Out for blood: causal inference in clinical transfusion research

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Chapter 2

Donor sex and recipient outcomes

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Abstract

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

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

Introduction

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

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

Donor sex and pregnancy in hematopoietic stem cell transplantation

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

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

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

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

Donor sex and pregnancy in solid organ transplant

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

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

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

Donor sex and pregnancy and red blood cell recipient mortality

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

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

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

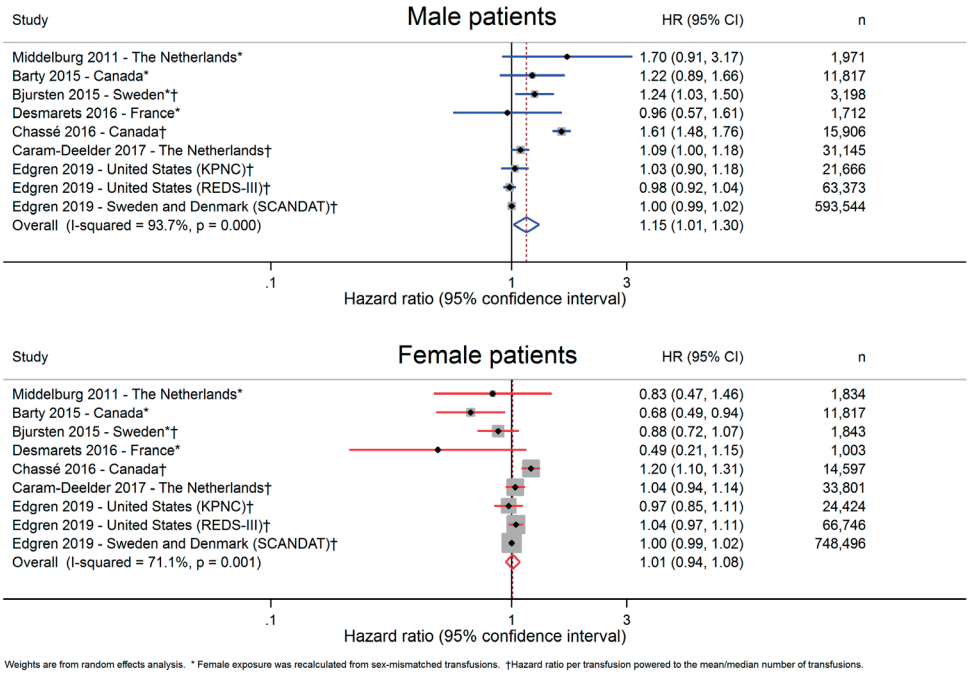

Figure 1 - Publications on the association of recipient mortality female blood donors, stratified by recipient sex

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

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

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

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

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

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

Hemoglobin

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

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

Cell-free DNA

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

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

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

Hormones

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

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

Conclusions and clinical implications

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

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

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

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

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Disclosure

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

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