The effects of storage of red blood cells on the development of postoperative infections after noncardiac surgery

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BACKGROUND: Prolonged storage of red blood cells (RBCs) is a potential risk factor for postoperative infections. The objective of this study was to examine the effect of age of RBCs transfused on development of postoperative infection.

STUDY DESIGN AND METHODS: In this prospective, double-blind randomized trial, 199 patients undergoing elective noncardiac surgery and requiring RBC transfusion were assigned to receive nonleukoreduced RBCs stored for not more than 14 days (“fresh blood” group, n = 101) or for more than 14 days (“old blood” group, n = 98). The primary outcome was occurrence of infection within 28 days after surgery; secondary outcomes were postoperative acute kidney injury (AKI), in-hospital and 90-day mortality, admission to intensive care unit, and hospital length of stay (LOS). As older blood was not always available, an “as-treated” (AT) analysis was also performed according to actual age of the RBCs transfused.

RESULTS: The median [interquartile range] storage time of RBCs was 6 [5-10] and 15 [11-20] days in fresh blood and in old blood groups, respectively. The occurrence of postoperative infection did not differ between groups (fresh blood 22% vs. old blood 25%; relative risk [RR], 1.17; confidence interval [CI], 0.71-1.93), although wound infections occurred more frequently in old blood (15% vs. 5%; RR, 3.09; CI, 1.17-8.18). Patients receiving older units had a higher rate of AKI (24% vs. 6%; p < 0.001) and, according to AT analysis, longer LOS (mean difference, 3.6 days; CI, 0.6-7.5).

CONCLUSION: Prolonged RBC storage time did not increase the risk of postoperative infection. However, old blood transfusion increased wound infections rate and incidence of AKI.

Transfusion of allogenic red blood cells (RBCs) is the most common treatment for acute anemia, although several studies have reported an association between this therapy and certain complications, in particular, the development of immunosuppression,1 which increases the risk of nosocomial infection, including pneumonia and bloodstream or wound infections.2,3 Moreover, host immune cells can

ABBREVIATIONS: AKI = acute kidney injury; AT = as treated; ICU = intensive care unit; IQR(s) = interquartile range(s); ITT = intention to treat; KDIGO = Kidney Disease/Improving Global Outcomes; LOS = length of stay; RR = relative risk; RRT = renal replacement therapy.

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react to donor blood as a result of several antigenic challenges, which may activate or down regulate the immune system, in the so-called transfusion-associated immunomodulation process.4

The mechanisms underlying the development of transfusion-associated immunomodulation have not yet been completely elucidated; however, studies in vivo and in vitro suggested that the storage time of RBCs may play a role in inducing immunosuppression.5 RBCs undergo morphologic and functional changes during prolonged storage (so-called “storage lesions”), including imbalance of the cell membrane expression of CD47 and phosphatidylserine, which can contribute to activate the immune system.3 Moreover, during storage, cytokines and other substances (e.g., histamine, immunologically active phospholipids, soluble CD40 ligand) with inflammatory and immunomodulatory properties accumulate in the supernatant and trigger the activation of immune cells.3 Although the biochemical effects of storage on RBCs are well documented, the clinical consequences of receiving RBCs of longer storage duration are currently a matter of debate.5 Three recent randomized clinical trials did not find significant differences between fresh and old blood in patients undergoing cardiac surgery6 or admitted to the intensive care unit (ICU)7 and in hospitalized patients.8 Immunosuppression induced by surgery and anesthesia may be aggravated by transfusion.9 Most studies in trauma and cardiac surgery reported that prolonged RBC storage was a risk factor for postoperative infections after cardiac surgery,10-12 but the same association was not found in liver transplantation13 or orthopedic surgery.14 A recent systematic review15 that analyzed four trials which investigated the nosocomial infections, showed no benefits in receiving fresh RBCs. However, the authors reported that the results are difficult to interpret because the studies involved different populations and there were considerable differences in the length of storage of fresh and old blood and in study methods.15

To our knowledge, the association of age of RBCs with infections in noncardiac surgery population has not been investigated by double-blind randomized studies. Accordingly, the aim of this study was to investigate the relationship between RBC storage time and postoperative infection after elective noncardiac surgery. In addition, we evaluated the effects of the age of RBCs on other outcomes such as postoperative acute kidney injury (AKI), hospital length of stay (LOS), and 90-day mortality.

MATERIALS AND METHODS

Study population
This prospective, randomized, controlled, double-blind clinical trial was conducted between August 2013 and July 2015 at Sant’Anna University Hospital in Ferrara (Italy).

The study protocol was approved by the local ethics committee (Protocol 85a/12) and registered on ClinicalTrials.gov (NCT01976234).

All patients undergoing scheduled noncardiac surgery were informed about the study protocol during the preoperative visit with the anesthesiologist. Patients declining to participate to the study were not considered eligible. Written informed consent was obtained for all randomized patients.

Patients were excluded if they had received a RBC transfusion in the 30 days before surgery; if they had suspected or documented infection in the 30 days before surgery (defined as need for antibiotic therapy in the past 30 days, regardless of the identification of an infectious site); if they were being treated with chronic corticosteroid therapy or other immunosuppressive therapy; or if they had active malignant hematologic illness or any other congenital or acquired immunodeficiency. For patients undergoing multiple surgical interventions, only the first procedure was considered in the study. All patients received the same antibiotic prophylaxis (Tables S1 and S2, available as supporting information in the online version of this paper) depending on the type of surgery, chosen according to the American Society of Health-System Pharmacists (ASHP) Clinical Practice Guidelines.16

According to local transfusion policy, postoperative patients in our department receive transfusions to keep their hemoglobin (Hb) concentration between 7 and 9 g/dL.17 Fresh-frozen plasma (FFP) is considered for: 1) correction of excessive bleeding in the presence of an international normalized ratio greater than 2.0 or an activated partial thromboplastin time greater than two times normal; 2) correction of excessive bleeding secondary to coagulation factor deficiency in patients transfused with more than one blood volume. Platelet (PLT) units are administered to bleeding patients when the PLT count is less than $50 \times 10^9$ cells/L.

When blood was sent from the included patients to the hospital transfusion service for crossmatching, the blood bank personnel randomly assigned the patient to one of two groups by opening a sealed and numbered envelope with randomly permuted block sizes of 4 and 6. One group exclusively received RBCs that had been stored for 14 days or less (“fresh blood” group), and the other group received units stored for more than 14 days (“old blood” group). This cutoff point was chosen considering other studies.10,11,18 Moreover, in a previous retrospective study conducted in our institution,19 the cutoff of 14 days identified the median values of blood storage in trauma patients. The old blood group received RBCs with the longest storage times, according to the usual policy followed by the local blood bank: this is the “first-in, first-out” policy, that is, the oldest units are used first, to minimize waste of blood units.17
Patients remained in the study group until hospital discharge or death, whichever came first, and received exclusively blood of the assigned group during the whole hospital stay. RBC products transfused during the study period were stored in SAGM up to 35 days. The RBC units were not leukoreduced, according to the policy of our hospital during the study period.

**Data collection**

Perioperative and postoperative clinical, laboratory, and transfusion data were recorded daily and retrieved from the patient records and the hospital electronic information systems. Information on the duration of RBC storage, as well as their ABO group, were provided by blood bank technologists. Microbiologic and clinical data on infections were collected daily during the study period. Wound classification was in accordance with Centers for Disease Control and Prevention (CDC) criteria (Appendix S1, available as supporting information in the online version of this paper). Chronic kidney disease was defined as abnormalities of kidney structure or function (glomerular filtration rate [GFR] < 60 mL/min/1.73 m²) present for at least 3 months. Preoperative anemia was defined according to the World Health Organization as a Hb level of less than 13 g/dL in men and less than 12 g/dL in women.

**Outcomes**

The primary outcome was the development of postoperative infections within the 28 days after surgery, including: 1) nosocomial pneumonia defined according to CDC’s National Healthcare Safety Network criteria; 2) sepsis according to Surviving Sepsis Campaign Criteria; 3) wound infections (defined as deep incisional surgical site infection, organ or surgical site infection, or wound dehiscence); 4) peritonitis defined according to the Dutch Peritonitis Study Group; or 5) urinary tract infections defined according to American College of Surgeons National Surgical Quality Improvement Program standardized definition of postoperative urinary tract infection. Details about diagnostic criteria for postoperative infections are given in Appendix S2 (available as supporting information in the online version of this paper). The diagnosis of infection was assessed by a specialist in infectious diseases who was blinded to the group assignment of the patient. The blood bank personnel were blinded to the development of infections and other outcomes of the studied patients. For patients discharged before 28 days, a postdischarge ambulatory visit was conducted on Day 28 after the initial surgery.

Secondary outcomes were in-hospital mortality, 90-day all-cause mortality, postoperative AKI, admission to the ICU, and hospital LOS. Postoperative AKI was defined according to the Kidney Disease/Improving Global Outcomes (KDIGO) criteria as an increase in serum creatinine of 0.3 mg/dL within 48 hours, an increase in serum creatinine to 1.5 times the baseline value present within the previous 7 days, or urine volume of less than 0.5 mL/kg/hr for 6 hours. We also evaluated AKI defined and staged using the AKI network criteria, with AKI defined as reduction in kidney function (within 48 hr) defined as an absolute increase in serum creatinine of at least 0.3 mg/dL, a percentage increase in serum creatinine of more than or equal to 50%, or a reduction in urine output (less than 0.5 mL/kg/hr for more than 6 hr). In patients with AKI, the use of renal replacement therapy (RRT) was also recorded.

**Statistical analysis**

Data analysis was primarily based on the intention-to-treat (ITT) principle. However, due to the high turnover of blood products in our hospital, we anticipated that RBCs stored for more than 14 days might not always be available. Therefore, to test the real effect of the exposure to RBCs stored for more than 14 days an “as-treated” (AT) analysis was also performed according to the age of the RBCs transfused. If the differences we observed between fresh and old groups are not due to chance, it is reasonable to expect that they increase when comparing more extreme groups. Therefore, we performed a post hoc subgroup analysis considering the patients who received blood stored for less than 7 days only or more than 21 days only. Categorical data are presented as numbers and percentages, while continuous data are expressed as means and standard deviations (SD) or medians and interquartile ranges [IQRs], as appropriate. Normality of data distribution was evaluated for each variable using the Kolmogorov-Smirnov test. Pearson’s chi-square test or Fisher’s exact test was used to compare categorical data, when appropriate (wound classification, intraoperative need for FFP PLTs, or vasoactive drugs). The t test or Mann-Whitney U test was also used to compare groups when data were normally (intraoperative Hb, hematocrit [Hct], pH, PaCO₂, HCO₃⁻, crystalloid, and lactate levels) or nonnormally distributed (intraoperative base excess, PaO₂/FIO₂, and colloids), respectively. Prespecified subgroup analyses for site of infections and site of surgery were also performed. Standardized differences were used to compare the randomized groups in ITT analysis on baseline variables. A covariate was considered imbalanced when the standardized differences were more than 0.28. Measures of effect of primary and secondary outcomes are presented as relative risk (RR) with 95% confidence interval (CI). A two-sided p value of less than 0.05 was considered significant. Data were analyzed using computer software (Version 19.0, SPSS, IBM Corp.).

**Sample size calculation**

We estimated that a total sample of 182 patients would be required to have 80% power and a Type I error rate of 5%.
(two-sided alpha) to detect an absolute 13% difference in the occurrence of the primary outcome between old blood and fresh blood groups; the rate of postoperative infections among patients receiving old blood was considered to be 18%. With an anticipated rate of loss to follow-up of 10%, the total number of patients to be randomized was 200.

RESULTS
Among patients undergoing elective surgery over the study period, 275 patients required transfusions and were assessed for eligibility. Finally, 199 met the inclusion criteria and were included in the study (Fig. 1). The adherence to the transfusion protocol was 93% for all RBCs transfused, with 100% of patients in the fresh blood group receiving only RBCs less than 14 days old and 83% of patients in the old blood group. The median [IQR] storage times of RBCs were 6 [5-10] days in the fresh blood group and 15 [11-20] days in the old blood group. According to the AT analysis, 118 patients received fresh blood and 81 old blood. In the AT analysis, the median [IQR] storage times of RBCs received were 6 [5-10] days in the fresh blood group and 18 [14-22] days in the old blood group. No significant differences at baseline were detected in demographics and clinical characteristics of the population for both ITT and AT analyses (Table 1). The numbers of RBC units or other blood components transfused during surgery are comparable between the treatment groups (Table 2). Postoperative laboratory variables are presented in Table S3 (available as supporting information in the online version of this paper).

Primary outcome
The total number of infections, as well as the site and the causative microorganisms, are summarized in Table 3. In the ITT analysis, the rate of infection was 22 of 101 [22%] in the fresh blood group versus 25 of 98 [25%] in the old
blood group (RR, 1.17; CI, 0.71-1.93; Figs. 2 and 3). Among the different subgroups of infections, wound infections occurred more frequently in the old blood than in the fresh blood group (15/98 [15%] vs. 5/101 [5%]; RR, 3.09; CI, 1.17-8.18).

There were no significant differences between groups in the occurrence of infection in specific surgical site. In the AT analysis, the rate of infection was 23 of 118 (19%) in the fresh blood group versus 24 of 81 (29%) in the old blood group (RR, 1.52; CI, 0.92-2.5); as in the ITT analysis, in the AT analysis wound infections also developed more frequently in the old blood than in the fresh blood group (14/81 [17%] vs. 6/118 [5%]; RR, 3.4; CI, 1.36-8.4).

Secondary outcomes
All-cause mortality rates, both in hospital and at 90 days, did not differ between groups in the ITT and AT analyses (Fig. 4). Patients in the old blood group received more postoperative RBC transfusions (Table 4). Postoperative AKI by KIDGO was detected more frequently in the old blood group, both in the ITT (6% vs. 24%; RR, 4.12; CI, 1.76-9.65) and in the AT analyses (6% vs. 28%; RR, 4.79; CI, 2.16-10.6; Table 4). There was no difference found for need of RRT between groups. There were no other differences in the secondary outcomes between groups in the ITT analysis. In the AT analysis, hospital LOS was longer in the old blood group than in the fresh blood group (11 [7-20] vs. 9 [6-16]; difference in means, –3.62; CI, –7.6 to –0.6; Table 4).

Subgroup analysis
The rate of infection in patients receiving only RBCs stored less than 7 days was seven of 49 (14%), while in patients receiving only RBCs more than 21 days old, the rate was 14 of 37 (38%; p = 0.02). Among the different subgroups of infection, only wound infections were more frequent in patients receiving RBCs more than 21 days old.

### TABLE 1. Demographics and clinical characteristics of the study groups at baseline*

| Variables                        | ITT analysis | AT analysis |
|----------------------------------|--------------|-------------|
|                                  | Fresh blood  | Old blood   | Standardized differences | Fresh blood  | Old blood   |
|                                  | (n = 101)    | (n = 98)    |                  | (n = 118)    | (n = 81)    |
| Patient characteristics          |              |             |                  |              |             |
| Age (years)                      | 72 ± 24      | 69 ± 18     | 0.14             | 71 ± 22      | 69 ± 26     |
| Female                           | 50 (49)      | 48 (49)     | 0.01             | 60 (51)      | 38 (42)     |
| BMI (kg/m²)                      | 23 ± 6       | 23 ± 4      | -0.10            | 23 ± 7       | 23 ± 8      |
| Blood group                      |              |             |                  |              |             |
| O                                | 42 (42)      | 43 (44)     |                  | 47 (40)      | 38 (47)     |
| A                                | 50 (49)      | 37 (38)     |                  | 58 (49)      | 29 (36)     |
| B                                | 6 (6)        | 12 (12)     |                  | 8 (7)        | 10 (12)     |
| AB                               | 3 (3)        | 5 (5)       |                  | 4 (4)        | 4 (5)       |
| ASA score                        |              |             | 0.15             |              |             |
| I                                | 2 (2)        | 3 (3)       |                  | 3 (3)        | 2 (2)       |
| II                               | 20 (20)      | 20 (20)     |                  | 26 (22)      | 14 (17)     |
| III                              | 64 (64)      | 67 (68)     |                  | 72 (61)      | 59 (73)     |
| IV                               | 15 (15)      | 8 (8)       |                  | 17 (14)      | 6 (7)       |
| Comorbidities                    |              |             |                  |              |             |
| Hypertension                     | 34 (34)      | 33 (34)     | 0.01             | 41 (35)      | 26 (32)     |
| Chronic kidney disease           | 15 (15)      | 14 (14)     | -0.01            | 17 (14)      | 12 (15)     |
| Peripheral vascular disease      | 8 (8)        | 14 (14)     | 0.20             | 10 (8)       | 12 (15)     |
| Diabetes                         | 38 (38)      | 26 (27)     | -0.23            | 41 (35)      | 23 (28)     |
| Anemia                           | 59 (58)      | 64 (65)     | 0.14             | 70 (59)      | 53 (65)     |
| Preoperative laboratory values   |              |             |                  |              |             |
| Hb (g/dL)                        | 11.4 ± 1.6   | 11.2 ± 1.8  | 0.11             | 11.3 ± 1.6   | 11.2 ± 1.8  |
| PLTs (×10⁹/L)                    | 244 [173-307]| 223 [177-223]| 0.06             | 244 [174-297]| 224 [177-288]|
| INR                              | 1.1 ± 0.3    | 1.2 ± 0.4   | -0.28            | 1.1 ± 0.4    | 1.2 ± 0.4   |
| Creatinine (mg/dL)               | 8 ± 3        | 8.3 ± 3.2   | -0.09            | 8 ± 3.5      | 8.4 ± 3.2   |
| Bilirubin (mg/dL)                | 0.5 [0.3-0.7]| 0.97 ± 0.6  | 0.13             | 1.01 ± 0.6   | 0.97 ± 0.5  |
| Glyceria (mg/dL)                 | 119 ± 21     | 120 ± 20    | -0.04            | 120 ± 27     | 119 ± 27    |
| Sodium (mmol/L)                  | 139 ± 3      | 139 ± 4     | 0                | 139 ± 4      | 139 ± 4     |
| Potassium (mmol/L)               | 4.3 ± 0.5    | 4.2 ± 0.5   | 0.2              | 4.3 ± 0.5    | 4.2 ± 0.5   |
| Type of surgery                  |              |             | -0.06            |              |             |
| Abdominal                        | 47 (47)      | 43 (44)     |                  | 54 (46)      | 36 (46)     |
| Orthopedic                       | 31 (31)      | 25 (26)     |                  | 35 (30)      | 21 (26)     |
| Vascular                         | 14 (14)      | 25 (26)     |                  | 19 (16)      | 20 (23)     |
| Urology                          | 9 (9)        | 5 (5)       |                  | 10 (8)       | 4 (5)       |

*Data are expressed as mean ± SD, number (%), or median [IQR]. Standardized differences are calculated as the difference in means or proportions divided by the pooled SD.

ASA = American Society of Anesthesiologists; BMI = body mass index; INR = international normalized ratio.
**TABLE 2. Intraoperative clinical characteristics***

|               | Fresh blood (n = 101) | Old blood (n = 98) | p value | Fresh blood (n = 118) | Old blood (n = 81) | p value |
|---------------|-----------------------|-------------------|---------|-----------------------|-------------------|---------|
|               |                       |                   |         |                       |                   |         |
| **Transfused blood components** |                       |                   |         |                       |                   |         |
| RBC units per patient | 2 [2-3]               | 2 [2-4]           | 0.721   | 2 [2-3]               | 2 [2-4]           | 0.661   |
| RBC storage (days) | 6 [5-10]              | 15 [11-20]        | <0.001  | 6 [5-10]              | 18 [14-22]        | <0.001  |
| FFP, number of patients (%) | 11 (11)              | 13 (13)           | 0.666   | 13 (11)               | 11 (14)           | 0.659   |
| PLTs, number of patients (%) | 0 (0)                | 1 (1)             | 0.489   | 0 (0)                 | 1 (1)             | 0.407   |
| **Intraoperative variables** |                       |                   |         |                       |                   |         |
| Lowest Hb (g/dL) | 8.4 ± 1.2             | 8.5 ± 1.0         | 0.909   | 8.4 ± 1.2             | 8.5 ± 1.0         | 0.921   |
| Hct (%)        | 24 ± 3                | 25 ± 3            | 0.862   | 24 ± 3                | 25 ± 3            | 0.902   |
| pH             | 7.35 ± 0.07           | 7.36 ± 0.06       | 0.710   | 7.36 ± 0.05           | 7.36 ± 0.06       | 0.709   |
| PaCO2 (kPa)    | 5.7 ± 0.9             | 5.7 ± 0.9         | 0.951   | 5.7 ± 0.9             | 5.7 ± 0.8         | 0.977   |
| HCO3 (mmol/L)  | 3 ± 2                 | 2 ± 2             | 0.607   | 3 ± 2                 | 2 ± 2             | 0.599   |
| PaO2/FiO2      | 39.3 [20.6-50.4]      | 39.2 [24.9-58.9]  |         | 38.3 [16.2-52.5]      | 42.1 [27.3-56.9]  |         |
| Crystallloid (mL) | 2300 ± 1100         | 2500 ± 1100       | 0.688   | 2240 ± 1000           | 2500 ± 1100       | 0.701   |
| Colloid (mL)   | 500 [0-500]           | 500 [0-500]       | 0.412   | 500 [0-500]           | 500 [0-500]       | 0.413   |
| Lactate (mmol/L) | 1.6 ± 0.4           | 1.6 ± 0.7         | 0.413   | 1.6 ± 0.5             | 1.5 ± 0.7         | 0.531   |
| Need for vasoactive drugs | 24 (24%)           | 22 (22%)          | 0.861   | 25 (21%)              | 21 (26%)          | 0.487   |
| Wound classification |                   |                   |         |                       |                   |         |
| Clean          | 75 (74)               | 70 (71)           | 0.772   | 86 (69)               | 59 (73)           | 0.995   |
| Clean-contaminated | 23 (23)            | 27 (28)           | 0.567   | 29 (25)               | 21 (26)           | 0.961   |
| Contaminated   | 3 (3)                 | 1 (1)             | 0.621   | 3 (3)                 | 1 (1)             | 0.647   |
| Dirty          | 0 (0)                 | 0 (0)             | 0.607   | 0 (0)                 | 0 (0)             | 0.607   |

* Data are expressed as median [IQRs], number (%), or mean ± SD, unless otherwise specified.

**TABLE 3. Postoperative infections and causative organisms**

| Variable                              | Fresh blood (n = 101) | Old blood (n = 98) | RR (CI) | Fresh blood (n = 118) | Old blood (n = 81) | RR (CI) |
|---------------------------------------|-----------------------|-------------------|---------|-----------------------|-------------------|---------|
| At least one infection, n (%)         | 22 (22)               | 25 (25)           | 1.17 (0.71-1.93) | 23 (19)               | 24 (29)           | 1.52 (0.92-2.5) |
| Sepsis, n (%)                         | 6 (6)                 | 8 (8)             | 1.37 (0.49-3.81) | 6 (6)                 | 8 (10)            | 1.94 (0.70-5.39) |
| *Staphylococcus aureus*               | 2                     | 2                 | 2        | 2                     | 2                 | 2       |
| *Streptococcus pyogenes*              | 1                     | 2                 | 1        | 1                     | 1                 | 1       |
| *Escherichia coli*                    | 2                     | 2                 | 2        | 2                     | 2                 | 2       |
| Other                                 | 1                     | 2                 | 1        | 1                     | 2                 | 2       |
| Pulmonary infections, n (%)           | 7 (7)                 | 3 (3)             | 0.44 (0.11-1.66) | 7 (7)                 | 3 (4)             | 0.62 (0.16-2.34) |
| *Streptococcus pneumoniae*            | 1                     | 1                 | 1        | 1                     | 1                 | 1       |
| *Pseudomonas aeruginosa*              | 1                     |                   | 1        |                       |                   |         |
| Undetected                            | 5                     | 5                 | 5        | 5                     | 5                 | 5       |
| Wound infections, n (%)               | 5 (5)                 | 15 (15)           | 3.09 (1.16-8.18) | 6 (5)                 | 14 (17)           | 3.4 (1.36-8.4) |
| Superficial SSI                       | 4 (75)                | 12 (80)           | 1.00 (0.37-2.70) | 5 (83)                | 11 (78)           | 1.00 (0.37-2.70) |
| Deep SSI                              | 1 (25)                | 3 (20)            | 1.00 (0.37-2.70) | 1 (17)                | 3 (21)            | 1.00 (0.37-2.70) |
| *Streptococcus pyogenes*              | 2                     | 5                 | 2        | 2                     | 2                 | 2       |
| *Escherichia coli*                    | 2                     | 4                 | 1        | 1                     | 1                 | 1       |
| *Staphylococcus hominis*              | 2                     | 1                 | 2        | 1                     | 1                 | 1       |
| *Pseudomonas aeruginosa*              | 2                     |                   | 2        |                       |                   |         |
| *Proteus vulgaris*                    | 1                     | 3                 | 1        | 3                     | 1                 | 3       |
| Peritonitis, n (%)                    | 4 (4)                 | 2 (2)             | 0.51 (0.09-2.75) | 4 (3)                 | 2 (3)             | 0.73 (0.13-3.88) |
| *Escherichia coli*                    | 1                     |                   | 1        |                       |                   |         |
| *Enterobacter cloaceae*               | 2                     | 1                 | 2        | 1                     | 1                 | 1       |
| *Pseudomonas aeruginosa*              | 1                     |                   | 1        |                       |                   |         |
| *Staphylococcus epidermidis*          | 1                     |                   | 1        |                       |                   |         |
| Urinary tract infections, n (%)       | 2 (2)                 | 1 (1)             | 0.51 (0.05-5.59) | 2 (2)                 | 1 (1)             | 0.73 (0.07-7.90) |
| *Escherichia coli*                    | 1                     |                   | 1        |                       |                   |         |
| *Pseudomonas aeruginosa*              | 1                     |                   | 1        |                       |                   |         |

SSI = surgical site infection.
(3/49 vs. 9/37; p = 0.03). Also, the rate of AKI was significantly higher when RBCs more than 21 days old were given (3/49 vs. 12/37; p = 0.003).

**DISCUSSION**

The main finding of this randomized trial is that the risk of developing postoperative infections was not different in patients receiving either fresh (stored for less than 14 days) or old (stored for more than 14 days) nonleukoreduced RBCs during elective noncardiac surgery (RR, 1.17; CI, 0.71-1.93). However, we detected a higher frequency of wound infections in the old blood group (RR, 3.1; CI, 1.2-8.2), which remained significant in the AT analysis. Among secondary outcomes, AKI was more frequent in the old blood group, in both ITT and AT analysis (Table 4).

During storage, RBCs undergo functional changes collectively known as the RBC storage lesion. These changes involve the accumulation of biologic response modifiers including cytokines such as monocyte chemo-attractant protein-1, RANTES, cell-free Hb, and RBC microparticles. Accordingly, washed blood components have been shown to reduce the inflammatory response in children undergoing cardiac surgery. The clinical relevance of this change was studied in different patient populations.

Recently, the effects of RBC storage duration have been evaluated in patients undergoing cardiac surgery and the rate of postoperative infections ranged from 4% to 15%. In one study, Koch and colleagues found that in patients receiving RBC transfusions during cardiac surgery, those treated with fresh blood (stored for ≤14 days) had a reduced occurrence of sepsis (2.8% vs. 4.0%, p = 0.01) and renal failure (1.6% vs. 2.7%, p = 0.003) than those receiving old blood, although differences in the baseline characteristics between the two groups could affect the ability to interpret the results. A higher rate of postoperative infections when RBCs older than 25 days were transfused was also detected in a trial focused on pediatric cardiac surgery. In contrast, two other studies found that a storage age of RBC transfusion up to 21 to 35 days was not correlated with increased postoperative adverse events in patients undergoing cardiac surgery.

Interestingly, the cutoff used to define fresh or old blood ranged from 14 to 25 days, resulting in high heterogeneity between studies and, consequently, a difficult comparison of results.

In noncardiac patients, such as cases of elective colorectal cancer surgery, two studies with different design investigated the effects of RBC storage on postoperative infections generating conflicting results. In the first study, infections occurred more frequently in patients receiving old blood (stored for more than 21 days) when compared to fresh blood (stored for <21 days; 46% vs. 32%). Nevertheless, the same authors showed in a second study that infection rates were similar in patients transfused with old blood (stored >21 days) and fresh blood (stored <21 days; 34% vs. 29%). However, the influence of old blood transfusion on outcomes of patients undergoing noncardiac surgery needs further investigation.

![Fig. 2. Occurrence of the primary outcome (at least one site of infection within 28 days after surgery) in the ITT and in the AT analysis. (■) Fresh blood group; (■) old blood group.](image)

![Fig. 3. Forest plot of RR and CIs related to the primary outcome.](image)
noncardiac surgery has not been defined in prospective randomized studies.

In this setting, the amount of transfused blood has been related with poor outcomes. In our study, a median of 2 RBC units were transfused during surgery. However, the groups were balanced for RBC units received intraoperatively so that our findings seem not to be dependent on the number of RBCs transfused (Table 2).

Another important issue is that we used nonleukoreduced RBCs. Leukoreduction helps to decrease the negative immunosuppressive consequence of blood transfusion. However, there are conflicting data in the literature regarding the occurrence of postoperative infections when the use of leukoreduced RBCs is compared with use of nonleukoreduced ones. In a trial conducted by Tartter and coworkers, patients receiving leukoreduced RBCs during gastrointestinal surgery had lower risk of postoperative infection. By contrast, another study showed no differences in postoperative infections after colorectal cancer surgery between patients receiving leukoreduced versus nonleukoreduced RBCs. Even if a recent review by Cata and colleagues argues that there is no strong evidence that a mitigation of the immunomodulatory effect of RBC transfusion can be ascribed to leukoreduction, three detailed meta-analyses provided the effectiveness of leukoreduction in reducing postoperative infection. Leukoreduction mitigates postoperative infection with a RR reduction of approximately 35%. Currently, leukoreduction has not been adopted worldwide and most transfused RBC units in Europe are not leukoreduced.

An interesting hypothesis is that prestorage leukoreduction may prevent some of the harmful effects of storage. Recently, Phelan and Aldy suggested that leukoreduction might decrease the negative effects of RBC storage on the risk of mortality, infection, and organ failure. In contrast, in an animal study, Callan and colleagues demonstrated that the proinflammatory response secondary to prolonged blood storage was independent of leukoreduction, suggesting that the potential association between storage time and an increased risk of
infection may be linked to the storage lesions per se rather than to the presence of residual white blood cells in the RBC units. Another potential source of confounding is the solution used for RBC storage. In Europe, it is almost exclusively SAGM, while in the United States it is based on other solutions (i.e., AS-1 or AS-3). However, two recent randomized clinical trials on this topic, which used different storage solutions, reached similar conclusions, suggesting only a marginal effect of the type of storage solution on transfusion-related outcomes.6,7

We observed that wound infections occurred more frequently in patients receiving old blood. Transfusions have traditionally been considered a risk factor for wound infections.44 Similar findings were also observed after cardiac11 and colorectal surgery.30 Although the reasons for such findings are not easy to elucidate, some studies have suggested that the reduction in 2,3-diphosphoglycerate (DPG) in transfused RBCs could increase oxygen affinity for Hb, which may limit oxygen release in the peripheral tissues and thus promote bacterial growth in postoperative wounds,45 although 2,3-DPG levels might decline very early in the storage period (1-2 weeks).

Another potential mechanism for an increased infection risk after transfusion of older RBCs is the release of plasma non–transferrin-bound iron after the ingestion of nonviable RBCs by the monocyte-macrophage system.27 This hypothesis has received experimental support in animals;27 however, we did not evaluate iron or transferrin levels in our study cohort and could not draw any conclusion on this issue. An observation, possibly related, is that blood from male donors older than 30 years or female donors older than 45 years exhibits increased susceptibility to hemolysis.46 If confirmed, this would add to the list of potential confounders. However, in our study, RBCs from older donors were equally distributed in both groups (50/101 fresh blood group vs. 50/98 for old blood group, p = 0.88).

Among secondary outcomes, we also detected an increased risk of AKI in patients receiving old blood. RBC transfusions have been associated with an increased risk of perioperative AKI, and the transfusion of older RBCs was suggested as an additional risk factor for AKI in susceptible patients.47 The mechanisms of renal injury caused by prolonged RBC storage are not clear. Many substances released during RBC storage can affect kidney function: histamine may contribute to the damage of glomerular capillaries, while plasma-free Hb may inhibit endothelial nitric oxide release, which can promote vasoconstriction and reduce regional perfusion.48 In an experimental study in guinea pigs, massive transfusion of RBCs stored for more than 28 days induced various adverse effects on renal function. Renal histopathology of animals transfused with old blood showed markers of tubular damage typically associated with tubular dysfunction. Proteomic analyses showed up regulation of Nrf-2–dependent antioxidant gene expression signatures, suggesting enhanced oxidative stress in the kidneys of animals transfused with old blood.49

In humans, the relationship between transfusion of older blood and AKI has been found in a retrospective study.10 On the other hand, in the RECESS trial, Steiner and coworkers6 found no difference in the maximum postoperative decline of renal function between patients receiving fresh or old blood after cardiac surgery. However, these results are not comparable with those from our study as renal function was defined using the multiple organ dysfunction score, which is less sensitive than the KDIGO criteria for detecting early renal dysfunction.20

Regarding the staging of AKI, few patients in our study had severe deterioration in renal function requiring RRT, while most of patients developed KDIGO Stage 1 AKI (60%). This fact suggests that the renal damage was mostly reversible and of limited clinical relevance. However, even patients with Stage 1 AKI usually have higher long-term mortality and prolonged LOS.50 Accordingly, we observed prolonged LOS in the old blood group in the AT analysis.

A higher rate of postoperative transfusion (Table 4) and bilirubin levels was detected in patients receiving old blood (Table S4, available as supporting information in the online version of this paper). Previous studies have reported similar findings.6,19

The interpretation of the results of our study should take into account some limitations. First, it was a single-center study and results may be dependent on local transfusion and surgical practice. It was previously suggested that adverse effects are associated with RBCs stored for more than 35 days,51 but in our hospital, RBCs are stored only up to 35 days. Thus, we were not able to explore the effects of RBCs stored for a longer period. Moreover, only a few patients in our study underwent a second or more surgical interventions. Data concerning those interventions were not analyzed. This particular population of patients would probably deserve a separate study. Second, if the infection rates in the fresh blood group and the old blood group were actually different but to a lesser degree than anticipated when the trial was designed, then our study would have been underpowered. Third, we did not measure serum biomarkers of infection, such as C-reactive protein or procalcitonin and this may have limited the diagnosis of postoperative infections. However, postoperative C-reactive protein levels have a limited role in the diagnosis of infection in this setting and the role of postoperative procalcitonin measurements is also under debate. Fourth, we did not perform specific laboratory tests to assess the presurgical and postoperative immunologic status of patients and could not directly evaluate the immunologic effects of RBC storage in this setting. Finally, 62% of patients in our study met the definition of preoperative anemia, which is higher than in the data reported in
other studies. Preoperative anemia is a relevant risk factor for postoperative mortality. However, this aspect should not have influenced our results since the two experimental groups did not differ in the preoperative anemia rate (Table 1). Of note, our data probably reflect the high prevalence of thalassemia minor in our province (7%-19%).

The strengths of this trial included the enrollment of a large spectrum of noncardiac surgery patients, ensuring wide applicability of our results; moreover, the bias was reduced by the high adherence to the transfusion protocol and the statistical design including an AT analysis. In conclusion, our study suggests that patients undergoing elective noncardiac surgery who receive nonleukoreduced RBC units stored for 14 days or longer might be at greater risk of developing postoperative wound infection and AKI.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s website.

**Table S1.** Recommendations for surgical antimicrobial prophylaxis (Adapted from ASHP report antimicrobial prophylaxis).

**Table S2.** Recommended doses and re-dosing intervals for commonly used antimicrobials for surgical prophylaxis (Adapted from ASHP report antimicrobial prophylaxis).

**Table S3.** Laboratory variables on the first day after surgery. ITT = intention-to-treat; INR = international normalized ratio; WBC = white blood cell.

**Table S4.** Laboratory variables on the second day after surgery. ITT = intention-to-treat; INR = international normalized ratio; WBC = white blood cell.