The age of red blood cells is associated with bacterial infections in critically ill trauma patients

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Background. Blood transfusion increases the risk of nosocomial infection in trauma patients. Specific patient- and transfusion-related risk factors are largely unknown. In this study, risk factors for developing a bacterial infection after transfusion of red blood cells (RBC) or platelets were determined in a cohort of transfused critically ill trauma patients.

Material and methods. A retrospective study was conducted in a mixed medical-surgical Intensive Care Unit (ICU) of a level-1 university trauma centre, in trauma patients who received a RBC or platelet transfusion. Patients who developed a bacterial infection after transfusion were compared to transfused controls who did not develop such an infection. Multivariable logistic regression was used to determine risk factors for infection.

Results. Of the 7,118 patients admitted to the ICU during the study period, 196 trauma patients met the inclusion criteria. An infection developed in 56 patients (29%). Infection occurred irrespective of the administration of antibiotics as part of selective digestive tract decontamination, surgery status or Injury Severity Score. Transfusion of RBC stored for more than 14 days was associated with infection in trauma patients (odds ratio 1.038, [95% CI: 1.01-1.07], p=0.036). Neither the amount of RBC nor that of platelets was associated with onset of infection.

Conclusions. Transfusion of RBC stored for more then 14 days is a risk factor for onset of bacterial infection after trauma, irrespective of the use of prophylactic antibiotics. Transfusion of platelets was not a risk factor. These results may contribute to designing prospective studies on transfusion of fresh RBC only in trauma patients.

Keywords: trauma, transfusion, storage time, immunomodulation, infection.

Introduction

Although life-saving at times, blood transfusion has clinically significant immuno-modulatory effects. Studies in trauma patients have demonstrated a clear association between transfusion and the onset of nosocomial infection[1-4]. Proposed mechanisms of the observed association include down-regulation of the recipient's immune function by leucocytes and release of soluble mediators during storage of red blood cells (RBC) and platelets[5-7]. In trauma patients, an association has been found between transfusion of stored blood and infectious complications[2,3,8]. Recently, Weinberg *et al.* showed that this association remained after implementation of leucoreduction[4], thereby contributing to adverse outcome[9]. This study was, however, conducted over a long inclusion period, during which transfusion practices changed significantly in the institution in which the study was performed[10]. Also, infections at sites other then the lung were not taken into consideration, nor was effect of aged platelets, which have considerable immuno-modulatory properties[5]. These confounders may have contributed to conflicting results on the effect of leucoreduction on infection in trauma patients and in other populations of surgical patients[7].

Besides transfusion-related risk factors, patient-related risk factors may play a role. It is unknown whether the association between stored

blood and infection persists in an intensive care setting in which antibiotics are given to prevent onset of infection as part of selective digestive tract decontamination (SDD). Patients with traumatic brain injury suffer a high incidence of bacterial infections, which occur in up to 60% of such patients[11]. Furthermore, undergoing surgery may contribute to a state of immune suppression, thereby affecting the risk of transfusion-related infectious complications. Specific patient-related risk factors for infection following transfusion are, however, largely unknown.

We hypothesised that the severity of trauma and storage time of blood products could influence the risk of developing an infectious complication after trauma. As modifying blood banking practices to supply only fresh blood may significantly impair blood availability, knowledge on which trauma patients could benefit from such a strategy is important. The aim of this study was to investigate patient- and transfusion-related risk factors for the onset of bacterial infection in a cohort of transfused, critically ill trauma patients in a SDD setting.

Materials and methods

The study was performed in a mixed medical-surgical ICU of a university hospital with a designated level-1 trauma centre in The Netherlands. The study was approved by the Ethical Committee of the hospital. All consecutive trauma patients (with multiple trauma and/or head injury) admitted to the ICU between the period of January 1, 2004 and November 30, 2007 who received a transfusion of RBC or platelets during or before their first ICU admission were included. Patients developing infection were compared with patients not developing an infection. Data on gender, age, surgical status, alcohol abuse, diabetes, human immunodeficiency virus infection, malignancy, use of immunosuppressive medications and Acute Physiology and Chronic Health Evaluation (APACHE II) score were retrieved from the patients' digital medical system (PDMS). The administration of SDD was scored. SDD is part of standard care for patients with an anticipated ICU stay of more than 48 hours and consists of 4 days of intravenous cefotaxime 1 g 6 hourly in combination with topical application of tobramycin, colistin, and amphotericin B in the oropharynx and stomach. Injury Severity Score (ISS) data were retrieved from the hospital's trauma registry.

Transfusion data were extracted from the hospital blood transfusion service computer system and included the date, number of units and storage time of the blood products administered. Blood products were leucoreduced by removal of the buffy coat followed by filtration. RBC were categorised as being ≤14 days old or >14 days old[12]. Platelets (as a pooled product from 5 donors) were categorised as being ≤4 days old or >4 days old[13]. Only transfusions given before the onset of an infection were included in the analysis. In this cohort, the majority of RBC and platelets were administered because of bleeding. As an ICU policy, RBC were administered to correct anaemia when the haemoglobin concentration dropped below 7 g/dL.

Infections were diagnosed based on a positive culture of samples of blood, sputum, bronchoalveolar lavage fluid or urine, which were taken when infection was suspected by the treating physician. Only bacterial infections were studied, as they are most common and most clinically relevant in the ICU[14]. When contamination from skin flora was considered, the pathogen had to be cultured from two samples before the culture was scored as positive.

Statistics

Differences between the infectious and non-infectious groups were described using the *t*-test (normally distributed variables), Mann-Whitney test (non-normally distributed variables) and χ^2-test or Fisher's exact test (categorical data). Multivariate logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between infection and blood products. Inclusion of variables in the model was based on results of univariate analyses and clinical significance. Co-viariates included were the ISS score, head trauma, surgery and use of SDD, which were entered in the model. Effects of RBC and platelet products were analysed separately. In both models, storage time and total units transfused were included separately because of co-linearity. First, the risk of infection after transfusion was investigated by entering the number of units of RBC or platelets, i.e. blood volume, in a crude model. We then analysed whether storage time influenced the crude risk per amount of blood product by adding age of RBC as an interaction term. The interaction term of old blood has a p value of 0.40, indicating that the relation between

RBC and infection is not different for various degrees of age of RBC and that one model was sufficient for analysis. Given that a significant interaction was not found, the model was examined for effect modification of other covariates and confounding factors. A confounding effect was defined as a ≥10% change in the coefficient of the first crude effect size per product as a consequence of adding a co-variate. Effect modification was defined as a significant p value for the interaction term added to the model. Co-variates included ISS score, head trauma, surgery and use of SDD. Pearson's correlation coefficient was used to determine the association between the amount of stored and fresh RBC with infection rate. Statistical analyses were conducted using SPSS 16. A p value of ≤0.05 was considered statistically significant.

Results

During the study period, 7,118 patients were admitted to the ICU, of which 339 (5%) were admitted with multiple trauma and/or head injury. Of these, 196 patients (58%) received a transfusion of RBCs or platelets (1,684 units of RBC and 163 units of platelets). This cohort was further analysed for risk factors.

Of the 196 transfused patients, 56 (29%) developed an infection, of whom 17 patients had infections at multiple sites (Table I). The most frequent nosocomial infection was pneumonia.

Table I - Infections following blood transfusion.

Site of infection	N=73
Pneumonia	34 (46%)
Wound infection	13 (18%)
Bacteraemia	14 (19%)
Urinary infection	10 (14%)
Deep-seated abscess	2 (3%)
Bacteria	
Gram-positive	45 (62%)
Gram-negative	28 (38%)

Infections with Gram-positive bacteria were most prevalent. Variables that may influence host response are shown in Table II. Specific patient-related risk factors, including ISS and surgery status, did not differ between groups. Notably, patients developing an infection more often received SDD compared to the controls who did not develop an infection.

As far as concerns transfusion-related risk factors, trauma patients developing an infection tended to receive a larger amount of blood products and RBC compared to controls (Table III). The median storage time of RBC did not differ between groups, but among the patients who had received RBC stored for >14 days, the patients who developed an infection had received more units than the patients who did not develop an infection. Neither the volume of platelets administered nor the storage time of platelets differed between groups.

Table II - Characteristics and patient-related risk factors for acquiring an infection after transfusion.

	Infection		
	Yes (n=56)	**No (n=140)**	**P value**
Age, median (IQR)	42 (25-59)	39 (25-56)	0.619
Male gender, n (%)	47 (84)	102 (73)	0.101
APACHE II, median (IQR)	16 (11-22)	14 (10-21)	0.37
Injury Severity Score, mean (SD)	25 (11)	24 (12)	0.642
Multiple trauma, n (%)	43 (77)	96 (69)	0.171
Head trauma, n (%)	12 (21)	44 (31)	0.382
Alcohol abuse, n (%)	6 (11)	10 (7)	0.399
Surgical, n (%)	42 (75)	108 (77)	0.749
Receiving SDD, n (%)	50 (89)	103 (74)	0.016
Diabetes, n (%)	4 (7)	7 (5)	0.513
Malignancy, n (%)	2 (4)	3 (2)	0.625
Immune suppressive medication, n (%)	12 (21)	26 (19)	0.648
HIV infection, n (%)	0 (0)	3 (2)	0.559

Legend: APACHE: Acute Physiology and Chronic Health Evaluation; SDD: selective digestive tract decontamination; HIV: human immunodeficiency virus.

Table III - Transfusion descriptives of trauma patients with and without infection.

	Infection		
	Yes (n=56)	**No (n=140)**	**P value**
Patients			
Patients receiving RBC + platelets	n=25	n=30	
Patients only receiving RBC	n=29	n=108	
Patients only receiving platelets	n=2	n=2	
Blood products			
Total amount of units (RBC + platelets)	7 (2-18)	4 (2-8)	0.05
RBC			
Total amount of RBC units	8 (2-16)	4 (2-8)	0.06
RBC storage time, days	22 (20-24)	22 (17-24)	0.33
N. of RBC units ≤14 days	3 (2-3) (n=5)	4 (2-5) (n=122)	0.33
N of RBC units >14 days	8 (2-16) (n=619)	4 (2-8) (n=938)	0.02
Platelets			
Total amount of platelet units	2 (1-3)	2 (1-3)	0.94
Platelet storage time, days	4 (2-5)	3 (3-5)	0.787
N. of platelets units ≤4 days	1 (1-4) (n=28)	2 (1-4) (n=68)	0.53
N. of platelet units >4 days	2 (1-3) (n=37)	2 (1-2) (n=30)	0.48

Legend: RBC: red blood cells. Data are expressed as median (interquartile range).

Table IV - Multivariable analysis of the risk of transfusion of RBC and platelets for infection unadjusted and adjusted for storage time.

	OR (95% CI) unadjusted for storage time	**OR (95% CI) adjusted for storage time#**
Units of RBC	1.02 (0.99-1.05)	1.04 (1.01-1.07)
Units of platelets	1.12 (0.96-1.29)	1.78 (0.63-5.10)

Legend: RBC: red blood cells; OR: odds ratio; CI: confidence interval; #Storage time: RBC >14 days, platelets >4 days.

Multiple logistic regression analysis showed that the amount of RBC transfused was not significantly associated with the onset of infection (Table IV). Also, there was no association between the amount of platelets transfused and the onset of infection. When the model was adjusted for storage time, an association was found between transfusion of RBC stored for >14 days and onset of infection (OR 1.04, [CI 1.01-1.07], p=0.036). The effect size is expressed per unit of transfused RBC.

The storage time of platelets showed no association with the onset of infection in trauma patients. Furthermore, patient-related variables were not associated with infection, as none of the patient-related variables had a confounding effect or modified the effect of RBC on the risk of infection.

The duration of mechanical ventilation was significantly longer in patients with an infection, with a median of 9 (3-15) days compared to 3 (1-8) days in patients without infection ($p<0.05$). The rate of deaths in the ICU tended to be higher in patients with an infection (4%) than in those without an infection (13%, p=0.07).

Discussion

This cohort study identified storage time of RBC, but not of platelets, as a possible risk factor for the onset of infection in critically ill trauma patients. The fact that RBC were leucoreduced before storage suggests that neither allogeneic white blood cells nor white blood cell-derived soluble factors are the mechanisms mediating transfusion-related

infections, as shown before[4,15]. Rather, the observed effect between storage time and risk of infection may be related to the red blood cell itself, which undergoes morphological and functional changes during storage. In accordance with this hypothesis, stored leucoreduced RBC have immuno-modulating effects after transfusion[16]. The clinical relevance of the association of stored RBC and onset of infection warrants discussion, as the increase in odds ratio is only modest. It should, however, be noted that the reported odds ratio relates to each unit of transfused blood. With a mean of seven units RBC transfused per patient in our trauma cohort, the odds ratio of infection is 1.21, thereby increasing the risk of onset of infection up to 30%.

Notably, the rate of infections was higher in the group receiving antibiotics as part of SDD. In line with this, most infections in this cohort involved Gram-positive bacteria, whereas SDD is primarily aimed at potentially harmful Gram-negative bacteria. As SDD is administered to patients with an anticipated ICU stay of longer than 48 hours, prolonged ICU stay may be a risk factor for acquiring infectious complications after trauma in this study, as suggested before[17]. More importantly, results suggest that antibiotics administered early do not offset the observed association between stored blood and the risk of infection after trauma.

The amount of RBC tended to be higher in patients with an infection, but this association disappeared in the multivariable analysis. This finding is in contrast with those of previous studies, in which an association was found between the number of (non)-leucoreduced RBC and the risk of infection in trauma patients[1,15,18] as well as in the general ICU population[19]. Differences in study design and in manufacturing processes may have contributed to divergent results. Alternatively, the association between the amount of transfused RBC and increased mortality found in trauma patients[4] may not be mediated by infectious complications, but by other complications such as acute lung injury.

We found no specific patient-related risk factors for transfusion-related infection. Although blood transfusion is associated with post-surgical bacterial infections[7], surgery was not a risk factor for infection in our trauma population. Furthermore, the infection rate is particularly high in traumatic brain injury patients[11]. In this study, head injury was not a risk factor for transfusion-related infection, nor was the ISS. These results suggest that the association between transfusion of stored blood and risk of infection may be present in a heterogeneous group of trauma patients, as found before[4,9].

This study has some limitations. The amount of transfused blood products is inherently interdependent with storage time. The more units of blood a patient receives the greater likelihood that the mean age of those units will be older. Unfortunately, this study did not allow for meaningful analyses limited to those patients who received exclusively old *versus* exclusively young blood. However, we accounted for an interaction between volume and storage time in the regression model. Furthermore, given that the number of units of young blood was low, the study may have been underpowered to detect a relation between transfusion of young blood and infection. Although a definite association between age of blood and risk of infection can only be determined in a prospective study, within the limits of a retrospective study design, our results suggest that the observed association between the transfusion of relatively older blood and infection is independent of transfusion volume. Another limitation is that receipt of (larger volumes of) blood probably reflects more serious illness and, therefore, a greater likelihood of any adverse association. Thus, whether blood transfusion causes immunosuppression and subsequent infection or is merely a confounder of critical illness, cannot be dissected out from this observational study, but needs to be determined in a prospectively designed trial. Whether shortening RBC storage time without hampering a steady blood supply is feasible, also remains to be determined. Lastly, to be sure that the patients had a bacterial infection, we only considered culture-positive infections, thereby possibly underestimating the actual number of (secondary) infections.

In conclusion, the storage time of transfused RBC may be associated with the onset of infection in critically ill, trauma patients, irrespective of the use of antibiotics to prevent infection. These results are in line with previous findings, suggesting that a prospective trial investigating whether only fresh blood benefits trauma patients is warranted.

The Authors declare no conflicts of interest.

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