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Clinical outcome and inflammatory response after transfusion of washed and unwashed red blood cells in children following cardiovascular surgery

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ABSTRACT

Children frequently require red blood cell transfusion after cardiac surgery, which can generate an inflammatory response that is associated with increased morbidity and mortality. The aim of this study was to evaluate the inflammatory response and clinical effect of RBC transfusion in children.

In a pediatric prospective controlled clinical trial 48 children were randomized to an unwashed or washed red blood cell transfusion group after cardiac surgery. Red blood cells were washed using an auto transfusion device with blood gas analyses of the packed red blood cells performed before transfusion. Vital parameters, blood analyses, and plasma concentrations of cytokines were measured before and 24 h after transfusion.

Forty-five children were included with 23 receiving unwashed and 22 washed red blood cells. Washing reduced hemoglobin, potassium, and glucose while increasing sodium concentration in the packed red blood cells. Increased levels of hemoglobin, hematocrit, and erythrocyte counts were observed in both groups after transfusion. Increased levels of cytokine expression (IL-1α, IL-6, IL-8, MCP-1, and TARC) were detected in the group receiving unwashed red blood cells, while IL-8 levels were reduced in patients receiving washed red blood cells. Also, the percentage increase of cytokine concentrations (IL-6, IL-8, MCP-1, and GP-VI) before in comparison to after transfusion was lower in patients receiving washed red blood cells.

No significant differences in clinical outcome parameters were observed between the two groups.

Transfusion of red blood cells induces a pro-inflammatory reaction which can be diminished by washing the red blood cells before transfusion. Further clinical trials with larger patient cohorts receiving multiple transfusions are needed to better evaluate the clinical outcome.

1. Introduction

Red blood cell (RBC) transfusion is one of the most important therapies in intensive care medicine. Almost 50% of critically ill adult and pediatric patients admitted to intensive care units receive at least one red blood cell transfusion [1]. Moreover, children with congenital heart diseases require red blood cell transfusions during corrective open-heart surgery involving cardiopulmonary bypass (CPB). The decision to transfuse red blood cells depends on many factors, including hemoglobin and hematocrit levels, as well as the clinical appearance of the child and complexity of the heart defect. Children suffering from cyanotic heart disease benefit from higher hemoglobin levels to increase oxygen carrying capacity. A noteworthy new investigation by Cholette et al. showed that infants undergoing cardiac operations can be also managed with a conservative RBC transfusion strategy, supporting the idea to reserve RBC transfusions for clinical indications, even in children with cyanotic heart disease undergoing cardiac repair or palliation [2]. Although a potential lifesaver treatment, red blood cell transfusion can also cause adverse effects in critically ill patients. Red blood cell transfusion has been shown to be independently associated with increased morbidity and mortality, as sicker patients are generally more likely to receive transfusions [3,4]. The risk of transfusion-transmitted infections is substantially reduced by the use of modern molecular assays. Therefore, current studies focus mainly on non-infectious risks. Even without obvious clinical indications, RBC transfusion is known to affect the immune system and generate an

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inflammatory response [5–8]. Children receiving red blood cells during cardiac surgery have increased levels of leukocytes, neutrophil granulocytes, and C-reactive protein (CRP). Additionally, clinical trials have shown that RBC transfusions lead to increased levels of cytokines, including IL-8, TGF-β1, TNF-α, IL-6, and IL-10 in adults [6] and increased levels of IL-1β, IL-8, TNF-α, and MCP-1 in preterm infants [8]. It remains to be elucidated whether the cells themselves, due to the so-called storage lesions or bioactive substances in the supernatant of packed red blood cells, are directly responsible for this immunological response. Barsthein et al. observed in a recent study that storage lesions leading to the deformability of transfused RBCs are potent effectors for transfusion outcome. They indicated that functionality testing for hemodynamic quality of the cells would significantly contribute to improving transfusion therapy [9]. Findings by Andreasen et al., further support the hypothesis that storage time of RBCs is positively associated with the risk of transfusion related severe postoperative infection in patients undergoing coronary artery bypass grafting [10]. In addition to these rheological changes in the RBCs the immunological response to foreign packed red blood cells may be another reason for increased morbidity and poorer outcome after RBC transfusion [11].

We hypothesize that washing RBCs with an auto transfusion device before transfusion can reduce the pro-inflammatory response in the recipients. The aim of this study was to evaluate in a prospective randomized controlled trial the inflammatory response and clinical effect in children receiving washed and unwashed RBC transfusions.

2. Materials and methods

2.1. Study participants

Children who have undergone cardiac surgery with CPB at the Deutsches Herzzentrum Berlin (DHZB, German Heart Institute Berlin) were consecutively enrolled in the study from the period of September 2009 to May 2011. The patients ranged in age from one month to 15 years old. The period between transfusion and end of the operation was at least six hours to reduce the possible influence of CPB, interaction with anesthetic and previous transfusions. Exclusion criteria were acute bleeding, immunosuppressive therapy, congenital immunodeficiency syndromes, simultaneous transfusion with fresh frozen plasma or platelets, extracorporeal membrane oxygenation, and lack of consent. The study protocol was approved by the ethics committee of the Charité - Universitaetsmedizin Berlin, Germany (institutional review body) (EA2/014/09).

2.2. Study design and methods

After written consent was obtained from the parents of the patients, enrolled children were randomized to an unwashed or washed RBC transfusion group. All packed RBCs were reduced of leukocytes and AB0 identical. The washing procedure of the packed RBCs was performed with an Electra autotransfusion device (Sorin Group, Mirandola, Italy) using 0.9% sodium chloride wash solution. One milliliter (mL) of the washed and unwashed packed RBCs was collected for blood gas analysis and all foreign red blood cells were transfused within 4 h. Vital parameters (systolic and diastolic blood pressure, heart rate, temperature, and oxygen saturation) were documented before and 24 h after transfusion. Blood samples from the patients were collected before and 24 h after transfusion and analysed for levels of hemoglobin, hematocrit, erythrocyte counts, coagulation parameters, creatinine, liver function, as well as cytokine levels. Blood gas analyzes of RBCs were carried out before and 6 h after start of transfusion. (Fig. 1).

Two methods were used to measure the level of cytokines: In a simultaneous analysis, the absolute cytokine concentration at time of the blood withdrawal was measured by “Dried Blood Spots “(DBS) whereas the cytokine concentration, in with stimulated lipopolysaccharide whole blood culture systems, was determined by the Instant Leukocyte Culture System (ILCS). DBS, normally used for neonatal screening, were blotted with two blood drops, dried for 24 h, and stored at − 80 °C until analysis.

The Instant Leukocyte Culture System (ILCS) is a whole blood cell culture system using special ILCS tubes from EDI GmbH (Reutlingen, Germany) containing cell culture medium, heparin as an anticoagulant, and lipopolysaccharide (LPS) for stimulation. The working LPS dose was based on an experimentally tested concentration curve with submaximal activation of the monocytes. 1 mL whole blood was collected in an ILCS tube and then incubated at 37 °C for 24 h on a special heating block. The stimulation was stopped by membrane filtration to separate the supernatant from the cells. Supernatant was aliquoted and stored at − 80 °C until cytokine analysis. All cytokine measurements were performed using a Luminex multiplex bead based immunoassay (Millipore, Billerica, MA, USA) following the manufacturer's instructions (Luminex Corp, Austin, TX, USA).

2.3. Function of selected cytokines

Activated monocytes produce cytokines, which can be vaguely sub classified according to their accentuated pro-inflammatory (e.g. IL-1, IL-6, IL-8, IL-12, and TNF-α) or anti-inflammatory (e.g. IL1 Ra and IL-10) effect. The effects of cytokines differ widely depending on the type of stimulus and stimulated target cells. To characterize the immunological reaction of unwashed and washed RBCs the following cytokines were analysed: IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-17A, IP-10, MCP-1, TARC, INF-y, TNF-α, GP-VI, PDGF, and AB/BB. Levels of the cytokines are presented in the results section and are explained in detail (Table 1) [12].

2.4. Data analysis

Blood counts, vital parameters, cytokine levels and their ratio (defined as the level of cytokine concentration after transfusion divided by the level of cytokine concentration before transfusion) were documented as number, percentage, median, and interquartile range (IQR) in all enrolled children. As the outcome data was not normally distributed, median values were compared using non parametric tests. The Wilcoxon signed-rank test was performed to evaluate differences between pre- and post-transfusion levels, and Mann-Whitney *U* Test was performed to compare vital parameters and blood counts between the two randomized groups. Cytokine levels for both groups were calculated as a percentage change of the levels between pre- and post-transfusion and also analysed for significant differences using Mann-Whitney *U* Test. Data were analysed using SPSS (IBM SPSS Statistics 20, Armonk, USA) and illustrated using PRISM (GraphPad PRISM version 4.03, San Diego, USA). A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

Forty-eight children were enrolled in the study and randomized into one of the two groups. Three children were excluded from our analysis, because two children received more than one red blood cell transfusion within a 24 h period and one patient suffered a cardiac arrest and was resuscitated during the study period. The remaining 45 children underwent the complete study procedure. Four female and six male patients were included twice in the study (period between the inclusion of the patients was at least seven days) and were analysed as two separate subjects. Twenty-three children were randomized into the unwashed and 22 into the washed RBCs group. In total, 22 females (49%) and 23 males (51%) were enrolled with a median age of 5 months (IQR 3.5–9.0), median weight of 5 kg (IQR 4.3–7.7) and median size of 62.5 cm (IQR 59.0–74.0). There were no significant differences in