11 at subgroup mean of 4 units transfused; $p<0.001$). Significant interaction was also present for donor sex. At first transfusion, the risk of death was higher after female donor transfusions, but the difference was not significant after adjustment.

DISCUSSION

Our large cohort study aimed at evaluating the effect of donor age and sex on recipient survival after RBC transfusion. We found a significant increase in the risk of mortality for recipients that received RBCs from young donors as well as from female donors. Our findings were not affected by statistical adjustment and were consistent across subgroups. Sensitivity analyses also supported our findings.

The fact that younger donors were associated with poor survival was unexpected. An animal study suggested improved cognitive function and synaptic plasticity in mice that were transfused young blood[18]. The authors suggested that there were rejuvenation effects of blood obtained from young donors. In a similar study in mice, it has been suggested that young blood may reverse age-related cardiac hypertrophy[19]. However, no clinical studies have supported such association. Five studies including a total of 586 recipients assessed donor age and its association with outcome[20–24]. Four of these studies reported no association with outcomes (Transfusion Related Acute Lung Injury, risk of HIV and HTLV transmission). One study[22] evaluated the risk of mortality in patients receiving plasma. In the subgroup of patients who received RBC transfusions, patients who died received blood from donors in average 6.24 younger (MD -6.24 [-12.43, -0.05], unpublished data, see Chapter 3).

Because of the observational nature of our study, our findings are not evidence that young age is causal in the survival pathway of transfusion recipients. It however suggests that biological or environmental factors affecting young donors, on average, may influence the resulting RBC products transfused. The resulting association with mortality may be caused

by such factors and that by removing those donors from the donor pool, this association could be mitigated, or even reversed. Therefore, using the current blood screening procedures in Canada, young donor blood is no better than older blood in regard to post-transfusion survival. Our observation that young blood, on average, may in fact be associated with increased mortality in transfusion recipients does not support the use of young blood for its therapeutic effects and warrants further epidemiological study to elucidate potential mechanisms.

One potential explanation for our findings may be related to the healthy-donor phenomenon[25]. This phenomenon is related to the fact that one must be in a relatively good state of health to consider donating or to be permitted to donate blood. Potential blood donors undergo a screening questionnaire that can lead to donation deferral if the provided responses are deemed inappropriate. Also, patients who are unwell are less likely to give blood, thus excluding the sickest patients from donating blood. It has also been shown that long-term blood donors are healthier than short-career donors[25]. It is therefore possible that our observation is related to the impact of a healthy-donor effect, where young donors may not be aware of an ongoing medical condition that may affect recipients, whereas with age, potential sicker donors exclude themselves from the donor pool.

We found that the transfusion of a cumulative number of female-donor units was also associated with worse survival. In normal physiologic conditions, male and female RBC are comparable regarding their O_2 delivery capacity, their deformability and composition[26,27]. Sex differences however exist when erythrocytes are exposed to adrenaline. It has been shown that the enzymatic activity (acetylcholinesterase) decreases in females but not in

males, there is an increased RBC membrane rigidity and a decreased affinity to oxygen[26]. These perturbations at the cellular level could contribute to explain the different tissues responses to stress between sexes[26]. Blood composition is also affected by sex. Female donor plasma has been associated with Transfusion-related lung injury (TRALI). In a recent systematic review of low-risk TRALI donor strategies that included male plasma donor only, such strategies were associated with a reduced odds ratio of TRALI (OR 0.61; 95% CI 0.29 to 0.90). Female sex, history of pregnancy and presence of Human Leucocytes Antibodies in the donor have been associated with TRALI outcome. These mechanism may contribute to the increased risk of death in recipients of female blood but unlikely to be the only factor as RBC products contain only a minimal portion of plasma and all units transfused were leukoreduced.

Our findings that transfusion outcome seems worse in recipients of young donor blood as well as from female donors, and that it may in fact be improved in recipients of blood from older donors, support two hypotheses: 1) characteristics of blood donors may indeed affect transfusion outcome and; 2) there are potential additional characteristics among young donors or female donors that negatively affect recipients survival.

Our study has some limitations. First, its observational design is subject to unmeasured confounding factors. We however believe that because of the current practices in blood collection, distribution and transfusion, unmeasured confounders will tend to be evenly distributed between groups. Indeed, donor characteristics are always strictly concealed from prescribing physicians and blood is distributed in a “random” manner across and within hospitals. Therefore, our exposures of interest are randomly distributed among

recipients. Because of the masking of characteristics of the donors, our study has similarities with features associated with randomized clinical trials such as allocation concealment and double-blinding[28,29] Furthermore, our robust statistical adjustments for recipients' characteristics and comorbidities did not alter our effect estimates (even though the selected covariates were strongly associated with outcome), suggesting a random distribution of donor characteristics. In our sensitivity analyses including only blood from donor characteristics categories (age and sex), the recipients characteristics were also comparable between the two groups, once again supporting the *at random* distribution. Another limitation of study is that it was impossible for us to further detail donor factors that may explain our findings. Our initial hypothesis focusing on donor age and sex, we did not design our study to examine other donor characteristics that may provide further details on the reasons for such associations. However, the framework we created will enable such exploratory analyses in the future. Meanwhile, we believe that even without definitive mechanistic explanation, the strength of our findings, its reproducibility across subgroups and sensitivity analyses suggest that the observed association is likely not due to chance alone, nor only to unmeasured confounders and further emphasizing the need to reassess current transfusion practices.

In conclusion, cumulative transfusion from young donor age and from female donors was strongly associated with increased risk of mortality in a large cohort of transfused patients. These findings suggest that blood donor characteristics may affect transfusion outcome and prospective clinical trials are warranted.

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TABLE 1 PATIENT CHARACTERISTICS AT TIME OF FIRST TRANSFUSION

	N=30,503
Age	
Mean (SD)	66.2 (18.2)
Median (IQR)	69.0 (56.0, 80.0)
Age Female	
Mean (SD)	65.8 (19.1)
Median (IQR)	69.0 (54.0, 81.0)
Age Male	
Mean (SD)	66.6 (17.2)
Median (IQR)	69.0 (58.0, 79.0)
Sex n (%)	
Female	15,906 (52.1)
Male	14,597 (47.9)
Blood Group n (%)	
A NEG	1,943 (6.4)
A POS	9,872 (32.4)
AB NEG	202 (0.7)
AB POS	1,037 (3.4)
B NEG	497 (1.6)
B POS	3,153 (10.4)
O NEG	2,207 (7.2)
O POS	11,450 (37.6)
No result available	16 (0.1)
Variable results	90 (0.3)
Charlson's Score (First available) n (%)	
0	8,812 (28.9)
1 – 2	8,155 (26.7)
3 – 4	4,436 (14.5)
5+	6,314 (20.7)
Not available	2,786 (9.1)
Comorbidities n (%)	
Cardiac disease	2,521 (9.1)
Congestive heart failure	3,313 (12.0)
Peripheral Vascular Disease	2,079 (7.5)
Cerebrovascular Disease	818 (3.0)

	Dementia	842 (3.0)
	COPD	1,820 (6.6)
	Connective Tissue Disease	308 (1.1)
	Peptic Ulcer Disease	1,020 (3.7)
	Mild Liver Disease	767 (2.8)
	Moderate or Severe Liver Disease	495 (1.8)
	Diabetes with no Organ Damage	2,448 (8.8)
	Diabetes with Organ Damage	4,830 (17.4)
	Hemiplegia	400 (1.4)
	Moderate or Severe Renal Failure	2,205 (8.0)
	Cancer without Metastases	7,246 (26.1)
	Cancer with Metastases	3,164 (11.4)
	HIV	92 (0.3)

TABLE 2 – DONOR CHARACTERISTICS AT TIME OF FIRST TRANSFUSION

	N=80,755
Sex	
Female n (%)	39,328 (48.7)
Male n (%)	41,427 (51.3)
Age	
Mean (SD)	40.4 (14.5)
Median (IQR)	42.0 (27.0, 52.0)
≤ 20 n (%)	10,578 (13.1)
20.1 – 30 n (%)	13,959 (17.3)
30.1 – 40 n (%)	12,802 (15.9)
40.1 – 50 n (%)	19,999 (24.8)
50.1 – 60 n (%)	17,506 (21.7)
60.1 – 70 n (%)	5,772 (7.1)
≥ 70 n (%)	139 (0.2)
Number of previous whole blood donations	
Mean (SD)	12.7 (19.0)
Median (IQR)	5 (1, 17)
Donor_ABO_Rh n (%)	
A+	24,860 (29.6)
A-	6,497 (7.7)
B+	7,053 (8.4)
B-	1,724 (2.1)
AB+	1,967 (2.3)
AB-	714 (0.9)
O+	32,271 (38.4)
O-	8,956 (10.7)

TABLE 3 – RBC UNITS CHARACTERISTICS

	N = 187,960
RBC units (all leukoreduced) n (%)	
AS-3	48,314 (25.7)
SAGM	138,328 (73.6)
CP2D	708 (0.4)
CPDA1	478 (0.3)
Deglycerolized	36 (0.02)
Washed	96 (0.1)
CMV n (%)	
Neg	105,240 (56.0)
Pos	74,469 (39.6)
Unknown	8,251 (4.4)
Irradiation n (%)	
No	173,316 (92.2)
Yes	14,644 (7.8)
Pack_type n (%)	
B1	60,334 (32.1)
B2	78,087 (41.4)
D2	24,733 (13.2)
Q1	478 (0.3)
Q2	52 (0.03)
T1	19,461 (10.4)
T2	4,815 (2.6)
Blood Groups n (%)	
A NEG	13,840 (7.4)
A POS	56,196 (29.9)
AB NEG	1,728 (0.9)
AB POS	3,770 (2.0)
B NEG	3,872 (2.1)
B POS	17,770 (9.5)
O NEG	19,063 (10.1)
O POS	71,721 (38.2)
Age of RBC*	
Mean (SD)	19.1 (17.7)
Median (IQR)	17 (13, 23)
***Includes deglycerolized**	

TABLE 4 – PROPORTION OF PATIENTS WHO RECEIVED OTHER BLOOD PRODUCTS

	N=12,343
Plasma n (%)	6,100 (49.4)
Platelets n (%)	5,177 (41.9)
Cryoprecipitates n (%)	1,326 (10.7)
Albumin n (%)	6,376 (51.7)
Immunoglobulin n (%)	794 (6.4)
Other (WBC, fibrinogen) n (%)	< 6 (<0.1)
Coagulation Factors n (%)	1,645 (13.3)

TABLE 5 – UNADJUSTED AND ADJUSTED SURVIVAL FOR DONOR AGE AND SEX, PER CUMULATIVE UNIT TRANSFUSED, INDEPENDENT OF TOTAL NUMBER OF UNITS TRANSFUSED

		Unadjusted			Adjusted*		
		HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**
Donor age (years)	**17 – 19.9**	1.11	1.09	1.13	1.10	1.08	1.12
	20 – 29.9	1.06	1.04	1.08	1.08	1.05	1.10
	30 – 39.9	1.01	0.99	1.03	1.01	0.99	1.03
	40 – 49.9	0.99	0.99	1.00	1.00	0.99	1.01
	50 – 59.9	1.00	0.99	1.01	1.00	0.99	1.01
	60 – 69.9	1.01	0.99	1.03	1.01	1.00	1.03
	≥ 70.0	0.90	0.83	0.96	0.91	0.84	0.98
Donor age (years)	**< 30**	1.09	1.08	1.10	1.09	1.08	1.10
	≥ 30	1.00	0.99	1.00	1.00	1.00	1.00
Donor sex	**Female**	1.06	1.05	1.07	1.06	1.05	1.07
	Male	0.98	0.97	0.99	0.98	0.98	0.99

HR: Hazard Ratio; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval

*Adjusted for Recipient age, Recipient sex, Charlson's index

TABLE 6 – UNADJUSTED AND ADJUSTED OVERALL SURVIVAL FOR DONOR AGE AND SEX, RATIO OF UNITS TRANSFUSED*

		Unadjusted			Adjusted**		
		HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**
Donor age (years)	**17 – 19.9**	2.76	1.78	4.28	2.25	1.43	3.53
	20 – 29.9	2.07	1.35	3.19	1.94	1.24	3.04
	30 – 39.9	1.56	1.35	3.19	1.34	0.85	2.11
	40 – 49.9	1.60	1.04	2.46	1.38	0.89	2.16
	50 – 59.9	1.66	1.07	2.59	1.43	0.90	2.26
	60 – 69.9	1.77	1.13	2.77	1.40	0.99	1.97
	≥ 70.0	Ref.			Ref.		
Donor age (years)	**100% < 30**	1.49	1.38	1.61	1.49	1.38	1.61
	100% ≥ 30	Ref.			Ref.		
Donor sex	**100% Female**	1.50	1.40	1.61	1.44	1.35	1.55
	100% Male	Ref.			Ref.		

HR: Hazard Ratio; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval

*Because of presence of interaction, data reported at the population mean of 6 transfusions, for a ratio of 1.0, compared to a ratio of 1.0 in the reference category.

**Adjusted for Recipient age, Recipient sex, Charlson's index

FIGURE 1 – FLOW CHART

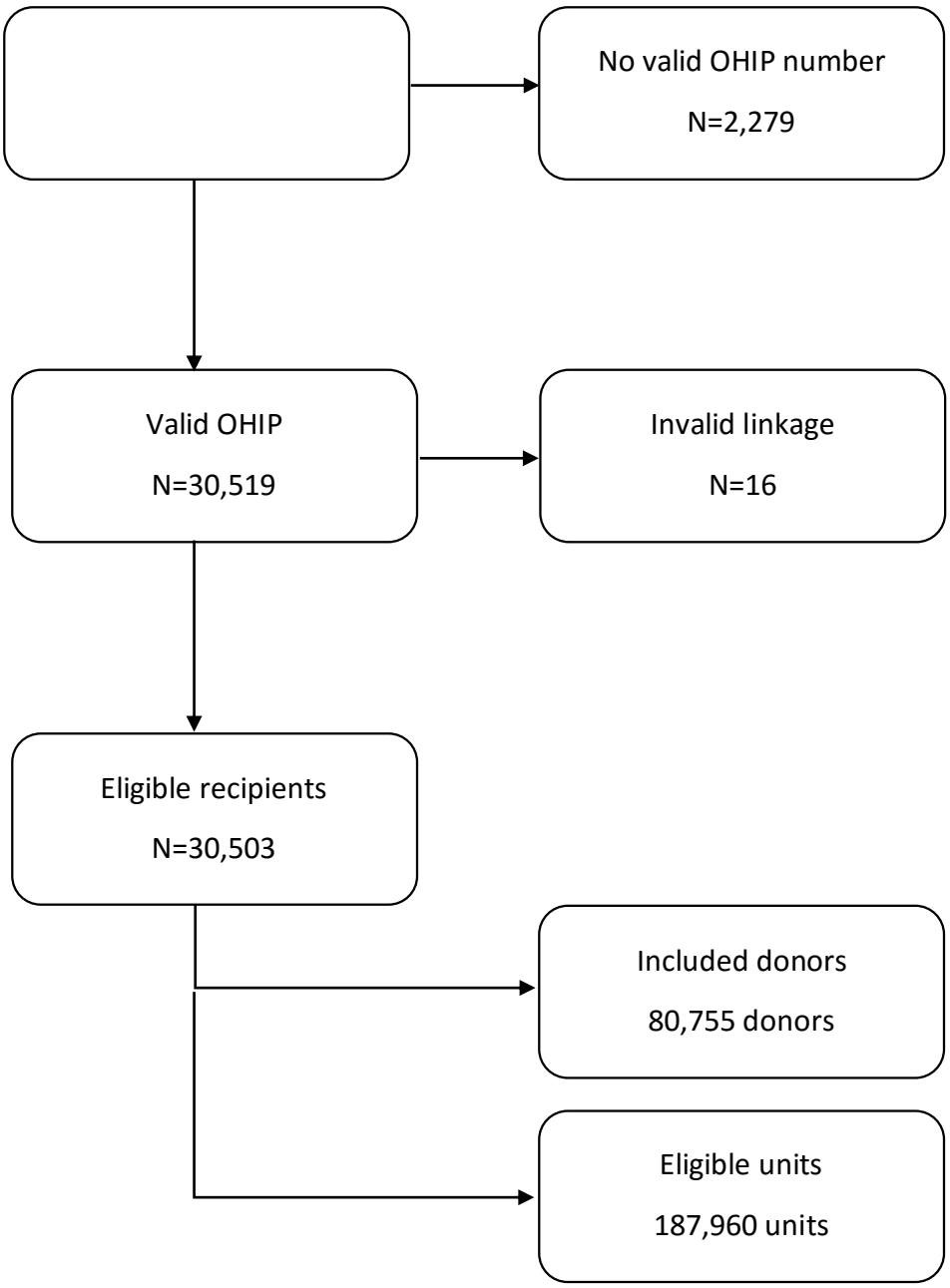
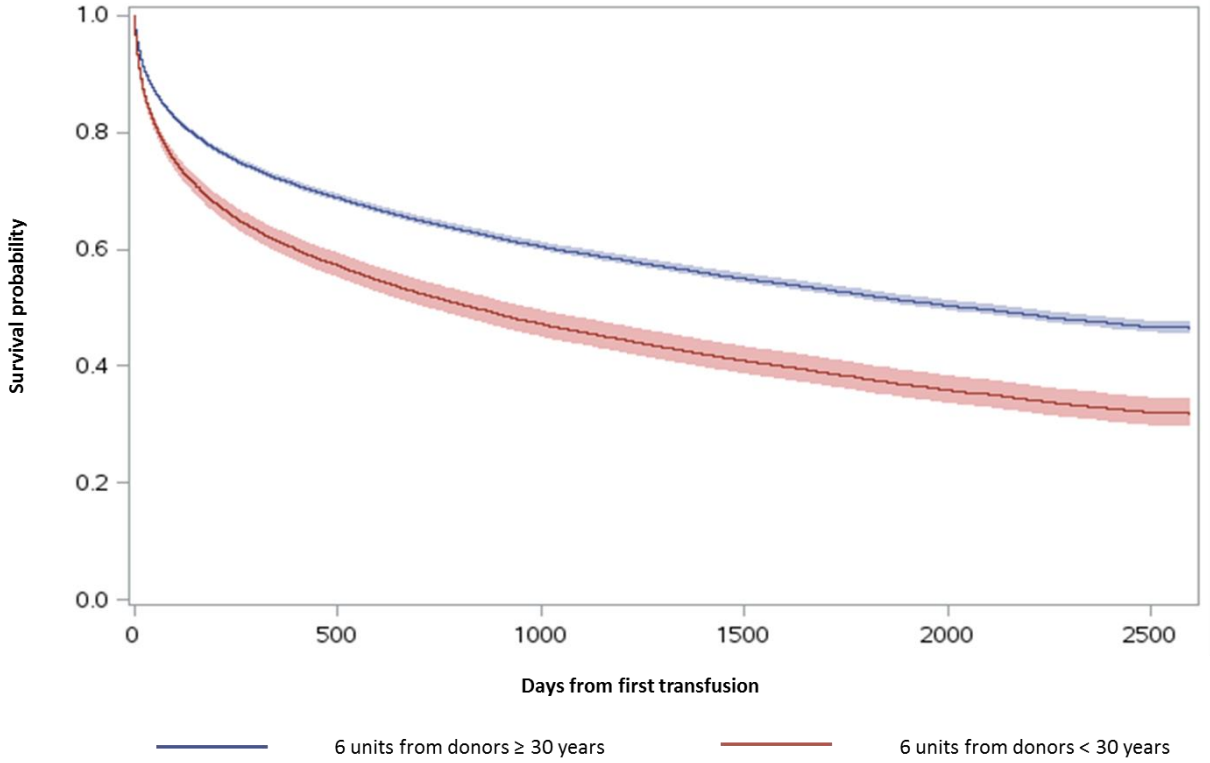


FIGURE 2 – PATIENT SURVIVAL ACCORDING TO DONOR AGE AND SEX USING A BASE CASE OF 6 TOTAL TRANSFUSIONS (STUDY MEAN) OVER THE STUDY PERIOD OF 2006-2013



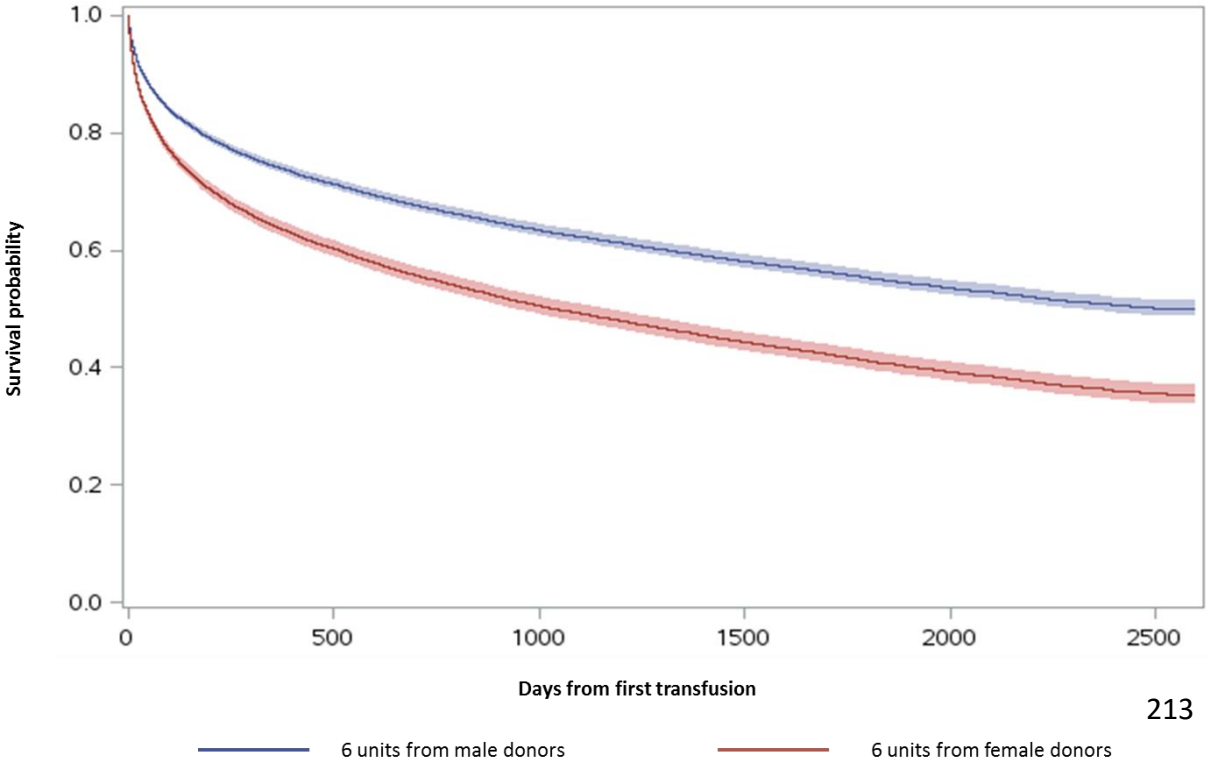
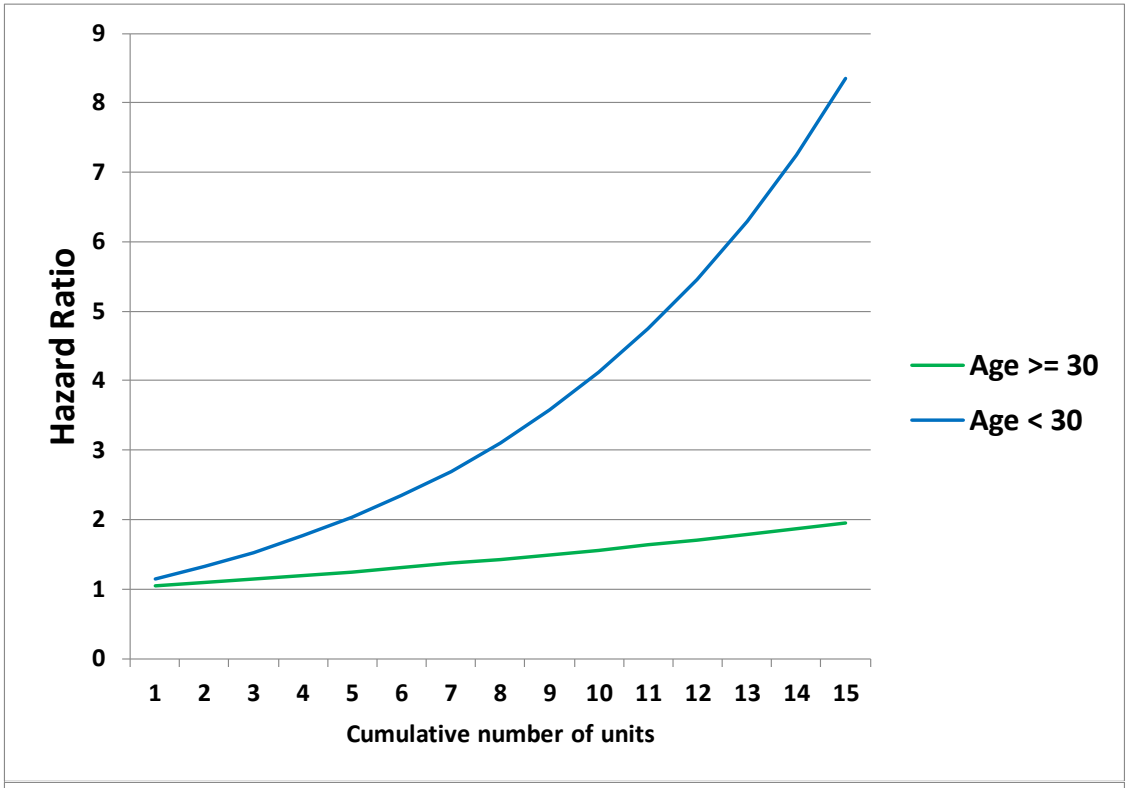


FIGURE 3 – HAZARD RATIO OF SURVIVAL BY CUMULATIVE NUMBER OF UNITS TRANSFUSED



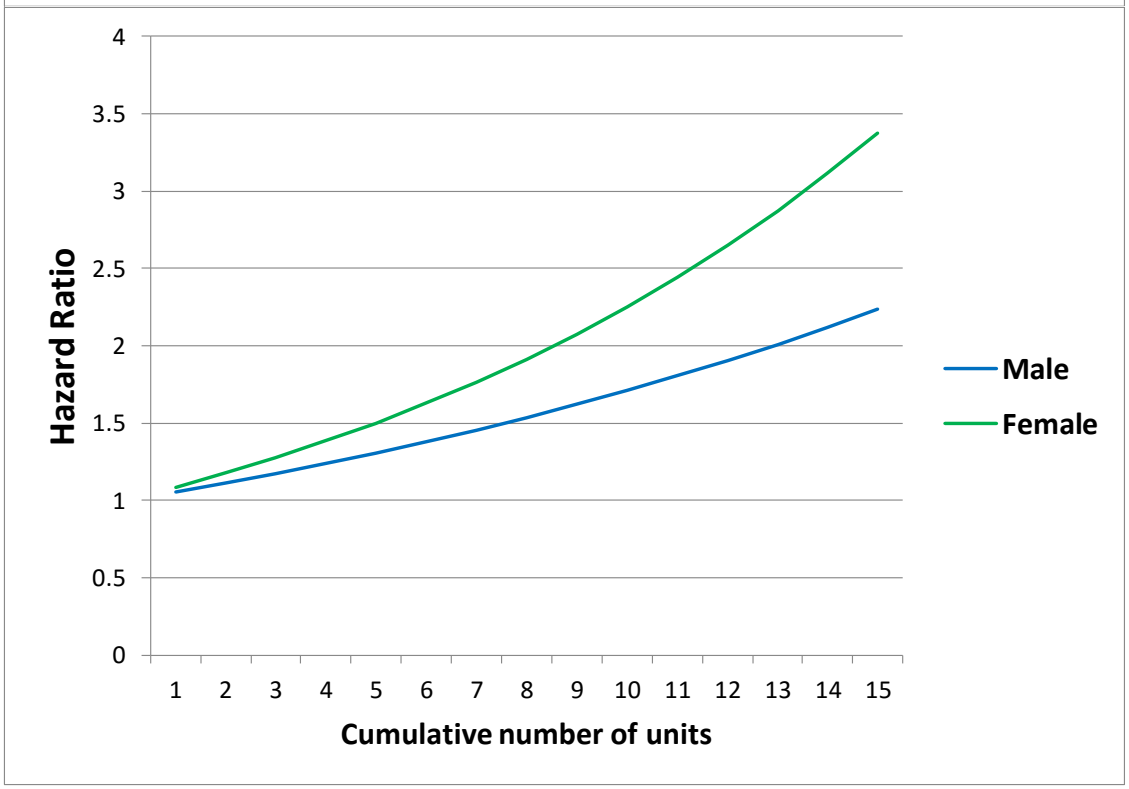


TABLE S1 – SUBGROUP ANALYSES

		Male Recipients			Female recipients		
		HR*	**LCI**	**UCI**	**HR***	**LCI**	**UCI**
Donor age (years)	**17 – 19.9**	1.14	1.11	1.16	1.04	1.02	1.07
	20 – 29.9	1.09	1.06	1.11	1.06	1.04	1.09
	30 – 39.9	1.02	1.00	1.04	0.98	0.96	1.01
	40 – 49.9	0.99	0.98	1.00	1.03	1.01	1.05
	50 – 59.9	1.00	0.99	1.01	0.98	0.97	1.00
	60 – 69.9	0.99	0.97	1.01	1.04	1.02	1.06
	≥ 70.0	0.92	0.85	1.00	0.91	0.82	1.01
Donor age (years)	**< 30**	1.12	1.11	1.13	1.06	1.04	1.07
	≥ 30	0.99	0.99	1.00	1.01	1.00	1.01
Donor sex	**Female**	1.07	1.06	1.08	1.04	1.03	1.05
	Male	0.98	0.97	0.98	1.00	0.99	1.00

		Recipient age < 1 year			Recipient age 1 - 17		
		HR*	**LCI**	**UCI**	**HR***	**LCI**	**UCI**
Donor age (years)	**17 – 19.9**	1.57	1.25	1.98	0.77	0.36	1.66
	20 – 29.9	1.58	1.29	1.93	0.51	0.33	0.81
	30 – 39.9	1.23	0.92	1.65	6.01	2.94	12.3
	40 – 49.9	1.67	1.37	2.03	1.72	1.04	2.86
	50 – 59.9	1.77	1.44	2.17	1.54	1.11	2.16
	60 – 69.9	0.88	0.38	2.04	0.48	0.26	0.90
	≥ 70.0	-	-	-	1.16	0.01	115.62
Donor age (years)	**< 30**	1.51	1.32	1.72	1.01	1.04	1.20
	≥ 30	1.51	1.29	1.76	1.12	1.04	1.20
Donor sex	**Female**	1.68	1.45	1.95	1.31	1.08	1.58
	Male	1.46	1.30	1.63	0.93	0.78	1.11

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval
*Adjusted for Recipient age, Charlson's index

		Recipient age 18 - 64			Recipient age 65 +		
		HR*	LCI	UCI	HR*	LCI	UCI
Donor age (years)	17 – 19.9	1.17	1.14	1.20	1.04	1.01	1.07
	20 – 29.9	1.09	1.06	1.11	1.07	1.05	1.09
	30 – 39.9	1.01	0.98	1.04	1.01	0.99	1.03
	40 – 49.9	1.00	0.98	1.01	1.02	1.00	1.03
	50 – 59.9	1.00	0.98	1.01	0.99	0.97	1.00
	60 – 69.9	0.99	0.96	1.02	1.03	1.01	1.05
	≥ 70.0	0.94	0.85	1.05	0.87	0.80	0.95
Donor age (years)	< 30	1.13	1.11	1.15	1.07	1.05	1.08
	≥ 30	0.99	0.99	1.00	1.00	1.00	1.00
Donor sex	Female	1.07	1.06	1.09	1.05	1.04	1.06
	Male	0.97	0.97	0.98	0.99	0.98	0.99

		Charlson Index = 0			Charlson Index = 1 - 2		
		HR**	LCI	UCI	HR*	LCI	UCI
Donor age (years)	17 – 19.9	1.08	1.01	1.15	1.08	1.04	1.12
	20 – 29.9	1.15	1.09	1.22	1.08	1.04	1.12
	30 – 39.9	1.01	0.95	1.07	1.07	1.03	1.11
	40 – 49.9	1.04	1.00	1.08	1.04	1.02	1.06
	50 – 59.9	1.03	1.00	1.06	1.01	0.98	1.03
	60 – 69.9	0.93	0.89	0.97	0.96	0.93	0.99
	≥ 70.0	0.91	0.77	1.08	1.06	0.93	1.21
Donor age (years)	< 30	1.17	1.13	1.20	1.12	1.10	1.13
	≥ 30	1.00	1.00	1.00	1.01	1.00	1.01
Donor sex	Female	1.09	1.08	1.11	1.09	1.08	1.10
	Male	0.98	0.98	0.99	0.99	0.98	0.99

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval
*Adjusted for Recipient sex, Charlson's index
** Adjusted for Recipient age, Recipient sex

		Charlson Index = 3 - 4			Charlson Index = 5 +		
		HR*	LCI	UCI	HR*	LCI	UCI
Donor age (years)	17 – 19.9	1.07	1.01	1.13	1.01	0.98	1.05
	20 – 29.9	1.05	1.01	1.10	1.04	1.01	1.07
	30 – 39.9	1.05	1.01	1.10	1.03	1.00	1.07
	40 – 49.9	1.02	0.99	1.05	1.05	1.03	1.07
	50 – 59.9	1.04	1.02	1.07	1.01	0.99	1.03
	60 – 69.9	1.03	0.99	1.07	1.03	1.00	1.06
	≥ 70.0	0.94	0.81	1.10	1.01	0.90	1.14
Donor age (years)	< 30	1.07	1.04	1.10	1.03	1.01	1.05
	≥ 30	1.03	1.03	1.04	1.03	1.02	1.03
Donor sex	Female	1.05	1.03	1.07	1.04	1.02	1.05
	Male	1.03	1.02	1.04	1.03	1.01	1.03
		No Charlson index (outpatient)					
		HR*	LCI	UCI			
Donor age (years)	17 – 19.9	1.09	1.05	1.13			
	20 – 29.9	1.07	1.04	1.10			
	30 – 39.9	1.01	0.98	1.04			
	40 – 49.9	1.00	0.98	1.02			
	50 – 59.9	0.99	0.97	1.00			
	60 – 69.9	1.02	0.99	1.05			
	≥ 70.0	0.95	0.83	1.08			
Donor age (years)	< 30	1.09	1.07	1.10			
	≥ 30	1.00	0.99	1.00			
Donor sex	Female	1.06	1.05	1.07			
	Male	0.98	0.97	0.98			

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval
*Adjusted for Recipient age and Recipient Sex

TABLE S2 – SENSITIVITY ANALYSES

Subcohort of recipients that received <30 donor age RBC only vs ≥ 30 donor age RBC only and of patients that received female RBC only or male RBC only (stratified by number of units transfused, by interaction term)

		Unadjusted			Adjusted*		
		HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**
Age < 30 vs ≥ 30 years **N=16,133 <30=1,388 ≥30=14,745**	**1 unit**	0.93	0.83	1.05	0.89	0.79	1.01
	2 units	1.15	1.02	1.29	1.31	1.16	1.47
	3 units	1.42	1.14	1.77	1.91	1.50	2.44
	4 units	1.75	1.24	2.47	2.80	1.92	4.11
Donor sex (female vs male) **N=11,176 F=4,175 M=7,001**	**1 unit**	1.08	0.99	1.17	0.99	0.91	1.07
	2 units	1.03	0.96	1.09	0.99	0.93	1.06
	3 units	0.98	0.99	1.08	0.99	0.90	1.10
	4 units	0.93	0.80	1.09	1.00	0.85	1.17

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval

*Adjusted for Recipient age, Recipient Sex and Charlson as time-varying

TABLE S3 – SENSITIVITY ANALYSES – DESCRIPTIVE STATISTICS

Descriptive statistics of the subcohort for **Donor Age**

	Donor age < 30 n = 1,388	**Donor age ≥ 30 n = 14,745**
Recipient age		
Mean (SD)	64.9 (22.2)	66.5 (19.0)
Female recipients (%)	54.4	54.9
Charlson's Index		
0 (%)	27.3	25.4
1-2 (%)	18.5	18.8
3-4 (%)	10.9	11.6
5 + (%)	14.8	15.9
N/A (%)	28.5	28.4
Mean follow-up days (SD)	807.3 (732)	853 (778)
Median N Transfusions	1 (1-2)	2 (1-3)

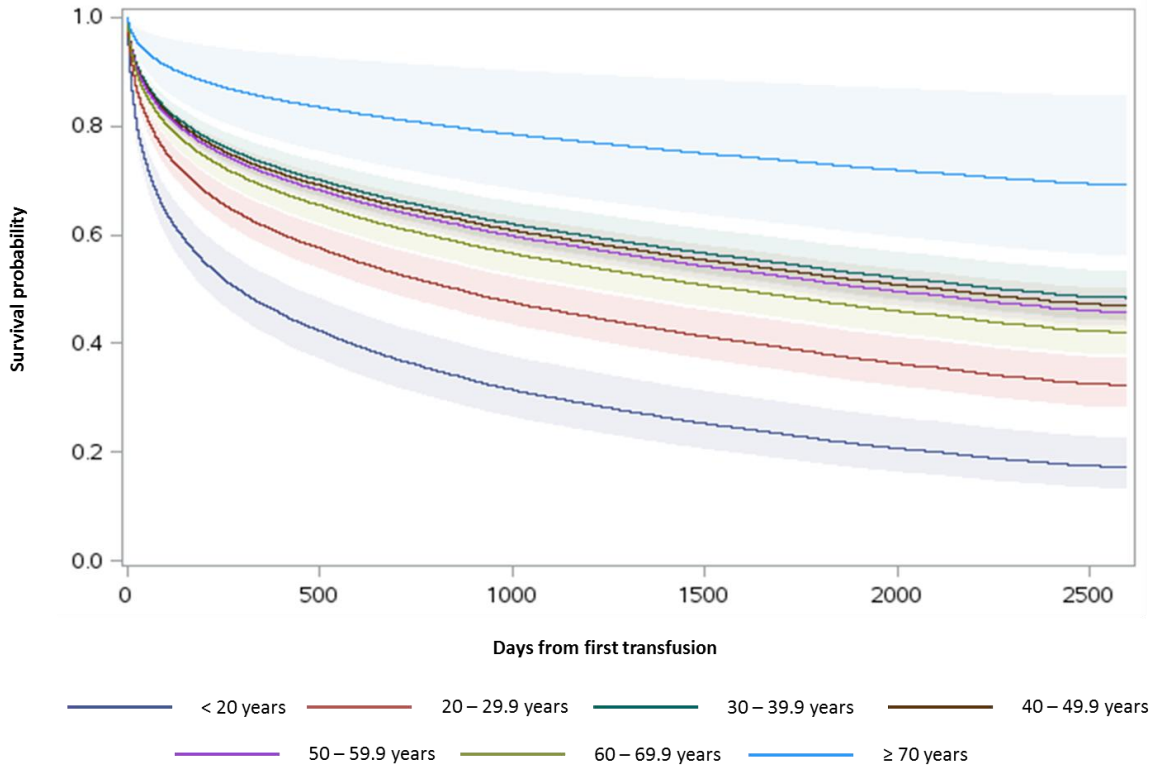
SD: Standard deviation; IQR: interquartile range

Descriptive statistics of the subcohort for **Donor Sex**

	Female donors n = 4,175	**Male donors n = 7,001**
Recipient age		
Mean (SD)	66.5 (20.3)	66.0 (19.6)
Female recipients (%)	55.0	55.5
Charlson's Index		
0 (%)	25.8	26.3
1-2 (%)	18.5	18.6
3-4 (%)	11.6	11.6
5 + (%)	15.5	15.5
N/A (%)	28.5	28.0
Mean follow-up days (SD)	845 (770)	841.4 (771)
Median N Transfusions (IQR)	2 (1-2)	2 (1-2)

SD: Standard deviation; IQR: interquartile rang

FIGURE S1 – PATIENT SURVIVAL ACCORDING TO DONOR AGE DECADE USING A BASE CASE OF 6 TOTAL TRANSFUSIONS (STUDY MEAN) OVER THE STUDY PERIOD OF 2006-2013



APPENDIX 1 – MODELLING APPROACH

CONSIDERATIONS

The selected model for my analyses had to correctly account for the possibility that each patient may receive RBC transfusions from different donors, with different characteristics, and over time. In addition, I had to allow for the variable follow-up time of each included patient, with start of follow-up at time of first transfusion, and end of follow-up at either death or end of study. The exposure to RBC transfusions from donors of one of the characteristics of interest was allowed to be repeated over time (time-varying). Since there were no reasons to a priori believe that the effect of one transfusion stops at the moment an additional unit of blood is transfused the exposure of interest had to consider cumulative exposure. Finally, the model had to allow for the consideration of a time-dependent effect of the exposures on the outcome.

We constructed an "extended Cox model", using the counting process described by Anderson and Gill[1] which considers each exposure occurrence (transfusion) as an individual entry in the dataset. The exposure of interest was the cumulative number of transfusions of a given characteristic at each transfusion exposure. For example, for donor sex, we included the cumulative number of female units transfused as well as the cumulative number of male donor units. We then regressed the incremental risk of an additional transfusion with a characteristics of interest, keeping all the other covariates fixed, over time. This allowed for the estimate of the effect of any increase in one unit of a characteristic, independently from the number of units transfused from the other types. The interaction between each

cumulative exposure covariates (male vs female for example) was also tested to estimate effect modification depending on the number of units already transfused from the other categories. Finally, we tested the interaction of each donor characteristic (donor age, donor sex) with recipient age and sex.

MAJOR ASSUMPTIONS

For our model to be valid, some assumptions had to be considered and tested. First, as for any survival model, censoring had to be non-informative. In our study, the outcome of interest (mortality) was collected from provincial databases. The death registry we used has been reported to have less than 2% to 3% missing values[2], we have no reason to believe that a significant number of patients censored had in fact had a mortality event before the end of the study, and thus incorrectly censored and misclassified.

Given the continuous nature of our exposures (cumulative numbers), the appropriate form (distribution) of the data had to be tested. The log, exponential, quadratic and cubic forms were tested, with only minimal changes in the model fit statistics (- 2 log likelihood statistics) and with minimal changes in the estimates. We opted for the simplest log-linear form.

The effect of exposures was also tested for proportionality over time. We first tested visually any evidence of departure from proportionality by plotting Kaplan-Meier survival curves for patients that received only one unit of blood of each characteristic. We did not observe any obvious or significant deviation from proportionality. To test whether the cumulative risk associated with one transfusion also respected the proportionality

assumption, we introduced a time interaction term between the cumulative exposure covariates and the time each patient was exposed to each level of covariates in each model. Given our very large sample size and long follow-up, we found statistically significant interaction between time and the cumulative number of each units, suggesting an increased effect of the exposure earlier during the follow-up period, with the log(time) form being the most appropriate. However, the changes in parameter estimates associated with the introduction of this interaction was only minimal, with variation less than 2% across the duration of the study. Given the difference was not clinically relevant, and to facilitate interpretation and dissemination of the results, we decided not to report this time interaction in our main presentation of the results.

REFERENCES

1. Andersen P, Gill R. Cox's regression model for counting processes: a large sample study. Ann Stat 1982;10(4):1100–11200.

2. Iron K, Zagorski B, Sykora K, Manuel D. Living and Dying in Ontario: An opportunity for Improved Health Information. Toronto: 2008.

CHAPTER 6 – ADDITIONAL RESULTS

In Chapter 5, our analysis used data from Canadian Blood Services, The Ottawa Hospital and the Registered Persons Database of Ontario. In this chapter, we conduct different epidemiological analyses for an additional type of exposure (donor-recipient mismatch) and additional outcomes across our three exposures (age, sex, mismatch). We will demonstrate the feasibility of using different sources of information, both on the donor and recipient sides, to perform the same types of analyses presented in Chapter 5.

6.1 DONOR-RECIPIENT BLOOD GROUP MISMATCH

For our analyses of blood group mismatch, the donor blood group was identified using the blood group identifier on each RBC unit transfused. The recipient blood group was identified using laboratory cross-match results obtained from the laboratory information system at each included site. For 16 patients, a blood group was not identified. For 90 patients, lab results returned more than one blood group result. It was not possible to determine the reasons for those disparities but we hypothesize that those patients were either bone marrow transplant patients, or those results are coding errors or lab errors. For the purpose of those analyses, we excluded those 106 patients (0.3%).

We counted the number of ABO-Rh mismatch occurrences for each transfused patient for the duration of the study and regressed the cumulative number of mismatch transfusions and matched transfusions over time using the approach described in Chapter 4. We considered a mismatch any transfusion that was not the exact same ABO-Rh phenotype for donor and recipient. We also measured the number of Rh mismatched transfusions, for

both Rh positive and Rh negative patients. We finally stratified by recipient blood group and recipient sex. Results are presented in Table 6.1.

Table 6.1: Risk of death for each cumulative transfused RBC unit

	Unadjusted			Adjusted*		
	HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**
Any mismatch	0.99	0.99	1.00	1.00	0.99	1.00
By Recipient Blood Group						
A Neg	1.01	0.99	1.02	1.03	1.01	1.05
A Pos	1.00	1.00	1.01	1.00	1.00	1.01
AB Neg	1.01	0.94	1.08	1.00	0.94	1.07
AB Pos	0.99	0.97	1.01	1.00	0.98	1.02
B Neg	1.01	0.98	1.03	1.01	0.99	1.04
B Pos	0.99	0.98	1.01	1.00	0.99	1.01
O Neg	1.07	1.04	1.10	1.07	1.04	1.10
O Pos	0.99	0.98	1.01	1.00	0.99	1.01
Female Recipient Rh Mismatch						
A Neg	0.97	0.92	1.02	0.99	0.95	1.05
A Pos	0.97	0.94	1.01	0.98	0.94	1.03
AB Neg	-	-	-	-	-	-
AB Pos	1.04	1.01	1.08	1.02	0.98	1.08
B Neg	1.00	0.68	1.49	0.89	0.58	1.34
B Pos	1.07	1.03	1.11	1.10	1.06	1.13
O Neg	1.21	1.11	1.33	1.13	1.03	1.24
O Pos	0.99	0.97	1.02	1.01	1.00	1.03
Male Rh Mismatch						
A Neg	1.09	1.03	1.16	1.09	1.02	1.16
A Pos	1.00	0.99	1.00	1.00	0.99	1.00
AB Neg	1.04	0.63	1.73	0.92	0.52	1.63
AB Pos	0.97	0.93	1.01	0.98	0.94	1.02
B Neg	1.29	1.08	1.55	1.51	1.25	1.82

B Pos	1.00	0.98	1.02	1.00	0.99	1.02
O Neg	1.06	1.03	1.09	1.06	1.03	1.09
O Pos	0.94	0.92	0.96	0.97	0.95	0.98

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval

*Adjusted for recipient Age, Sex (when not stratified) and Charlson index

In this analysis, we see that overall, the transfusion of mismatched compatible RBC was not associated with survival. However, in O negative females and males, such intervention was strongly associated with a reduced survival (adjusted HR 1.13; 95% CI 1.03 to 1.24 for each additional Rh positive RBC unit transfused in females; and adjusted HR 1.06; 95% CI 1.03 to 1.09 for each additional Rh positive RBC unit transfused in males). The B negative blood group in male and B positive group in females was also associated with decreased survival.

For the blood group characteristic, the "mismatch" exposure is not concealed and not at random as it was the case for donor age and donor sex. The reasons for transfusion of mismatched blood, such as urgent transfusion or a reduced blood bank can be significant confounding factors. The analytical strategy proposed in this research can be used to perform epidemiological analyses of such exposures although careful consideration of those confounding factors must be done to reduce the risk of bias estimates.

6.2 Exposures: Donor Age and Sex – Outcome: Cancer

To study the association of donor age and sex with the risk of cancer in the recipient, we linked the Ontario Cancer Registry (OCR)[1] to our clinical and administrative data. In the OCR, all cancer events, date of diagnosis and the type of cancers are encoded centrally using the standardized ICD-O-3 classification[2]. For the purpose of this analysis, a patient

was eligible for inclusion if he had no entry in the OCR prior to first transfusion during the time period of the study. A cancer outcome was defined as any occurrence of cancer, of any type, after first RBC transfusion. Patients were censored at end of follow-up or death.

Table 6.2: Risk of cancer for each cumulative transfused RBC unit

		Unadjusted			Adjusted		
		HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**
Donor age (years)	**17 – 19.9**	1.07	1.03	1.11	1.06	1.03	1.10
	20 – 29.9	1.05	1.01	1.08	1.05	1.01	1.08
	30 – 39.9	1.05	1.00	1.07	1.04	0.99	1.07
	40 – 49.9	0.99	0.97	1.01	0.99	0.97	1.01
	50 – 59.9	0.99	0.97	1.02	0.99	0.97	1.02
	60 – 69.9	1.02	0.98	1.06	1.02	0.98	1.06
	≥ 70.0	0.93	0.81	1.09	0.96	0.83	1.10
Donor age (years)	**< 30**	1.07	1.05	1.09	1.06	1.04	1.09
	≥ 30	1.00	0.99	1.00	1.00	1.00	1.00
Donor sex	**Female**	1.03	1.01	1.05	1.03	1.01	1.04
	Male	0.99	0.98	1.00	1.00	0.98	1.01

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval

*Adjusted for recipient age, sex and Charlson's index.

Here, we observed a strong association of donor age and sex with the risk of new cancer diagnosis in the RBC recipients. Young donor age below 30 years conferred an adjusted HR for cancer diagnosis of 1.06; 95% CI 1.04 to 1.09 for each additional RBC unit transfused. The HR for female sex was 1.03; 95% CI 1.01 to 1.04 for each additional female RBC unit transfused. We found no increased risk with the use of older donor RBCs or male RBCs.

6.3 Exposures: Donor Age and Sex – Outcome: Myocardial Infarction

We identified the occurrence of myocardial infarctions from the Discharge Abstract Database (DAD). This database collects among other things the main and secondary diagnoses of all patients hospitalized and discharged in Ontario. Myocardial infarctions were identified using the ICD-10 code I21 (ST elevation MI and non-ST elevation MI). A recent systematic review reported that this code had a very good sensitivity (above 86% in more than 50% of studies) and specificity (89 to 99%)[3]. A patient was included if he was hospitalized with a main diagnosis of MI after the occurrence of first transfusion during the study period. Here again, patients were censored at end of follow-up or death.

Table 6.3: Risk of myocardial infarction for each cumulative transfused RBC unit

		Unadjusted			Adjusted*		
		HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**
Donor age (years)	**17 – 19.9**	1.04	0.97	1.12	1.04	0.97	1.12
	20 – 29.9	0.98	0.91	1.05	0.98	0.90	1.05
	30 – 39.9	1.06	0.99	1.14	1.06	0.99	1.14
	40 – 49.9	1.00	0.95	1.05	1.00	0.95	1.05
	50 – 59.9	1.00	0.96	1.05	1.00	0.95	1.05
	60 – 69.9	0.98	0.90	1.06	0.99	0.92	1.07
	≥ 70.0	0.80	0.58	1.11	0.79	0.57	1.09
Donor age (years)	**< 30**	1.02	0.96	1.07	1.01	0.96	1.07
	≥ 30	1.00	0.99	1.01	1.00	0.99	1.01
Donor sex	**Female**	1.01	0.98	1.05	1.01	0.98	1.05
	Male	0.99	0.97	1.02	1.00	0.98	1.01

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval

*Adjusted for recipient age, sex and Charlson's index.

In this analysis, we found no indication that donor age and sex was associated with subsequent hospitalizations with myocardial infarctions.

6.4 Exposures: Donor Age and Sex – Outcomes: C Difficile and Methycilline Resistant Staphylococcal Aureus Infections

To study the risk of new infections after RBC transfusions we selected two highly prevalent infections in our hospitals: *C Difficile* infection and *Methycilline Resistant Staphylococcal Aureus* (MRSA). The infections were identified from microbiological results in hospital health records. We considered any positive stool toxin for our C Difficile analysis, and for MRSA, any new positive screening test or lab culture. Patients were censored at end of follow-up or death.

Table 6.4: Risk of new *C Difficile* infection for each cumulative transfused RBC unit

		Unadjusted			Adjusted*		
		HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**
Donor age (years)	**17 – 19.9**	1.07	1.02	1.12	1.07	1.02	1.12
	20 – 29.9	1.09	1.03	1.15	1.09	1.04	1.15
	30 – 39.9	1.00	0.94	1.06	1.00	0.94	1.06
	40 – 49.9	0.98	0.95	1.02	0.98	0.95	1.02
	50 – 59.9	1.00	0.96	1.04	1.00	0.97	1.03
	60 – 69.9	1.05	0.99	1.11	1.05	1.00	1.10
	≥ 70.0	1.09	0.94	1.27	1.09	0.95	1.24
Donor age (years)	**< 30**	1.08	1.05	1.11	1.08	1.05	1.11
	≥ 30	1.00	1.00	1.01	1.01	1.00	1.01
Donor sex	**Female**	1.05	1.03	1.06	1.05	1.02	1.08
	Male	0.99	0.99	1.00	1.00	0.98	1.01

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval

*Adjusted for recipient age, sex and Charlson's index.

Table 6.5: Risk of new *MRSA* infection for each cumulative transfused RBC unit

		Unadjusted			Adjusted*		
		HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**
Donor age (years)	**17 – 19.9**	0.97	0.92	1.03	0.97	0.91	1.01
	20 – 29.9	1.11	1.05	1.18	1.11	1.06	1.17
	30 – 39.9	1.01	0.95	1.07	1.01	0.95	1.07
	40 – 49.9	0.99	0.96	1.02	0.99	0.98	1.03
	50 – 59.9	1.00	0.97	1.04	1.00	0.97	1.04
	60 – 69.9	0.99	0.934	1.05	0.99	0.94	1.05
	≥ 70.0	1.06	0.90	1.24	1.04	0.89	1.22
Donor age (years)	**< 30**	1.07	1.03	1.11	1.07	1.03	1.11
	≥ 30	1.00	0.99	1.01	1.00	0.99	1.01
Donor sex	**Female**	1.05	1.02	1.07	1.04	1.02	1.07
	Male	0.99	0.97	1.00	0.99	0.97	1.01

HR: Hazard Ration; LCI: Lower 95% confidence interval; UCI: Upper 95% confidence interval

*Adjusted for recipient age, sex and Charlson's index.

Similarly to our previously studied outcomes of survival and cancer occurrence, in this section we observed that RBC transfusions from young donors and female donors was associated with two infectious complications. Although the events did not occur in the same patients, the effect estimates are similar for the two outcomes, further strengthening our hypothesis that donor age and sex are important risk factors in RBC transfusion.

6.5 CONCLUSION

In this Chapter, we demonstrated our capacity to perform various epidemiological analyses for an additional donor characteristic (ABO-Rh mismatch) as well as additional outcomes (cancer, myocardial infarction, C Difficile infection and MRSA infection). We did so by using data from various sources, including administrative and clinical databases.

In addition to showing the feasibility of studying the donor-recipient continuum using epidemiological methods, we found that young donor age and female sex were not only associated with increased mortality but also cancer and infectious outcomes.

6.6 REFERENCES

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CHAPTER 7 - DISCUSSION

7.1 SUMMARY OF FINDINGS

In this thesis project, we first conducted a thorough review of the scientific evidence regarding the clinical effect of blood donor characteristics on RBC recipient outcomes. We then developed a methodologically robust framework allowing the appropriate study of blood donor characteristics in regard to their effect on blood donor recipients. We finally reported the main results of our epidemiological analysis.

Before the conduct of this project, the evidence supporting the association of donor characteristics and RBC transfusion outcome was very limited. Of the 17 different donor characteristics reported in the 58 eligible papers, only 9 characteristics were non-infectious. Of those 9 characteristics, female donor sex, positive white blood cells antibodies, mismatched HLA-DR donor-recipient status were potentially associated with RBC recipients' outcome. Survival outcomes were reported in only 7 studies. The quality of the evidence was very low to low for all exposures and outcomes.

Regarding our main exposure of interest, donor age, the only study[1] we found that provided some effect estimates for survival was conducted in patients that all received plasma. The results reported in the systematic review were obtained after communications with the main author and from secondary analyses performed on that study database. Albeit several methodological and analytical limitations, this study found that patients who died in hospital after a transfusion were likely to receive blood from younger donors than

those who survived (MD -6.24 [-12.43, -0.05]). Donor age was associated with no other outcome among the 5 included papers.

For donor sex, 2/17 studies reported survival outcomes. From our post-hoc analysis of the Gajic data[1], we found a non-significant reduced risk of hospital mortality for recipients of male only plasma (OR 0.57 [0.20, 1.61]). The other study also found an association between donor sex and survival, but only in men recipients. Most studies reported TRALI as there outcome of interest for donor sex. Male-only or low risk plasma policies seemed to affect the risk of TRALI in RBC recipients after the implementation of such policies (RR 0.50 [0.35, 0.72]). However, such findings were not found when including studies designed to study only RBC transfused patients, as opposed to changes in policies (with a before-and-after design), strongly suggesting contamination with plasma products, given the fact that the policy changes were not affecting donors of RBC. All studies had important methodological limitations and it was therefore impossible to draw definitive conclusions.

Another consistent finding of the review was the risk of allo-immunization when transfusing HLA-DR mismatched blood to a transplantation population (OR 0.39 [0.29 to 0.88]). Although HLA-DR mismatch is not the same as ABO-Rh compatibility, it suggests that at least in one specific clinical setting, transfusion of mismatched RBC can have impact on the recipient. The impact of allo-immunization on long-term outcomes is less certain. In the transplant population, the transfusion of HLA-DR mismatched blood was associated with an increased risk of graft rejection, with no effect on overall survival[2–5].

The findings of our systematic review provided some very low quality evidence that donor age, donor sex and blood mismatch may have an impact on the transfusion recipient. However, the heterogeneous study designs, the poor methodological quality and the designs were most of the time inappropriate to correctly assess the impact of donor characteristics on RBC transfusion outcome.

To investigate if donor age, donor sex and donor-recipient ABO-Rh mismatch was associated with RBC transfusion outcomes, we created an analytical framework allowing the robust epidemiological analyses of donor characteristics, and their effect on transfusion outcome[6]. Using the transfused patients as index cases, we were able to identify from hospital administrative and clinical databases blood products transfused, including the dates and time when such transfusions occurred. With the collaboration of Canadian Blood Services, and using the unique blood unit numbers associated with each transfusion, we linked each RBC unit with its respective blood donor. The donor characteristics of interest were added to each unit and the resulting dataset was securely transferred to ICES for linkage with hospital data and ICES data for clinical outcomes.

To accurately model the risk associated with transfusion of RBC from different donors, we built extended Cox regression models with time-dependent stratification. For any given patient, each strata represented one transfusion. We then computed for each subsequent strata the cumulative number of transfusions of the studied donor characteristic and regressed the survival function over time. The dataset and computing method used was the counting process described by Andersen[7]. The resulting hazard function was therefore a representation of the incremental risk of death associated with each additional transfusion

of a RBC unit bearing the studied characteristic, when keeping the other characteristics stable, over time. This model also allowed us to adjust for time-varying covariates such as comorbidities at time of transfusion using the Charlson index, or recipient age. Finally, an additional strength of this model is that in addition to study the risk of any given characteristic, it enables us to assess the dose-response relationship.

Our main analysis investigating the association of donor age with survival after RBC transfusion suggests a strong inverse relationship. The risk of dying was the highest after receiving RBC transfusion from young donors, especially under 30 years (adjusted HR 1.09; 95% CI 1.08 to 1.10 for each additional RBC unit transfused, p<0.001). The risk was not significant for donors between 30 and 69.9 years. RBC transfusion from donors aged above 70 years seemed to confer a protective effect compared to receiving blood from other age groups. Although the selected covariates for adjustment were also strongly associated with mortality, the inclusion of those covariates did not change the effect estimates.

For donor sex, we found that each additional female donor transfusion was associated with decreased survival in the recipient (adjusted HR 1.06; 95% CI 1.05 to 1.07 for each additional RBC unit transfused, p=<0.001). Again, the estimates were not affected by our statistical adjustments and it was seen in both male and female recipients, with no evidence of increased risk of sex mismatched transfusions.

We also conducted analyses that included an additional exposure, donor-recipient ABO-Rh mismatch, as well as 4 different secondary outcomes: new diagnoses of cancer, myocardial infarction, C Difficile and MRSA infections.

Overall, transfusion of ABO-Rh compatible but mismatched RBC was not associated with worse survival (adjusted HR 1.00; 95% 0.99 to 1.00). However, when examining blood groups, recipients from the group O negative had a worse outcome compared to the other group when receiving mismatched transfusions (adjusted HR 1.07; 95% CI 1.04 to 1.10 for each additional RBC unit transfused, p=<0.001). Further analyses revealed that Rh mismatch was associated with a reduced survival among patients in the B negative and O negative groups in males, and O negative and B negative groups in females.

Finally, out of our 4 secondary outcomes, young donor age and female sex was associated with three: occurrence of cancer, new C Difficile infections and new MRSA infections. Those findings support our main hypothesis that donor characteristics are of importance in transfusion medicine.

7.2 Potential impacts

This PhD project will have impact in two main domains. First, we showed the feasibility of linking blood donor and recipient data from various sources and to perform advanced epidemiological analyses that would never have been possible before. Second, if the results of our analyses are independently confirmed, this project will open a whole new area of research in blood transfusion, and potentially affect blood transfusion policies.

In transfusion medicine, the study of RBC transfusion has most often been limited to the quality of the product itself, and its impact on, for example RBC survival or potassium levels in the bag. Some recent epidemiological studies evaluated more clinically relevant outcomes such as survival or risk of infection, for characteristics of the products such as the

age of blood[8,9] or leukoreduction[10]. One large database, the SCANDAT database from Sweden, allows limited analyses of the donor-recipient continuum, but is limited to only administrative information, with few clinical information and with limited capacity for adjustment[11]. The infrastructure resulting from our study has the potential to revolutionize the way we study blood transfusion. A similar analytical framework could be used to study any other donor characteristics. It could also be adapted to different blood manufacture processes. As long as the characteristic of interest is distributed at random among blood recipients, the estimates remain at limited risk of bias. When the distribution is not at random, as it is the case for example when ordering irradiated blood, then usual adjustment should be performed, but with an increased risk of unmeasured confounding factors.

Regarding the impact on blood collection agencies policies, the results of this thesis have the short-term potential to reassure current practices and policy changes regarding older donors. CBS has for example recently announced that it would reduce the minimal age for first time blood donation at 61 years of age[12]. Knowing that transfusion of such blood is safe supports this decision, at least on the RBC recipient side.

Our findings that younger donors may be associated with worse survival after transfusion is concerning. It would however be premature to act at the health-care level on those findings as this is the first study to show such an association.

For donor age, preliminary animal data suggested benefits rather than harm from young mice blood transfusions[13]. This area of research is really at its beginning and our

epidemiological findings should be taken as hypothesis generating only. In the solid organ transplantation literature, the current evidence suggest that older donors are associated with poorer outcomes in recipients[14]. It has been suggested that such poor outcomes may be related to the immune tolerance occurring with advanced age and that it may be harder to induce immunomodulation in the oldest recipients[15]. In the transfusion population, no immunosuppression is required in the recipients. However, the blood from older donors may be less immunogenic than the blood from younger patients, a phenomenon called the immunosenescence[16,17]. In our study, the effect estimates for the youngest donor RBC transfused to the oldest (>65 years) was still associated with a poorer survival, but with lower absolute estimates (adjusted HR 1.04; 95% CI 1.01 to 1.06 for each additional RBC unit transfused, p=0.002). On the other hand, transfusion of blood from the oldest patients seemed associated with better survival (adjusted HR 0.81; 95% CI 0.74 to 0.88 for each additional RBC unit transfused, p<0.001). Those findings may suggest that the transfusion of a more or less immunogenic blood impact on transfusion recipients. We cannot affirm that the observed associations between donor age and survival is causal as the real cause of harm from those donors may well be environmental, behavioral or other unknown factors associated to donor age, rather than age itself. However, the observed association is strong enough to warrant 1) independent confirmation; and 2) further epidemiological and mechanistic studies to try and explain the observed effect. If confirmed, such findings would change transfusion practice worldwide.

For donor sex, our findings that RBC transfusions from female donors was associated with a decreased survival in recipients may also change blood donation practices. The risk of

transfusing female RBC was present to both male and female recipients as well as for all recipient age subgroups. For plasma transfusion, current Canadian policies already exclude female plasma from the plasma pool in hope of reducing the risk of transfusion reactions such as TRALI[18]. Transfusion of female RBC has however never been associated with adverse outcomes, especially survival. One of the hypothesized mechanism for the increased risk of TRALI in recipients of female plasma is the presence of anti-leukocyte antibodies in the plasma of the donor. However, this explanation is unlikely for RBC as only a minimal amount of plasma remains in RBC units, and that all units transfused in our study were leukoreduced. Until independent confirmation, or better understanding of mechanism, one has to weight the impact on the blood supply of limiting the source of RBC to male only, like this is the case for plasma.

7.3 LIMITATIONS

This PhD program has several limitations that need to be acknowledged. Although we designed our systematic review to be as detailed and inclusive as possible, the quality of the evidence gathered is limited by the quality and design of the included studies. We provided pooled estimates from studies of using different designs, inclusion and exclusion criteria or type of estimators. Therefore, any estimate provided is only hypothesis generating. In addition, a significant number of studies were excluded because it was impossible to extract estimates for RBC transfusions. This affects mainly the effect estimates for infectious characteristics as only 8 excluded studies investigated non-infections characteristics. Those exclusions are very unlikely to influence the conclusions of our review for non-infectious donor characteristics. We however believe that the limited evidence gathered by our

systematic review supports a research program designed to investigate the main hypotheses of this thesis, and that the limitations uncovered by our systematic review are in fact positive for the program.

Regarding our analytical framework, several limitations must be noted. First, the validity of the results is highly dependent on the validity of the variables used to perform the different analyses. For this project, we used only variables that have been shown to be of high validity (Charlson[19], recipient age and sex, mortality outcomes from national registry) or that may be considered as gold standards (donor characteristics obtained from Canadian Blood Services databases). One has however to use caution when using less validated variables in the analytical models as it may bias estimates[20,21]. As for any observational and retrospective studies, such analyses describe what is observed in a given cohort, but the results are associations only, and may not be generalizable to a different population. Our study included more than 30,000 recipients and 80,000 donors, but all recipients were from the same geographic location in Canada. Our observations may not hold in different donor-recipient populations. As discussed in Chapter 5, although observational studies are subject to unmeasured confounders, even when extensive adjustments are performed, we reiterate our findings that the donor characteristics of interest were distributed evenly among our two subgroups of recipients of male versus female donors and young versus old donors. Given the nature of blood distribution in Canada, our study shares some similarities with randomized controlled trials. With the current available data, we are also limited on the donor characteristics we can analyze. We do have the capabilities to study basic demographic donor characteristics and blood characteristics, but the data collected by CBS

from donor is rather limited. Further donor linkages and data collection will need to be performed to study in more details donor characteristics, especially if mechanistic studies are to be conducted using the presented methodology. For now, we are limited to study the different recipient subpopulations and donor-recipients interactions in regard to the available donor characteristics.

7.4 FUTURE DIRECTIONS

Our results need to be confirmed

The next natural step of this program will be to independently confirm our main results. This can be performed in a variety of manners. First, the same analyses could be independently performed using a different donor-recipient population. For example, the Province of Quebec has a different blood collection agency. Héma-Québec possesses similar donor databases than Canadian Blood Services and The Province of Quebec has mortality registries. The missing link between donor and recipients would therefore be the collection of recipient comorbidities and transfusion events. With the ongoing development of electronic records, such analyses will be possible in the nearby future.

We however believe that the most valid strategy to confirm our results would be through prospective interventional studies. One could easily imagine a study where a given donor characteristic, for example donor sex, be randomized at the blood bank level prior to transfusion. Such randomization could be performed at the patient level using a standard randomized controlled trial design. It could also be conducted at the institution level, using

clustered designs, where half centers would randomly receive blood from males and the other half from females, with outcomes measured centrally using provincial registries.

Additional analyses

Our framework readily allows for additional epidemiological analyses. Available for future analyses are different manufacturing characteristics such as the packaging type for the RBC products, CMV and irradiation status of the units, preservative agents and age of RBC. We also have the capacity to study donor-recipient compatibility. Several secondary outcomes are also immediately available such as the risk of cancer in the recipient, including the type of cancer, the risk of *C. difficile* and *Methicillin-resistant Staphylococcus aureus* infections, new myocardial infarction, new acute or chronic renal failure post transfusion of RBC and rehospitalizations. Each of these additional analyses will require careful data validation, analyses and interpretation. They may provide more information regarding potential mechanisms explaining the main findings of this thesis.

Additional data linkage

This project demonstrated that it was possible to robustly investigate the donor-recipient continuum. Even more powerful and detailed analyses are however possible. The number of covariates available for analyses in the current cohort remains limited if one wishes to perform more detailed analyses. Further linkages with other databases will however be possible. On the donor side, the information gathered by Canadian Blood Services regarding the donors may allow direct linkage of donors with hospital clinical and administrative databases and to provincial registries. Such linkages would allow for more detailed analyses

on the donor side as it would provide information regarding donor comorbidities and outcomes. Data from bio banks could also be linked to both donors and recipients, thus providing potential mechanistic explanations for observed associations. Donor characteristics in plasma and platelet transfusion may also be significant. Our methods can be applied to other blood products that are not a pool of multiple donors. It may therefore be worth including other non-pooled blood products such as platelet apheresis in further linkage initiatives.

Eventually, the linkage of blood donor to their recipients will become possible in other institutions with the development of computerized health systems, thus increasing the total sample size and diversity of our cohort. Ultimately, by performing regular updates and analyses of such a donor-recipient cohort, it may allow for improved hemovigilance and improved safety of our transfusion system.

7.5 CONCLUSIONS

In summary, before this study, there was limited low quality evidence suggesting that donor characteristics may affect outcomes after RBC transfusion. The evidence was even more scare regarding survival. We demonstrated the feasibility to develop a donor-recipient analytical framework allowing the robust and powerful study of blood donor characteristics. Using this framework, we found that young donor age and female donor sex matter in regard to survival after RBC transfusion.

The developed framework has the potential to improve significantly our abilities to study blood transfusion in Canada. It allows for robust short and long-term epidemiological

analyses that are at low risk of confounding given the concealed nature of donor characteristics in blood transfusion.

Our findings suggesting a reduced survival in RBC transfused patients from female donor blood and younger donor blood create new hypotheses regarding potential explanation for adverse long-term outcomes after blood transfusion. If independently confirmed, our findings may significantly change transfusion practices and potentially improve transfusion recipient outcomes.

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APPENDIX 1: THE OTTAWA HOSPITAL RESEARCH ETHICS BOARD APPROVAL

Ottawa Health Science Network Research Ethics Board/ Conseil d'éthique de la recherche du Réseau de science de la santé d'Ottawa

Civic Box 411, 725 Parkdale Avenue, Ottawa, Ontario, K1Y 4E9 613-798-[illegible] ext. 14602 Fax: 613-761-4311
http://www.ohsn-reb.ca

February 12, 2015

Dr. Dean Fergusson
The Ottawa Hospital Research Institute
Division of Clinical Epidemiology Program
Centre for Practice Changing Research, Box 201B
501 Smyth Rd., Room # 1296
Ottawa, ON K1H 8L6

Dear Dr. Fergusson:

Re: Protocol # 20140111-01H Short and long-term clinical effects of blood donors characteristics in transfusion recipients

Thank you for the OHSN-REB protocol amendment report dated January 22, 2015, as well as the revised Protocol dated January 22, 2015, extending the parameters from "January 1, 2003 and December 31, 2012" to "October 25, 2006 and December 31, 2013".

The above mentioned documents are approved

Study approval remains in effect until March 23, 2015.

OHSN-REB complies with the membership requirements and operates in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans; the International Conference on Harmonization - Good Clinical Practice: Consolidated Guideline; and the provisions of the Personal Health Information Protection Act 2004.

Yours sincerely,

Raphael Saginur, M.D.
Chairman
Ottawa Health Science Network Research Ethics Board

/cb

Ottawa Hospital Research Institute
Institut de recherche

uOttawa

University of Ottawa Heart Institute
Institut de cardiologie de l'Université d'Ottawa

Ottawa Health Science Network Research Ethics Board/ Conseil d'éthique de la recherche du Réseau de science de la santé d'Ottawa
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23 February, 2015

Dr. Dean Fergusson
The Ottawa Hospital Research Institute
Division of Clinical Epidemiology Program
Centre for Practice Changing Research, Box 201B
501 Smyth Rd., Room # 1298
Ottawa, ON K1H 8L6

Dear Dr. Fergusson:

RE: Protocol# - 20140111-01H **Short and long-term clinical effects of blood donors characteristics in transfusion recipients**

Renewal Expiry Date - 23 March, 2016

I am pleased to inform you that your Annual Renewal Request was reviewed by the Ottawa Health Science Network Research Ethics Board (OHSN-REB) and is approved. No changes, amendments or addenda may be made in the protocol or the consent form without the OHSN REB's review and approval.

Renewal is valid for a period of one year. Approximately one month prior to that time, a single renewal form should be sent to the REB office.

OHSN REB complies with the membership requirements and operates in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, the International Conference on Harmonization - Good Clinical Practice: Consolidated Guideline; and the provisions of the Personal Health Information Protection Act 2004.

Yours sincerely,

Rochard Sauvageau, M.D.
Chairperson
Ottawa Health Science Network Research Ethics Board

/km

APPENDIX 2: CANADIAN BLOOD SERVICES RESEARCH ETHICS BOARD APPROVAL

Canadian Blood Services
Société canadienne du sang

1800 Alta Vista Drive
Ottawa, ON K1G 4J5
T 613.739.2300 F 613.731.1411
www.blood.ca www.sang.ca

Research & Innovation

PROTOCOL REFERENCE # **2014.004** March 28, 2014

Dr. Michaël Chassé
The Ottawa General Hospital
501 Smyth Road
Ottawa, ON K1H 8L6

Dear Dr. Chassé:

Re: **"Short and long-term clinical effects of blood donors characteristics in transfusion recipients"**

ETHICS APPROVAL	**Approval Date**	**:2014-03-21**
	Annual Renewal Date	**:2015-03-21**

We are writing to advise you that the CBS Research Ethics Board has granted approval to the above-named research study, for a period of **one year with the following conditions**:

1. The principal investigator is required to provide a *progress report* for the period indicated on attached Research Study Report. Renewal requests will only be processed when a progress report is provided.
2. Upon completion of the study, the principle investigator will provide a ***final report*** summarizing findings conclusions and results.

The REB's decision to approve the ethical aspect of a research proposal depends on the initial information submitted. The REB must be informed about any changes/amendments to the project. No change can be made without prior permission, except in an emergency when subject safety is in question.

Best wishes for the successful completion of your project.

Yours sincerely,

Cilla Perry

Cilla Perry
Manager, Research & Innovation

cc: Francis Rolleston, Chair, CBS Research Ethics Board

Canadian Blood Services
Société canadienne du sang

1800 Alta Vista Drive
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PROTOCOL REFERENCE #2014.004 March 10, 2015

Dr. Michaël Chassé
The Ottawa General Hospital
501 Smyth Road
Ottawa, ON K1H 8L6

Dear Dr. Chassé:

Re: **"Short and long-term clinical effects of blood donors characteristics in transfusion recipients"**

ETHICS APPROVAL	**Renewal w/Amendment Approval**	**: 2015-03-10**
	Next Renewal Date	**: 2016-03-21**

We are writing to advise you that the CBS Research Ethics Board has granted approval to the amendment request for the above-named research study.

The REB's decision to approve the ethical aspect of a research proposal depends on the initial information submitted. The REB must be informed about any changes/amendments to the project. No change can be made without prior permission, except in an emergency when subject safety is in question.

Best wishes for the successful completion of your project.

Yours sincerely,

Cilla Perry

Cilla Perry
Manager, Centre for Innovation, Canadian Blood Services

cc: Francis Rolleston, Chair, CBS Research Ethics Board