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Transfusion of stored red blood cells in critically ill trauma patients: a retrospective study

S. SPADARO, R. REVERBERI[1], A. FOGAGNOLO, R. RAGAZZI, N. NAPOLI[2], E. MARANGONI, T. BELLINI[3], C.A. VOLTA

Department of Morphology, Surgery and Experimental Medicine, Anesthesia and Intensive Care Unit. University of Ferrara, Ferrara, Italy
[1]Blood Transfusion Service. Sant'Anna Hospital, Ferrara, Italy
[2]Health Statistics Unit. Sant'Anna Hospital, Ferrara, Italy
[3]Department of Biomedical and Specialist Surgical Sciences, University of Ferrara, Ferrara, Italy

Abstract. – OBJECTIVE: The many published studies on the effects of the transfusion of stored red blood cells on clinical outcomes yielded discordant results. Therefore, we chose to study patients with severe trauma. The clinical outcomes considered included in-hospital mortality, the occurrence of sepsis, length of stay in intensive care unit and in hospital, and days of mechanical ventilation.

PATIENTS AND METHODS: We selected all patients with traumatic injury, who received at least 2 red cell units in the first day of admission. Patients were divided into two groups: those who had received fresh red cells only (fresh group) and those who had received at least one "old" red cell unit (old group). The red cells were considered fresh if they had been stored <14 days.

RESULTS: The fresh and old groups included 376 and 321 patients, respectively. Baseline demographic and clinical characteristics were comparable between the groups. However, old group received more red cell and plasma units during whole hospital stay (red cells: 11 ± 7 vs 6 ± 4, $p < 0.001$; plasma: 7 [0-9] vs 3 [0-6]). Among outcomes, only length of stay in intensive care unit (old vs fresh: 18 ± 9 vs 12 ± 8 days, $p < 0.001$) and in hospital (77 ± 35 vs 45 ± 30 days, $p < 0.001$) differed significantly between groups. The association remained statistically significant in a multivariate analysis including known confounding factors.

CONCLUSIONS: Patients with major trauma transfused with old (≥14 days) red cells had a longer length of stay in intensive care unit and in hospital, without any difference in mortality, occurence of sepsis or days of mechanical ventilation.

Key Words:
Storage of red cells, Blood transfusion, Trauma patients and outcome.

Introduction

During storage, red blood cells (RBC) undergo a series of changes, which are collectively known as "storage lesion"[1,2]. Many studies, most of them retrospective, examined the relationship between storage age of transfused RBC and several clinical outcomes, such as short term and long term mortality, multiorgan failure, nosocomial infections, duration of mechanical ventilation, length of stay (LOS) in hospital and in intensive care unit (ICU)[1-4]. However, the clinical relevance of the storage lesion remains unclear, because adverse effects were documented in some studies, whilst no association was reported in others[3-6].

Some retrospective studies failed to account for known confounders and in some of them, but not all, the inclusion in the statistical model of covariates representing transfusion risk factors, such as the total number of RBC units transfused[7] or preoperative haemoglobin and renal function[8], abolished the apparent association. A nationwide retrospective cohort study yielded inconclusive results, interpreted by the authors as probably reflecting a residual indication bias[9]. Another possible (partial) explanation for the discrepant results of clinical studies is the two-hit hypothesis, which speculates that a second insult is necessary to predispose the patient to the adverse effects of stored RBC[10]. In accordance with the two-hit hypothesis, patients with critical illness or trauma would be the appropriate and relevant study population; indeed, in previous studies adverse outcomes related to stored blood are more often detected in trauma patients[11,12]. The aim of our study was to evaluate the association between the storage time of

Corresponding Author: Savino Spadaro, MD; e-mail: savinospadaro@gmail.com

transfused RBC and clinical outcomes in trauma patients admitted to an intensive care unit.

Patients and Methods

Patients

This is a single-centre study. Hospital discharge records were consulted to identify the patients with traumatic injury admitted to the Sant'Anna University Hospital of Ferrara from July 2005 through January 2013. Among them, we selected all adult patients (at least 18 years of age old) admitted to ICU who had received at least 2 RBC units during the first 24 hours after hospital admission.

Clinical and Laboratory data

Patient's age at admission, LOS in hospital and ICU, major morbidity and duration of mechanical ventilation were obtained from the hospital discharge records. Injury severity score (ISS) data was retrieved from the emergency admission notes. Haemoglobin, platelet count, serum creatinine, lactate, base deficit, international normalized ratio (INR) and bilirubin were obtained from the laboratory information system. Two measurements of haemoglobin were considered: at admission and the first value in the second day after admission.

Transfusion Data

Transfusion data was derived from the hospital blood transfusion service information system and included the date of transfusion, number of units of RBC, fresh frozen plasma (FFP), platelets transfused, and storage age of RBC. FFP units were quarantined plasma obtained from whole blood, from male donors exclusively (in order to avoid transfusion-related acute lung injury: TRALI). The blood components were not leukoreduced. The RBC concentrates were stored in saline-adenine-glucose-mannitol for a maximum of 35 days. The blood transfusion service provided the oldest available unit for each request. Transfused RBC were categorized as being less than 14 days old ("fresh") or 14 or more days old ("old"). Such a cutoff was chosen in analogy with many previous studies[9,10] and also because it allowed us to divide our study population into two groups of similar numerosity: those who received "fresh" red blood cell only and those who received at least one "old" RBC unit.

Outcomes

The aim of the present study was to evaluate whether the transfusion of old RBC had an influence on the following clinical outcomes: in-hospital mortality, the occurrence of sepsis, LOS in the ICU, LOS in hospital, and days of mechanical ventilation.

Statistical Analysis

Statistical analysis was carried out with the software package IBM SPSS 19. The normality of data distribution was evaluated for each parameter using the Kolmogorov-Smirnov test. The clinical characteristics of the two groups were compared with the *t*-test (continuous data, normally distributed), Mann-Whitney U-test (continuous data, not normally distributed), and χ^2-test (categorical variables). Differences in the outcomes of the two experimental groups were initially sought in a univariate analysis. The outcomes which resulted as significantly associated in the initial analysis were studied in a multivariate regression analysis, also including as covariates age of the patient, year of discharge, ISS score, sex, total number of transfused RBC units, total number of transfused FFP units, and storage age of the oldest RBC unit. All data is reported as means±1 SD or as medians with the interquartile range in square brackets. The differences between the two groups were considered statistically significant for probability (p) values < 0.05.

Results

During the study period 6204 trauma patients were admitted to our hospital. 4834 patients were not admitted to the ICU and, therefore, were excluded. Of the remaining, 697 received at least 2 units of RBC in the first day of admission and were included. 376 received fresh RBC only ("fresh" group), while 321 patients received at least one "old" RBC unit ("old" group). Due to the rapid turnover of RBC in our hospital, only 58 patients received old RBC units exclusively, too few to consider them as a separated group. Table I displays baseline demographic data and clinical characteristics at admission. No significant differences regarding demographics and specific patient-related risk factors were detected between the two groups. In particular, trauma characteristics, base deficit, lactate and haemoglobin at admission and comorbidities were similar between groups.

Table I. Baseline demographic data and clinical characteristics at admission.

	Patients receiving fresh RBC (N = 376)	Patients receiving old RBC (N = 321)	*p*
Age	55 [35-76]	53 [34-74]	
Sex	249 M – 127 F	209 M – 112 F	0.76
Trauma			
ISS	23.7 ± 2.2	24.2 ± 2.7	0.6
Systolic pressure (mmHg)	118 ± 16	115 ± 19	0.71
Head trauma	63 (16.7%)	70 (21.8%)	0.09
Pelvic fracture	52 (13.8%)	54 (16.8%)	0.26
Rib or sternal fracture	84 (22.3%)	80 (24.9%)	0.7
Laboratory data			
Hb at admission (g/dL)	11 ± 2.4	10.7 ± 2.4	0.14
Hb in the second day (g/dL)	10.3 ± 1.5	10 ± 1.5	0.2
Base deficit at admission (mEq/L)	-3.4 ± 1.4	-3.8 ± 2	0.19
Lactate at admission (mmol/L)	2.6 ± 1.1	2.9 ± 1.4	0.21
Serum creatinine at admission (mg/dL)	1.23 ± 1	1.16 ± 1	0.85
Bilirubin at admission (mg/dL)	0.7 ± 0.7	0.73 ± 0.7	0.87
Platelets at admission ($\cdot 10^3/\mu L$)	210.8 ± 77.1	182.88 ± 74.2	0.07
Platelets at ICU admission ($\cdot 10^3/\mu L$)	149.2 ± 73	126 ± 64.1	0.08
INR at admission	1.55 ± 1	1.67 ± 0.5	0.59
INR at ICU admission	1.67 ± 0.6	1.59 ± 0.5	0.56
Patient comorbidity			
COPD	10 (2.6%)	4 (1.24%)	0.12
Hypertension	37 (9.8%)	25 (8.4%)	0.8
Diabetes mellitus	19 (5.0%)	13 (4.0%)	0.57
Cancer	12 (3.1%)	8 (2.5%)	0.6
Chronic Kidney Disease	5 (1.3%)	5 (1.5%)	0.81

Values reported as n (%), mean (± SD), median [IQR] as appropriate. ISS: Injury severity score; Hb: haemoglobin; COPD: Chronic obstructive pulmonary disease; INR: International normalized ratio; BE: Base excess.

Transfusion data are summarized in Table II. The number of RBC units and FFP units transfused during the first 24 h was somewhat higher in the old group but the difference did not reach statistical significance. However, the old group received significantly more RBC and FFP units during the whole hospital stay (RBC: 1.1 ± 6.6 vs 6.4 ± 3.7, $p < 0.001$; FFP: 2.5 [0-6] vs 7.15 [0-9]). As shown in Figure 1, the percentage of patients who received 2-4 RBC units only was 2-3 times higher in the fresh group. Conversely, the percentage of patients who received more than 10 RBC units was higher in the old group (35,8% vs. 13,5%). The old group was transfused for more days (5 [2-16.8] vs 2 [0-8]; $p < 0.001$). Pooling all the patients together, the average storage age of transfused RBC was about 10 days, irrespective of the total number of RBC units transfused (Figure 2). However, the minimum and the maximum age decreased and increased, respectively, as the total number of RBC units increased (Figure 2). This expected behaviour explains in all likelihood the higher prevalence of the more heavily transfused patients among the old group. The frequency of blood group O was similar in both patient groups: fresh, 43.1%; old, 39.9% ($p = 0.398$, not significant).

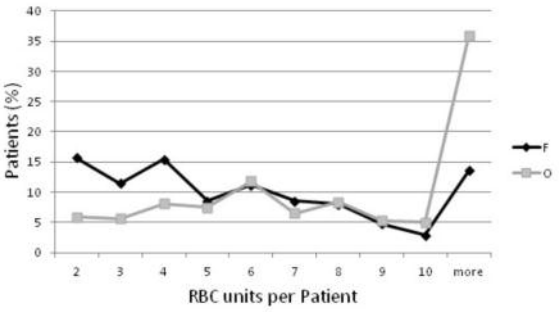

Figure 1. Number of Red-Cell Units transfused in relation to the percentage of patients. F = Fresh Group; O = Old Group.

Table II. Transfusion data during recovery in hospital.

	Patients receiving fresh RBC	Patients receiving old RBC	*p*
Average RBC storage age (days)	6.8 ± 1.7	14.1 ± 4	
Old RBC units received	0	4.2 + 2.8	
FFP transfused in the first 24 h	2.0 [0-4]	2.0 [0-6]	0.08
FFP transfused during the whole hospital stay	2.5 [0-6]	7.2 [0-9]	<0.001
Patients who received platelet concentrates	26 (6.9%)	34 (10.5%)	0.08
RBC transfused in the first 24 h	3 [2-5]	4 [2-7]	0.07
RBC transfused during the whole hospital stay	6.4 ± 3.7	10.1 ± 6.6	<0.001
Crystalloid received in the first 24 h (L)	4.8 [2-7]	5.2 [2-8]	0.19

RBC: Red Blood Cell; FFP: Fresh Frozen Plasma.

Among the outcomes, only LOS in ICU and in hospital were significantly different between the groups (LOS in ICU: 18.4±9.3 (old) vs 11.7±80.0 days (fresh), $p < 0.001$; LOS in hospital: 77 ± 35 vs 44 ± 29 days, $p < 0.001$) (Table III). The correlation between the number of old RBC units transfused and LOS in ICU is statistically significant ($p < 0.001$).

The difference in LOS between the experimental groups was confirmed in the multivariate analysis (Table IV). (Table IV shows the results of the analysis regarding the LOS in ICU only. The results for the LOS in hospital were similar). In particular, the difference remained statistically significant ($p = 0.003$), notwithstanding the inclusion in the model of the total number of transfused RBC. The latter is significantly associated both to the risk of receiving old RBC (hazard ratio [HR]: 1.12; 95% confidence interval [CI]: 1.08-1.15; $p < 0.001$) and to a prolonged LOS (standardized regression coefficient: 0.546, $p < 0.001$). Among the other covariates considered in the model, ISS was statistically significant ($p = 0.012$), while age of the oldest RBC unit was not.

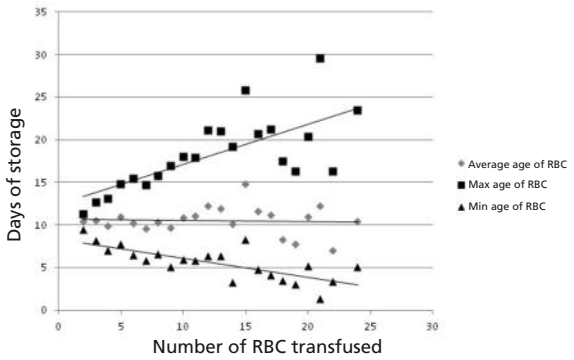

Figure 2. Days of storage of Red-Cell Units related to the number of units transfused.

If the experimental groups are classified considering the transfusions received during the whole hospital stay, as in our main analysis, it is entirely possible that the old RBC units were transfused after the occurrence of the outcomes. Therefore, we repeated the analyses only using the first two or the first three days in order to classify the patients. However, the differences in LOS between the groups were confirmed (old vs fresh groups, first two days used for classification: LOS in ICU, 17.4±7.5 vs 13.1±9 days, $p = 0.002$; first three days used for classification: LOS in ICU, 17.3±8.1 vs 13.1±8.6, $p = 0.002$).

Discussion

The main finding of the present study is that patients with a major trauma, which were transfused with old (≥14 days) RBC had a longer LOS in ICU and in hospital, but not in-hospital mortality, than those transfused with fresh RBC only. Similar results in trauma patients were reported by Murrell et al[13] who, however, used as age parameter the product of the average storage age and the total number of RBC units transfused. The total number of transfused RBC units is a well known confounder, as it is associated both with the risk of receiving an old RBC unit and, obviously, with the severity of trauma. This notwithstanding, the association between storage age and LOS remained quite significant after the inclusion of the total number of transfused RBC units in the statistical model.

Baseline clinical characteristics, particularly injury severity and type, and haemoglobin concentration, were well balanced between the experimental groups, testifying to the quasi-random occurrence of the transfusion of an old RBC unit.

Table III. Clinical outcomes.

	Fresh Group	Old Group	p
ICU length of stay	11.7 ± 8.0	18.4 ± 9.3	<0.001
Hospital length of stay	44.7 ± 29.7	76.8 ± 35.3	<0.001
Days of mechanical ventilation	9.4 ± 2.3	11.2 ± 1.7	0.09
Sepsis	287 (76.3%)	239 (74.4%)	0.56
Mortality	90 (23.9%)	69 (21.4%)	0.41

ICU: Intensive Care Unit.

The higher prevalence of the more heavily transfused patients among the old group is expected and has been noted in other studies[3]. Figure 2 illustrates that point nicely: the curve showing the maximum storage age crosses the cut off age (14 days) at approximately 4-5 RBC units. Comparing the graph of Figure 2 with the analogous one (Figure 1) in reference 13, it is apparent that the RBC units transfused in our Institution are generally fresher. In fact, the group including the patients who only received fresh RBC was more numerous than the other. In our province there is a high prevalence of transfusion-dependent patients with homozygous beta-thalassemia and their transfusion needs, together with those of the rest of the population, exceed the blood units collected locally by approximately 3-15% annually. The difference is provided by the Regional Blood Centre when necessary. Therefore, blood components are used after a short period of storage and very rarely reach the expiry date (35 days in our Institution).

The most obvious alternative explanation for the apparent association between storage age and LOS is that patients who are bleeding more severely receive more blood components and, because of this, have a higher probability of receiving old RBC. In this case, however, storage age should not be independently associated with LOS and the association should not remain significant after the correction for the number of RBC transfused. Moreover, the baseline characteristics of the patients in the two experimental groups should presumably differ, which this not the case in our study. Another hypothetical explanation is that patients do not differ initially but, because of the individual preferences of the attending physicians, some of them are administered more fluids (including perhaps more blood components). It has long been suspected that a vigorous treatment of hypovolemia before haemostasis has been reached may be detrimental[14] and recent evidence confirms it[15].

Presumably, the consequent increase in the blood pressure aggravates bleeding through clot disruption, and dilutional coagulopathy may also contribute. Those patients would then receive more blood components and a spurious association between storage age and LOS would ensue. The latter hypothesis fits neatly with some of our findings and cannot be discounted altogether, but it is hardly compatible with the results of the multivariate analysis.

Table IV. Multivariate analysis results regarding the LOS in ICU.

Variables	Standardized regression coefficient	p
Number of RBC received	0.546	<0.001
Fresh or old group	0.160	0.003
ISS score	0.112	0.012
Age of the oldest unit given	-0.077	0.148
Sex	0.053	0.173
INR	0.137	0.248
Age of the patient at admission	0.036	0.354
FFP given	0.047	0.362
Year of discharge	0.032	0.407

ISS: Injury severity score; ICU: Intensive Care Unit

Our study is apparently free of most, if not all the known pitfalls of retrospective studies on the clinical impact of the transfusion of stored RBC[16]. Apart from the inclusion of the total number of transfused RBC in the multivariate analysis and the balanced distribution of baseline clinical characteristics, the difference in the LOS was also confirmed when only the transfusions in the first 2-3 days were used to classify the patients into the fresh and old groups. Moreover, in the case of the LOS in hospital it is not possible that the association be confounded by transfusions performed after the outcome has occurred[16]. The imbalance in the distribution of ABO blood groups between recipients of fresh and old RBC, normally the excess of O in the fresh group[17], was not present in our patient population. The preferential assignment of older RBC units to the most severely affected patients, which is a non-written but reasonable policy aimed at avoiding the discarding of units close to the expiry date[16], is not applied in our Institution. RBC units are assigned just before transfusion (the blood transfusion service is located inside the hospital), the return rate is less than 6%, and the expiration rate is less than 0.8%.

Therefore, our findings raise the suspicion that the association between the transfusion of old RBC and LOS hides a causal relationship and is not only the effect of unaccounted residual confounding factors. An association between the transfusion of old RBC and increased mortality and morbidity has been reported in a large retrospective study where the two experimental groups had been transfused at a similar rate[17].

Certainly, an increased LOS is just a surrogate for worse clinical conditions. Unfortunately, our results do not offer any clue as to the possible mechanism(s), if any, for the putative adverse effects of the transfusion of stored RBC on clinical outcomes. Apart from the prolonged LOS, all the other clinical outcomes at our disposal did not show any significant worsening in recipients of stored RBC.

The oxygen delivery function of stored RBC is impaired by at least two mechanisms. One is the depletion of 2,3-DPG, which is almost complete by the second week of storage[18]. This condition is reversible *in vivo* but a complete recovery requires 48-72 hours[19] and may be too late in an acute situation. The second mechanism is the impaired modulation of microcirculatory blood flow, which depends on the local release of nitric oxide[20,21] or ATP[20] by RBC. The decline of S-nitrosothiol-haemoglobin occurs early during storage and is not reversible *in vivo*[20,21]. The loss of deformability, which takes place more gradually, may further hinder microcirculation and aggravate organ ischaemia[20]. It is not known whether the loss of deformability is reversible *in vivo* or not.

RBC units contain variable amounts of leukocytes and platelets which are very dependent on the technicalities of their preparation: whether the buffy coat is removed or platelets are prepared and by what technique (platelet-rich plasma or buffy-coat method), whether they are leukoreduced and, if they are, whether leukocyte removal is pre- or post-storage. These details could be not negligible, as residual leucocytes release a variety of substances (enzymes, cytokines, etc.) which produce biologically active lipids, attract monocytes and prime neutrophils[3,5]. The clinical importance of these phenomena is at present unknown. An intriguing observation may be related to these aspects, however: that detrimental effects of the transfusion of stored RBC are found in North American but not in European studies, suggesting a role for differences in the preparation of RBC concentrates[2]. In this regard, our study is an exception. At first sight, the leukocyte content of our RBC concentrates could be blamed for the difference. In fact, contrary to what is generally performed in Europe, in our Institution the RBC concentrates are not buffy-coat-poor nor leukoreduced. However, two recent pediatric studies which showed a significant association with multiple organ dysfunction syndrome, used RBC units which had been leukoreduced pre-storage[23,24].

The limitations of our study are primarily due to its retrospective nature. One can argue that our results might be partially explained by differences of severity of the enrolled patients. Although we cannot exclude *a priori* this possibility, we are confident that the two groups had similar clinical characteristics (Table I), as pointed out by the ISS score, vital parameters, lactate and base excess.

Conclusions

Patients with major trauma transfused with old (≥14 days) red cells had a longer length of stay in intensive care unit and in hospital, without any difference in mortality, incidence of sepsis or days of mechanical ventilation. Drawing firm conclusions from a retrospective study is notori-

ously difficult. Prospective studies will certainly help clarify the issue by establishing whether RBC storage is associated with worse clinical outcomes and, it is hoped, by suggesting possible mechanisms for the adverse effects. Several prospective studies can partly support the deleterious effects of RBC storage[25,26]. However, if the relevant storage lesion is not identified in advance and its temporal pattern is not taken into account in the design phase, the statistical power of a prospective clinical trial could turn out to be much lower than expected[27].

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Hess JR. Red blood cell storage. J Proteomics 2010; 73: 368-373.
2) Gerber DR. Risks of packed red blood cell transfusion in patients undergoing cardiac surgery. J Crit Care 2012; 27: 737.e1-9.
3) Van de Watering LMG, Brand A. Effects of storage of red cells. Transfus Med Hemother 2008; 35: 359-367.
4) Tinmouth A, Fergusson D, Yee IC, Hébert PC for the ABLE Investigators and the Canadian Critical Care Trials Group. Clinical consequences of red cell storage in the critically ill. Transfusion 2006; 46: 2014-2027.
5) Zimrin AB, Hess JR. Current issues relating to the transfusion of stored red blood cells. Vox Sang 2009; 96: 93-103.
6) Lelubre C, Piagnerelli M, Vincent JL. Association between duration of storage of transfused red blood cells and morbidity and mortality in adult patients: myth or reality? Transfusion 2009; 49: 1384-1394.
7) Van de Watering LMG, Lorinser J, Versteegh M, Westendord R, Brand A. Effects of storage time of red blood cell transfusions on the prognosis of coronary artery bypass graft patients. Transfusion 2006; 46: 1712-1718.
8) Dardashti A, Ederoth P, Algotsson L, Brondén B, Lührs C, Bjursten H. Blood transfusion after cardiac surgery: is it the patient or the transfusion that carries the risk? Acta Anaesthesiol Scand 2011; 55: 952-961.
9) Edgren G, Kamper-Jørgensen M, Eloranta S Rostgaard K, Custer B, Ullum H, Murphy EL, Busch MP, Reilly M, Melbye M, Hjalgrim H, Nyrén O. Duration of red blood cell storage and survival of transfused patients. Transfusion 2010; 50: 1185-1195.
10) Sanders J, Patel S, Cooper J, Berryman J, Farrar D, Mythen M, Montgomery HE. Red blood cell storage is associated with length of stay and renal complications after cardiac surgery. Transfusion 2011; 51: 2286-2294.
11) Lelubre C, Vincent JL. Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review. Crit Care 2013; 17: R66.
12) Vandromme MJ, McGwin G Jr, Weinberg JA. Blood transfusion in the critically ill: does storage age matter? Scand J Trauma Resusc Emerg Med 2009; 17: 35.
13) Murrell Z, Haukoos JS, Putnam B, Klein SR. The effect of older blood on mortality, need for ICU care, and the length of ICU stay after major trauma. Am Surg 2005; 71: 781-785.
14) Gauvin F, Spinella PC, Lacroix J, Choker G, Ducruet T, Karam O, Hébert PC, Hutchison JS, Hume HA, Tucci M; Canadian Critical Care Trials Group and the Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network. Association between length of storage of transfused red blood cells and multiple organ dysfunction syndrome in pediatric intensive care patients. Transfusion 2010; 50: 1902-1913.
15) Hussmann B, Lefering R, Waydhas C, Touma A, Kauther MD, Ruchholtz S, Lendemans S; Trauma Registry of the German Society for Trauma Surgery. Does increased prehospital replacement volume lead to a poor clinical course and an increased mortality? A matched-pair analysis of 1896 patients of the Trauma Registry of the German Society for Trauma Surgery who were managed by an emergency doctor at the accident site. Injury 2013; 44: 611-617.
16) Van de Watering L, for the Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Pitfalls in the current published observational literature on the effects of red blood cell storage. Transfusion 2011; 51: 1847-1854.
17) Koch CG, Li L, Sessler DI, Hoeltge GA, Mihaljevic T, Blackstone EH. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med 2008; 358: 1229-1239.
18) Hogman CF, Meryman HT. Storage parameters affecting red blood cell survival and function after transfusion. Transfus Med Rev 1999; 13; 275-296.
19) Heaton A, Keegan T, Holme S. *In vivo* regeneration of red cell 2,3-diphosphoglycerate following transfusion of DPG-depleted AS-I, AS-3 and CPDA-1 red cells. Br J Haematol 1989; 71: 131-136.
20) Reynolds JD, Ahearn GS, Angelo M, Zhang J, Cobb F, Stamler JS. S-nitrosohaemoglobin deficiency: a

mechanism for loss of physiological activity in banked blood. Proc Natl Acad Sci USA 2007; 104: 17058-17062.

21) Bennett-Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Mulherin MA, Zhu H, Buck RD, Califf RM, McMahon TJ. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci USA 2007; 104: 17063-17068.

22) Isbell TS, Sun CW, Wu LC, Teng X, Vitturi DA, Branch BG, Kevil CG, Peng N, Wyss JM, Ambalavanan N, Schwiebert L, Ren J, Pawlik KM, Renfrow MB, Patel RP, Townes TM. SNO-haemoglobin is not essential for red blood cell-dependent hypoxic vasodilation. Nat Med 2008; 14: 773-777.

23) Weinberg JA, MacLennan PA, Vandromme-Cusick MJ, Magnotti LJ, Kerby JD, Rue LW 3rd, Angotti JM, Garrett CA, Hendrick LE, Croce MA, Fabian TC, Barnum SR, Patel RP. The deleterious effect of red blood cell storage on microvascular response to transfusion. J Trauma Acute Care Surg 2013; 75: 807-812.

24) Karam O, Tucci M, Bateman ST, Ducruet T, Spinella PC, Randolph AG, Lacroix J. Association between length of storage of red blood cell units and outcome of critically ill children: a prospective observational study. Critical Care 2010; 14: R57.

25) Roberts I, Evans P, Bunn F, Kwan I, Crowhurst E. Is the normalisation of blood pressure in bleeding trauma patients harmful? Lancet 2001; 357: 385-387.

26) Baek JH, D'Agnillo F, Vallelian F, Pereira CP, Williams MC, Jia Y, Schaer DJ, Buehler PW. Hemoglobin-driven pathophysiology is an in vivo consequence of the red blood cell storage lesion that can be attenuated in guinea pigs by haptoglobin therapy. J Clin Invest 2012; 122: 1444-1458.

27) Pereira A. Will clinical studies elucidate the connection between the length of storage of transfused red blood cells and clinical outcomes? An analysis based on the simulation of randomized controlled trials. Transfusion 2013; 53: 34-40.