THE INFLUENCE OF LEUKOCYTE FILTRATION DURING CARDIOPULMONARY BYPASS ON POSTOPERATIVE LUNG FUNCTION

A clinical study

The accumulation of activated leukocytes in the pulmonary circulation plays an important role in the pathogenesis of lung dysfunction associated with cardiopulmonary bypass. Animal studies have demonstrated that the elimination of leukocytes from the circulation reduces postoperative lung injury and improves postoperative pulmonary function. We conducted a prospective randomized clinical study to evaluate whether postoperative lung function could be improved by use of a leukocyte filter during cardiopulmonary bypass. Elective coronary artery bypass grafting was done with a leukocyte-depleting arterial blood filter incorporated in the extracorporeal circuit (14 patients, leukocyte filter group) or without the filter (18 patients, control group). Blood samples collected at intervals before, during, and after operation were used for analysis of blood cell counts, elastase concentrations, and arterial blood gases. The use of the leukocyte filter caused no significant reduction in leukocyte count ($p = 0.86$). There were no differences in postoperative lung function between the groups, as assessed through (1) oxygenation index (290 for leukocyte filter group compared with 329 for control group, 95% confidence interval, 286 to 372, $p = 0.21$), (2) pulmonary vascular resistance ($p = 0.10$), and (3) intubation time (16.6 hours for leukocyte filter group versus 15.7 hours for control group, 95% confidence interval, 12.1 to 19.1 hours, $p = 0.72$). The levels of neutrophil elastase were significantly higher at the end of cardiopulmonary bypass in the leukocyte filter group (460 $\mu\text{g/L}$ in leukocyte filter group versus 230 $\mu\text{g/L}$ in control group, 95% confidence interval, 101 to 359 $\mu\text{g/L}$, $p = 0.003$). We conclude that the clinical use of the present form of leukocyte-depleting filter did not improve any of the postoperative lung function parameters analyzed in this study. (J THORAC CARDIOVASC SURG 1995;109:1138-45)

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Pulmonary dysfunction after cardiopulmonary bypass remains an important clinical problem despite refinements in techniques of extracorporeal circulation and improvements in postoperative intensive care.

The pathogenesis of postoperative lung injury consists of several steps. The initial step is a complement activation and release of inflammatory mediators as a result of blood exposure to the surface

of the cardiopulmonary bypass circuit.¹⁻⁵ This causes the up-regulation of leukocyte adhesive receptors CD11b/CD18 (known as Mac-1 or CR3).⁶ Furthermore, the inflammatory cytokines induce the synthesis of ligand intercellular adhesion molecules on the endothelial cells, which leads to enhanced adhesiveness of endothelium for neutrophils.⁷ In the phase of lung reperfusion at the termination of cardiopulmonary bypass, neutrophils adhere to the endothelial surface of the pulmonary circulation creating a microenvironment that permits high concentration of toxic agents (such as elastase and oxygen radicals) released by activated neutrophils.^{8,9}

Although the experimental studies showed that elimination of leukocytes by means of leukocyte depletion could interrupt the sequence of these events and reduce the postoperative pulmonary injury, no clinical data support these observations.¹⁰⁻¹²

The purpose of this clinical study was to deter-

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mine whether mechanical filtration of leukocytes during cardiopulmonary bypass improves the postoperative pulmonary function in human beings.

Patients and methods

Enrollment of patients. We enrolled 32 male patients in the study between September 1992 and March 1993. All patients underwent elective coronary artery bypass grafting. The criteria for eligibility included normal preoperative pulmonary function, absence of chronic or acute pulmonary disease, absence of cardiac valvular disease, absence of congenital heart disease or pulmonary hypertension, and age less than 75 years. There was no significant difference between the groups regarding age (57 ± 8 years for leukocyte-depleted group; 62 ± 6 years for control group, $p = 0.07$) or preoperative heart ejection fraction ($64\% \pm 12\%$ for leukocyte-depleted group; $62\% \pm 8\%$ for control group, $p = 0.63$).

Informed consent was obtained from all patients. The study was approved by the institutional ethical committee.

Study design. We randomly assigned the participating patients to receive a leukocyte filter or to undergo an operation using the standard cardiopulmonary bypass circuit. A randomization scheme with permuted blocks design and additional stratification according to the surgeon was used. Leukocyte filtration during cardiopulmonary bypass was done for 14 patients, and 18 patients comprised the control group.

The patients' pulmonary and hemodynamic parameters were monitored after operation. These determinations included arterial blood pressure, central venous blood pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, body temperature, arterial blood gases, and oxygen saturation of arterial and venous blood. Furthermore, blood cell count analyses and measurements of elastase- α_1 -antiprotease complex were done.

Anesthesia, operation, and conduct of bypass. Identical anesthetic (flunitrazepam, fentanyl, pancuronium) and monitoring techniques (electrocardiogram, central venous and arterial catheter, pulmonary artery catheter, urinary catheter, and temperature probes) were used in both groups of patients. No patient received steroids before or during the operation.

The cardiopulmonary bypass circuit used in all patients was composed of a disposable membrane oxygenator (Dideco, Mirandola, Italy) and Stöckert roller pumps (Stöckert, Munich, Germany) primed with crystalloid solution containing 2 million KIU of aprotinin (Trasylol, Bayer, Leverkusen, Germany). The leukocyte-depleting arterial blood filter for extracorporeal service (Leuko-Guard-6, Pall Biomedical Products Corp., East Hills, N.Y.) was incorporated instead of a standard arterial filter in circuits of 14 patients.

After median sternotomy and heparinization (300 IU/kg body weight, Liquemin, F. Hoffmann-La Roche & Co., Basel, Switzerland), standard cannulation techniques of the ascending aorta and the right atrium were used to complete the cardiopulmonary bypass. After the institution of cardiopulmonary bypass all patients were cooled to 26° to 28° C (blood temperature). Nonpulsatile pump flow

was adjusted to 1.7 to 1.8 L/min per square meter during hypothermia. The mean systemic pressure was maintained between 40 and 60 mm Hg. Heparin was added to maintain an activated clotting time of more than 480 seconds during bypass (Medtronic HemoTec, Inc., Englewood, Colo.). During cardiopulmonary bypass lung ventilation was discontinued; ventilation was subsequently resumed at the end of the period of aortic crossclamping.

The management of myocardial protection was identical in both groups. Cold intermittent blood cardioplegia resulting from mixing blood with high potassium cardioplegic solution was administered in an antegrade fashion into the aortic root or in a retrograde fashion via the coronary sinus, or by both methods.

After operation all patients received mechanical ventilation of the lungs. Pain was controlled with nicomorphine (0.5 mg/10 kg) on the request of the patient. No sedatives were given. Continuous infusions of vasodilators (nitroglycerin, phentolamine, nifedipine) were used when necessary to keep the mean arterial pressure between 60 and 80 mm Hg. As soon as the rectal temperature reached 36° C, attempts to wean the patient from the ventilator were initiated. The criteria for extubation of the trachea were adequate spontaneous ventilation, cooperation of the patient, stable hemodynamics, and absence of significant blood loss.

Hemodynamic measurements and analysis of blood samples. Bolus thermodilution measurements of cardiac output and calculations of standard hemodynamic parameters were made before cardiopulmonary bypass, after closure of the sternum, 3 and 9 hours after the end of cardiopulmonary bypass, and on the morning of the first postoperative day.

Intraoperative and postoperative blood samples for blood cell counts and elastase determination were collected the day before operation (baseline), before cardiopulmonary bypass, during cardiopulmonary bypass (15 and 30 minutes), and at the end of cardiopulmonary bypass. Postoperative samples were obtained 3 and 9 hours after the end of cardiopulmonary bypass and on the first, second, and third days. Blood cell counts were determined on samples containing ethylenediaminetetraacetic acid with use of a Technikon H 1 counter (Terry Town, N.Y.).

Blood for elastase determination was immediately centrifuged (1600 rpm for 10 minutes) and plasma samples frozen until the assay was done. Elastase bound to the α_1 -protease inhibitor was determined by an enzyme-linked immunosorbent assay (PMN Elastase IMAC, Merck Diagnostica, Darmstadt, Germany).

Separate blood samples for arterial blood gas analysis (Automatic Blood Gas System, AVL Medical Instruments AG, Schaffhausen, Switzerland) were obtained before the operation, after the closure of the sternum, and at 2, 4, 7, 10, 13, 16, 19, and 22 hours after the operation.

Statistical analysis. All values are expressed as mean plus or minus the standard error. Confidence intervals were calculated for the control group.

Differences in clinical characteristics among the groups were assessed with the unpaired *t* test or Mann-Whitney test according to the distribution of the data. Serial measurements of blood cell counts and pulmonary vascu-

Table I. Operative data

	Group		<i>P</i> Values
	Leukocyte filter	Control	
CPB duration (min)	82 ± 9	88 ± 8	0.67
Aortic crossclamp time (min)	50 ± 10	53 ± 6	0.26
No. of performed anastomoses	3.3 ± 0.4	4.2 ± 0.4	0.07

CPB, Cardiopulmonary bypass.

lar resistance were analyzed with analysis of variance repeated measures. An unpaired two-tailed *t* test corrected with the Bonferroni method was used in analysis of differences in cell counts between the groups at particular time points. The two-step method for serial measurements described by Matthews and associates¹³ was used for comparison of oxygenation index values in both groups. An unpaired *t* test was used to analyze differences between postoperative infusion rates of phentolamine and nitroglycerine. Simple regression was used to analyze the influence of elastase levels on oxygenation index. Differences were considered statistically significant at a probability level of $p < 0.05$. Data were analyzed with the StatView 4.0 software (Abacus Concepts, Inc., Berkeley, Calif.) on a Macintosh IIfx computer (Apple Computer, Cupertino, Calif.).

Results

There was no statistically significant difference between groups considering duration of cardiopulmonary bypass, aortic crossclamp time, or number of coronary artery anastomoses performed (Table I).

Blood cell counts. All values were corrected for hemodilution according to hematocrit changes.

A slight decrease in the total leukocyte count during cardiopulmonary bypass was followed by leukocytosis in the early and late periods after bypass ($7.0 \pm 0.4 \times 10^9/L$ before bypass versus $14.9 \pm 1.3 \times 10^9/L$ after bypass for the leukocyte filter group; $p < 0.001$, and $7.1 \pm 0.6 \times 10^9/L$ before bypass versus $14.8 \pm 1.1 \times 10^9/L$ after bypass for the control group; $p < 0.001$, Fig. 1). The use of the leukocyte-depleting filter during cardiopulmonary bypass did not cause significant reduction in circulating leukocytes at any time during or after filtration. The analysis of the time course of total leukocyte count did not reveal any significant difference between the groups ($p = 0.86$).

The analysis of the peripheral neutrophil counts revealed a pattern similar to those described by leukocytes (Fig. 2). After cardiopulmonary bypass, the number of neutrophils increased significantly

compared with numbers before bypass ($3.7 \pm 0.3 \times 10^9/L$ before bypass versus $11.8 \pm 1.1 \times 10^9/L$ after bypass for the leukocyte filter group; $p < 0.001$, and $3.6 \pm 0.28 \times 10^9/L$ before bypass versus $12.3 \pm 1.0 \times 10^9/L$ after bypass for the control group, $p < 0.001$). There was no statistical difference between the groups considering neutrophil dynamics ($p = 0.94$).

The lymphocyte counts (Fig. 3) showed a slight decline after the beginning of cardiopulmonary bypass without the rebound effect in the period after bypass, which was observed by neutrophil count. On the contrary, there was a sharp and significant decline of the lymphocyte count 9 hours after the end of cardiopulmonary bypass (baseline value of $2.20 \pm 0.17 \times 10^9/L$ versus $0.73 \pm 0.06 \times 10^9/L$ 9 hours after cardiopulmonary bypass for the leukocyte filter group, $p < 0.001$; baseline count of $1.68 \pm 0.12 \times 10^9/L$ versus $0.86 \pm 0.15 \times 10^9/L$ 9 hours after cardiopulmonary bypass for the control group, $p < 0.001$). There was no significant difference in the lymphocyte counts between the groups during the observed period ($p = 0.15$).

Elastase levels. The plasma values of elastase rose gradually during cardiopulmonary bypass in both groups (Fig. 4). The peak levels occurred after the end of cardiopulmonary bypass, with significantly higher levels in the leukocyte filter group ($460 \mu g/L$ in leukocyte filter group versus $230 \mu g/L$ in control group, 95% confidence interval, 101 to 359 $\mu g/L$, $p = 0.003$; laboratory reference range 2 to 42 $\mu g/L$). The fast decrease of the elastase values was observed after the end of cardiopulmonary bypass. Six hours after the operation the elastase levels were just moderately higher than the baseline values.

Assessment of postoperative lung function. There was no difference in intubation time between the groups (16.2 hours for leukocyte filter group versus 15.6 hours for control group, 95% confidence interval, 12.1 to 19.1 hours, $p = 0.72$). The time course of oxygenation index (arterial oxygen tension/inspired oxygen fraction) values for both groups is shown in Fig. 5. The calculation of area under the curve revealed no statistically significant difference between the groups (290 area under the curve for oxygenation index of leukocyte-depleted group versus 329 area under the curve for oxygenation index of control group, 95% confidence interval, 286 to 372, $p = 0.21$). The regression coefficient of oxygenation index versus logarithm of elastase values ($R^2 = 0.20$) was statistically significant ($p = 0.016$) but clinically irrelevant, indicating that just 20% of variations of oxygenation index could be explained through the changes in elastase values.

Fig. 1. Total white blood cell counts (mean $\pm$ standard error of mean). L, Leukocyte filter group; C, control group; CPB, cardiopulmonary bypass.

Fig. 2. Time course of neutrophil counts (mean $\pm$ standard error of mean). L, Leukocyte filter group; C, control group; CPB, cardiopulmonary bypass.

The postoperative pulmonary vascular resistance values did not differ between the leukocyte filter and control groups ($p = 0.10$; Fig. 6). The dosage of vasodilators administered in the same period was

similar in both groups (leukocyte filter versus control: phentolamine, $150 \pm 19 \mu\text{g}/\text{min}$ versus $228 \pm 67 \mu\text{g}/\text{min}$, $p = 0.41$; nitroglycerin, $75 \pm 13 \mu\text{g}/\text{min}$ versus $89 \pm 15 \mu\text{g}/\text{min}$, $p = 0.48$).

Fig. 3. Lymphocyte counts (mean $\pm$ standard error of mean). L, Leukocyte filter group; C, control group; CPB, cardiopulmonary bypass.

Discussion

The use of a leukocyte-depleting filter for extracorporeal circulation did not cause a significant reduction in the number of circulating leukocytes during cardiopulmonary bypass. There was no improvement of postoperative lung function in the leukocyte filter group as assessed by oxygenation index, pulmonary vascular resistance, and intubation time. The higher plasma levels of elastase that occurred as a consequence of leukocyte filtration did not significantly influence the lung function parameters measured in this study.

Accumulated knowledge regarding the mechanism of neutrophil-mediated lung dysfunction after bypass has led to several unspecific pharmacologic interventions directed toward the inhibition of granulocyte activation and aggregation. Administration of anisodamine (M-cholinergic receptor blocker) reduced pulmonary granulocyte sequestration and lung water content after cardiopulmonary bypass in a canine experimental model.¹⁴ Treatment with nifedipine and deferoxamine in clinical trials resulted in partial inhibition of granulocyte activation during cardiopulmonary bypass, even in the presence of complement activation.^{15,16} However, the potential clinical benefit of those treatments was not documented.

The experimental use of leukocyte filtration posed an attractive alternative to the pharmacologic

interventions. The reduction in the number of circulating leukocytes preserved arterial oxygenation, minimized pulmonary leukocyte sequestration, and prevented postoperative changes in pulmonary vascular resistance in a canine model.¹⁰ The protective effects of leukocyte depletion on postoperative lung function were demonstrated in a bovine model of heart-lung transplantation as well.^{11,12}

The efficacy of the new leukocyte-depleting arterial blood filter for extracorporeal service (Leuko-Guard-6) was documented in an experimental setting with the use of fresh heparinized bovine blood perfusate.¹⁷ The leukocytes present in the small volume of bovine blood (1100 ml) were efficiently filtered after several passes through the filter during 90 minutes of recirculation at high flow rates (4.5 L/min). However, such an experimental design does not entirely simulate clinical conditions. The analysis of leukocyte dynamics in our study showed increased mobilization of leukocytes during the last phase of cardiopulmonary bypass, which corresponds with the data from previous studies.^{6,18-20} The separate analysis of neutrophil and lymphocyte counts showed that postoperative leukocytosis is the consequence of neutrophilia, which may be the result of enhanced mobilization of granulocytes from the marginated pool or release of new cells from the bone marrow. The discrepancy between the neutrophil depletion rate of

Fig. 4. Plasma elastase values (mean $\pm$ standard error of mean). L, Leukocyte filter group; C, control group; CPB, cardiopulmonary bypass; asterisk, $p = 0.003$.

Fig. 5. Oxygenation index (mean $\pm$ standard error of mean) for leukocyte filter (L) and control group (C).

70% observed in the experimental setting and the failure of the filter in the clinical trial could be explained through the additional load of newly mobilized cells, which exceeded the capacity of the filter.

Elastase is a major constituent of azurophilic granules of human polymorphonuclear leukocytes and can be used as a specific marker of granulocyte activation.^{15,18} Elevated plasma levels of elastase during

Fig. 6. Pulmonary vascular resistance (PVR). L, Leukocyte filter group; C, control group; CPB, cardiopulmonary bypass.

cardiopulmonary bypass reflect neutrophil injury or activation and positively correlate with the duration of extracorporeal circulation.²¹ In the current study the elevated elastase levels in the leukocyte filter group could be considered as a side effect of filtration caused by the activation of neutrophils after the contact with the nonwoven polyester fiber surface of the filter.

The capacity of neutrophils to damage endothelial cells and cause lung injury has been noted both in vitro and in vivo, and neutrophil elastase has been implicated in this endothelial cytotoxicity.^{22, 23} The correlation between elastase levels and the oxygenation index in the current study was statistically significant, but not clinically relevant, indicating that just 20% of variations of oxygenation index can be explained through the changes in elastase levels. This finding correlates well with those of recent clinical studies and indicates a multifactorial origin of pulmonary dysfunction after cardiopulmonary bypass.²¹ Furthermore, there was no obvious clinical consequence regarding intubation time, which did not differ between the groups.

Cell death is not the obligatory outcome of elastase-endothelial cell interaction. In vitro studies with lower concentrations of elastase revealed its reversible effect on endothelial prostaglandin I₂ production in response to extracellular adenosine triphosphate, which suggests the vasoregulatory properties of this protease.²⁴ The reduction in pul-

monary vascular resistance in patients after esophageal cancer operations after infusions of the neutrophil elastase inhibitor ulinastatin indicated the clinical importance of experimental observations.²⁵ We were not able to demonstrate any measurable effect of higher elastase concentrations in the leukocyte filter group on pulmonary vascular resistance, which indicates low clinical relevance of in vitro findings.

The clinical use of a leukocyte filter has not fulfilled the expectations suggested by the results from experimental studies. The assessment of various aspects of lung function (oxygenation index, pulmonary vascular resistance, and intubation time) did not show any improvement in these aspects in the leukocyte filter group. The question of whether the release of elastase as a consequence of neutrophil filtration poses a real danger to the lungs remains open. Even a slight degree of filtration, such as presumably occurred in our study, resulted in the large increase in elastase plasma concentration. It could be easily postulated that more efficient mechanical filtration would induce even higher elastase peaks. On the other hand, the high degree of leukocyte depletion resulted in excellent postoperative lung function in the experimental setting. Unfortunately, elastase levels were not measured in any of these studies.¹⁰⁻¹²

The future development of leukocyte filters with

higher capacity could provide the definite answer considering clinical benefits associated with leukocyte depletion. Further studies are necessary to prove whether the elevation of elastase levels could be reduced by the selective use of the filter just before the reperfusion of the lungs.

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