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Effect of red blood cell transfusion on parameters of inflammation and oxidative stress in critically ill patients

Efeito da transfusão de concentrado de hemácias sobre parâmetros de inflamação e estresse oxidativo em pacientes criticamente enfermos

ABSTRACT

Introduction: Red blood cell transfusions are common in intensive care units. For many years, transfusions of red blood were thought to have obvious clinical benefits. However, in recent years, the risks and benefits of blood transfusions have been examined more carefully, including the risk of increased morbidity and mortality due to transfusion-related immunomodulation effects.

Objectives: To evaluate red blood cell transfusion effects and the relationship of this procedure to the production of inflammatory cytokines and oxidative damage in critically ill patients admitted to an intensive care unit.

Methods: For 6 months in 2008, we evaluated patients admitted to an intensive care unit who underwent packed red blood cell transfusions. Pre- and post-transfusion levels of interleukin-6, carbonylated proteins and thiobarbituric acid reactive substances were assessed.

Results: Serum post-transfusion interleukin-6 levels were reduced, and thiobarbituric acid reactive substances and carbonylated proteins were significantly increased. No statistically significant relationship was found between the levels of pre- and post-transfusion interleukin-6 and thiobarbituric acid reactive substances and the mortality rate. However, there was a significant relationship between levels of post-transfusion carbonylated proteins and mortality.

Conclusion: Red blood cell transfusion is associated with increased oxidative damage markers and reduced interleukin-6 levels in critically ill patients.

Keywords: Erythrocytes; Erythrocyte transfusion; Oxidative stress; Interleukin-6; Intensive care units

INTRODUCTION

Anemia is common in patients admitted to intensive care units (ICU). Approximately 95% of ICU patients will have anemia before the 3rd day of admission.[1] To combat this anemia, red blood cell (RBC) transfusions are frequently prescribed in ICUs.[2]

Several epidemiological trials evaluated blood transfusions in ICUs during the last decade.[3] The European 'ABC trial' shows that 37% of the patients were transfused (average 4.8 ± 5.2 blood units (U)) during their ICU stay. Additionally, in patients who stay longer than one week, these figures increase to 73%.[4] This statistic is confirmed by another trial, the 'CRIT study', which reported that 44% of American patients received red blood cells (average 4.6 ± 4.9 U) during their ICU stay.[1]

For a large portion of the last century, red blood cell transfusion was thought to have obvious clinical benefits. However, in the past 20 years, blood transfusions have been examined more carefully, initially because of concerns related to blood transfusion transmitted infections, particularly human immunodeficiency virus (HIV).[5] Consequently, a dramatic and progressive drop in this type of complication was observed. The decrease in infection is thought to results from extensive research to characterize transfusion transmitted microbes, the development of strategies to assess infection rates of blood donors and recipients, the initial characterization of viremia dynamics, the implementation of more restrictive donation eligibility criteria, and the improved sensitivity of the screening tests.[6]

Although transfusion-related infections (TRI) were initially the focus of studies on transfusion safety, other factors, such as immunomodulation and the age of the blood, are gaining attention. Transfusion risk assessment, in turn, has led to investigations of the benefits of this procedure.[7] It has become evident that transfusion-related immunomodulation effects may increase the risk of diseases, such as nosocomial infections, the relapse of neoplasms and possibly the development of autoimmune diseases.[8]

Although transfusion-related immunomodulation mechanisms (TRIM) are widely debated, this phenomenon is not yet fully understood. Among the proposed mechanisms, three have been investigated most extensively: clone deletion, anergy induction and immunosuppression. Clone suppression refers to the inactivation and removal of alloreactive lymphocytes, which, for example, cause allograft rejection. Anergy describes the unresponsiveness and suppression of the immune system and is related to impaired cell response due to a cell mechanism or cytokines.[9] In addition to the above mentioned mechanisms, blood storage could lead to physical and chemical changes in red blood cells, such as a loss of organic phosphates, membrane changes and the production and release of inflammatory cytokines.[10]

Interleukin-6 (IL-6) is produced by many types of cells, including B and T lymphocytes, macrophages, fibroblasts, endothelial cells, neurons and glial cells. Among its functions are fever induction, the promotion of B lymphocyte maturation and differentiation, the stimulation of T lymphocyte proliferation and differentiation, the induction of acute phase protein production (e.g., C reactive protein) by hepatocytes and the stimulation of the hypothalamic-pituitary-adrenal axis.[11] Oxidative stress is a key element in several physiological and pathological phenomena. Although erythrocytes contain an extensive antioxidant defensive system, membrane proteins and lipid oxidative damage contribute to normal cell aging, resulting in shorter cell lifespans due to cell injury.[12,13] Because oxidative damage has been related to poorer prognosis in critically ill patients, measurements of oxidative stress in this population have become clinically relevant.[14,15]

Therefore, this study was designed to evaluate the effects of red blood cell transfusion and its relationship with the production of inflammatory cytokines and oxidative damage in critically ill patients admitted to an ICU.

METHODS

This cohort study was approved by the Institution's Ethics Committee (approval number CAAE - 0004.0.379.139-08), Santa Catarina, Brazil. All patients above 18 years old admitted within a 6 month period during 2008 to the 20 beds in the general ICU were evaluated, and those receiving red blood cell transfusions and consenting to participate were included. This study only evaluated red blood cell transfusions with non-leukodepleted, non-irradiated red blood cells, and the investigators had no influence on the attending doctors' prescription of transfusion. The patients were followed for 28 days or until death to determine patient mortality rates. Within this period, relevant clinical information was collected for analysis.

Before and 12 hours after the transfusion, a blood sample was drawn from each patient to determine serum interleukin (IL)-6 and oxidative stress. IL-6 levels were assessed by ELISA, as recommended by the manufacturer (R&D systems, Minneapolis, MN, USA), and were used as proinflammatory response markers.

Thiobarbituric acid reactive substances (TBARS) were measured as a measure of oxidative stress. In short, the samples were mixed with 1 mL of 10% trichloroacetic acid and 1 mL of 0.67% thiobarbituric acid, and the samples were then heated in boiling water bath for 15 minutes. TBARS were determined by 535 nm absorption, using 1,1,3,3-tetrametoxypropane as an external standard. The results were expressed as malondialdehyde equivalent per protein milligram.[16]

Oxidative damage to proteins was evaluated by carbonyl groups, based on the dinitrophenylhydrazine reaction. The proteins were precipitated by adding 20% trichloroacetic acid and were then dissolved in dinitrophenylhydrazine, and then the absorbance was read at 370 nm.[17]

The data were analyzed with the Statistical Package for the Social Sciences (SPSS) software. Categorical variables

were compared using the chi-square test. The continuous variables were compared using the t-test or the Mann-Whitney U test, according to the variables' distribution. Comparisons of the pre- and post-transfusion IL-6 and oxidative stress markers levels were made using the t-test for dependent variables. The values are expressed as frequency (%), mean ± standard deviation or median with interquartile intervals. For all analyses, the significance level was established as $p < 0.05$.

RESULTS

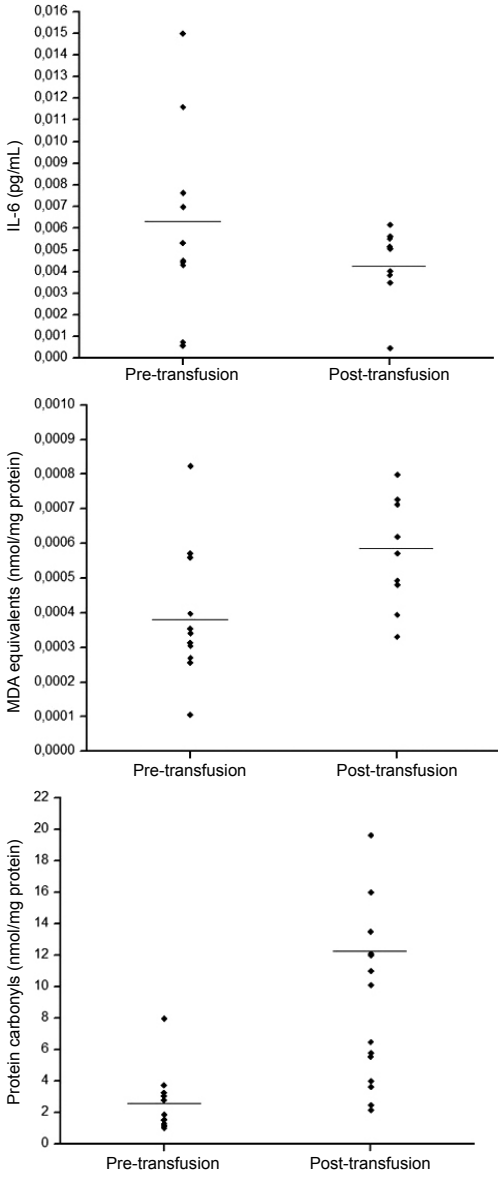
During the study period, 322 patients were admitted to the General ICU of the Hospital São José. Of these, 40 patients who received red blood cell transfusions were analyzed. Table 1 shows the clinical and demographical characteristics for the groups that did or did not receive blood transfusions. The groups had no significant differences in demographic variables or Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores on the day of admission. The mean pre-transfusion hemoglobin was 6.8 ± 3.2 g/dL, and the hematocrit was 20.8 ± 9.9%, which is characteristic of a restrictive transfusion strategy.[18] As shown in Table 1, of the 40 evaluated patients, 14 patients eventually died (35%), and 26 were discharged from the hospital (65%). When this group is compared with the patients who did not receive a transfusion, the transfused group had significantly higher mortality ($p<0.05$).

Table 1 – Clinical and demographic characteristics of the patients included in the study

	Transfusion		p value
	Yes (N=40)	No (N=292)	
Age	51 ± 17	57 ± 16	0.1
Male	25	220	0.6
APACHE II	11 (5 – 20)	10 (5 – 18.5)	0.8
SOFA	6.3 ± 4.4	4.2 ± 3.8	0.2
Reason for admission			
Post-operative monitoring	13	100	0.4
Sepsis	5	25	
Cardiovascular	10	80	
Neurologic	4	40	
Respiratory	5	50	
Others	3	57	
Deaths (%)	14 (35)	75 (25)	<0.05

APACHE - Acute Physiology and Chronic Health Evaluation; SOFA - Sequential Organ Failure Assessment. Data shown as mean ± standard deviation or median (25-75).

Regarding the pre- and post-transfusion IL-6 levels, this inflammatory parameter was significantly reduced ($p=0.002$). Conversely, when oxidative parameters were compared, significant increases were observed for both TBARS and carbonylated proteins ($p < 0.001$ for both) (Figure 1). However, there was no significant



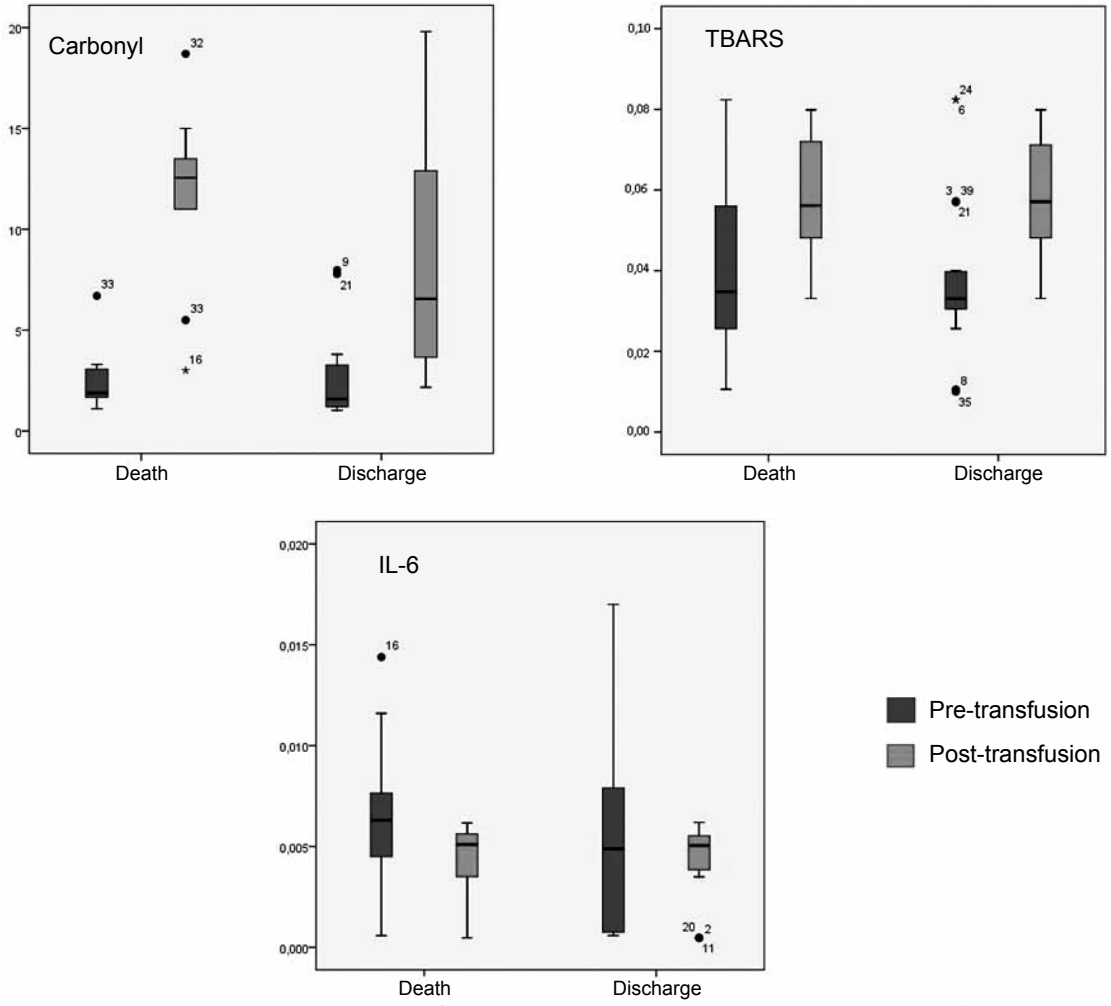
A total of 322 patients were admitted to a general ICU, of whom 40 patients received packed red blood cells. Before the transfusion and 12 hours after, a blood sample was collected for a determination of serum interleukin (IL)-6 levels and an assessment of oxidative stress. The pre- and post-transfusion levels were compared using the t-test. After transfusion, we observed a significant decrease in IL-6 ($p=0.002$) reduction and significant increases in both TBARS and carbonylated proteins ($p < 0.001$ for both). IL-6 – interleukin-6; MDA – malondialdehyde; TBARS – thiobarbituric acid reactive substances.

Figure 1 – Comparison of pre- and post-transfusion oxidative and inflammatory markers in critically ill patients.

relationship between serum IL-6 levels before (p=0.783) or after (p=0.970) RBC transfusion and mortality. Equally, pre- (p=0.663) and post-transfusion (p=0.887) TBARS levels were not significantly associated with mortality. Additionally, carbonyl levels before transfusion (p=0.808) were not significantly associated with mortality. However, a significant association (p=0.047) was found for the post-RBC transfusion levels of carbonylated proteins and mortality (Figure 2). The different post- versus pre-transfusion deltas showed no significant association with mortality for IL-6 (p=0.725) and TBARS (p=0.633), but the delta change was significantly associated with mortality for carbonylated protein (p=0.04).

DISCUSSION

This study evaluated the influence of RBC transfusions in patients admitted to a general ICU and the production of IL-6, a cytokine closely associated with inflammatory response and produced by immune and other cells, such as endothelial cells, keratinocytes and intestinal epithelium cells.[11] Significantly reduced serum IL-6 levels were found post-transfusion, implying a transfusion immunosuppressive effect. However, there was no significant association between IL-6 serum levels and mortality rate. We also analyzed the pre- and post-transfusion levels of oxidative damage markers, TBARS and protein carbonyls. TBARS and carbonyl levels were elevated after red blood cell transfusions, and a positive



A total of 322 patients were admitted to a general ICU, of whom 40 patients who received packed red blood cells were evaluated. Before the transfusion and 12 hours after, a blood sample was collected to determine interleukin (IL)-6 levels and to assess oxidative stress, as detailed in Materials and Methods. The differences between the pre- and post-transfusion markers were compared to the patients' outcome, using the t-test separately according to the outcome. A significant association was found only for the level of carbonylated proteins level after transfusion (p = 0.047) with mortality. TBARS – thiobarbituric acid reactive substances; IL-6 – interleukin-6.

Figure 2 – Association of oxidative and inflammatory markers with mortality in critically ill patients who received blood transfusions.

relation with mortality was shown in this population. These results suggest that blood transfusions in critically ill patients may have harmful acute effects, which may influence patient outcomes. This information helps to explain the relationship between transfusion and mortality described in systematic literature reviews.[5]

Allogeneic blood transfusions result in an infusion of considerable amounts of exogenous antigens, either dissolved or associated with cells. The persistence of these antigens in the circulation may provide favorable conditions for the development of immune suppression.[19] Several studies have suggested that white blood cells may be responsible for these effects, but the etiology of transfusion-related immunosuppression is poorly understood.[20] Transfusion-related immunomodulation has shown some benefits, including increased survival following kidney transplant, a reduced risk of recurrent spontaneous abortion and a reduced severity of autoimmune diseases, such as rheumatoid arthritis[21] or Crohn's disease relapse.[22] In contrast, possible harmful effects as a result of TRIM have been described, including increased cancer relapse, post-surgery infections, multiple organ failure and overall mortality.[21] The reduced IL-6 levels may reflect immunosuppression, adding new evidence to the literature regarding the immunomodulatory effects of blood components. In contrast to our findings, Avall et al. found IL-6 levels to be significantly increased after surgery, both in patients who received total autologous blood transfusions and in patients receiving allogeneicallogeneic blood transfusions.[23] However, in their study, no comparisons were made with patients who did not receive any blood transfusions.

Even though red blood cells possess high antioxidant properties, their antioxidant demand is increased during storage because of exposure to several factors such as light, shaking, high glucose concentrations and free radicals release from leukocytes, among others.[24] During storage, red blood cells undergo chemical-structural changes that may interfere with post-transfusion performance.[25] These changes are associated with a series of modifications, including adenosine triphosphate and 2,3-diphosphoglycerate (DPG) depletion, flexibility loss, vesiculation, phospholipid loss, protein oxidation and red blood cell lipid membrane peroxidation. These associations may contribute to adverse clinical consequences, such as reduced oxygen transportation.[26,27] Wardle et al. found increased urinary levels of malondialdehyde after transfusion in preterm newborns, indicating a relationship between transfusion and lipid peroxidation.[28]

Some important limitations should be highlighted. Although our sample was sufficient to compare pre- and post-transfusion markers, our sample size is not appropriate for a definitive analysis of the variation of these markers and mortality in critically ill patients. For such an analysis, larger studies should definitely clarify this relationship. Additionally, we conducted the study in one site that uses a restrictive transfusion strategy, so our results may not be representative of average ICUs. In addition, we collected only one blood sample, and marker kinetics were not evaluated ; this limitation could influence the results, as IL-6 levels show time-dependent changes following the initial inflammatory challenge, as previously shown in the literature.[27,29]

CONCLUSION

This study has shown increased oxidative damage markers and reduced IL-6 after RBC transfusion. However, only post-transfusion serum levels of carbonylated proteins was significantly associated with mortality.

RESUMO

Introdução: Transfusão de concentrado de hemácias é freqüentemente prescrita nas unidades de terapia intensiva. Durante muito tempo a transfusão de hemácias era vista como tendo benefícios clínicos óbvios. Entretanto nos últimos anos a prática de transfusão sanguínea tem sido examinada de uma forma mais cautelosa, levando a investigações a respeito dos benefícios transfusionais, incluindo aqui o fato de os efeitos imunomoduladores relacionados à transfusão podem aumentar o risco de morbimortalidade dos pacientes.

Objetivos: Avaliar o efeito da transfusão de concentrado de hemácias e sua relação com a produção de citocinas inflamatórias e dano oxidativo em pacientes criticamente enfermos admitidos em uma unidade de terapia intensiva.

Métodos: Foram analisados durante 6 meses, no ano de 2008, pacientes internados na unidade de terapia intenvia que realizaram transfusão de concentrado de hemácias. Foram analisados os níveis séricos pré e pós transfusionais de interleucina-6 (IL-6), proteínas carboniladas e substâncias reativas ao ácido tiobarbitúrico (TBARS).

Resultados: Houve diminuição dos níveis séricos de IL-6 pós-transfusionais e um aumento significativo tanto para TBARS quanto para proteínas carboniladas. No entanto não houve significância estatística entre os níveis séricos de IL-6, TBARS antes e após transfusão de concentrado de hemácias e a taxa de mortalidade. Contudo ocorreu significância da relação dos níveis pós transfusionais de proteínas carboniladas e mortalidade.

Conclusão: Transfusão de concentrado de hemácias é associada a aumento dos marcadores de dano oxidativo e diminuição de IL-6 em pacientes criticamente enfermos.

Descritores: Eritrócitos; Transfusão de eritrócitos; Estresse oxidativo; Interleucina-6; Unidades de terapia intensiva

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