The Effect of Sex-Mismatched Red Blood Cell Transfusion on Endothelial Cell Activation in Critically Ill Patients

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Keywords

Sex-mismatched transfusion · Red blood cell · Blood donor · Transfusion outcomes · Endothelial activation · Critically ill patients

Abstract

Background: Observational studies suggest that sex-mismatched transfusion is associated with increased mortality. Mechanisms driving mortality are not known but may include endothelial activation. The aim of this study is to investigate the effects of sex-mismatched red blood cell (RBC) transfusions on endothelial cell activation markers in critically ill patients. **Study Design and Methods:** In patients admitted to the intensive care unit who received a single RBC unit, blood samples were drawn before (T_0), 1 h after (T_1), and 24 h after transfusion (T_{24}) for analysis of soluble syndecan-1, soluble intercellular adhesion molecule-1, soluble thrombomodulin (sTM), von Willebrand factor antigen, interleukin-6 (IL-6), and tumor necrosis factor- α (TNF α). Changes in the levels of these factors were compared between sex-matched and sex-mismatched groups. **Results:** Of 69 included patients, 32 patients were in the sex-matched and 37 patients were in the sex-mismatched group. Compared to baseline, sex-matched transfusion was associated with significant reduction in sTM level (p value = 0.03). Between-group comparison showed that levels of syndecan-1

and sTM were significantly higher in the sex-mismatched group compared to the sex-matched group at T_{24} (p value = 0.04 and 0.01, respectively). Also, TNF α and IL-6 levels showed a statistically marginal significant increase compared to baseline in the sex-mismatched group at T_{24} (p value = 0.06 and 0.05, respectively), but not in the sex-matched group. **Discussion:** Transfusion of a single sex-mismatched RBC unit was associated with higher syndecan-1 and sTM levels compared to transfusion of sex-matched RBC unit. These findings may suggest that sex-mismatched RBC transfusion is associated with endothelial activation.

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Introduction

Red blood cell (RBC) transfusion is a common lifesaving therapy for patients hospitalized in the intensive care unit (ICU), with nearly half of ICU patients receiving at least one RBC unit during their stay [1–3]. Despite its widespread usage, several studies have linked RBC transfusion to adverse clinical outcomes, particularly in critically ill patients [4–6]. Recently, observational studies using data from various transfusion registries have suggested that donor-specific characteristics may impact patient outcome. More specifically, a mismatch between the sex of blood donors and recipients was found to be associated

with increased mortality of recipients of RBC transfusions [7–12]; however, results from other studies have been inconsistent [13]. As the majority of studies on the effect of sex-mismatched transfusions have only focused on mortality as the main outcome, specific mechanisms driving transfusion-related mortality are not known.

Critically ill patients often display evidence of endothelial activation, characterized by upregulation of circulating cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1). In addition, there are indications for loss of endothelial glycocalyx integrity, characterized by releasing syndecan-1 into the circulation [14–18]. It can be hypothesized that in the critically ill patients the presence of an activated endothelium interacts with RBCs, which is in line with previous studies showing that RBC transfusion is associated with changes in ICAM-1 and syndecan-1 concentrations [19]. A recent study has also shown that blood donor age and sex can impact secretion of endothelial markers after incubating blood product supernatant with endothelial cells [20]. In terms of potential mediators of endothelial activation following RBC transfusion, an increase in levels of circulating inflammatory cytokines, such as interleukin-6 (IL-6) and necrosis factor- α (TNF α) [6, 21], have been suggested to promote endothelial activation [22]. Other mediators that can induce endothelial activation are the presence of circulating hemoglobin and free heme [23–27].

Studying the course of endothelial activation may serve as a predictive marker for adverse outcomes associated with blood transfusion. However, there are currently no data on the effects of donor-recipient, sex-mismatched transfusions on endothelial activation. The aim of this study is to investigate the effects of sex-mismatched transfusion on markers of endothelial activation. We hypothesized that RBC transfusion from sex-mismatched donors to critically ill patients would result in higher levels of circulating endothelial markers compared to sex-matched donors.

Materials and Methods

Study Design

This study aims to investigate the effects of RBC transfusion on the host response of patients admitted to the ICU of the Amsterdam University Medical Centre in Amsterdam, the Netherlands. The study cohort was restricted to adult patients who received a single RBC unit to correct anemia (given at a hemoglobin level of 7 g/dL) during their ICU stay. Actively bleeding patients were excluded. Blood donor demographics, including sex, age, weight, and height, in addition to RBC storage duration, were obtained from Sanquin Blood Supply in Amsterdam. These characteristics of the donors were linked to their recipients via a product identification number. All blood donors were at least 18 years old, and all RBC units were leukocyte-depleted and stored in SAGM (saline-adenine-glucose-mannitol) additive solution. The study was

divided into two groups, a donor-recipient, sex-matched transfusion group, and a donor-recipient, sex-mismatched transfusion group. Ethical approval of this study was obtained from the Medical Ethical Committee of the Amsterdam Medical Centre, Amsterdam (NTR 6596; NL61833.018.17). Written informed consent was obtained from all participants or their legal representatives.

Blood Sampling and Testing

Blood samples were drawn from an indwelling arterial catheter into EDTA, citrate, and heparin collection tubes at 3 time points; prior to transfusion (T_0), 1 h after transfusion (T_1), and 24 h after transfusion (T_{24}). Blood samples were centrifuged at $1,500 \times g$ for 20 min, and the supernatant was frozen at -80°C for further analysis of endothelial activation markers including soluble syndecan-1, soluble ICAM-1, soluble thrombomodulin (sTM), and von Willebrand factor antigen (vWF:Ag). Mediators of endothelial activation included IL-6, TNF α , free Hb, and free heme. Syndecan-1, soluble ICAM-1, sTM, IL-6, and TNF α levels were measured in EDTA plasma with a Luminex[®] assay (R&D systems). The percentage of vWF:Ag was measured from citrate plasma using an enzyme-linked immunosorbent assay (ELISA) (R&D systems). Cell free Hb and free heme levels were determined from the plasma of heparinized blood using a chromogenic assay (Quanti-Chrom, Bioassay Systems).

Statistical Analysis

Data of endothelial activation markers and mediators of endothelial activation were not normally distributed according to Shapiro-Wilk test. Therefore, non-parametric tests were used to compare within/between groups in this study. Due to baseline differences on each outcome at T_0 , two statistical approaches were applied. A within-group comparison was used to measure differences between pre- and post-transfusion within each group using Friedman analysis of variance at the three time points (T_0 , T_1 , and T_{24}). Subsequent pairwise comparisons were performed to identify the contribution of each testing point. Subsequently, a between-groups analysis was used to measure differences between sex-matched and sex-mismatched transfusion groups by generating two timing points of absolute change: from the baseline to 1 h after transfusion (ΔT_1) and from the baseline to 24 h after transfusion (ΔT_{24}). An independent Mann-Whitney U test was used to assess between-group differences. Correlation between storage duration and endothelial activation markers were tested using Spearman correlation. Unless stated otherwise, medians and interquartile ranges (IQR) were reported. A p value of less than 0.05 was considered statistically significant. For the pairwise comparisons, reported p values have been adjusted by Bonferroni correction for multiple tests. Statistical analyses were performed using SPSS[®] version 26.00 software. Graphical representation was generated using GraphPad Prism[®] version 8.3.0.

Results

Study Population

The characteristics of the patients included in this study are summarized in Table 1. Less than half of the patients were female and the median age was 63 years. A little more than half of patients were admitted to ICU for surgical needs, which included cardiac surgery, neurosurgery, and general surgery, while the remaining patients were admitted for specialized medical care in car-

Fig. 1. Box plots of endothelial activation markers of soluble syndecan-1 (**A**), soluble ICAM-1 (sICAM-1) (**B**), soluble thrombomodulin (sTM) (**C**), and von Willebrand factor antigen (vWF:Ag) (**D**) of sex-matched and sex-mismatched transfusion prior to transfusion (T_0), 1 h after transfusion (T_1), and 24 h after transfusion (T_{24}).

The box at each testing time point represents the inter-quartile ranges (IQR), the bar inside the box is the median, and the top and bottom of the whiskers represent the full range of the minimum and maximum limit of all of the data. * Significant difference (p value < 0.05) when compared with the baseline testing (T_0).

Table 1. Characteristics and outcome of critically ill patients receiving either a sex-matched or sex-mismatched transfusion

	All patients ($n = 69$)	Sex-matched transfusion ($n = 32$)	Sex-mismatched transfusion ($n = 37$)	p value
Sex				
Female, % (n)	43.5 (30)	46.9 (15)	40.5 (15)	0.60
Age, years				
Median (IQR)	63 (57–74)	63.5 (51.3–75)	63 (59.5–71.5)	0.63
Ethnicity, % (n)				
Asian	4.3 (3)	3.1 (1)	5.4 (2)	0.89
Caucasian	89.9 (62)	90.6 (29)	89.2 (33)	
African	5.8 (4)	6.3 (2)	5.4 (2)	
Admission specialty, % (n)				
Medical	44.9 (31)	31.3 (10)	56.8 (21)	0.03
Surgical	55.1 (38)	68.8 (22)	43.2 (16)	
SOFA score at admission				
Median (IQR)	8 (6.5–11)	8 (7–10.8)	7 (5–11)	0.33
Duration of ICU stay, days				
Median (IQR)	11 (3.3–16.8)	11 (4–19)	11 (3–16)	0.69
ICU mortality, % (n)	24.6 (17)	28.1 (9)	21.6 (8)	0.53
SOFA, sequential organ failure assessment score.

Fig. 2. Post-transfusion differences between sex-matched and sex-mismatched groups of endothelial markers of soluble syndecan-1 (**A**), soluble ICAM-1 (sICAM-1) (**B**), soluble thrombomodulin (sTM) (**C**), and von Willebrand factor antigen (vWF:Ag) (**D**). ΔT_1 is the difference between the baseline testing (T₀) and 1 h after transfusion (T₁). ΔT_{24} is the difference between the baseline testing (T₀) and 24 h after transfusion (T₂₄). Zero line represents no

change from T₀. The box at each testing time point represents the interquartile ranges (IQR), the band inside the box is the median, and the top and bottom of the whiskers represent the full range of the minimum and maximum limit of all of the data. * Significant difference (p value <0.05) between sex-matched and sex-mismatched groups.

diology, internal medicine, and neurology. Of the 69 included patients, 32 patients received blood from donors of the same sex (sex-matched transfusion), and 37 patients received blood from donors of different sex (sex-mismatched transfusion). There were no significant differences in the baseline characteristics between the two groups with the exception of admission specialty (Table 1). Since the two groups differed in admission specialties, the Kolmogorov-Smirnov sensitivity test [28] was carried out for all measurements to rule out this potential confounder. Sensitivity testing showed no significant differences between medical and surgical admission specialties in all measurements. Online supplementary Table 1 shows the characteristics of all the blood donors included in the study (for all online suppl. material, see www.karger.com/doi/10.1159/000520651). Slightly more than half of the blood transfused in this study was drawn from female donors ($n = 53$, 6%) with a median

age of 49 years (IQR, 36.8–56.3). Storage duration of blood used in transfusions did not differ between groups (13 days, IQR, 6.5–23).

Effects of Sex-Mismatched Transfusion on Endothelial Activation Markers

Figure 1 shows the box plot of endothelial activation markers: soluble syndecan-1, sICAM-1, sTM, and vWF:Ag. There was a significant decrease of sTM over time in the sex-matched transfusion group between 6.3×10^3 pg/mL (IQR, 5.3 – 11.2×10^3) at T₀ to 6.2×10^3 pg/mL (IQR, 4.8 – 10.5×10^3) at T₂₄ (p value = 0.02). This decline was not noted in the sex-mismatched transfusion group (p value = 0.76). Additionally, the percentage of vWF:Ag significantly increased over time in the sex-mismatched transfusion group from 442% (IQR, 326.3–583.3) at T₀ to 524% (IQR, 372.5–603.5) at T₂₄ (p value = 0.03), and there was a marginally significant increase in the sex-matched

transfusion group from 495% (IQR, 401–640) at T_0 to 520% (IQR, 432–669.5) at T_{24} (p value = 0.06). Soluble syndecan-1 and sICAM-1 showed no significant difference within groups over time.

Differences between groups were compared using the absolute change from baseline to either 1 h (ΔT_1) or 24 h after transfusion (ΔT_{24}), as shown in Figure 2. Syndecan-1 endothelial marker was significantly higher in the sex-mismatched transfusion group compared to the sex-matched transfusion group at ΔT_{24} (p value = 0.04). Similarly, sTM of the sex-mismatched transfusion group was significantly increased at ΔT_{24} as compared to the sex-matched transfusion group (p value = 0.01). sICAM-1 and vWF:Ag showed no significant difference between the two groups.

Effect of Sex-Mismatched Transfusion on Mediators of Endothelial Activation

Mediators that precipitate endothelial activation are shown in online supplementary Figure 1. Median levels of TNF α showed no difference over time within the sex-matched transfusion group (p value = 0.80), while it tended to increase within the sex-mismatched transfusion group from 10.88 pg/mL (IQR, 7.54–13.61) at T_0 to 12.54 pg/mL (IQR, 8.49–14.51) at T_{24} (p value = 0.06). The differences between the two groups (sex-matched transfusion and sex-mismatched transfusion) with regards to the presence of post-transfusion endothelial activation mediators are shown in online supplementary Figure 2. Levels of IL-6 showed no difference at ΔT_1 (p values = 0.47) but showed a marginally significant increase at ΔT_{24} in the sex-mismatched group when compared to the sex-matched group (p values = 0.05).

Relationship between Storage Duration and Endothelial Activation Markers

Correlations between storage duration and endothelial activation markers were tested for all patients as well as for the study groups to assess if storage duration affects the differences between sex-matched and sex-mismatched groups (online supplementary Table 2). Endothelial activation markers did not correlate with storage duration.

Discussion

This study investigates the effect of donor-recipient, sex-mismatched transfusion on endothelial activation markers. Results showed that sex-mismatched transfusion was associated with increases in syndecan-1 and sTM levels compared to sex-matched transfusion. The percentage of vWF:Ag increased over time in both study groups, reaching statistical significance only in the sex-mismatched transfusion group. These findings suggest

that endothelial activation may play a role in the previously observed relationship between increased mortality and sex-mismatched transfusion [7–12].

Syndecan-1 and sTM elevations following sex-mismatched transfusion may indicate endothelial glycocalyx breakdown. The glycocalyx is a carbohydrate-rich layer with anti-adhesive and anticoagulant characteristics, covering the luminal side of endothelial cells [29]. Syndecan-1 is the main proteoglycan constituent of the glycocalyx. Thrombomodulin is an integral protein and also part of superficial glycocalyx but is embedded deeper within the endothelial cells [30]. Hence, the release of syndecan-1 in the circulation may reflect superficial damage to the endothelial glycocalyx, and TM shedding may reflect the disruption of the glycocalyx and endothelial cells [31]. In either case, circulating levels of syndecan-1 and sTM proportionally represent the degree of damage to the glycocalyx and/or endothelial cells [30]. It has been demonstrated that high levels of syndecan-1 and sTM are significant predictors of in-hospital mortality for critically ill patients [31–33]. This is in conjunction with findings from this study, suggesting that blood from sex-mismatched donors transfused to critically ill patients may result in more glycocalyx breakdown followed by endothelial disruption compared to sex-matched transfusions. As a consequence, glycocalyx disruption decreases the endothelial cell barrier, leading to edema and enhanced coagulation response, which may mediate organ failure, and eventually death [32]. This may explain the high mortality rate of sex-mismatched transfusion described by other studies [7–12].

Despite the relatively small difference in syndecan-1 and sTM between sex-matched and sex-mismatched groups, a recent study has related such relatively minor changes to disease severity [34]. In addition, all of the changes noted in the current study pointed towards the same direction, which is endothelial activation associated with sex-mismatched transfusions. Not to mention, these small differences were already found following a single RBC transfusion. Whether multiple RBC units would yield greater differences remains to be determined. Another possible explanation for the decline of the soluble syndecan-1 and sTM levels in sex-matched transfusion might be related to the natural course of these markers during an ICU stay. To clarify, sex-matched group is protective, or relatively protective, and the sex-mismatched transfusion “prevents” this natural decline.

The percentage of vWF:Ag was already increased prior to transfusion and increased further over time following RBC transfusion. During endothelial activation or in case of inflammation, vWF is released substantially by endothelial cells [35, 36]. Thereby, the high vWF:Ag level probably reflects an already activated endothelial cell re-

sponse in this cohort of critically ill patients, as found before [37, 38]. However, as there was no difference between sex-matched and sex-mismatched recipients, vWF does not appear to be influenced by donor sex.

It has been postulated that free hemoglobin and free heme are associated with adverse outcome of RBC transfusion [23, 24, 39]. Previous studies evaluating donor sex on the RBC storage quality observed that male sex was strongly associated with storage hemolysis, suggesting that donor sex may cause post-transfusion intravascular hemolysis in the recipient [40, 41]. However, in this study, there were no differences in free hemoglobin and free heme between the two groups suggesting that this is not a relevant mechanism in sex-mismatched transfusion, at least not following a single RBC transfusion. Also, changes in pro-inflammatory markers were small. Of note, a small difference between the sex-matched and sex-mismatched groups in IL-6 levels was observed. It is unclear whether these small differences impact clinical outcome of sex-mismatched transfusion.

The impact of storage duration on development of post-transfusion outcomes has been a controversial and much disputed subject within the field of blood transfusion [5, 42–45]. The current study assessed the relationship between endothelial markers and storage duration. However, no significant association was found.

Currently, there is no established mechanism to explain how sex-mismatched blood transfusion results in adverse outcomes of critically ill patients. One hypothesis is related to the residual plasma in the RBC products, which can cause transfusion-related acute lung injury (TRALI) [46, 47]. Female sex is a well-established risk factor for TRALI for plasma transfusion [48–50]. Exclusion of females from plasma donation has led to a lower incidence of TRALI [49, 51]. In line with this explanation, a recent cohort study showed that male recipients of RBCs from female donors who had been pregnant had a higher risk of mortality than those receiving RBCs either from female donors who had never been pregnant or from male donors [52]. Another hypothesis, which we postulate, is a high proportion of young RBCs in female donor blood, which may have immunomodulatory effects [53].

A strength of the current study is that patients randomly received the transfusion product, and the caregivers are not aware of donor sex, rendering this a double-blinded study. However, the current study is subject to several limitations. This is an observational study, therefore changes to endothelial activation and pro-inflammatory markers may be due to residual confounding that were not captured. Additionally, this study is subject to a risk of finding false-positive results (type 1 error) due to uncontrolled multiplicity in the statistical comparisons. The two groups of sex-matched and sex-mismatched

were compared in four endothelial activation markers and another four mediators of endothelial activation. However, the *p* value was not corrected for these multiple comparisons. Therefore, it is important to bear in mind that this is an explorative work raising a safety signal that should be considered cautiously. Another limitation is that this study featured a limited cohort from one singular setting, which hampers extrapolation to other sites. Some of the changes in the levels of these factors did not reach statistical significance, suggesting a too small sample size. However, all changes point towards the same direction of activation of endothelium by sex-mismatched transfusion.

In conclusion, sex-mismatched transfusion is associated with higher levels of circulating syndecan-1 and sTM levels compared to sex-matched transfusion in ICU patients. Notwithstanding the small differences and the likelihood of confounding in this study, increased endothelial activation markers may be a relevant mechanism mediating the association between sex-mismatched transfusion and increased mortality as found in other studies. As matching the gender of RBC donor with the recipient will have consequences for ensuring adequate blood supply with minimum waste, results need to be confirmed in other studies prior to changes in transfusion practices.

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Statement of Ethics

Ethical approval of this study was obtained from the Medical Ethical Committee of the Amsterdam Medical Centre, Amsterdam (NTR 6596; NL61833.018.17). Written informed consent was obtained from all participants or their legal representatives.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.A. and N.P.J. conceived and planned the study. L.v.M. and M.B. collected data for the study. A.A. performed data analyses and drafted the manuscript. L.v.M., M.B., R.v.B., J.P.A., and N.P.J. provided critical feedback and helped shape the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available due to patient and blood donor privacy but are available from the corresponding author on reasonable request.

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