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Influence of Erythrocyte Concentrate Storage Time on Postsurgical Morbidity in Cardiac Surgery Patients

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Background: **The transfusion of erythrocytes that have been stored for long periods of time can produce visceral ischemia and favor the acquisition of postsurgical infections. To estimate the role of the duration of storage of erythrocytes on morbidity in cardiac surgery, we performed an observational study.**

Methods: **All patients (n = 897) undergoing cardiac surgery during three consecutive years were included. Morbidity (main outcome measure) was evaluated by means of four surrogate measures: duration of stay in the intensive care unit longer than 4 days, mechanical ventilation time longer than 1 day, perioperative myocardial infarction rate, and severe postoperative infection rate. The mean duration of storage of all erythrocytes transfused and the duration of storage of the oldest unit transfused were used as storage variables.**

Results: **After considering multiple confounding variables related to patient severity, illness, and surgical difficulty, the duration of storage of erythrocytes was found to be associated neither with a more prolonged stay in the intensive care unit or mechanical ventilation time nor with increased rates of perioperative infarction, mediastinitis, or sepsis. However, each day of storage of the oldest unit was associated with an increment of the risk of pneumonia of 6% (95% confidence interval, 1–11; *P* = 0.018). The cutoff point of maximum sensitivity and specificity (54.8 and 66.9%) associated with a greater risk for pneumonia corresponded to 28 days of storage for the oldest unit (odds ratio, 2.74; 95% confidence interval, 1.18–6.36; *P* = 0.019).**

Conclusions: **Prolonged storage of erythrocytes does not increase morbidity in cardiac surgery. However, storage for longer than 28 days could be a risk factor for the acquisition of nosocomial pneumonia.**

CARDIAC surgical procedures consume a large proportion of the blood supply. The maximum duration of storage of erythrocyte units is 42 days,[1] so these patients may receive blood that has been stored for long periods of time.

During storage, erythrocytes undergo corpuscular changes, including depletion of 2,3-diphosphoglycerate,[2] increased rigidity,[3] and progressive depletion of nitric oxide.[4] The depletion of 2,3-diphosphoglycerate may result in a significant leftward shift in the oxyhemoglobin curve and may have an adverse clinical consequence on oxygen delivery in patients whose balance is precarious. Marik and Sibbald,[5] in a study on septic patients receiving mechanical ventilation, found that patients receiving "old" transfused blood (stored > 15 days) developed splanchnic ischemia. Prolonged duration of storage can increase the rate of postinjury multiple organ failure,[6] mortality,[7] and especially postoperative infection rate. The transfusion of stored blood can cause alterations in the recipient's immune system, leading to a state of immunosuppression[8] called allogeneic blood transfusion–associated immunomodulation (TRIM effect).[9] A recent study reported that the risk of postoperative pneumonia increased by 1% per day of the increase in the mean storage time of transfused erythrocytes.[10] The mechanism(s) of the TRIM effect(s) and the specific constituent(s) of allogeneic blood that mediates the TRIM effect remain unclear.[8,9] Allogeneic plasma, allogeneic leukocytes, and substance that accumulate in blood components during storage have been implicated in the pathogenesis of TRIM. However, the immunosuppressive effects of allogeneic transfusion still remain as a controversial subject since the association between transfusion of old erythrocytes and increased morbidity has not been corroborated by other studies.[11,12] In fact, the implementation of universal prestorage leukodepletion of erythrocyte units in France has not diminished the rate of postoperative infections.[13]

In spite of the high number of patients receiving transfusions in cardiac surgery, the influence of duration of storage of erythrocyte units on postoperative morbidity has not been widely studied, and the need for further research has been recognized.[9] Our working hypothesis was that the duration of storage of erythrocytes has a negative effect on the outcome of cardiac surgery patients. We therefore designed this study to analyze whether the duration of storage of erythrocyte units had a deleterious effect on postoperative morbidity in cardiac surgery patients.

Materials and Methods

Study Population

This study was performed between January 1, 1998 and December 31, 2000 in the public, 2,000-bed University Hospital "Virgen del Rocío" in Seville, Spain. All patients (n = 897) undergoing cardiac surgery were admitted to the postoperative intensive care unit (ICU)

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and were initially included. Cardiac surgery performed in this center includes coronary artery bypass grafting, valve replacement, correction of congenital cardiopathies in children and adults, and cardiac transplantation. The institutional review board committee approved this study and waived the need for informed consent.

Patients with any of the following criteria before surgery were excluded: age less than 16 years (n = 10), hemoglobin less than 110 g/l and/or coagulation disorder (n = 12), previous history of transfusions (n = 15), and presence of fever or any symptom of infection during the patient's stay in the hospital (n = 10). Patients who died during the first 48 h of their ICU stay or remained in the ICU for less than 48 h (n = 40) were also excluded. The reasons for this were, first, because they conceptually could not acquire a nosocomial infection, and second, because those factors related to early death might act as strong confounding variables on the association between storage time and morbidity. Patients who received transfusions with autologous blood were also excluded (n = 15). A total of 795 patients were eligible.

Study Design

This was a single-center, cohort study. All the data were collected in a prospective fashion. All of the variables related to the aim of the study were carefully collected and then introduced into a database, previously designed according to the aim of the study, prior to the patients' discharge from the postoperative care unit. All cardiac procedures were performed by the same staff members (five cardiovascular surgeons, five anesthesiologists, and three intensive care specialists) throughout the entire study period, with no differences in the results of the five surgeons. First-generation cephalosporins were used for antibiotic prophylaxis just before the onset of surgical intervention and were discontinued after 24 h in the ICU. Gastric protection was performed routinely with famotidine (20 mg/12 h intravenously) during the first 24 h of admission to the ICU, and, if a patient required further treatment, famotidine was replaced by sucralfate (1 g orally or through nasogastric tube every 8 h).

The objective of the study was to investigate the influence of duration of storage of erythrocytes on postsurgical morbidity, considering also the variables that can affect the relation between duration of storage and morbidity. Three sets of variables were considered: those related to duration of storage, outcome measure (morbidity), and confounding variables.

Storage Time–Related Variables. Two variables were used to study the effects of duration of storage of transfused erythrocytes for each patient in the perioperative period: the mean duration of storage of all erythrocytes transfused and the duration of storage of the oldest unit. Additionally, the duration of storage of the newest unit was introduced as a surrogate measure for polytransfusion.

Perioperative transfusions were performed during the surgical intervention and/or in the postoperative period. All patients who received transfusions received allogeneic packed erythrocytes that were depleted of the buffy coat but without additional leukocyte depletion. In all of the cases, the additive solution consisted of saline, adenine, glucose, mannitol, and an anticoagulant preservative such as citrate–phosphate–dextrose. All the units were handled in the same way. Some patients also received fresh frozen plasma and/or platelet concentrates. The criteria for transfusion were based on previously published regulations acknowledged by the anesthesiologist, the intensive care specialist, and the surgeons involved in the care of these patients, adapted by our institution.[14]

Outcome Measure (Morbidity). Morbidity was evaluated by the duration of stay in the ICU, the need for prolonged mechanical ventilation time (MVT), development of perioperative myocardial infarction (POMI), and acquisition of severe postoperative infections. Duration of stay in the ICU (in days) has been used as a global surrogate measure of morbidity. Prolonged stay in the ICU was considered when the patient remained for more than 4 days (duration of stay in the ICU > 4 days), which is the usual stay in our ICU. Our hospital's policy is to keep cardiac surgery patients in the ICU for a prolonged period since there are no intermediate units and patients are transferred from the ICU to the general hospital ward. The need for prolonged MVT and POMI rate were used as markers of perioperative pulmonary and cardiac dysfunction, respectively. As in other studies,[10,11] prolonged MVT was considered when it exceeded the first 24 h of the patient's arrival to the ICU (MVT > 1 day). Perioperative myocardial infarction was suspected on the basis of clinical, electrocardiographic, and enzymatic criteria and was confirmed through echocardiography and/or scintigraphy with pyrophosphate. Only severe infections were considered: pneumonia, mediastinitis, and/or sepsis. In our experience, urinary tract infections and catheter-related bloodstream infections had a very low incidence because of our premature removal of bladder and venous catheters. Pneumonia was diagnosed in the presence of new pulmonary infiltrate on chest radiograph, fever, leukocytosis, and positive quantitative culture of secretions obtained in tracheal aspirate of more than 10^6 colony forming units (cfu)/ml and/or protected specimen brush (> 10^3 cfu/ml).[15] Mediastinitis was diagnosed if the surgical wound was infected, requiring reintervention for its treatment, with positive cultures obtained at the sternotomy level.[16] Patients without evidence of pneumonia or mediastinitis but with criteria for sepsis or septic syndrome[17] were classified as patients with sepsis.

Confounding Variables. The following variables were evaluated for their possible confounding effect on the association between duration of storage and postsurgical morbidity:

1. Patient's data: age and sex
2. Chronic health status: defined by the presence of the following variables: New York Heart Association score greater than III, chronic obstructive pulmonary disease (treated with corticosteroids and/or bronchodilators), arterial hypertension (medically treated), obesity (more than 20% greater than ideal weight), and diabetes mellitus (medically treated)
3. Factors related to cardiac function: myocardial infarction prior to surgery, cardiac function before surgery, expressed as a fraction of ejection (evaluated by echocardiography), and either left main vessel or three coronary vessels disease
4. Factors related to the difficulty of surgical intervention: type of surgery (coronary artery bypass grafting, valve replacement or others), emergency or elective surgery, need for intraaortic balloon pump, time of cardiopulmonary bypass, time of ischemia, and intervention performed without cardiopulmonary bypass
5. Factors related to the patient's illness severity in postoperative period: acute physiology, age, and chronic health evaluation (APACHE II) score measured in the first 24 h of the patient's admission to ICU, central nervous system dysfunction, MVT, reintubation, and need for enteral feeding and/or nasogastric tube
6. Factors related to blood transfusion: number of erythrocyte units, fresh frozen plasma and platelet concentrates per patient, and amount of mediastinal bleeding

Statistical Analysis

Variables associated ($P < 0.05$) simultaneously with the main outcome measure (morbidity) and the use of erythrocyte unit transfusion were identified as potential confounding factors of the relation of interest.[9] Variables related to the duration of storage of erythrocyte units were also analyzed in regard to the main outcome (four surrogate measures). Univariate analysis was accomplished by using a two-sample unpaired *t* test for parametric continuous variables after correction for equality of variance (Levene test), Mann–Whitney U rank sum test for nonparametric continuous variables, and Pearson chi-square test or Fisher exact test for categorical variables. Relative risks and their corresponding 95% confidence intervals (95% CIs) were calculated. Statistical significance was considered when *P* was less than 0.05. Those *P* values related to the main outcomes must be cautiously interpreted because of the presence of multiple comparisons.

Multivariate analysis with logistic regression analysis was used to evaluate the independent contribution of the variables. The model was constructed using a forward stepwise method with the likelihood ratio test. Those factors found significant in the univariate analysis were included in the multivariate analysis, with variables associated simultaneously with the main outcome measure and the use of erythrocyte transfusion as potential confounders of the relations of interest. The duration of storage of erythrocyte units was tested for entry in the models after the inclusion of the identified confounding factors. Two multivariate analyses were conducted. In the first analysis, all patients (n = 795) were included. We tested the association between these potential confounding variables and the main outcome measure. Allogeneic transfusion was tested for entry into logistic regression models predicting the occurrence of postoperative morbidity. The second analysis was restricted to patients who had received erythrocytes (n = 585). The duration of storage of the erythrocytes transfused to each patient was tested for association with postoperative morbidity. The presence of colinearity in the model was evaluated using tolerance and variance inflation factor analysis followed by testing the condition indices. No condition index greater than 15 was found, so the variance proportions were not examined. Odds ratios (ORs) and their corresponding 95% CIs for each variable were also calculated. Data were collected and analyzed with the SPSS 10.0 software package (SPSS Inc., Chicago, IL).

Results

Of the 795 patients evaluated, 53.7% underwent valve replacement surgery, 37.5% underwent coronary artery bypass grafting, 4% underwent closure of an atrial septal defect, and 4.8% underwent mixed surgery (coronary artery bypass grafting and valve replacement). Five hundred eighty-five patients (73.6%) received allogeneic transfusions of erythrocytes, 260 (32.7%) received fresh frozen plasma, and 135 (16.9%) received platelet concentrates. Table 1 shows the categorical and continuous variables related to erythrocyte transfusions. Duration of stay in the ICU, mechanical ventilation time, and the rates of infection and perioperative infarction were significantly higher in patients who had undergone transfusions (table 2).

Duration of Stay in Intensive Care Unit Longer Than 4 Days

Significant variables ($P < 0.05$) simultaneously related to transfusion (table 1) and a duration of stay in the ICU of more than 4 days (confounding variables) were age, APACHE II score, MVT, cardiopulmonary bypass time, ischemia time, reintubation, intraaortic balloon pump, and enteral nutrition. These variables were introduced in the logistic regression model together with the number of units transfused (the first multivariate analysis). Each unit increased the risk of ICU duration of stay more than 4 days by an average of 10.2% (95% CI, 5.8–14.7).

Table 1. Categoric and Continuous Variables Associated with Transfusion of Red Blood Cell Concentrates

	No Transfusion (n = 210)	Transfusion (n = 585)	
Categoric Variables	n, %	n, %	*P*
POMI			0.0001
No	202 (96.2)	505 (86.3)	
Yes	8 (3.8)	80 (13.6)	
Reintubation			0.001
No	205 (97.6)	532 (91)	
Yes	5 (2.4)	53 (9)	
Intraaortic balloon pump			0.004
No	206 (98.1)	542 (92.6)	
Yes	4 (1.9)	43 (7.4)	
Left main or three coronary vessels disease			0.0018
No	179 (85.2)	429 (73.3)	
Yes	30 (14.8)	156 (26.7)	
Enteral feeding			0.001
No	208 (99)	550 (94)	
Yes	2 (1)	35 (6)	
CNS dysfunction			0.001
No	204 (97.1)	526 (90)	
Yes	6 (2.9)	59 (10)	
Surgery without CPB			0.021
No	175 (83.3)	523 (89.4)	
Yes	35 (16.7)	62 (10.6)	
Continuous Variables	Mean, SD	Mean, SD	*P*
Age, yr	57.8 (± 12.3)	61.3 (± 12.0)	0.0001
APACHE II score	9.4 (± 3.4)	12.0 (± 4.5)	0.0001
VMT, h	10.0 (± 18.0)	18.4 (± 31.5)	0.0001
Mediastinal bleeding			
24 h, ml	627.8 (± 364.5)	1,296 (±1 235.3)	0.0001
CPB time, min	101.0 (± 32.5)	121.0 (± 43.3)	0.0001
Ischemia time, min	69.3 (± 26.3)	79.4 (± 32.4)	0.001

APACHE II score = acute physiology, age, and chronic health evaluation; CNS = central nervous system; CPB = cardiopulmonary bypass; CPBT = cardiopulmonary bypass time; POMI = perioperative myocardial infarction; VMT = ventilation mechanical time.

A second multivariate analysis was performed exclusively in patients who received transfusions and introduced the variables related to duration of storage that were significant in the preliminary analysis (table 3), showing that duration of storage was not related to a prolonged stay in the ICU (> 4 days; table 4).

Mechanical Ventilation Time Longer Than 24 Hours

The confounding variables were APACHE II score, 24-h mediastinal bleeding, cardiopulmonary bypass time (when performed), ischemia time, POMI, intraaortic balloon pump, and central nervous dysfunction. Each unit increased the risk of MVT more than 1 day by an average of 17.6% (95% CI, 11.7–23.9). However, duration of storage was not associated with mechanical ventilation time longer than 24 h (table 4).

Perioperative Myocardial Infarction

No differences were found between patients with and without POMI with regard to the number of units transfused and those variables evaluating the duration of storage (table 3). Therefore, these variables were not related to the presence of POMI.

Perioperative Severe Infection

When performing internal comparisons, only pneumonia presented significant differences between both groups of patients (7.5 *vs.* 1.4%; RR, 5.27; 95% CI, 1.65–16.78; $P = 0.001$; table 2). Thus, the presentation of variables associated with postoperative infection refers exclusively to variables associated with postoperative pneumonia.

The confounding variables were age, APACHE II score, MVT, 24-h mediastinal bleeding, cardiopulmonary bypass and ischemia times, POMI, reintubation, enteral nutrition, and central nervous dysfunction. The risk of pneumonia increased 11.7% for each unit transfused (95% CI, 7.0–16.5; $P < 0.001$).

A second multivariate analysis was performed exclusively in patients who received transfusions (n = 585). The results of this second multivariate analysis were similar to the first except that the duration of storage of the oldest unit was added as an independent factor of the acquisition of pneumonia. Each day of storage of the oldest unit increased the risk of pneumonia by 6% (95% CI, 1.0–11.0; $P = 0.018$). The cutoff points of maximum sensitivity and specificity (54.8 and 66.9%) to predict the development of pneumonia corresponded to 28 days of storage of the oldest unit (table 5). This

Table 2. Relation between Morbidity and Transfusion

Morbidity	No Transfusion (n = 210), %	Transfusion (n = 585), %	P
Postoperative infection	15 (7.1)	82 (14)	0.009
Pneumonia	3 (1.4)	44 (7.5)	0.001
Mediastinitis	9 (4.3)	21 (3.6)	NS
Sepsis	3 (1.4)	17 (2.9)	NS
POMI	8 (3.8)	80 (13.7)	0.0001
VMT > 1 d	6 (2.9)	76 (12.9)	0.0001
ICU-LOS > 4 d	47 (22.4)	265 (45.3)	0.0001

ICU-LOS = length of stay at intensive care unit; NS = not significant; POMI = perioperative myocardial infarction; VMT = ventilation mechanical time.

continuous variable (storage time of the oldest unit) was converted into a categorical one (storage time of the oldest unit > 28 days) by using this cutoff point and was then reintroduced in the multivariate analysis. We found that the transfusion of blood units with a storage time longer than 28 days was associated with an increased risk of pneumonia (OR, 2.74; 95% CI, 1.18–6.36; P = 0.019), without observing important changes in the coefficients of the rest of the variables. However, neither the mean duration of storage of all erythrocytes transfused to each patient nor that of the newest unit were related to the development of pneumonia.

Discussion

The major finding of this study was that duration of storage of erythrocytes does not increase the duration of stay in the ICU, mechanical ventilation time, the rate of POMI, or the rates of mediastinitis and sepsis. In fact, there are few differences between the multivariate analysis performed with the whole population (n = 795) and that performed only with patients who received transfusions (n = 585), in which variables related to the duration of storage were included (table 4). However, the transfusion of erythrocytes stored for more than 28 days was associated with the development of nosocomial pneumonia.

Duration of storage decreases the erythrocyte's ability to deform[3] and unload oxygen in the peripheral tissues, possibly favoring the degree of visceral ischemia.[5] In addition, leukocytes present in erythrocyte units might have deleterious effects on the recipient's immune system,[8,9,18,19] provoking a state of immunosuppression that favors the development of postoperative infections (TRIM effect). The bioactive substances released by leu-

Table 3. Length of Storage of Red Blood Cell Concentrates and Postsurgical Morbidity

		Length of Storage of Transfused RBCs, d		
Morbidity	No. RBC Units*	All†	Oldest‡	Youngest§
ICU stay > 4 d				
Yes: 265	7.0 ± 5.0¶	16.6 ± 5.8	24.0 ± 9.1¶	11.5 ± 5.8¶
No: 320	4.6 ± 4.2	17.3 ± 7	22.3 ± 9.0	13.5 ± 7.2
VMT ≥ 24 h				
Yes: 74	11.5 ± 7.7¶	16.2 ± 5.3	26.8 ± 9.1¶	9.5 ± 4.9¶
No: 511	4.8 ± 3.5	17.1 ± 6.6	22.6 ± 8.9	13.0 ± 6.8
POMI				
Yes: 80	5.6 ± 5.2	17.0 ± 6.8	23.0 ± 9.0	12.7 ± 7.1
No: 505	5.7 ± 4.7	17.0 ± 6.4	23.1 ± 9.0	12.5 ± 6.6
Postoperative infection				
Yes: 83	7.7 ± 4.7¶	16.5 ± 5.9	25.3 ± 9.5¶	10.5 ± 5.7¶
No: 502	5.3 ± 4.7	17.1 ± 6.6	22.7 ± 9.0	13 ± 6.8
Pneumonia				
Yes: 44	8.8 ± 4.7¶	16.5 ± 5.2	27.7 ± 8.8¶	9.8 ± 4.8¶
No: 541	5.4 ± 4.7	17.0 ± 6.6	22.7 ± 9.0	12.8 ± 6.8
Mediastinitis				
Yes: 21	7.0 ± 5.1	18.2 ± 7.0	24.2 ± 8.6	13.0 ± 7.4
No: 564	5.6 ± 4.8	17.0 ± 6.4	23.1 ± 9.0	12.6 ± 6.6
Sepsis				
Yes: 17	5.8 ± 3.4	14.3 ± 5.3	20.4 ± 11.0	9.0 ± 4.2
No: 568	5.7 ± 4.8	17.0 ± 6.4	23.2 ± 9.0	12.7 ± 6.7

Results are expressed as mean ± SD.

* Mean of all units RBCs transfused to each patient. † Mean length of storage of all RBCs transfused to each patient. ‡ Mean length of storage of the longest-stored RBCs transfused to each patient. § Mean length of storage of the youngest-stored RBCs transfused to each patient. ¶ $P < 0.05$, at least.

ICU = intensive care unit; POMI = perioperative myocardial infarction; RBC = red blood cell concentrate; VMT = ventilation mechanical time.

Table 4. Multivariate Analyses of Relation between Transfusion and Morbidity

Morbidity	OR	95% CI	*P*
ICU-LOS > 4 d (total of sample; n = 795 patients)			
Reintubation	4.52	2.08–9.83	0.0001
CNS dysfunction	3.87	1.82–8.23	0.0001
No. RBC units	1.10	1.06–1.15	0.0001
Age	1.03	1.01–1.04	0.0001
MVT	1.01	1.00–1.02	0.0001
ICU-LOS > 4 d (transfused patients only; n = 585 patients)			
Reintubation	7.77	2.73–19.35	0.0001
CNS dysfunction	3.42	1.47–7.97	0.002
APACHE II	1.07	1.02–1.13	0.004
No. RBC units	1.05	1.00–1.10	0.034
MVT	1.01	1.00–1.01	0.013
MVT > 1 d (total of the sample; n = 795 patients)			
CNS Dysfunction	6.11	3.09–12.09	0.0001
APACHE II	1.24	1.11–1.33	0.0001
No. units	1.17	1.12–1.24	0.0001
MVT > 1 d (Only transfused patients. N: 585 patients)			
CNS Dysfunction	6.15	2.96–12.78	0.0001
APACHE II	1.23	1.14–1.32	0.0001
Number of units	1.20	1.13–1.28	0.0001
POMI (total of sample; n = 795 patients)			
Surgery without CPB	4.20	1.74–10.15	0.002
Mediastinal bleeding	3.73	2.08–6.66	0.0001
APACHE II	1.10	1.05–1.15	0.0001
CPB Time	1.01	1.00–1.01	0.030
POMI (transfused patients only; n = 585 patients)			
Mediastinal bleeding	3.37	1.81–6.27	0.0001
Surgery without CPB	2.22	1.13–4.37	0.026
APACHE II	1.09	1.04–1.15	0.001
Pneumonia (total from sample; n = 795 patients)			
Reintubation	20.85	9.55–45.54	0.0001
CNS Dysfunction	3.83	1.71–8.60	0.001
APACHE II	1.11	1.03–1.20	0.006
MVT	1.02	1.01–1.02	0.0001
Pneumonia (transfused patients only; n = 585 patients)			
Reintubation	19.68	8.43–45.95	0.0001
CNS Dysfunction	4.47	1.88–10.63	0.001
APACHE II	1.11	1.02–1.20	0.011
MVT	1.01	1.00–1.02	0.016
Oldest of RBC unit	1.06	1.01–1.11	0.018

APACHE = acute physiology, age, and chronic health evaluation; CI = confidence interval; CNS = central nervous system; ICU-LOS = length of stay at intensive care unit; MVT = mechanical ventilation time; OR = odds ratio; POMI = perioperative myocardial infarction; RBC = red blood cell concentrate.

kocytes in a time-dependent manner, accumulating in blood components during storage, might be responsible for the TRIM effect. If all of these effects were clinically important, the transfusion of leukocyte-depleted blood or fresh blood components could diminish morbidity. Also, prestorage leukocyte depletion would have advantages on poststorage leukocyte depletion, given that in the first case, fewer bioactive substances would accumulate in the units of erythrocytes.

Table 5. Sensitivity and Specificity of Different Cutoff Points to Predict Pneumonia Using the Variable Days of Storage of the Oldest Unit

Length of Storage	Sensitivity, n (%)	Specificity, n (%)
21 d	31/42 (73.8)	225/531 (42.4)
28 d	23/42 (54.8)	355/531 (66.9)
35 d	11/42 (26.2)	460/531 (86.6)

Duration of Stay in Intensive Care Unit as a Global Surrogate Morbidity Marker

The deleterious effects of storage can be evaluated by duration of stay in the ICU as a surrogate measure of global postoperative morbidity. Given that stored blood can have adverse effects, a randomized controlled trial evaluating the consequences of transfusing blood stored for long periods of time would not be ethically defendable, so the relation between duration of blood storage and duration of ICU stay should be taken from observational studies. This association has recently been evaluated in an observational study of 268 patients undergoing cardiac surgery,[11] after adjusting for the effect of numerous confounding variables related to the severity of patients' illnesses and surgery difficulties. The study was unable to prove an association between storage and hospital stay. In our study, patients with a stay of more than 4 days had a mean duration of storage of the oldest unit that was significantly higher than that of patients with a stay of 4 days or less (table 4; $P = 0.023$). However, this association was not significant in the multivariate analysis (table 4). None of the rest of the variables that measure duration of storage were associated with a prolonged stay in the ICU.

The results of leukocyte depletion on hospital stay in cardiac surgery are contradictory. Two randomized controlled trials have evaluated the relation between leukocyte depletion and hospital stay. Gott *et al.*[20] designed four strategies to minimize the deleterious effects of extracorporeal circulation in 400 patients with high, middle, and low risk before surgery. The transfusion of leukocyte-depleted blood was one of the interventions studied. The stay diminished 1 day ($P = 0.05$) only in low-risk patients, with no benefits being found in patients at higher risk. In a randomized controlled trial by van de Watering *et al.*,[21] there were no differences in duration of stay between patients who received transfusions with blood containing leukocytes and those who received transfusions with leukocyte-depleted blood. Prestorage leukocyte depletion did not have any advantages over poststorage leukocyte depletion. However, mortality was lower in patients who received transfusions with leukocyte-depleted blood when the number of units transfused was more than three.

As in other studies,[22] there is a relation between the number of units of erythrocytes transfused and the duration of stay in the ICU (table 4). Our results suggest that this association is independent of the prolonged storage of erythrocytes.

Mechanical Ventilation for Longer Than 24 Hours and Increase in Perioperative Myocardial Infarction Rate

It is possible that the deleterious effect of storage could be small enough not to be detected through an increase in hospital stay. Therefore, we measured the effect of duration of storage on two organs frequently affected in cardiac surgery, the lungs and the heart, hypothesizing that the deleterious effect of storage would be more evident in these organs. As surrogate measures, we used MVT of more than 1 day and POMI rate.

Our study demonstrates that prolonged mechanical ventilation is associated with the number of units transfused but not with a prolonged duration of storage of the erythrocytes (table 4). Various observational studies with multivariate analysis have shown that the transfusion of allogeneic blood is associated with prolonged mechanical ventilation,[23,24] acting even as a predictor of late extubation.[23] Intraoperative administration of erythrocytes can be the most important independent variable in predicting prolonged MVT and ICU stay.[24] It is possible that allogeneic transfusion may cause other deleterious effects that have not been recognized and may explain the association between number of transfused units and prolonged ventilation (table 4) but not between duration of storage and prolonged ventilation.

The POMI rate was higher in patients who received transfusions (table 2). Spiess *et al.*[25] performed a multicenter study with 2,202 patients undergoing coronary artery bypass grafting. Other authors[26] found that liberal transfusion has adverse consequences on cardiac surgery. Our study does not support an association between blood transfusion and perioperative ischemia (table 4). If this relation exists, our data suggest that it is not caused by duration of storage of erythrocytes (table 3). Our results do not support the hypothesis that prolonged duration of storage of erythrocytes alters cardiorespiratory function in patients undergoing cardiac surgery.

Severe Postoperative Infection

Finally, we studied the relation between duration of storage and severe postoperative infection. Previous studies have documented a close relation between transfusion and postoperative infection in patients undergoing cardiac surgery.[10,27,28] In our hospital, a case–control study showed that the transfusion of 4 or more units of blood components was independently associated with the acquirement of nosocomial pneumonia.[28] This association seems to be exclusive between erythrocytes and pneumonia and not between blood components and other severe infections.[14] Although many researchers have observed an association between transfusion and infection, only the study performed by Vamvakas *et al.*[10] has studied the influence of duration of storage on the development of postoperative infection in cardiac surgery patients. Our study differs from that of Vamvakas *et al.* in that only duration of storage of the oldest unit was a predictor for pneumonia ($P = 0.0001$) but not the mean duration of storage of all the transfused units ($P = 0.566$; table 3). This suggests that only units stored for prolonged periods have the capacity to be associated with pneumonia.

It is possible that more severely ill patients require a higher number of erythrocytes, thus having a higher probability of receiving transfusions with older units. If this is so, the oldest unit would only be a surrogate marker of polytransfusion or illness severity. For this reason, duration of storage of the newest unit was included in the analysis since polytransfused, severely ill patients would also have a high probability of receiving transfusions with newer units. In fact, table 3 clearly indicates that all the morbidities associated with receiving an oldest unit of blood were also associated with receiving a newest unit of blood. However, multivariate analysis showed that only the duration of storage of the oldest unit and not that of the newest one influenced the acquisition of pneumonia, suggesting that prolonged storage may be an independent factor for acquiring nosocomial pneumonia. Unfortunately, in multivariate analysis of a single study population, it is nearly impossible to correct for such correlations. Several studies[9,29,30] have shown that bioactive substances released from leukocytes accumulate in a time-dependent manner, and our results suggest that units older than 4 weeks can have a negative effect on the recipient's immune system, which allows the patient to acquire nosocomial pneumonia. On the contrary, recent studies have not demonstrated a decrease in the infection rate when leukocyte depletion was performed in erythrocytes.[13,21]

We must point out that this study has limitations inherent to an observational design in establishing causal relations. Pneumonia is the most frequent nosocomial infection in cardiac surgery,[31] with a high attributable mortality that increases the duration of hospital stay and is associated with prolonged mechanical ventilation.[31–32] However, in this study, duration of storage was associated with pneumonia but not with prolonged mechanical ventilation and/or duration of stay. This contrast could be explained because the influence of storage on pneumonia, although present (table 4), is small. The duration of storage can have negative effects not considered with the surrogate variables of morbidity used in this study. Other multiple factors related to the difficulty of surgery, staff, equipment, manipulation, and duration of stay may have been involuntarily discarded. Finally, the strategy of including only the significant variables in the multivariate analysis carries the risk of excluding some potential confounding relations, but also minimizes unexplainable or spurious associations.

In summary, in this study, we failed to demonstrate an association between increased morbidity and the transfusion of erythrocytes stored for prolonged periods in patients undergoing cardiac surgery. However, we did observe an increase in the rate of pneumonia when erythrocytes had been stored for more than 4 weeks. We recommend continued research into the possible deleterious effects of the transfusion of "old" erythrocytes.

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