Effects of allogeneic leukocytes in blood transfusions during cardiac surgery on inflammatory mediators and postoperative complications*

Yavuz M. Bilgin, MD; Leo M. G. van de Watering, MD, PhD; Michel I. M. Versteegh, MD; Marinus H. J. van Oers, MD, PhD; Anneke Brand, MD, PhD

Objective: **To investigate whether the higher prevalence of postoperative complications in cardiac surgery after transfusion of leukocyte-containing red blood cells can be related to inflammatory mediators.**

Design: **Analysis of inflammatory markers interleukin-6, interleukin-10, interleukin-12, and procalcitonin in patients participating in a randomized trial comparing leukocyte-depleted with leukocyte-containing, buffy-coat-depleted red blood cells.**

Setting: **Two university-affiliated hospitals in the Netherlands.**

Subjects: **A total of 346 patients undergoing cardiac valve surgery with a complete series of pre- and postoperative blood samples.**

Measurements and Main Results: **There were no differences in the cytokines and procalcitonin concentrations between both study arms when the patients arrived in the intensive care unit. In subgroups, patients who received zero to three red blood cell transfusions showed similar cytokine concentrations in both study arms, whereas patients with ≥4 red blood cell transfusions had significantly higher interleukin-6 concentrations in the leukocyte-containing, buffy-coat-depleted red blood cell group. Patients who developed postoperative infections and multiple organ dysfunction syndrome showed, respectively, increased concentrations of interleukin-6 and interleukin-12 in the leukocyte-containing, buffy-coat-depleted, red blood cell group. The interaction tests in these subgroups showed significantly different reaction patterns in the leukocyte-containing, buffy-coat-depleted red blood cell group compared with leukocyte-depleted red blood cell group for interleukin-6 and interleukin-12. Multivariate analysis showed a high interleukin-6 concentration with multiple organ dysfunction syndrome and both high interleukin-6 and interleukin-10 concentrations with hospital mortality.**

Conclusions: **Allogeneic leukocyte-containing blood transfusions compared with leukocyte-depleted blood transfusions induce dose-dependent significantly higher concentrations of proinflammatory mediators in the immediate postoperative period after cardiac surgery. High concentrations of interleukin-6 are strong predictors for development of multiple organ dysfunction syndrome, whereas both interleukin-6 and interleukin-10 are associated with hospital mortality. These findings suggest that leukocyte-containing red blood cells interfere with the balance between postoperative proinflammatory response, which may further affect the development of complications after cardiac surgery. (Crit Care Med 2010; 38:546–552)**

KEY WORDS: blood transfusions; cardiopulmonary bypass; complications; infections; multiple organ dysfunction syndrome; mortality

Cardiac surgery is associated with tissue trauma, ischemia-reperfusion injury, and blood surface contact. These conditions induce systemic effects and release of inflammatory mediators, which are presumed to play a role in the development of postoperative complications, such as systemic inflammatory response syndrome, multiple organ dysfunction syndrome (MODS), and infections (1, 2). Moderate systemic inflammatory response syndrome often develops after cardiac surgery and usually resolves with supportive care. However, severe systemic inflammatory response syndrome can evolve to MODS, which causes higher morbidity and mortality after cardiac surgery (3, 4).

During cardiac surgery, despite blood-saving developments, allogeneic red blood cells (RBCs) are often transfused. Allogeneic RBC transfusions are dose dependent and associated with an increased risk of postoperative infections and mortality after cardiac surgery (5–9). However, it is not clear whether this relationship is causal or what the possible mechanisms could be (10).

In two randomized, controlled trials in patients undergoing cardiac surgery, we found a transfusion dose-dependent increased rate of postoperative infections and mortality (due to MODS) in the patient group receiving buffy-coat-depleted red blood cells (BCD-RBCs) as compared with filtered leukocyte-depleted red blood cells (LD-RBCs) (11, 12). These findings suggest that allogeneic leukocytes in BCD-RBCs may have played a causal role, possibly by enhancement of the inflammatory response after cardiac surgery. In this study, we compare blood samples collected from patients randomized to either BCD-RBCs or to LD-RBCs. The aim of this laboratory analysis is to investigate a possible relationship between the presence of leukocytes in blood transfusions and some inflammatory mediators with postoperative complications, such as morbidity and mortality.

We selected four key mediators that represent the inflammatory response after surgery. The proinflammatory cytokine, interleukin (IL)-6, has been shown to be an early predictor for nonsurviving patients in cardiac surgery and it has been previously reported that intraoper-

***See also p. 720.**

From the Department of Hematology (MHJvO), Academical Medical Center, Amsterdam, Netherlands; Department of Immunohematology and Blood Transfusion (AB), Leiden University Medical Center, Leiden, Netherlands; Department of Cardiothoracic Surgery (MIMV), Leiden University Medical Center, Leiden, Netherlands; and Sanquin Blood bank Southwest region (YMB, LMGvdW, AB), Leiden, Netherlands.

The authors have not disclosed any potential conflicts of interest.

For information regarding this article, E-mail: bilginyavuz@hotmail.com

DOI: 10.1097/CCM.0b013e3181c0de7b

ative blood transfusions in cardiac surgery caused an increase of IL-6 levels (13, 14). IL-10, an anti-inflammatory cytokine, has been found to be increased after perioperative allogeneic blood transfusions in orthopedic surgery and associated with prolonged hospital stay (15). IL-12 reflects activation and proliferation of lymphocytes and natural killer cells, which are relevant for the defense against nosocomial infections (16, 17). Procalcitonin has been shown to be an early marker for sepsis and bacterial infections after major surgery and has been found to be increased on the first postoperative day after cardiac surgery in patients who developed organ dysfunction and severe complications (18).

MATERIALS AND METHODS

Study Design. A randomized, controlled trial was performed in two university hospitals in the Netherlands. After written informed consent was obtained, 474 patients undergoing cardiac valve surgery with or without coronary artery bypass graft were randomized to receive BCD-RBCs or LD-RBCs. The end points of the study were postoperative infections, MODS, and 90-days and in-hospital mortality. The local ethics committees of both hospitals approved the trial protocol. The design and outcome of the study have been described previously (12). To assess preoperative risk of the patients, we applied the score model described by Parsonnet et al (19). We conducted anesthetic and surgical procedures according to the hospital standards. After endotracheal intubation, patients were ventilated to normocapnia with an air-oxygen mixture. Heparin sulfate was administered before the start of cardiopulmonary bypass at a dose of 3 mg/kg and subsequently at doses to maintain the activated clotting time above 400 secs. After arterial and venous cannulation, cardiopulmonary bypass was commenced, using a membrane oxygenator (Baxter, Uden, Netherlands). After termination of cardiopulmonary bypass, heparin was antagonized by protamine sulfate at a 1:1 ratio. A nonpulsatile roller pump was used for all operations. A nonpulsatile flow rate of about 2.4 L/min/m^2 was set to maintain arterial pressure. Patients were cooled to 27°C to 30°C. For myocardial protection, cardioplegia with cold crystalloids was administered. In one hospital, patients considered to be at high risk for bleeding received aprotonin. All patients received prophylactic antibiotics postoperatively for 48 hrs. Postoperatively the patients were monitored in the intensive care unit (ICU) until there was no need for positive inotropes and mechanical ventilation.

Postoperative Complications. Postoperative infections were defined according to the criteria of the Centers of Disease Control and Prevention (20). The following infections were scored: respiratory tract infection (defined as positive sputum culture and pulmonary infiltrate on the radiograph); urinary tract infection (defined as positive urine culture with clinical signs of urine tract infection); wound infection (defined as positive wound culture with clinical symptoms); and bacteremia (defined as positive blood culture and fever).

The development of postoperative organ dysfunction was assessed on the basis of the daily medical records in the ICU, using the model described by Knaus et al (21). The dysfunction of the following organ systems were scored postoperatively: respiratory dysfunction (defined as respiratory frequency $\leq$5 breath/min or $\geq$49 breath/min, or $Paco_2$ $\geq$50 torr [$\geq$6.5 kPa], or $P(A\text{-}a)o_2$ $\geq$349 torr [$\geq$46.5 kPa], or >72 hrs dependency of mechanical ventilation); cardiovascular dysfunction (defined as heart frequency $\leq$54 beat/min or $\geq$150 beat/min, or mean arterial pressure $\leq$49 mm Hg, or serum pH $\leq$7.24 in combination with $Paco_2$ <50 torr [<6.6 kPa], or dependency of positive inotropes); renal dysfunction (defined as urine production $\leq$479 mL/24 hrs or $\leq$159 mL/8 hrs, or creatinine concentration $\geq$3.4 mg/dL, or blood urea nitrogen concentration $\geq$50 mg/dL, or dependency of dialysis); hematologic dysfunction (defined as white blood cell count $\leq 1.0 \times 10^9$/L, or platelet count $\leq 20 \times 10^9$/L, or hematocrit $\leq$0.20); and insufficiency of the central nervous system (defined as Glasgow Coma Score $\leq$6). MODS was defined as the failure of $\geq$2 organ systems. The trial coordinators collected all information on infections, MODS, and hospital mortality from the patient records or electronically from the hospital computer system.

Blood Products. The blood products had been prepared and controlled, according to the procedures of the Dutch blood banks. Within 20 hrs of withdrawal, RBCs were prepared by centrifugation of whole blood at $3000\times g$ for 10 mins. Buffy-coat and plasma were removed from donated blood and BCD-RBCs were reconstituted with 100 mL of saline-adenine-glucose-mannitol. The average leukocyte count ($\pm$standard deviation) in BCD-RBCs was $0.7 \pm 0.4 \times 10^9$ per unit. LD-RBCs were prepared by prestorage filtration of RBCs within 24 hrs after collection of blood. BCD-RBCs were filtered subsequently through a leukocyte filter (Cellselect-Optima, NPBI International-Fresenius HemoCare, Netherlands), resulting in a mean residual leukocyte count in the LD-RBCs of $0.15 \pm 0.02 \times 10^6$ per unit. All platelet concentrates were prestorage leukoreduced by filtration.

Inflammatory Markers. Blood samples were obtained preoperatively, at the end of perfusion, at the time admission to the ICU, and in case of prolonged ICU stay, in total 2 days during ICU stay. Blood samples were immediately centrifuged and aliquoted; the serum sample was stored at −80°C. The concentrations of procalcitonin, IL-6, IL-10, and IL-12 were measured in all patients preoperatively and on arrival in the ICU, and in case of prolonged ICU stay (>2 days), additionally on days 1 and 2 of ICU stay. All concentrations of the mediators were measured in duplicate, using enzyme-linked immunoassay technique (PeliKline Compact, Sanquin Diagnostics, Amsterdam, Netherlands). The lower detection limit was for all cytokines 5.0 pg/mL. Procalcitonin was measured by an immunoluminometric assay (LumiTestPCT, Brahms Diagnostica, Berlin, Germany). The analytic sensitivity of the assay was 0.1 ng/mL.

Statistical Analysis. All patients with at least a preoperative blood sample and a blood sample at the time of arrival in the ICU were analyzed in this study. Descriptive results of continuous variables were expressed as mean $\pm$ standard deviation. Categorical variables were reported as frequency distributions (%). Complications were defined as postoperative infections, MODS, or hospital mortality. For comparison of qualitative parameters, Fisher's exact test or chi-square test was used and, for comparison of quantitative parameters, Student's *t* test or Mann-Whitney *U* test was used. The concentrations of the cytokines were expressed as the median with interquartile ranges (IQR) and were compared between randomization arms and correlated with complications. Tests were performed to search for interaction on cytokine concentrations between the randomization arms and the subpopulations with and without complications. Differences between groups analyzed by the Mann-Whitney *U* test were reported as odds ratios with 95% confidence intervals. Multivariate analysis of the risk factors was performed, using a logistic regression model to estimate the risk factors for each of the postoperative complications. For this purpose, the following variables were considered to be included in the model: gender; type of surgery; Parsonnet score; use of preoperative statins; cardiopulmonary bypass time; use of perioperative aprotonin and corticosteroids; randomization arm; number of RBC transfusions; storage times of transfused RBCs. The risk factors with $p < .20$ from the univariate analysis for the specific complications were entered into the model. The measured concentrations of IL-6, IL-10, IL-12, and procalcitonin on arrival in the ICU were forced into the final model. The multivariate analysis was performed for all postoperative complications separately. For this analysis, the concentrations of the cytokines, the Parsonnet score, and cardiopulmonary bypass time were transformed into quartiles. The storage time was divided into three groups: patients receiving only RBCs stored for <17 days; patients receiving only RBCs stored for $\geq$17 days; and patients receiving RBCs with storage times <17 days and $\geq$17 days. In the final model, the independent risk factors for infections, MODS, and hospital mortality were analyzed. The exponent with odds ratios and 95% confidence intervals are reported. All *p* values are

two-tailed. Because of the hypothesis-generating character of this study, we did not correct for multiple testing. All analyses were performed using SPSS version 15.0 for Windows (SPSS, Chicago, IL).

RESULTS

Patient Characteristics

Of the 474 evaluable patients randomized within the previously described study (12), due to logistic reasons (the majority of which concerned procedures outside office hours), complete serial blood samples could not be collected or processed properly from 128 patients (no pre- or postoperative blood samples could be collected from 106 patients; no pre- and postoperative blood samples were collected from 22 patients). As shown in Table 1, the patient and transfusion characteristics of the 346 included patients were balanced between the BCD-RBC and LD-RBC groups. In the BCD-RBC group, significantly more postoperative infections were found as compared with the LD-RBC group ($p = .02$). In the cohort available for laboratory analysis, the in-hospital mortality was not significantly different between both randomization arms ($p = .19$). The prevalence of MODS was similar in both randomization arms (Table 1). The patient characteristics of the subgroup of 54 patients with prolonged ICU stay were also well balanced between both study arms (Table 1). There were no differences in patient characteristics in the subgroups of patients with postoperative complications between both randomization arms (data not shown).

Inflammatory Markers on ICU Arrival

The preoperative concentrations of all cytokines and procalcitonin levels were all below the detection limits. The concentrations of cytokines and procalcitonin at the time of arrival in the ICU were not associated with patient age, Parsonnet score, use of preoperative statins and perioperative aprotinin, duration of cardiopulmonary bypass and the analysis according to storage times of transfused RBCs. Patients who received corticosteroids perioperatively had lower concentrations of IL-6 at the time ICU arrival than patients who did not (median = 70; IQR = 39–152 vs. median = 118; IQR = 52–245 pg/mL, $p < .001$), whereas the concentrations of IL-10 were higher in the patient group who received corticosteroids (median = 54; IQR = 14–93 pg/mL vs. median = 18; IQR = 6–76 pg/mL, $p < .001$) at the time ICU arrival. The concentrations of procalcitonin were increased at the time of ICU arrival (median = 0.16; IQR = 0.13–0.21 ng/mL). A postoperative increase of the leukocyte count was observed in all patients at the time of their arrival in the ICU arrival (median = 2; IQR = 1.4–3.6 $\times$ 10^9/L).

Table 1. Characteristics of patients

	All Patients			Patients Staying >2 Days in ICU		
	BCD-RBC (n = 171)	LD-RBC (n = 175)	*p* (n = 24)	BCD-RBC (n = 24)	LD-RBC (n = 30)	*p*
Preoperative characteristics						
Age in years (mean ± SD)	67.1 ± 11.8	66.8 ± 13.9	.81	73.3 ± 6.1	71.5 ± 9.5	.42
Valve + CABG, n (%)	60 (35.0)	63 (36.0)	>.90	15 (62.5)	15 (50.0)	.42
Female, n (%)	71 (41.5)	82 (46.8)	.33	10 (41.6)	17 (56.6)	.41
Parsonnet score	13.1 ± 8.3	13.9 ± 8.0	.33	16.5 ± 8.3	15.5 ± 8.2	.66
Use of statins, n (%)	39 (22.8)	46 (26.2)	.46	6 (25.0)	6 (20.0)	.75
Perioperative characteristics						
Corticosteroid use, n (%)	52 (30.4)	55 (31.4)	>.90	10 (41.6)	12 (40.0)	>.90
Aprotonin use, n (%)	79 (46.1)	80 (45.7)	>.90	14 (58.3)	20 (66.6)	.58
Cardiopulmonary bypass, in minutes (mean ± SD)	134 ± 51	139 ± 58	.37	159 ± 67	160 ± 70	>.90
Transfusion characteristics						
RBC transfusions, median (IQR)	3 (2–7)	3 (2–6.5)	.56	7.5 (3.2–9.7)	7 (4–12.2)	.10
Storage time of all transfused RBCs in days, mean ± SD	17.1 ± 5.7	17.0 ± 5.3	>.90	16.7 ± 4.7	15.4 ± 4.6	.35
Number of patients receiving only <17 days stored RBCs, n (%)	58 (33.9)	57 (32.5)	.82	7 (29.1)	12 (40.0)	.56
Number of patients receiving only >17 days stored RBCs, n (%)	63 (36.8)	65 (37.1)	>.90	8 (33.3)	7 (23.3)	.54
Number of patients receiving RBCs stored for <17 days and ≥17 days, n (%)	29 (16.9)	35 (20.0)	.49	8 (33.3)	11 (36.6)	>.90
Number of RBCs, n (%)						
0	21 (12.3)	18 (10.3)	.61	1 (4.2)	0	.44
1–3	66 (38.6)	59 (33.7)	.37	4 (16.7)	8 (26.7)	.52
≥4	84 (49.1)	98 (56.0)	.24	19 (79.2)	22 (73.3)	.75
Postoperative characteristics						
All complications, n (%)	66 (38.6)	55 (31.4)	.18	18 (75.0)	17 (56.6)	.25
Infections, n (%)	51 (29.8)	33 (18.9)	.02	16 (66.7)	11 (36.7)	.05
MODS, n (%)	29 (16.9)	33 (18.9)	.68	9 (37.5)	9 (30)	.58
Hospital mortality, n (%)	14 (8.2)	8 (4.6)	.19	7 (29.2)	2 (6.7)	.06

ICU, intensive care unit; BCD-RBC, Buffy-coat depleted red blood cells; LD-RBC, leukocyte-depleted red blood cells; SD, standard deviation; CABG, coronary artery bypass graft; RBC, red blood cells; IQR, interquartile range; MODS, multiple organ dysfunction syndrome.

Data presented as mean ± SD, n (%) or median with 25th and 75th quartiles (IQR).

Inflammatory Markers and Randomization to Different Blood Products

Between patients randomized to receive BCD-RBC or LD-RBC, no differences were measured at the time of their arrival in the ICU in the concentrations of IL-6, IL-10, IL-12, and procalcitonin. In the subgroup of patients receiving 0 to 3 units of RBCs, the concentrations of cytokines were not different (Table 2). There were 182 (52.6%) patients who had received ≥4 units of RBCs: 84 (49.1%) patients in the BCD-RBC group and 98 (56.0%) patients in the LR-RBC group. In this subgroup, only the concentration of IL-6 was significantly higher in the BCD-RBC group as compared with the LD-RBC group (median = 152; IQR = 74–340 pg/mL vs. median = 96, IQR = 61–249 pg/mL, $p = .02$). The concentrations of IL-10 and IL-12 were at the time of ICU arrival not different in the analysis, according to the type of blood products and the number of RBC transfusions (Table 2).

The concentration of IL-6 at the time of ICU arrival was higher in the BCD-RBC group than in the LD-RBC group, especially in patients who developed postoperative infections. The concentration of IL-12 was higher in patients who developed MODS in the BCD-RBC group. The concentrations of procalcitonin, postoperative increase of the leukocyte count (data not shown), and IL-10 were not different between BCD-RBC and LD-RBC groups, also not in subgroups with or without postoperative complications (Table 2). The interaction tests showed a significant interaction on IL-6 concentrations between the type of blood product and subsequent complications and postoperative infections. The interaction test on IL-12 showed significant interaction between the type of blood products and the MODS subgroups (Table 2).

Table 2. Cytokine concentrations at the time of ICU arrival in patients with and without complications

	BCD-RBC	LD-RBC	*p*	*p* for Interaction
IL-6 (pg/mL)[a]				
All patients (n = 346)	113 (50–250)	85 (45–228)	.07	
Transfusions: 0–3 RBCs (n = 164)	77 (36–167)	72 (37–194)	.64	.14
≥4 RBCs (n = 182)	152 (74–340)	96 (61–249)	.02	
Complications: None (n = 225)	71 (39–152)	72 (40–146)	>.90	.04
Any (n = 121)	198 (98–360)	183 (72–267)	.10	
Infections: No (n = 262)	85 (40–184)	77 (45–212)	.83	.006
Yes (n = 84)	190 (97–390)	99 (52–250)	.03	
MODS: No (n = 284)	97 (47–190)	73 (41–188)	.20	.17
Yes (n = 62)	258 (120–350)	237 (90–278)	.23	
Hospital mortality: No (n = 324)	99 (47–215)	81 (44–217)	.26	.07
Yes (n = 22)	303 (159–381)	206 (73–260)	.06	
IL-10 (pg/mL)[a]				
All patients (n = 346)	25 (6–77)	25 (9–93)	.35	
Transfusions: 0–3 RBCs (n = 164)	18 (6–56)	19 (9–94)	.35	.74
≥4 RBCs (n = 182)	43 (7–84)	33 (9–90)	.75	
Complications: None (n = 225)	25 (6–66)	26 (10–82)	.30	.52
Any (n = 121)	24 (7–91)	22 (7–115)	.74	
Infections: No (n = 262)	26 (7–69)	26 (9–91)	.40	.92
Yes (n = 84)	17 (6–85)	24 (10–110)	.24	
MODS: No (n = 284)	25 (6–74)	25 (10–82)	.29	.68
Yes (n = 62)	19 (9–128)	32 (6–165)	>.90	
Hospital mortality: No (n = 324)	24 (6–77)	24 (9–83)	.34	.71
Yes (n = 22)	64 (10–123)	161 (62–210)	.20	
IL-12 (pg/mL)[a]				
All patients (n = 346)	40 (23–64)	37 (23–58)	.48	
Transfusions: 0–3 RBCs (n = 164)	36 (21–54)	31 (18–50)	.66	.82
≥4 RBCs (n = 182)	44 (26–87)	44 (27–64)	.47	
Complications: None (n = 225)	34 (21–63)	34 (22–51)	>.90	.22
Any (n = 121)	48 (30–75)	45 (25–63)	.29	
Infections: No (n = 262)	37 (21–64)	34 (21–51)	.70	.76
Yes (n = 84)	51 (30–75)	49 (30–64)	>.90	
MODS: No (n = 284)	37 (21–64)	38 (23–58)	.80	.03
Yes (n = 62)	50 (39–88)	36 (24–60)	.02	
Hospital mortality: No (n = 324)	39 (22–64)	37 (23–58)	.56	.65
Yes (n = 22)	55 (44–89)	38 (24–49)	.10	

ICU, intensive care unit; BCD-RBC, buffy-coat-depleted red blood cells; LD-RBC, leukocyte-depleted red blood cells; IL, interleukin; RBCs, red blood cell; MODS, multiple organ dysfunction syndrome.

[a]Data presented as median with 25th and 75th quartiles (interquartile range).

Table 3. Results of univariate analyses of risk factors associated with postoperative complications

	All Postoperative Complications *p*	Infections *p*	MODS *p*	Hospital Mortality *p*
Type of surgery, valve or valve + CABG	.05	.15	.69	.30
Gender, male or female	.91	.58	.49	.14
Preoperative statin use	.85	.85	.94	.47
Parsonnet-score	<.001	.003	.04	<.001
Randomization arm, BCD-RBC or LD-RBC	.10	.01	.64	.16
Perioperative corticosteroid use	.16	.79	.96	.29
Perioperative aprotinin use	.29	.51	.48	.69
Cardiopulmonary bypass time, min	.001	.001	.01	.53
Number of RBC transfusions	<.001	<.001	<.001	<.001
Storage time of transfused RBC units (<17 days, ≥17 days, or both)	.01	.02	.01	.07

MODS, multiple organ dysfunction syndrome; CABG, coronary artery bypass graft; BCD-RBC, buffy-coat-depleted red blood cells; LD-RBC, leukocyte-depleted red blood cells; RBC, red blood cell.

Multivariate Analyses of Risk Factors for All Postoperative Complications

The association between cytokine concentrations on arrival in the ICU and subsequent development of postoperative complications was analyzed separately for each complication (infections, MODS, and hospital-mortality) in a multivariate logistic regression model. Based on the results of the univariate analysis for the postoperative complications (Table 3), the risk factors with a $p < .20$ were included in the multivariate analysis. The multivariate analysis showed that all complications were associated independently with the number of RBC transfusions (Table 4). Furthermore, the concentration of IL-6 was independently associated with the composite of postoperative complications. Postoperative infections were also associated independently with the study arm in favor of LD-RBCs and the cardiopulmonary bypass time. MODS was also associated in-

Table 4. Multivariate analyses of risk factors associated with postoperative complications

	All Postoperative Complications OR (95% CI)	p	Infections OR (95% CI)	p	MODS OR (95% CI)	p	Hospital Mortality OR (95% CI)	p
Parsonnet score	1.18 (0.95–1.46)	.13	1.16 (0.92–1.47)	.20	0.98 (0.76–1.31)	.98	1.70 (1.04–2.78)	.04
Gender							2.09 (0.70–6.22)	.19
Randomization arm	1.46 (0.87–2.49)	.15	2.01 (1.15–3.54)	.01			1.92 (0.67–5.51)	.22
Type of surgery	1.08 (0.60–1.92)	.80	0.86 (0.46–1.60)	.63				
Use of corticosteroids	0.54 (0.29–1.05)	.06						
Cardiopulmonary bypass time	1.25 (0.95–1.64)	.10	1.46 (1.09–1.96)	.01	0.95 (0.69–1.31)	.74		
Number of RBCs	1.22 (1.13–1.31)	<.001	1.14 (1.08–1.21)	<.001	1.26 (1.17–1.36)	<.001	1.12 (1.05–1.19)	.001
Storage time RBCs	0.97 (0.68–1.37)	.84	0.99 (0.68–1.45)	>.90	0.79 (0.49–1.27)	.33	1.05 (0.50–1.93)	>.90
Procalcitonin concentrations	0.28 (0.05–1.57)	.15	0.99 (0.24–4.11)	>.90	0.87 (0.15–5.05)	.87	0.02 (0.01–20.2)	.27
IL-6 concentrations	1.54 (1.21–1.94)	<.001	1.05 (0.79–1.39)	.74	1.87 (1.36–2.57)	<.001	2.18 (1.27–3.71)	.004
IL-10 concentrations	1.14 (0.87–1.50)	.34	0.92 (0.69–1.22)	.54	1.31 (0.93–1.85)	.12	1.70 (1.06–2.73)	.03
IL-12 concentrations	1.03 (0.77–1.37)	.84	1.31 (0.97–1.76)	.07	0.92 (0.63–1.33)	.64	1.17 (0.66–2.10)	.59

OR, odds ratio; CI, confidence interval; MODS, multiple organ dysfunction syndrome; RBCs, red blood cells; IL, interleukin.

dependently with IL-6 concentrations and the hospital mortality was associated independently with the Parsonnet score and with the concentrations of IL-6 and IL-10.

Kinetics of Cytokines in Patients With Prolonged ICU Stay

In this group of 346 patients, 54 patients had a prolonged ICU stay of at least 2 days. From these patients, from the end of perfusion until postoperative day 2, the kinetics of the cytokines IL-6, IL-10 and IL-12 were measured. These patients had a higher age, had longer duration of surgery, received more blood transfusions, and had higher hospital mortality than the whole study group of 346 patients (Table 1). The concentration of IL-6 increased at the end of perfusion and reached its peak on arrival in the ICU, after which it declined. This peak was higher in patients who received BCD-RBC as compared with patients who received LD-RBC transfusions (median = 188; IQR = 111–330 pg/mL vs. median = 104; IQR = 66–193 pg/mL, $p = .02$). The increase of IL-10 preceded the increase of IL-6 and reached immediately after the end of perfusion significantly higher peak concentrations in the LD-RBC group than in the BCD-RBC group (median = 168; IQR = 81–270 pg/mL vs. median = 94; IQR = 19–234 pg/mL, $p = .05$). The IL-10 concentrations had already decreased on arrival in the ICU. IL-12 concentrations only showed a transient rise on the arrival in the ICU and were similar in both study arms (Fig. 1).

DISCUSSION

In a randomized study comprising 474 patients with high-risk heart valve surgery with or without coronary artery bypass graft, we found leukocyte-containing transfusions (BCD-RBC) dose dependently associated with more postoperative infections and higher in-hospital mortality compared with patients receiving LD-RBC transfusions (12). We assumed that inflammatory mediators associated with transfusion of allogeneic leukocytes during cardiac surgery might play a role. We investigated the concentrations of inflammatory cytokines (IL-6, IL-10, and IL-12) and procalcitonin in patients undergoing cardiac valve surgery in relationship with transfusion of BCD-RBCs or LD-RBCs. From 346 patients, pre- and postoperative blood samples were available to investigate cytokine and procalcitonin concentrations after surgery. In the analysis of the total patient population, we found no differences between randomization arms in the concentrations of the selected cytokines IL-6, IL-10, and IL-12 or procalcitonin on arrival in the ICU. However, significantly higher IL-6 concentrations at the time of arrival in the ICU were found in patients after transfusion of ≥4 units of BCD-RBCs compared with LD-RBCs. Higher IL-6 and IL-12 concentrations after leukocyte-containing transfusions were present in patients who developed infections and MODS, respectively. The significant interaction strongly suggests that the type of blood product has played a causal role in the increased cytokine concentrations and the development of postoperative complications. IL-10 and procalcitonin concentrations were not associated with the number and type of transfusions in patients with or without complications. The relevance of the cytokine concentrations for postoperative complications was evaluated in multivariate analyses, confirming the important role of IL-6 concentrations and the number of blood transfusions. This is the first study showing higher proinflammatory markers after leukocyte-containing transfusions in cardiac surgery, in particular in subgroups later developing serious clinical complications.

In several studies, IL-6 concentrations have been found to be increased in association with a proinflammatory response after cardiac surgery (22, 23). In these studies, the possible relationship with allogeneic blood transfusions was not analyzed. Furthermore, other studies found an association between allogeneic blood transfusions and postoperative morbidity and mortality (5–9); however, these studies did not perform laboratory analysis. Only one study reported that blood transfusions contributed to the release of inflammatory markers in cardiac surgery, without investigating the effect of different blood products (14). The purpose of this laboratory analysis was to evaluate a possible relationship between the concentrations of inflammatory markers after cardiac surgery and leukocyte-containing blood products, and to assess whether an association with postoperative complications, such as infections, MODS, and hospital mortality could be found. The interaction between allogeneic blood transfusions and specific postoperative complications has been questioned in the literature (24). Our results suggest that stimulation of a proinflammatory response by allogeneic leukocytes in transfusions enhances susceptibility for complications.

Because the complex cardiac surgery, longer cardiopulmonary bypass time, and

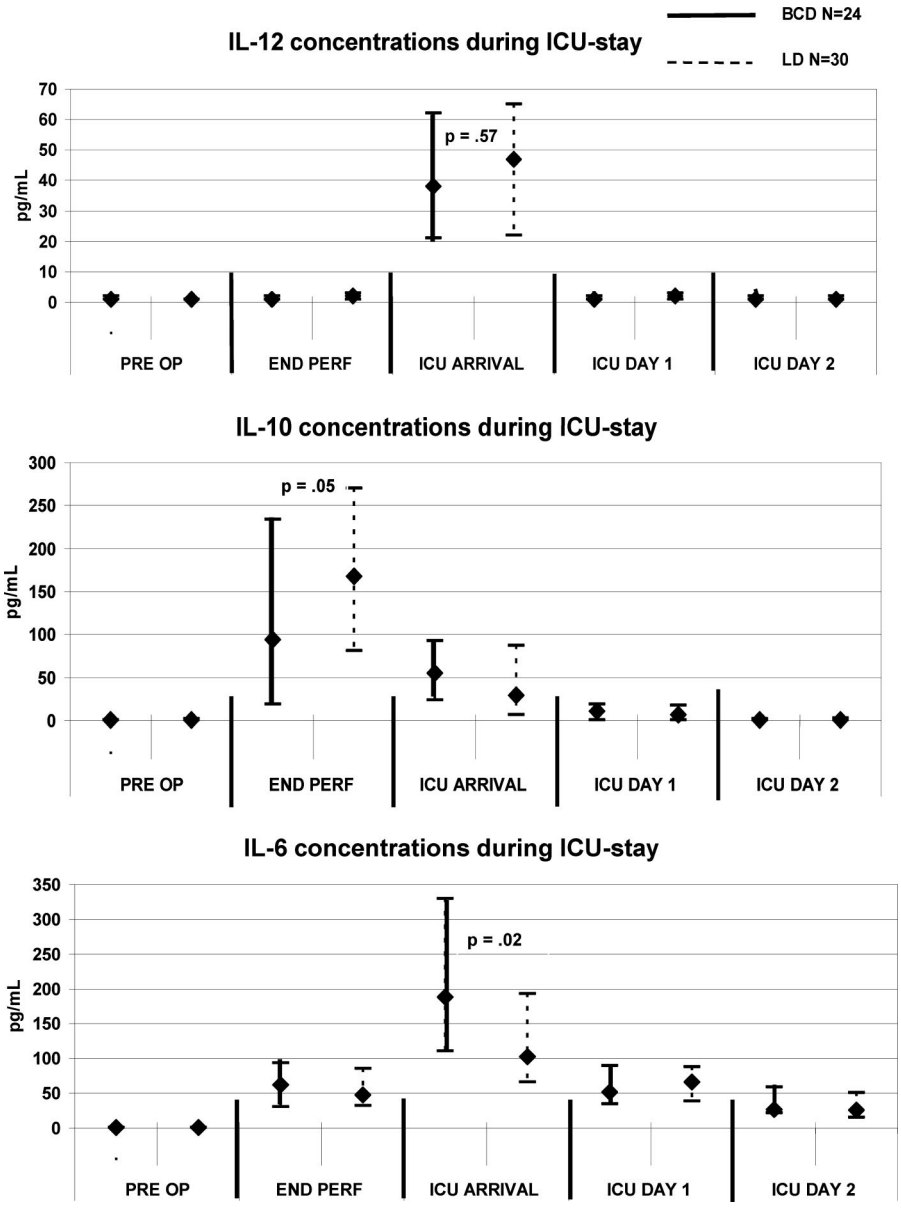

Figure 1. The cytokines interleukin (*IL*)-6, IL-10, and IL-12 concentrations (pg/mL) in patients measured preoperatively (*PRE OP*), at end of perfusion (*END PERF*), arrival in the intensive care unit (*ICU*) (*ICU ARRIVAL*), ICU stay day 1 (*ICU DAY 1*) and day 2 (*ICU DAY 2*) in patients staying >2 days in the ICU. The comparisons are made between buffy-coat-depleted red blood cells (*BCD-RBC*) and leukocyte-depleted red blood cells (*LD-RBC*).

the number of blood transfusions all can influence the cytokine concentrations and adverse outcomes, we performed multivariate analyses to identify the independent risk factors. The multivariate analysis in this patient cohort revealed that, besides the strong effect of the number of blood transfusions on all postoperative complications, an association existed between the type of blood product (BCD-RBC) and the development of postoperative infections. This was in accordance with clinical observations of detailed analysis of causes of deaths observed in two randomized studies (11, 12), which revealed that the higher mortality in patients receiving BCD-RBCs was caused by a combination of infections and MODS. Death due to cardiac complications and noninfectious reasons were comparable in patients after BCD-RBC or LD-RBC transfusions (25).

In contrast to prior reports, we found no differences in procalcitonin concentrations immediately after cardiac surgery predicting complications in patients (26). On the other hand, our study confirms that higher concentrations of IL-6 are associated with complications after cardiac surgery (27, 28) and that higher IL-6 and IL-10 concentrations are related to mortality (29, 30). Furthermore, we observed that the higher the number of blood transfusions, the more outspoken the differences in cytokine concentrations between randomization arms emerged. We found in two randomized trials a transfusion-dose-dependent association with postoperative infections and mortality with leukocyte-containing blood transfusions (11, 12). Our laboratory analysis suggests that the presence of leukocytes in blood transfusions are transfusion-dose-dependent associated with the concentrations of some inflammatory mediators, which could be further related with the development of more postoperative complications in highly transfused patients.

Furthermore, we evaluated the time course of the cytokine concentrations in 54 multiple transfused patients with prolonged ICU stay. In this more heavily transfused (median = 7 units of RBCs) subgroup with a prolonged stay in the ICU (>2 days), on arrival in the ICU, IL-6 levels were higher and IL-10 levels significantly lower in patients who received BCD-RBCs as compared with LD-RBCs. The increased concentrations of IL-6, IL-10, and IL-12 occurred immediately after cardiac surgery and generally decreased within 24 hrs. The anti-inflammatory cytokine IL-10 was the first to increase immediately after perfusion and was higher in the LD-RBC group compared with the BCD-RBC group. In both study arms, the concentration of IL-10 decreased during ICU stay. The increase of inflammatory cytokine IL-6 was observed later and reached higher levels in the BCD-RBC group than in LD-RBC group. These immediate shifts in pro- and anti-inflammatory cytokines seem typical for cardiac surgery and have been reported also in patients with uneventful recovery (13, 29, 30).

Cardiac surgery causes an initially anti-inflammatory response followed by a proinflammatory response, leading to production and release of inflammatory mediators (13, 29, 30). The immediate postoperative balance between these proinflammatory and anti-inflammatory responses determines the clinical course. The higher proinflammatory cytokine IL-6 and lower anti-inflammatory cytokine IL-10 in the BCD-RBC group suggests that leukocyte-containing blood transfusions aggravate this proinflammatory pattern, which is more pronounced

than in the leukocyte-depleted blood transfusions. These findings support that leukocyte-containing allogeneic blood transfusions amplify an inflammatory response in addition to an ongoing systemic inflammatory response induced by cardiac surgery. This transfusion-related immunomodulation could enhance the development of postoperative adverse events, which may result in severe infections and aggravation of MODS. These adverse events could influence recovery and could eventually lead to higher frequency of mortality.

CONCLUSIONS

This laboratory analysis derived from a large patient population undergoing cardiac surgery provides for the first time detailed information about differences between leukocyte-depleted and leukocyte-containing transfusion-related immune responses. The results support that leukocyte-containing transfusions interfere with the balance between immediate postoperative pro- and anti-inflammatory responses toward the proinflammatory direction. The subsequent course of this balance between proinflammatory and anti-inflammatory mediators has not been covered by our data. In addition to blood transfusions, other postoperative factors may influence this balance, leading to susceptibility for postoperative complications. Future studies are needed to investigate the long-term effects of allogeneic blood transfusions in cardiac surgery.

ACKNOWLEDGMENTS

We thank J. Lorinser for his technical assistance and contribution, and Prof. R. Brand for his critical statistical comments.

REFERENCES

1. Levy JH, Tanaka KA: Inflammatory response to cardiopulmonary bypass. *Ann Thorac Surg* 2003; 75:S715–S720
2. Franke A, Lante W, Fackelday V, et al: Proinflammatory cytokines after different kinds of cardio-thoracic surgical procedures: Is what we see what we know? *Eur J Cardiothoracic Surgery* 2005; 28:569–575
3. de Mendonca-Filho HTF, Pereira KC, Fontes M, et al: Circulating inflammatory mediators and organ dysfunction after cardiovascular surgery with cardiopulmonary bypass: A prospective observational study. *Critical Care* 2006; 10:46–53
4. Guidet B, Aergerter P, Gauzit R, et al: Incidence and impact of organ dysfunctions associated with sepsis. *Chest* 2005; 127: 942–951
5. Michalopoulos A, Tzelepis G, Dafni U, et al: Determinants of hospital mortality after coronary artery bypass grafting. *Chest* 1999; 115:1598–1603
6. Chelemer SB, Prato S, Cox PM, et al: Association of bacterial infection and red blood cell transfusion after coronary artery bypass surgery. *Ann Thorac Surg* 2002; 73:138–142
7. Koch CG, Li L, Duncan AI, et al: Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. *Crit Care Med* 2006; 34:1608–1616
8. Murphy GJ, Reeves BC, Rogers CA, et al: Increased mortality, postoperative morbidity and cost after red blood cell transfusion in patients having cardiac surgery. *Circulation* 2007; 116:2544–2552
9. Scott BH, Seifert FC, Grimson R: Blood transfusion is associated with increased resource utilisation morbidity and mortality in cardiac surgery. *Ann Card Anaesth* 2008; 11: 15–19
10. Vamvakas EC, Blajchman MA: Transfusion-related immnomodulation (TRIM): An update. *Blood Reviews* 2007; 21:327–348
11. van de Watering LMG, Hermans J, Houbiers JGA, et al: Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: A randomized clinical trial. *Circulation* 1998; 97:562–568
12. Bilgin YM, van de Watering LMG, Eijsman L, et al: Double-blind, randomized controlled trial on the effect of leukoreduced erythrocyte transfusions in cardiac valve surgery. *Circulation* 2004; 109:2755–2760
13. Sablotzki A, Dehne MG, Friedrich I, et al: Different expression of cytokines in survivors and non-survivors from MODS following cardiovascular surgery. *Eur J Med Res* 2003; 8:71–76
14. Fransen E, Maessen J, Dentener M, et al: Impact of blood transfusions on inflammatory mediator release in patients undergoing cardiac surgery. *Chest* 1999; 116:1233–1239
15. Kirkley SA, Cowles J, Pellegrini VD, et al: Blood transfusion and total joint replacement surgery: T helper 2 (TH2) cytokine secretion and clinical outcome. *Transfus Med* 1998; 8:195–204
16. Ertel W, Keel M, Neidhardt R, et al: Inhibition of the defense system stimulating IL-12 Interferon-gamma pathway during critical illness. *Blood* 1997; 89:1612–1620
17. Kang BY, Kim TS: Targeting cytokines of interleukin-12 family in autoimmunity. *Curr Med Chem* 2006; 13:1149–1156
18. Falcoz PE, Laluc F, Toubin MM, et al: Usefulness of procalcitonin in the early detection of infection after thoracic surgery. *Eur J Cardiothorac Surg* 2005; 27:1074–1078
19. Parsonnet V, Dean D, Bernstein AD: A method of uniform stratification of risk evaluating the results of surgery in acquired adult heart disease. *Circulation* 1989; 79(Suppl I):I3–I12
20. Garner JS, Jarvis WR, Emori TG, et al: CDC definitions for nosocomial infections, 1988. *Am J Infect Control* 1988; 16:128–140
21. Knaus WA, Draper EA, Wagner DP, et al: Prognosis in acute organ system failure. *Ann Surg* 1985; 202:685–693
22. Mitchell JD, Grocott HP, Philips-Bute B, et al: Cytokine secretion after cardiac surgery and its relationship to postoperative fever. *Cytokine* 2007; 38:37–42
23. Tonnesen E, Christensen VB, Toft P: The role of cytokines in cardiac surgery. *Int J Cardiol* 1996; 53(Suppl):S1–S10
24. Vamvakas EC, Blajchman MA: Transfusion-related mortality: the ongoing risks of allogeneic blood transfusion and the available strategies for their prevention. *Blood* 2009; 113:3406–3417
25. Bilgin YM, van de Watering LMG, Eijsman L, et al: Is increased mortality associated with postoperative infections after leukocytes containing red blood cell transfusions in cardiac surgery? *Transfus Med* 2007; 17:304–311
26. Dörge H, Schöndube FA, Dörge P, et al: Procalcitonin is a valuable prognostic marker in cardiac surgery but not specific for infection. *Thorac Cardiovasc Surg* 2003; 51:322–326
27. Hauser GJ, Ben-Ari J, Colvin MP, et al: Interleukin-6 levels in serum and lung lavage fluid of children undergoing open heart surgery correlate with postoperative morbidity. *Intensive Care Med* 1998; 24:481–486
28. Hall RI, Smith MS, Rocker G: The systemic inflammatory response to cardiopulmonary bypass: Pathophysiological, therapeutic and pharmacological considerations. *Anesth Analg* 1997; 85:766–782
29. Sablotzki A, Dehne MG, Welters I, et al: Alterations of the cytokine network in patients undergoing cardiopulmonary bypass. *Perfusion* 1997; 12:393–403
30. Risnes I, Ueland R, Mollness TE, et al: Changes in the cytokine network and complement parameters during open heart surgery. *Interact Cardiovasc Thorac Surg* 2003; 2:19–24