Transfusion Medicine and Hemotherapy

Review Article · Übersichtsarbeit

Transfus Med Hemother 2008;35:359–367
DOI: 10.1159/000155221

Received: June 6, 2008
Accepted: September 9, 2008
Published online: September 18, 2008

Effects of Storage of Red Cells

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Key Words
Red cell storage · Red cell storage lesion · Transfusion reactions · Adverse transfusion effects · Old blood

Summary
During storage, red blood cells intended for transfusion undergo progressive changes affecting survival and function. Some of these in vitro changes are partly restored in vivo after transfusion, and their clinical effects are largely unknown. We evaluated publications of clinical studies comparing storage times in connection with red blood cell transfusion using physiological or clinical outcomes. A few prospective randomised studies in humans investigated physiological outcomes or oxygen kinetics. Sixteen observational studies comparing clinical outcome yielded contradictory results regarding the effect of red cell storage on mortality, length of intensive care and hospital stay, infections, organ failure, and composite adverse effects. The use of different red blood cell products further obscures the issue. Available studies provide no evidence that longer stored red cells are more harmful than younger red cells. However, such an effect may occur under extreme clinical conditions of severe anaemia or septicaemia, but this can only be answered by randomised studies controlling for confounding factors.

Schlüsselwörter
Erythrozytenlagerung · Erythrozytenlagerungsschaden · Transfusionsreaktionen · Schädliche Transfusionseffekte · Altes Blut

Zusammenfassung
Auswirkungen der Erythrozytenlagerung
Für die Transfusion vorgesehene Erythrozyten durchlaufen während ihrer Lagerung ständige Veränderungen, die das Überleben und die Funktion der Zellen beeinflussen. Einige dieser In-vitro-Veränderungen werden nach der Transfusion in vivo teilweise rückgängig gemacht, und ihre klinischen Auswirkungen sind größtenteils unbekannt. In der vorliegenden Arbeit werden Publikationen klinischer Studien, die Erythrozytenlagerungszeiten anhand des physiologischen bzw. klinischen Outcome vergleichen, bewertet. Einige am Menschen durchgeführte prospektive, randomisierte Studien haben das physiologische Outcome oder die Sauerstoffkinetik untersucht. Sechzehn Beobachtungsstudien, in denen das klinische Outcome verglichen wurde, erbrachten widersprüchliche Ergebnisse bezüglich des Einflusses der Erythrozytenlagerung auf die Mortalität, die Länge der intensivmedizinischen Behandlung und des Krankenhausaufenthalts, Infektionen, Organversagen sowie verschiedene Nebenwirkungen. Die Verwendung unterschiedlicher Erythrozytenpräparate ist dabei ein zusätzlicher Störfaktor. Vorliegende Studien liefern keine Hinweise darauf, dass länger gelagerte Erythrozyten gesundheitsschädlicher als jüngere Zellen sind. Dies schließt nicht aus, dass unter extremen klinischen Bedingungen wie schwerer Anämie oder Sepsis ein nachteiliger Effekt auftreten kann. Diese Frage kann jedoch nur durch randomisierte Studien, in denen Störgrößen überprüft werden, beantwortet werden.

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Introduction

The primary function of red blood cells (RBC) is uptake, transport, and delivery of oxygen. In addition, RBC contribute to the colloid osmotic pressure, to platelet-endothelium interactions, and transport of several molecules such as drugs or immune complexes. The aim of RBC transfusions is to treat or prevent tissue hypoxia. Virtually all oxygen is transported by the haemoglobin (Hb). The oxygen supply is further determined by cardiac output and by oxygen uptake capacity in the lung. Under resting circumstances, the oxygen supply exceeds the demand. Overall, only 25–35% of the available oxygen is extracted by the tissues, although some organs such as the heart, kidney, and brain have a higher basic oxygen extraction ratio of 55–70% and have less capacity to increase oxygen extraction in response to anaemia [1]. Oxygen supply is not synonymous with oxy Figgen delivery as this requires passage of erythrocytes through the microcirculation and unloading of oxygen, determined by the oxygen dissociation curve, which depends among other factors on acidosis and the red cell enzyme 2,3-diphosphoglycerate (2,3-DPG) (table 1).

During ex-vivo storage, red cells undergo changes affecting function and viability, often collectively referred to as 'storage lesions'. These raised concern for appropriate oxygen delivery, increased adhesion of erythrocytes to endothelium, and impaired deformability necessary for passage through small capillaries; however the clinical relevance is unknown [2–5]. We discuss available clinical studies investigating effects of red cell storage.

Table 1. Factors determining tissue oxygenation relevant for erythrocyte transfusions

Determinants of oxygen supply and delivery	Erythrocyte factors
Oxygen supply	
Hb/Ht level	post transfusion RBC recovery
Cardiac output	blood viscosity (Ht)
Hb oxygen uptake	COPD; oxygen supply/artificial ventilation
Oxygen delivery	
Vasodilatation	nitric oxide (endothelium, RBC)
RBC deformability	RBC surface:volume ratio; spectrin (un)folding
Oxygen off-loading	oxygen dissociation curve; pH; 2,3-DPG
Oxygen extraction ratio	
Organ dependent	exercise; fever

COPD = Chronic obstructive lung disease; Ht = haematocrit.

Red Cell Products

For the production of red cells intended for transfusion, in the Western world 450 or 500 ml of whole blood is collected in 63 or 70 ml of CPD-A (citrate-phosphate-dextrose with adenine), respectively. The process of preparing RBC products varies considerably. Variations concern holding time and temperature before centrifugation by a hard or a soft spin, buffy coat removal, filtration of whole blood or of buffy coat-depleted RBC to remove leucocytes, or no leucocyte depletion at all. The resulting RBC products have various volumes of residual plasma, platelets, and leucocytes. Red cells can be stored in PVC bags at 2–6 ºC for a period of 5 weeks or longer depending on the red cell preservation solution used. PVC storage bags contain the plasticiser di-ethylhexyl phthalate (DEHP) which accumulates in the red cell membrane and slows down the process of haemolysis and microvesicle formation, and improves red cell survival [6, 7]. Regulations require that 24 h after transfusion more than 75% of the cells are recovered in the circulation [8, 9]. At expiration, the plasma Hb may not exceed 0.8% (Europe) or 1% (USA) of the RBC mass.

It is prudent to realise that available databases which are used in retrospect for clinical studies on the role of aged red cells used different RBC products. These studies include packed RBC containing all leucocytes and platelets, RBC after removal of platelet-rich plasma leaving the majority of leucocytes in the product, buffy coat-depleted RBC in which > 90% of the platelets and 60–70% of the leucocytes are removed, and pre-storage filtered whole blood or filtered buffy coat-depleted RBC, virtually depleted from all leucocytes and platelets. Moreover, a variable volume of residual plasma and different preservation solutions (SAG-M, Adsol 1,2,3,5) have been present in these red cell products.

Red Cell Storage Lesions

Storage lesions, of which the most notable changes are discussed, not only affect red cell properties but also the red cell product suspended in various solutes and containing other residual cells.

Vasodilatory Capacity

Blood flow in the microcirculation is regulated by nitric oxide (NO) produced by endothelial cells and induced by erythrocytes. When the environmental oxygen tension is low, this is sensed by Hb, and within seconds an adjusted amount of NO is produced causing a dosed vasodilatation for erythrocyte passage through the vessels, bringing blood flow in line with metabolic demand [10]. Reynolds et al. [11] and Bennett-Guerrero et al. [12] showed an important role for S-nitrosothiol-Hb (SNO-Hb) for the release of NO. Because SNO-Hb is almost immediately decayed after blood withdrawal and is not restored in vivo, they argue that stored RBC rather act as a sink for NO, enhancing vasoconstriction. In contrast, Isbell et al. [13] showed SNO-Hb is not essential for erythrocyte-dependent vasodilation, and suggest an adenosine triphosphate (ATP)-dependent mechanism.

Depletion of 2,3-Diphosphoglycerate (2,3-DPG)

This enzyme binds to de-oxy Hb forming a complex with low O_2 affinity. In the case of anaemia, red cell 2,3-DPG increases after 16–36 h, albeit that this increase is reduced in critically ill septic patients [14]. In RBC stored for 2 weeks, 2,3-DPG is virtually depleted, resulting in a shift of the O_2 dissociation curve to the left, impairing oxygen delivery [12]. The reported speed of in vivo restoration of 2,3-DPG after transfusion varies. One hour after transfusion, 25–30% of 2,3-DPG was measured [15], after 24 h recovery it is 50%, but full restoration may take up to 3 days [16]. This led to concerns regarding impaired oxygen delivery after transfusion of large amounts of stored RBC. However, studies in baboons [17] and rats [18] found no support for the role for 2,3-DPG as a key factor for off-loading oxygen.

Sodium Potassium Pump

At 2–6 °C, the Na^+/K^+ pump is paralysed, and K^+ leaves the cell while sodium enters it [19]. After 3 days of storage, potassium leaks progressively from the erythrocyte, and the extracellular concentration can increase to 50 mEq/l. After transfusion, the red cell sodium content normalises within 24 h, while complete K^+ recovery should take at least 4 days [20]. Danger of arrhythmia due to high potassium levels is mainly present with large volume transfusions in newborns and small infants in whom lethal cardiac arrest has been reported [21]. Washing or simply removal of the supernatant are alternatives to reduce potassium toxicity for high-risk recipients [22, 23].

Gamma Irradiation

Gamma irradiation to prevent transfusion-associated graft versus host disease liberates reactive oxygen species, damages red cell band 3 proteins, increases K^+ leakage, enhances haemolysis, and impairs at all shear rates the erythrocyte deformability [24, 25]. Gamma irradiation does not enhance RBC aggregation and the adherence of red cells to endothelial cells [26, 27]. Because of lower levels of oxygen radical scavengers, older cells are more susceptible to gamma irradiation-induced damage [28].

Morphology, Deformability, and Viability

The lipid molecules of the red cell membrane are anchored to different skeletal proteins whose folding and unfolding is essential for cell deformability. The large surface-to-volume ratio of the disc-shaped RBC enables adaptation of shape, minimising the resistance of the 8 μm-sized red cells during changing flow conditions and passage through smaller capillaries of 3–8 μm. During their in vivo lifespan, red cells lose area, volume, and Hb through vesiculation of 50–200 nm particles [29]. After leaving the bone marrow, 10–14% of membrane area is lost during reticulocyte maturation, followed by 16–17% during the remaining lifespan [30, 31]. Extrusion of oxidative waste material of denatured Hb, along with surface area loss, seems a finite process to protect the RBC from premature removal [31, 32]. Exhaustion of this potential may herald the end of red cell lifespan as accumulated denatured Hb in the red cell membrane may be a sign for binding to natural auto-antibodies which in concert with complement promote partial phagocytosis causing further surface area loss [33–35]. Phosphaditylserine (PS/Annexin V), the death signal, is expressed on 30–70% of the microvesicles, and 50% are coated with immunoglobulins resulting in prompt removal by the liver Kupffer cells [30, 36].

During in vitro storage, besides micro-vesiculation in particular of the younger cells [37], red cells also undergo a shape change to echinocytes because of ATP depletion [38]. Initially, this is a reversible process, but towards the end of the in vitro shelf life, irreversible spheroechinocytes are formed, probably due to depletion of the total available adenine nucleotide pool consisting of ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) [39, 40]. On stored red cells, the expression of CD47, a 50-kDa surface transmembrane glycoprotein widely expressed on all cells, reduces by 10–65% depending on the type of product and assay used [41, 42]. CD47 is presumed to serve as a marker for self, and when expression falls below 50%, the erythrocytes become susceptible for phagocytosis [41, 43]. Of red cells transfused at the end of the storage time, 20–30% are non-viable and are removed from the circulation within a few hours. Cells that survive after 24 h, show a normal lifespan, irrespective of storage duration [44].

The Role of Residual Leucocytes and Platelets during Storage

Residual leucocytes and platelets influence not only the RBC storage lesion but also, by release of factors, the red cell product. Potassium leakage and haemolysis is less in leucocyte-depleted RBC products [45, 46]. Filtered leucocyte-depleted stored RBC show virtually no increase in PS expression [12], whereas PS becomes expressed in leucocyte-containing RBC products stored as little as 2 weeks. PS-expressing red cells exert pro-coagulant effects and increased adherence potential to endothelium [47, 48], inducing endothelial activation facilitating transendothelial migration of monocytes [49].

In the supernatant of stored non-leucocyte- and platelet-reduced RBC, histamine, complement, lipids, and cytokines can be detected [50]. Cytokines, Il-1β, IL-6, IL-8, TNF-α, are produced by residual leucocytes, and remain below detection levels in leucocyte-reduced RBC [12, 51–54]. On the other hand, filtration has been reported to increase the levels of neutrophil elastase and TGF-β1 in RBC [55]. From 2 weeks of storage onwards, the supernatant of leucocyte-containing RBC, but not of leucocyte-depleted RBC, can induce expression of CD11b (C3bi) and CD16 (FCR III) on neutrophils in vitro, with both markers being associated with priming of neutrophils [49, 54].

Residual platelets and red cells themselves release factors during RBC storage, such as the pro-inflammatory CD40Ligand and bio-active lipid peroxidases, respectively. Both can prime polymorph-nuclear cells (PMN), and have been proposed as

Table 2. Clinical studies on storage time and mortality

Reference	Design	Population	Patients, n	Storage time variables	Adjusting for confounders[a]	Results
Purdy et al., 1997 [64]	retrospective cohort	sepsis	31	mean	no	17 vs. 25 days, $p < 0.0001$
Edna et al., 1998 [65]	retrospective cohort	colorectal	336	mean	yes	NS
Mynster and Nielsen, 2001 [66]	prospective cohort	colorectal	452	< 21 vs. > 21 days	no	NS
Gajic et al., 2004 [67]	retrospective cohort	ICU	181	< 15 vs. 15–20 vs. > 20 days	no	NS
Murrell et al., 2005 [68]	retrospective cohort	trauma	275	dose (= mean number of RBC)[a]	yes	NS
Hebert et al., 2005 [69]	RCT	cardiac + ICU	57	< 8 days (median 4 days) vs. standard (median 19 days)	no	NS
Basran et al., 2006 [70, 84]	retrospective cohort	cardiac	321	mean + oldest RBC	yes	both show increased in-hospital mortality
Van de Watering et al., 2006 [71]	retrospective cohort	cardiac	2,732	mean + oldest + youngest + < 18 vs. > 18 days[b]	yes	NS
Leal-Noval et al., 2008 [72]	prospective cohort	trauma	66	< 10 vs. 10–14 vs. 15–19 vs. > 19 day	no	NS
Koch et al., 2008 [73]	retrospective cohort	cardiac	6,002	< 14 vs. > 14 days	no	1.7 vs. 2.8%, $p = 0.004$
Yap et al., 2008 [74]	retrospective cohort	cardiac	670	mean + oldest + (y/n) > 30 days	yes	NS
Weinberg et al., 2008 [75]	retrospective cohort	trauma	1,813	< 14 vs. ≥ 14 days	yes	NS[c]

NS = No significant independent association found between storage time and mortality; RCT = randomised clinical trial; y/n = yes/no.
[a]Adjustments for confounders like number of transfused RBC were performed in the analyses on mortality.
[b]1,895 patients in analyses < 18 days (945) vs. > 18 days (950).
[c]Only in subgroup analysis significant associations were found.

(co-)factors causing transfusion-related acute lung injury (TRALI) [56–58]. RBC supernatants harvested after 15 days storage from filtered leucocyte-depleted as well as from non-filtered RBC increase the oxidative potential of PMN in response to N-formyl-methionyl-leucyl-phenylalanine (fMLP), a chemotactic peptide stimulating the oxidative burst [54]. The role of storage of red cells causing febrile non-haemolytic transfusion reactions (FNHTR) and TRALI has indeed often been questioned, but randomised studies have not yet been performed [59, 60]. Heddle et al. [59], by application of multivariate regression analysis in a prospective study, identified the number of contaminating leucocytes and the age of the RBC component as the most significant factors associated with FNHTR [59].

Although RBC expressing PS show enhanced endothelial adherence and pro-coagulant activity, and RBC supernatants can induce priming of PMN – factors that may play a role in causing TRALI, the exact contribution of aged RBC products to TRALI is not yet known.

Physiological Studies in Humans

Oxygen Kinetics

Hebert and Chin-Yee [4] reviewed 14 observational studies in critically ill patients estimating the effect of RBC transfusions on oxygen delivery, oxygen consumption, and lactate levels. A striking number of studies showed no improvement in these parameters. Again, the use of different blood products may explain some of the discordant outcomes. Marik et al. [61] found in a prospective study measuring gastric mucosal oxygenation that 3 units of (leucocyte-containing) RBC stored longer than 15 days, impaired gastric mucosal pH indicating lower oxygen release. This study was repeated in a double-blinded randomised trial, assigning patients to 2 units of RBC stored < 5 days (mean 2) or > 20 days (mean 28), which had been leucocyte-reduced before storage and stored in SAG-M solution. This study found no support of any difference in oxygenation index between the storage groups, but also no improvement as compared to baseline values [62]. In an elegant

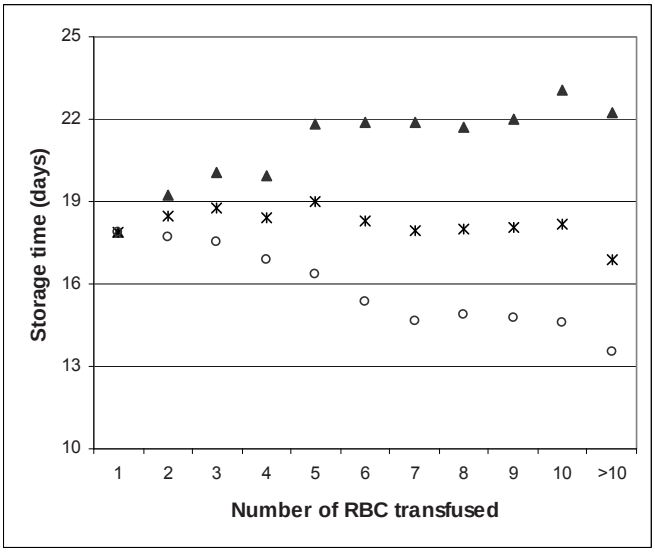

Fig. 1. Correlation of total number of transfused RBC with storage time variables.
Correlation of the total number of RBC transfused with the 3 storage time variables: (▲) mean storage time of the oldest RBC/patient; (x) mean storage time of all RBC/patient; (○) mean storage time of the youngest RBC/patient.
Reproduced with permission from van de Watering L, Lorinser J, Versteegh M, Westendorp R, Brand A: Effects of storage time of red blood cell transfusions on the prognosis of coronary artery bypass graft patients. Transfusion 2006;46(10):1712–8. ©Transfusion, 2006.

study in healthy volunteers, Weiskopf et al. [63] showed that absence of 2,3-DPG in non-leucocyte-reduced RBC stored up to 2–3 weeks improved cognitive functions in acute normovolaemic anaemia with Hb of 5–6 g/dl.

Studies with Clinical (Surrogate) Endpoints

Dozens of studies have been performed investigating the association between clinical outcome and the transfusion of stored RBC. However, only a minority of these have actually investigated the role of the storage time of the RBC. The clinical setting was most often in trauma, intensive care unit (ICU), cardiac, or (colo)rectal surgery patients. Clinical outcome was analysed using endpoints like mortality [64–75], ICU/hospital stay [67, 68, 70–72, 74, 76–79], infectious complications [67, 73, 74, 79–82], intubation days [77–79], (multiple) organ failure [69, 70, 74, 83], and composites of specific adverse outcomes [69, 73, 79, 80]. Nearly all studies are observational, and therefore mostly associations are reported.

Storage Time and Mortality

The most investigated association is that between the storage time of RBC and mortality (table 2). In 1997, Purdy et al. [64] reported, in a population of 31 transfused septic patients, a significant longer mean storage time of transfused RBC in patients that died (25 days) versus survivors (17 days), $p < 0.0001$. One year later, Edna et al. [65] could not see any independent association between mean storage time and survival. And Mynster and Nielsen [66], in 2001, reported even a small beneficial effect of prolonged (> 21 days) storage in transfused colorectal surgery patients, mostly due to significantly less cancer recurrence. Gajic et al. [67] stratified his patient population according to the mean storage time of the transfused RBC into '< 15 days', '15–20 days', '> 20 days'. These strata showed similar mortality rates (0.30 vs. 0.30 vs. 0.26; $p = 0.869$). Two studies published in 2005 on this subject, both failed to find an association with mortality. Murrell et al. [68] did not find an association between the dose of aged blood and mortality in major trauma patients. Neither did Hebert et al. [69] reporting on a randomised pilot study in 57 transfused cardiac/ICU patients receiving either standard RBC (mean storage time: 19 days) or RBC stored < 8 days (mean 4 days). Then, in 2006, Basran et al. [70] reported mortality to be associated with both the mean storage time and the maximum storage time of the transfused RBC, in transfused cardiac surgery patients. However, the reported results showed some inconsistencies which could not be corrected, as the used dataset had been lost [84]. Also in 2006, van de Watering et al. [71] reported analyses on 2,732 coronary artery bypass graft (CABG) patients that showed an association in univariate analyses between mortality and the storage time of both the oldest and the youngest RBC transfused. However, both these storage time variables were strongly correlated with the total number of RBC transfused, as shown in figure 1, and the multivariate analyses adjusting for this imbalance showed absolutely no independent correlation of storage times with mortality (both $p > 0.95$). The mean storage time and the subgroups with all RBC stored < 18 days vs. all RBC stored > 18 days were in none of the analyses associated with mortality. In the study of Leal-Noval et al. [72] in patients receiving 1 or 2 RBC, the storage time was stratified into '< 10 days', '10–14 days', '15–19 days', and '> 19 days'. The analyses showed no association between these strata and mortality. In 2008, Koch et al. [73] reported on 6,002 cardiac surgery patients, receiving all their RBC either ≤ 14 days or all > 14 days old. A significant association of mortality with storage time was reported ($p = 0.004$), but shortcomings in this analysis (e.g. no adjusting for confounders like the number of RBC transfused) lead to several letters in response [85]. Yap et al. [74] analysed mean storage time, the oldest RBC and if any RBC stored > 30 days had been transfused, in 670 cardiac surgery patients. None of these variables were independently associated with mortality. Weinberg et al. [75] analysed storage, stratifying the number of filtered RBC < 14 days and ≥ 14 days into 0, 1–2, or ≥ 3 RBC in 1,813 transfused trauma patients. Both 'young 'and 'old' RBC increased the odds of death, but this was stronger with old blood. The unadjusted association of the storage time of transfused RBC with mor-

Table 3. Clinical studies on storage time and (ICU and/or hospital) length of stay

Reference	Design	Population	Patients, n	Storage time variables	Adjusting for confounders[a]	Results
Martin et al., 1994 [76]	retrospective cohort	ICU	698	< 14 vs. > 14 days	yes	ICU length of stay p = 0.003
Vamvakas and Carven, 2000 [77]	retrospective cohort	cardiac	269	mean + oldest + mean of 2 oldest	yes	NS
Keller et al., 2002 [78, 86]	retrospective cohort	trauma	86	number of RBC > 14 days	yes	NS
Leal-Noval et al., 2003 [79]	prospective cohort	cardiac	585	mean + oldest RBC	yes	NS
Gajic et al. 2004 [67]	retrospective cohort	ICU	181	< 15 vs. 15–20 vs. > 20 days	no	NS
Murrell et al., 2005 [68]	retrospective cohort	trauma	275	dose (= mean × number of RBC)	yes	dose aged blood ⇒ increased ICU length of stay[b]
Basran et al. 2006 [70, 84]	retrospective cohort	cardiac	321	mean + oldest RBC	yes	oldest RBC ⇒ increased both ICU + hospital length of stay[c]
Van de Watering et al. 2006 [71]	retrospective cohort	cardiac	2732	mean + oldest + youngest + < 18 vs. > 18 days[d]	yes	NS
Leal-Noval et al., 2008 [72]	prospective cohort	trauma	66	< 10 vs. 10–14 vs. 15–19 vs. > 19 days	no	NS
Yap et al., 2008 [74]	retrospective cohort	cardiac	1,813	mean + oldest + (y/n) > 30 days	yes	NS

NS = No significant independent association found between storage time and ICU length of stay.
[a]Adjustments for confounders like number of transfused RBC were performed in the analyses on ICU length of stay.
[b]Relative risk 1.15, 95% confidence interval (CI): 1.11–1.20.
[c]Inconsistencies in reported hazard ratios, 95% CIs, and p values could not be corrected due to loss of the dataset [84].
[d]1,895 patients in analyses < 18 (945) vs. > 18 days (950).

tality, as initially reported in a small (n = 31) study, was not reliably confirmed as independent association in any of the 11 succeeding larger studies.

Storage Time and Length of Stay

An association between storage time of RBC and length of ICU stay (table 3) was reported by Martin et al. [76] in 1994. In 698 ICU patients, the transfusion of RBC stored > 14 days was independently associated with the length of ICU stay. Vamvakas and Carven [77] investigated in CABG patients the association with both ICU and hospital length of stay, using mean storage time of all RBC, the storage time of the oldest RBC, and the mean of the 2 oldest RBC. After adjustment for confounding factors, none of the storage time variables were associated with either length of ICU stay or hospital stay. Keller et al. [78] investigated both these associations in trauma patients, using the number of RBC stored > 14 days as variable. In all their models adjusting for confounding, no association was found with length of ICU stay. Length of hospital stay was only independently associated in the multivariate models not including the number of RBC transfused [86]. Leal-Noval et al. [79] reported in 2003 their study in cardiac surgery patients. Neither the mean storage time of all RBC nor the storage time of the oldest RBC was associated with length of ICU stay. Gajic et al. [67] stratifying his population of mechanically ventilated patients on mean RBC storage into '< 15 days', '15–20 days', '> 20 days' also found no association of these strata with length of ICU stay. The study by Murrell et al. [68] in trauma patients reported that the dose of aged blood (defined as the average age of received RBC multiplied by the number of RBC received) was significantly correlated with longer ICU stay. From the 2 studies in 2006 on this topic in cardiac surgery patients, Basran et al. [70, 84] reported an association of the length of both ICU and hospital stay with the storage time of the oldest RBC, but not with the mean storage time or even the number of transfusions. The study by van de Watering et al. [71] showed strong associations between the storage time of both the youngest and the oldest RBC in the unadjusted univariate analyses, that completely disappeared in the multivariate analyses adjusting for confounders. Again, no associations were seen with the mean storage time or in the subgroups with all RBC < 18 days vs. all RBC > 18 days,

analysing 2,732 and 1,895 (945 vs. 950) patients, respectively. The study by Leal-Noval et al. [72] stratifying brain trauma patients on RBC storage time into '< 10 days', '10–14 days', '15–19 days', and '> 19 days' showed no association between these strata and length of ICU or hospital stay. Yap et al. [74] reported that neither the mean storage time nor the oldest RBC or any RBC > 30 days old were independently associated with the postoperative ICU length of stay. Like with the studies on the association with mortality, the initially reported association of the storage time of transfused RBC with ICU/hospital length of stay was not reliably confirmed as independent association in any of the succeeding studies.

Storage Time and Infectious Complications

A possible association between storage time of RBC and infectious complications is investigated using several different endpoints. Vamvakas and Carven [80], investigating transfused CABG patients, reported both their composite outcome (wound infection or pneumonia) and pneumonia by itself to be associated with both the mean length of storage of all RBC and the mean length of storage of the 2 oldest RBC. Mynster and Nielsen [82] found in rectal cancer patients 60% of RBC were stored for > 20 days in patients with postoperative infections versus only 25% of RBC in patients without infections. Offner et al. [81] investigated in trauma patients the association between infection and the number of RBC stored > 14 days or > 21 days. To correct for the total number of RBC transfused, the analyses were performed in 3 strata (6–10 RBC; 11–15 RBC; 16–20 RBC). An independent association with infection was seen in 1 of the 3 strata analysed for both the number of RBC > 14 days (in 6–10 RBC) and > 21 days (in 16–20 RBC). Leal-Noval et al. [79] saw in cardiac surgery patients no independent association between the mean or maximum storage time and their composite endpoint infection (pneumonia, sepsis, mediastinitis). However, in subgroup analyses, storage of the oldest RBC longer than 28 days was identified as risk factor for nosocomial pneumonia. The study by Gajic et al. [67] comparing patients with a mean RBC storage < 15, 15–20, or > 20 days found no association of these strata with the occurrence of sepsis. Yap et al. [74] found no association between postoperative pneumonia and mean storage time, oldest RBC, or storage time > 30 days. Although both the type of infectious complications investigated and the way storage time was analysed differed between the studies, an association between storage time and infectious complications was repeatedly reported.

Storage Time and Organ Failure

Other studies reporting on associations between storage time of RBC and clinical endpoints mostly report on some type of organ failure. Zallen et al. [83] reported in their study in trauma patients an association between the mean storage time, the number of RBC stored > 14 days, and the number of RBC stored > 21 days with the occurrence of multiple organ failure. Respiratory failure (period of intubation or acute lung injury) was analysed in 5 studies. Vamvakas and Carven [77] found no association with the mean storage time of all RBC, the storage time of the oldest RBC, or the mean of the 2 oldest RBC, Keller et al. [78] found no association with the number of RBC stored > 14 days, Leal-Noval et al. [79] reported no association with the mean storage time or the storage time of the oldest RBC, Gajic et al. [67] found no difference in their analyses stratified on mean storage time, and Hebert et al. [69] found no difference in their randomised controlled trial in patients receiving either standard RBC (mean storage time: 19 days) or RBC stored < 8 days (mean 4). This last study also found no differences in cardiac or renal support. Renal dysfunction was reported to be associated with both the mean and the maximum storage time by Basran et al. [70], although the precise magnitude of this association remains unclear [84]. Koch et al. [73, 85], comparing patients receiving either all their RBC stored ≤ 14 days or all > 14 days, reported associations with respiratory failure, renal failure, and multiple organ failure, only in their unadjusted analyses. Yap et al. [74] found, after adjusting for number of transfusions and Euroscore, no association between renal failure and mean storage time, oldest RBC, or storage time > 30 days.

Conclusions

There is no clear consensus on possible associations between storage time and morbidity or mortality. The results on infectious complications are most consistent, but with the other endpoints there seem to be additional variables, maybe unreported or even unrecorded, that play a major role. Publication bias may have played some role as more of the older, smaller, studies report independent associations. Furthermore, some studies failed to correct for known confounders, like the number of RBC transfused. Another explanation may be sought with the fact that apart from infectious complications, associations are reported in most of the North American studies and in none of the European studies. Differences in production techniques, storage media, or cellular composition of the blood products used in these studies may be part of the explanation. The registered randomised controlled trials on storage time (*ClinicalTrials.gov* identifiers: NCT00141674 & NCT 00458783) may come up with answers for North America, but if their results will also be applicable for Europe will need further, intercontinental research.

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