Red blood cell transfusion results in adhesion of neutrophils in human endotoxemia and in critically ill patients with sepsis

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BACKGROUND: Red blood cell (RBC) transfusion is associated with adverse effects, which may involve activation of the host immune response. The effect of RBC transfusion on neutrophil Reactive Oxygen Species (ROS) production and adhesion ex vivo was investigated in endotoxemic volunteers and in critically ill patients that received a RBC transfusion. We hypothesized that RBC transfusion would cause neutrophil activation, the extent of which depends on the storage time and the inflammatory status of the recipient.

STUDY DESIGN AND METHODS: Volunteers were injected with lipopolysaccharide (LPS) and transfused with either saline, fresh, or stored autologous RBCs. In addition, 47 critically ill patients with and without sepsis receiving either fresh (<8 days) or standard stored RBC (2-35 days) were included. Neutrophils from healthy volunteers were incubated with the plasma samples from the endotoxemic volunteers and from the critically ill patients, after which priming of neutrophil ROS production and adhesion were assessed.

RESULTS: In the endotoxemia model, ex vivo neutrophil adhesion, but not ROS production, was increased after transfusion, which was not affected by RBC storage duration. In the critically ill, ex vivo neutrophil ROS production was already increased prior to transfusion and was not increased following transfusion. Neutrophil adhesion was increased following transfusion, which was more notable in the septic patients than in non-septic patients. Transfusion of fresh RBCs, but not standard issued RBCs, resulted in enhanced ROS production in neutrophils.

CONCLUSION: RBC transfusion was associated with increased neutrophil adhesion in a model of human endotoxemia as well as in critically ill patients with sepsis.

Red blood cell (RBC) transfusions in the critically ill are associated with nosocomial infections,[1–4] organ dysfunction and mortality in observational studies.[5,6] In a meta-analysis, a reduced risk of infection after transfusion was found in a restrictive transfusion strategy compared with a liberal transfusion strategy.[7] This may support the notion that transfusion modulates the

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The sponsors of this work had no role in this work including study design, the collection, analysis, and interpretation of data, the writing of the report, or the decision to submit the manuscript for publication. RVB has received a grant by the Dutch Ministry of Health (grand number PPOC-13-018).

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Received for publication March 8, 2019; revision received September 23, 2019, and accepted October 14, 2019.

doi:10.1111/trf.15613

TRANSFUSION 2020;60;294–302

recipient immune response, also referred to as transfusion related immune modulation (TRIM). Multiple TRIM effects may exist. RBC transfusion is associated with nosocomial infections, implying an immunosuppressive effect of transfusion. On the other hand, RBC transfusion is also associated with the induction of a pro-inflammatory cytokine response, with aggravation of the underlying inflammation of the recipient and the occurrence of organ failure, in particular lung injury.[8–11] This process involves the interaction of immune cells such as neutrophils with endothelial cells.

Neutrophils are essential in orchestrating an inflammatory response.[12] Neutrophils migrate to the site of infection by adhesion to activated endothelium, followed by extravasation and chemotaxis into the tissues, where they phagocytose and kill the disease-causing pathogen by the release of antibacterial proteases or by the production of reactive oxygen species (ROS) by the NADPH oxidase.[13]

Neutrophil activation can result in increased production of ROS production to certain stimuli, such as the bacterial peptide N-Formylmethionyl-leucyl-phenylaline (fMLP),[14] a process termed "priming." In addition, adhesion of neutrophils to the endothelium is increased.[15,16] Inflammatory conditions such as sepsis are characterized by an increased ability to induce priming of neutrophil ROS production as well as upregulation of neutrophil activation markers.[17–19] Enhanced neutrophil activation was found to be associated with the development of organ failure as well as with an increased risk of mortality.[20–22]

Experimental data suggest that RBC transfusion can result in activation of neutrophils. Supernatant from stored RBC products has been found to activate neutrophils in vitro[23–25] and in a rat model.[26] Possibly, this may be due to soluble bioactive substances accumulating during storage, such as CD40 ligand,[27] lipids,[28–32] cytokines,[33–35] microparticles[36,37] or free heme,[38–41] which are all able to prime neutrophil ROS production in experimental conditions. Besides accumulation of activating agents in the RBC product related to storage duration, also the inflammatory status of the recipient may modulate effects of RBC transfusion on neutrophil function. In an in vitro flow model, increased adhesion of RBCs to endothelial cells was found when cells were stimulated with endotoxin.[42,43] In addition to the upregulation of adhesion molecules on the endothelium, activation of the vascular endothelium by endotoxin can result in the release of chemokines, which can rapidly induce neutrophil activation.[28,44] Also, critically ill patients seem particularly susceptible for the adverse effects of RBC transfusion.[6] This indicates that the underlying immune response of the host plays a role.

This study investigated whether a RBC transfusion induces priming of neutrophil ROS production and adhesion. To this end, plasma samples of endotoxemic human volunteers receiving an autologous RBC transfusion as well as plasma samples of critically ill patients that received a RBC transfusion were tested for the capacity to induce ex vivo priming of ROS production and adhesion of human neutrophils of healthy controls. We hypothesized that RBC transfusion results in changes in the plasma of the recipient that increase priming of ROS production and adhesion of neutrophils. Moreover, we hypothesized that the effects on neutrophil activation are modulated both by storage duration as well as by the inflammatory status of the recipient.

MATERIAL AND METHODS

Both study protocols adhere to the declaration of Helsinki and have been approved by the Medical Ethical Committee of the Academic Medical Center. Written informed consent was obtained from all subjects before entry in the study.

Endotoxemia model[45]

Healthy male volunteers (18-35 years of age) donated a unit of whole blood, which was collected in a citrate-phosphate-dextrose bag and stored for 16-22 hours. The RBC transfusion products were produced according to the national standards of Sanquin Blood Supply Foundation, Amsterdam, The Netherlands. After centrifugation, the units were processed into a RBC unit, a unit of plasma, and a buffy coat. The RBC unit was leukoreduced by filtration (Fresenius Kabi) and Saline-adenine-glucose-mannitol (150 mM NaCl, 1.25 mM adenine, 50 mM glucose, 29 mM mannitol) (SAGM, Fresenius Kabi) was added as storage solution. On the day of the experiment, volunteers (n = 18) were injected intravenously with *Escherichia coli* lipopolysaccharide (2 ng/kg [National Institutes of Health Clinical Center]). Two hours after LPS infusion, the volunteers were transfused with an autologous RBC unit. The subjects received either 2-day stored (2D) autologous RBCs, 35-day stored (35D) autologous RBCs, or an equal volume of NaCl 0.9% (saline) (all groups n = 6). Heparin anticoagulated blood samples were taken before LPS (baseline), 2 hours after LPS but prior to transfusion, and 4 hours after transfusion. Samples were centrifuged at 1800 G for 10 minutes at 4°C and plasma was stored at −80°C until further use.

Clinical study in critically ill patients

Between November 2011 and October 2015, critically ill patients in need of a single RBC unit to correct for anemia, were consecutively included. Patients were subdivided into septic and non-septic at the time of transfusion. Sepsis was diagnosed according to the SEPSIS-3 criteria[46]; Sequential Organ Failure Assessment (SOFA) score ≥2 in combination with a suspected or proven infection which was treated with antibiotics. Exclusion criteria were 1) actively bleeding patients; 2) patients who received multiple RBC units; and 3) patients who received RBC, plasma, or thrombocyte transfusion in the 24 hours prior to inclusion. As per protocol, critically ill patients were transfused with a single RBC unit when their hemoglobin level was 4.3 mmol/L. In The Netherlands the maximum storage time of RBCs is 35 days, which is shorter than in the Unites States where bank

polices allow storage for up to 42 days; and to reduce waste, RBCs which have been stored the longest are dispensed first. The patients were divided into groups receiving either fresh RBC (<8 days) or standard stored RBC (2-35 days). Heparin anticoagulated blood samples were drawn from an indwelling arterial catheter prior to, straight after, and 24 hours after transfusion, centrifuged at 1500 g for 20 minutes at room temperature and plasma was stored at −80°C until further use.

Neutrophil isolation

Venous blood was collected from healthy volunteers in heparin anticoagulant blood tubes. Neutrophil isolation was performed using a 1.076 g/mL Percoll-based (Pharmacia) density gradient. Blood samples were mixed gently with an equal volume of 10% PBS/ TNC (tri sodiumcitrate, Merck), layered over Percoll, and centrifuged (1125 G, 20 min). After removing the peripheral blood mononuclear cell (PBMC), lysis of RBCs was performed twice by adding ice-cold lysis buffer (NH_4Cl [0.155 M], KCO_3 [0.01 M], and EDTA (triplex III) [0.1 mM] (Merck-Millipore). The isolated PMNs were washed and resuspended at 5 x 10^6/mL in Hepes buffered saline solution (containing 132 mM NaCl, 6 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 1.2 mM K_2HPO_4, 20 mM Hepes, 5.5 mM glucose (Merck-Milipore) and 0.5% (w/v) human serum albumin, pH 7.4 (Brocacef).[47]

Priming of neutrophil ROS production

Isolated neutrophils (1 x 10^6/ml stimulation) were incubated with 25% plasma from both study cohorts and healthy controls in buffer at 37°C for 30 minutes in a shaking bath. After the incubation, neutrophils were spun down and resuspended in 1 mL Hepes buffer. Neutrophils (0.25 x 10^6/mL) in the presence of Amplex Red (25 μM) and horseradish peroxidase (0.5 U/mL) were incubated for 5 minutes at 37°C (Amplex Red kit, Molecular Probes) and activated by formyl-methionyl-leucyl-phenylalanine (fMLP) (1 μM, Sigma Aldrich). The hydrogen peroxide production was measured with the HTS7000plus plate reader (Tecan). Fluorescence was measured at 30-second intervals for 30 minutes. Hepes buffer and 20 ng/mL *E. coli* lipopolysaccharide (LPS)(Sigma- Aldrich)/LPS- binding protein (R&D Systems) served as a negative and positive control. The priming properties were assessed by the amount of hydrogen production (H_2O_2) 5 minutes after fMLP addition in nmol/ 10^6 cells. The concentration of H_20_2 was calculated from a calibration curve. The medium control was deducted from the fMLP measurement and the value corrected by the neutrophil count was used for analysis.

Neutrophil adhesion assay

Neutrophils were fluorescently labeled with Calcein-AM (1 μM, 30 min) (Molecular Probes).[48] Calcein-labeled neutrophils (1 x 10^6/ml) were incubated in an uncoated Maxisorp plate (Nunc) and 25% plasma was added. After incubation of 30 minutes at 37°C in an incubator, the plate was washed twice with PBS. Cells were lysed with Triton (0.5% X-100, Sigma Aldrich) for 5-10 minutes at room temperature. Adhesion was assessed by the fluorescence of calcein-labeled neutrophils in the infinite F200-pro plate reader (Tecan) and was determined as a percentage of the total input of calcein-labeled cells (100%).

To reduce bias due to the donor effect in the priming of ROS production and adhesion assays, neutrophils from two different donors were used for each patient plasma sample and all assays were performed in duplicate.

Statistical analysis

Data were tested for normality using histograms and density plots. For categorical data, the Chi- square test was used; for continuous data, the t-test was used; or when data were skewed, the Mann-Whitney U test was used. For comparisons between treatment groups (e.g., RBC vs. saline, septic vs. non-septic patients, fresh vs. standard RBCs), we a priori determined to use the measurements at a single timepoint or the change between baseline and the last time point as our primary (independent) outcome in the statistical analysis. These were analyzed using an unpaired t-test for normally distributed data and a Mann–Whitney U test when the data were skewed. Comparisons within treatment groups were carried out using a paired samples t-test or when data were skewed the Wilcoxon Signed Ranks test. Double-sided p values of <0.05 were considered to be statistically significant. Since we consider our study to be explorative (given the sample sizes), we chose not to correct for multiple testing or repeated measurements. All statistical analyses were performed in IBM SPSS Statistics version 24.

Power statement

Sample sizes were based on previous experimental studies.[23,25] For the present outcomes, the sample sizes used (6 vs. 12 & 22 vs. 25) will have 80% power to detect an effect size of 1.5 and 0.84, respectively using a t-test with a two-sided significance level of 0.05.

RESULTS

The effect of RBC transfusion on neutrophil function in a human endotoxemia model

To investigate the effect of an autologous RBC transfusion on neutrophil activation we tested the activating properties of plasma from transfused endotoxemic individuals on freshly isolated neutrophils of healthy volunteers. As previously reported,[45] LPS infusion induced a systemic inflammation reaction syndrome (SIRS), including fever, tachycardia, and leucocytosis. These symptoms subsided several hours following LPS administration. In this model, the ex vivo capacity of plasma taken after LPS to prime neutrophil ROS production was not significantly different compared to plasma taken

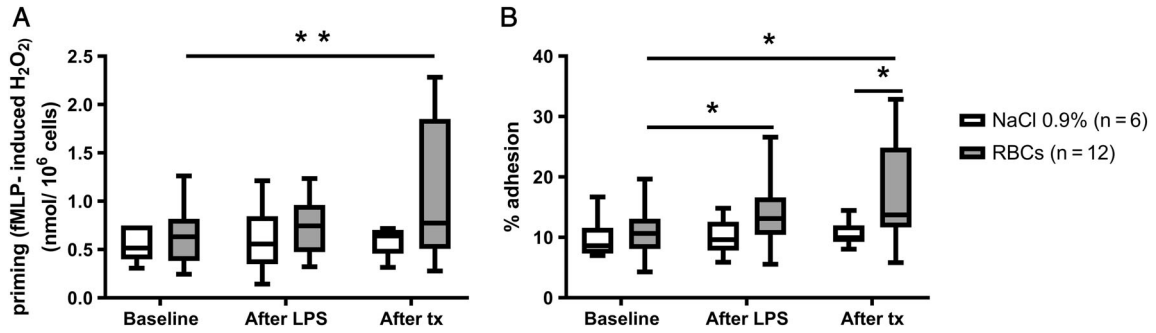

Fig. 1. Neutrophil ROS production (A) and adhesion (B) activity in the endotoxemia model. "Baseline" is before the LPS is given, "After LPS" is 2 hours after LPS was infused and "After tx" is 4 hours after the volunteers received either saline or an autologous RBC transfusion. LPS: lipopolysacheride, RBC: red blood cell. Data are shown as median [IQR]. *p < 0.05, **p < 0.01.

before LPS infusion. Following RBC transfusion, ex vivo priming of the neutrophil ROS production was significant higher versus baseline levels (0.77 nmol/10^6 cells [IQR 0.51-1.85] vs. 0.63 nmol/10^6 cells [IQR 0.38-0.81], p = 0.002 Fig. 1). When compared to the control volunteers who received LPS and saline, the priming of the ROS production was not statistically different at the last time point compared to the volunteers who received a RBC transfusion (0.64 nmol/10^6 cells [IQR 0.50-0.70] vs. 0.77 nmol/10^6 cells [IQR 0.51-1.85], p = 0.213, Fig. 1A).

In the volunteers who received a RBC transfusion, the capacity of plasma to induce ex vivo neutrophil adhesion was increased following LPS infusion in healthy volunteers (13.1% [IQR 10.4- 16.6]) and further increased by RBC transfusion when compared to baseline levels (13.7% [IQR 11,6- 24,9] vs. 10.6% [IQR 8.0-13.1], p = 0.015 Fig. 1). When compared to the saline control recipients at the last time point, the adhesion was significant higher in the volunteers who received a RBC transfusion (13.7% [IQR 11.6-24.8] vs. 9.95% [IQR 9.3-12.0] in controls, p = 0.024, Fig. 1B). Transfusion of fresh (2 days of storage) as well as of stored (35 days of storage) RBC products induced the priming of neutrophil ROS production and adhesion ex vivo, without a difference between these

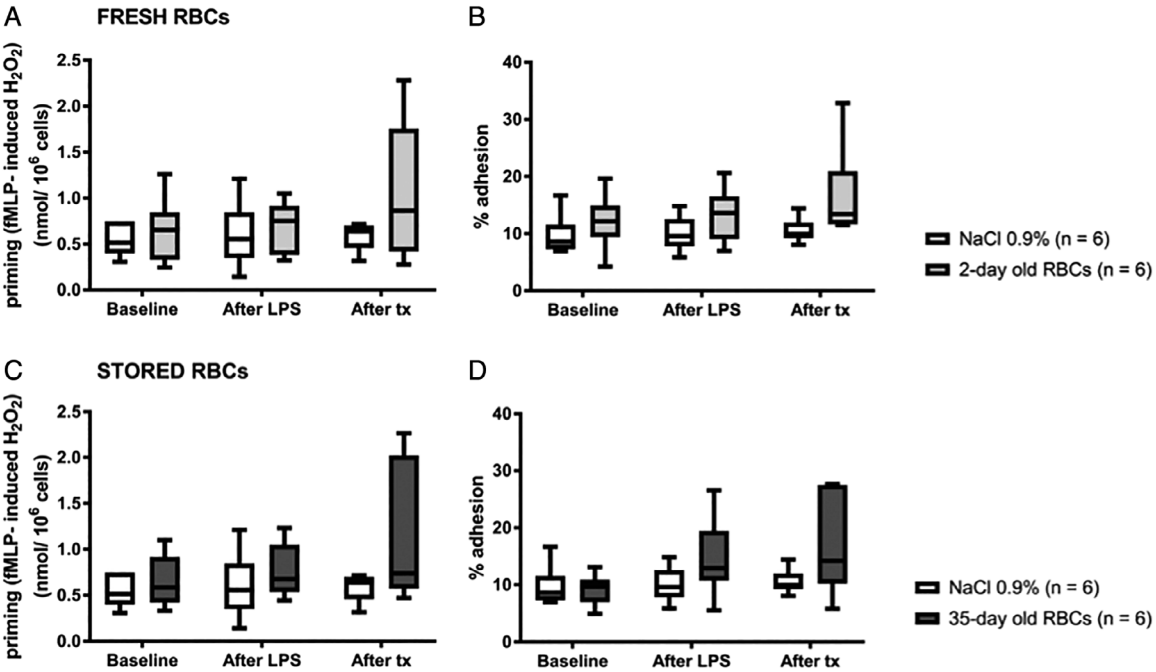

Fig. 2. Effect of storage time on priming of neutrophil ROS production and adhesion activity in the endotoxemia model. "Baseline" is before the LPS is given, "After LPS" is 2 hours after LPS was infused and "After tx" is 4 hours after the volunteers received either saline or an autologous RBC transfusion stored for 2 days (2D RBCs, Fig. 2A,B) or 35 days (35D RBCs, Fig. 2C,D)). LPS: lipopolysaccharide, RBCs: red blood cells. Data are shown as median [IQR].

TABLE 1. Critically ill patient characteristics

	All patients (n = 47)	Septic patients (n = 26)	Non-septic patients (n = 21)	p value
Age (years) *median (IQR)*	63 [57-73]	64 [56-70]	61 [58-70]	0.676
Sex				
Male, n (%)	25 (53)	10 (38)	15 (71)	0.024
Specialty, n (%)				
Cardiology	9 (19)	3 (12)	6 (29)	0.121
Cardiothoracic surgery	10 (21)	5 (19)	5 (24)	
Internal medicine	11 (23)	7 (27)	4 (19)	
Neurology	1 (2)	0 (0)	1 (5)	
Surgery	12 (26)	10 (38)	2 (10)	
Traumatology	4 (9)	1 (4)	3 (14)	
SOFA on transfusion day *median (IQR)*	8 [5-9]	8 [6-9]	7 [5-9]	0.425
RBC storage time (days) *median (IQR)*	8 [6-22]	8 [5-22]	12 [7-20]	0.434
Pre-transfusion Hemoglobin (mmol/l) *median (IQR)*	4.3 [3.9-4.6]	4.1 [3.8-4.6]	4.3 [4.1-4.6]	0.103
Pre-transfusion Leukocytes (1 x 10^9/l) *median (IQR)*	16.3 [10.7-22.8]	18.9 [13.9-27.1]	14.8 [10.7-17.9]	0.064
Hospital mortality, n (%)	15 (33)	11 (42)	4 (19)	0.068

SOFA = sequential organ failure assessment; RBC = red blood cell.

groups (Fig. 2). Thus, in a human endotoxemia model, RBC transfusion is associated with an increase in ex vivo neutrophil adhesion, which was not affected by RBC storage duration. In the priming of neutrophil ROS production there seems to be a trend after RBC transfusion, albeit with a large variation.

The effect of RBC transfusion on neutrophil function in critically ill patients

Given that critically ill patients receive RBC transfusion very regularly, this patient population was selected to investigate the effects of allogeneic RBC transfusion in a clinical setting. As the underlying immune status of the recipient may affect the neutrophil activation, we made a distinction between septic and non-septic patients. From all sampling time points, 5 samples were missing due to logistical problems (9.6%) and those patients were excluded. Eventually, 47 patients receiving an RBC transfusion were included in this study, of which 26 patients were diagnosed as septic at the time of transfusion. The median duration from intensive care unit (ICU) admission until RBC transfusion was 11 days [IQR 4-16]. The baseline characteristics of each group are presented in Table 1. Of note, at the time of RBC transfusion the disease severity (as assessed by the SOFA score) was similar, probably because septic patients had reached the convalescent phase of sepsis. In the group with non-septic patients, significantly more men were present compared to the septic group. Age, hemoglobin levels before transfusion, and storage time of the RBC units were not different between the groups. Prior to transfusion, the capacity of plasma from critically ill patients to induce ex vivo neutrophil ROS production was already increased when compared to plasma from healthy controls (0.82 nmol/10^6 cells [IQR 0.64-1.19] vs. 0.57 nmol/10^6 cells [IQR 0.45-0.86], p = 0.003, Fig. 3A) and was not further augmented following RBC transfusion. In septic patients, the ability of plasma to prime neutrophil ROS production at baseline was not significantly different compared to the plasma from the non-septic patients (0.81 nmol/10^6 cells

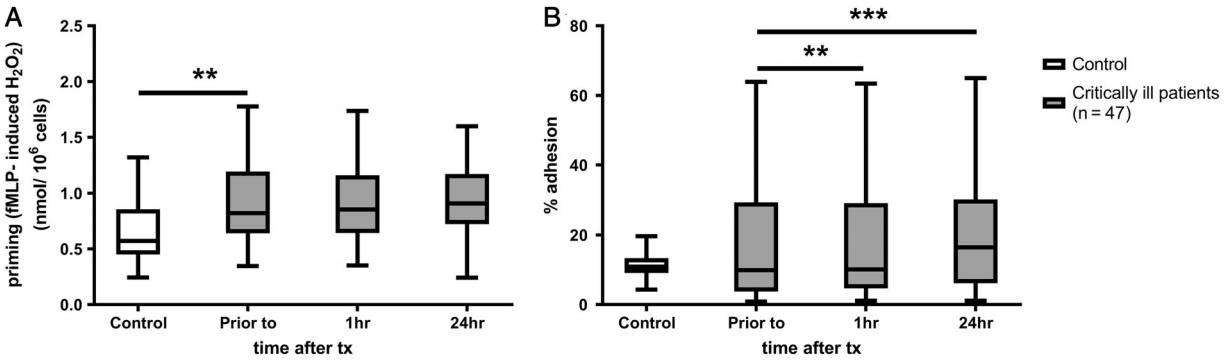

Fig. 3. Priming of neutrophil ROS production (A) and adhesion (B) activity in the critically ill patients. "Prior to" is before the RBC transfusion is given, "1 hr" is 1 hour after and "24 hr" is 24 hours after critically ill patients received an allogeneic RBC transfusion. RBC: red blood cell. Data are shown as median [IQR]. *p < 0.05, **p < 0.005, ***p < 0.001.

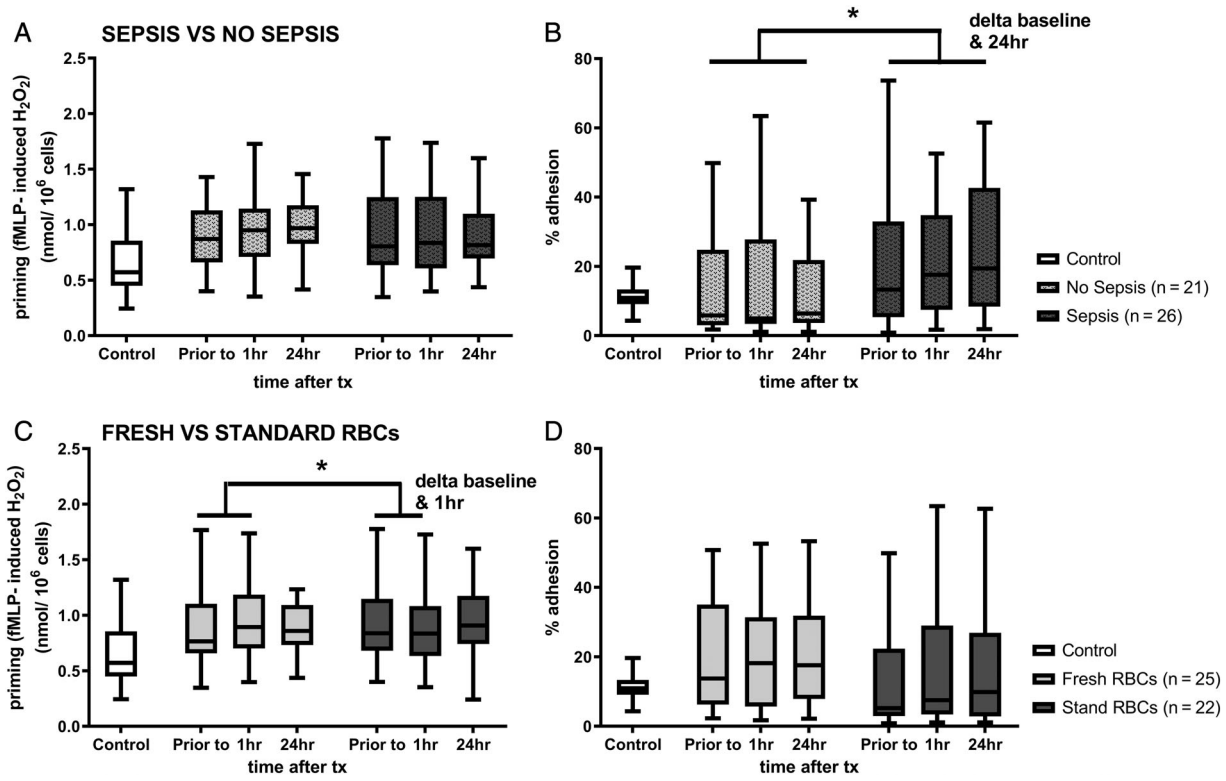

Fig. 4. Priming of neutrophil ROS production (A) and adhesion (B) activity in septic and non-septic patients, and effect of storage time on neutrophil ROS production (C) and adhesion (D) activity in the critically ill patients. "Prior to" is before the RBC transfusion is given, "1 hr" is 1 hour after and "'24 hr" is 24 hours after critically ill patients received an allogeneic RBC transfusion. RBC: red blood cell. Data are shown as median [IQR]. *p < 0.05.

[IQR 0.63-1.25] vs. 0.87 nmol/10^6 cells [IQR 0.66-1.13], Fig. 4A). Following RBC transfusion, the post transfusion change to prime neutrophil ROS production did not differ in septic patients 24 hours after transfusion when compared to the non-septic patients (+0.05 nmol/10^6 cells [IQR 0-0.25] vs. 0 nmol/10^6 cells [IQR -0.19-0.17], p = 0.141, Fig. 4A). When testing the effects on adhesion, the capacity of plasma from critically ill patients to induce neutrophil adhesion was not increased prior to transfusion when compared to plasma from healthy controls. After RBC transfusion the capacity of plasma to induce neutrophil adhesion is significantly higher compared to pre-transfusion levels (10.1% [IQR 4.8-29.0] and 16.5% [IQR 6.2-29.0] vs. 9.9% [IQR 3.8-27.6], p < 0.01, Fig. 3B). When patients were divided into septic and non-septic patients, the post transfusion change to induce neutrophil adhesion was significantly higher in septic patients 24 hours after RBC transfusion when compared to non-septic patients (+3.2% [IQR 0.9-5.7] vs. +0.8% [IQR -0.7-2.6], p = 0.039, Fig. 4B), while at baseline septic and non-septic patients did not differ in the adhesion assay. In critically ill patients receiving fresh blood, storage duration was 6 days [IQR 3-8], which was significantly shorter than from the patients who received standard issued RBCs (21 days [IQR 15-31]). In patients who received fresh RBC transfusion, the post transfusion change in priming of the ROS production ex vivo was larger after transfusion compared to the patients who received a standard issued RBC (+0.11 nmol/ 10^6 cells [IQR -0.06-0.42] vs. −0.06 nmol/10^6 cells [IQR -0.2-0.36], p < 0.05, Fig. 4C). This difference is not found for adhesion, where there are no differences in the post-transfusion change between patients who received fresh RBCs compared to patients who received standard RBCs (Fig. 4D).

DISCUSSION

In this study, the effect of RBC transfusion on priming of neutrophil ROS production and adhesion was investigated in human endotoxemia as well as in critically ill patients. The main findings are that 1) in human endotoxemia, RBC transfusion is associated with an increase in ex vivo neutrophil adhesion, which was not affected by RBC storage duration; 2) in critically ill patients, RBC transfusion is associated with an increase in the ex vivo neutrophil adhesion capacity in septic recipients compared to non-septic recipients; and 3) in critically ill patients, fresh RBC transfusion, but not standard issued RBC transfusion, is associated with augmentation of ex vivo priming of neutrophil ROS production.

RBC transfusion resulted in a higher neutrophil adhesion-inducing capacity in septic recipients than in non-septic recipients, the plasmas had an equal ROS priming capacity. This may suggest that the inflammatory status of the recipient plays an important role in the association between RBC transfusion and ex vivo neutrophil adhesion. This finding is in line with results from experimental studies, in which endotoxin increased the ability of endothelial cells to adhere to neutrophils in vitro.[49] We hypothesize that during inflammation, elevated levels of pro-inflammatory substances mediate RBC-induced neutrophil adhesion.[50] Of note, neutrophils play a key role in the pathogenesis of transfusion related acute lung injury (TRALI). In this syndrome, it is thought that the presence of an inflammatory status primes pulmonary neutrophils and activates the endothelium, which increases the risk of a TRALI reaction following transfusion. Our results are in line with this hypothesis. Taken together, our results suggest that the immunomodulatory effects of an RBC transfusion are more increased when an inflammatory status is present in the recipient.

At the start of this study, our initial hypothesis was that increased storage duration of RBCs was associated with increased effects on neutrophil function. However, we found the contrary; transfusion of fresh RBCs, but not standard issued RBCs, resulted in priming of neutrophil ROS production in critically ill patients. Large clinical trials have provided strong evidence that a prolonged storage time is not related to complications of RBC transfusions.[51,52] On the contrary, a meta-analysis suggest that fresh RBC may even be more harmful,[53] which is suggested to be related to the manufacturing method.[54] However, the manufacturing method is not likely to be the explanation for the findings in this study, as all patients received red cell filtered RBC products that were manufactured the same way. Given that storage duration did not play a role in the endotoxemia model which used autologous blood, we suggest that inflammatory mediators accumulating during storage do not play a role. We hypothesize that allogeneic properties of the transfusion have an interaction with the recipient. Of note, we previously showed that an autologous RBC transfusion does not induce immune tolerance in endotoxemic recipients.[55] Again, this points toward a role for allogenic factors in the RBC product. An alternative explanation may be donor related factors like sex mismatch,[56] which has also been associated with adverse outcome. Sex mismatch was not corrected for in this study in critically ill patients and cannot be dissected from our findings. However, it is unknown whether sex mismatch may occur only with fresh RBC products.

This study has several limitations. The effect of RBC transfusion was investigated by comparison to pre-transfusion baseline levels, and not to a negative control. Thereby, effects may have occurred in time or by something other than the RBC product itself. Also, samples were not spun down to lose any residual platelets. The ability to prime neutrophil ROS production and adhesion was tested using neutrophils from healthy subjects. The endogenous neutrophils in critically ill patients receiving a RBC transfusion may react differently. In addition, due to the incubation time of 30 minutes, we may have missed transient effects of other agents on the neutrophil ROS production and adhesion. Also, although a power calculation was done, the study in critically ill patients could have been underpowered to find a significant difference in priming of the neutrophil ROS production after transfusion due to confounders unaccounted for, such as shock. However, SOFA scores between groups were similar and large variation between individuals was also found in the endotoxemia model. In addition, we tested effects of a single RBC unit. Possibly, multiple RBC units may induce more immunomodulatory effects. However, the use of one single RBC unit to correct anemia is comparable to the clinical practice at the ICU[51] and makes the study clinically relevant. Of note, the results in this may not apply to other countries where other manufacturing methods to produce RBC units are used.

In conclusion, RBC transfusion results in an increased capacity of plasma from the recipients to induce ex vivo neutrophil adhesion in a model of human endotoxemia as well as in a cohort of critically ill patients with sepsis. Fresh RBC transfusion, but not standard issued RBC transfusion, is associated with ex vivo priming of neutrophil ROS production. Therefore, neutrophil activation may be a mechanism of transfusion-induced adverse outcome in individuals with severe systemic inflammation. Future research should focus on which factors in the RBC product are responsible for priming of neutrophil ROS production and adhesion, as well as on the influence of short storage duration.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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