Strategic leukocyte depletion reduces pulmonary microvascular pressure and improves pulmonary status post-cardiopulmonary bypass

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Cardiopulmonary bypass (CPB) precipitates inflammation that causes marked pulmonary dysfunction. Leukocyte filtration has been proposed to reduce these deleterious effects. Other studies show an improvement with aprotinin. We proposed that a combination of these two therapies would synergistically improve pulmonary outcomes. Two hundred and twenty-five patients participated in a randomized prospective trial comparing pulmonary microvascular function and pulmonary shunt fraction post coronary artery bypass grafting (CABG). The study group underwent leukocyte depletion with aprotinin during the procedure. Pulmonary microvascular function was assessed by pulmonary microvascular pressure (PMVP), a measure of pulmonary capillary edema, and pulmonary function was evaluated by comparing pulmonary shunt fractions. Elevated PMVP and increased pulmonary shunting compromise pulmonary performance. The leukocyte-depleted group had significantly reduced PMVP and pulmonary shunt fraction for at least the first 24 hours post bypass. The combination of strategic leukocyte filtration and aprotinin therapy can effectively reduce postoperative decline in pulmonary function.

Cardiopulmonary bypass precipitates a variety of inflammatory effects that can cause marked pulmonary dysfunction to the point of respiratory failure, necessitating prolonged mechanical ventilation. Leukocyte filtration has been investigated previously and appears to be beneficial in improving pulmonary outcome by preventing direct neutrophil-induced inflammatory injury. Recent studies of leukocyte reduction profiles suggest that leukoreduction via leukofiltration is short lived with filter saturation occurring 30–45 minutes after onset of filtration. This phenomenon may explain the limited utility observed with higher risk patients. These patients typically require longer pump runs, so leukocyte reduction capability is suboptimal at the time of pulmonary vascular reperfusion. To more effectively protect the lung from reperfusion injury, leukocyte filtration can be delayed so that reduction of activated neutrophils is maximal at the time of pulmonary vascular reperfusion. It is, thus, conceivable that a timely use of arterial line leukoreducing filters may improve, more substantially, pulmonary function post bypass.

Two hundred and twenty-five isolated coronary revascularization patients participated in this prospective, randomized trial. The patients received moderately hypothermic CPB alone (control group: n = 110) or combined with leukocyte depletion, initiated 30 minutes before crossclamp release, with filters placed in the bypass circuit (study group: n = 115). All patients also received full Hammersmith aprotinin dosing during the operation.

Pulmonary microvascular pressures were lower in the study group at three hours post bypass, and continued to fall until 24 hours post bypass. In contrast, the control group measured a rise in PMVP and a continued plateau throughout 24 hours post bypass (p < 0.028). The calculated pulmonary shunt fraction also was reduced significantly throughout the study interval, with the greatest reduction occurring approximately three to six hours post-CBP (p < 0.002). Shunt fractions eventually converged at 24 hours post bypass. Outcome measures included hospital charges and length of stay, which were also markedly reduced in the treatment group.

Increasing PMVPs are a direct reflection of pulmonary capillary edema, which, in conjunction with increased pulmonary shunt ratio, lead to an overall worsening of pulmonary function. Intraoperative strategic leukocyte filtration combined with aprotinin treatment improves post-CBP lung performance by reducing significantly the reperfusion inflammatory response and its sequelae. These benefits are manifested by reductions in ventilator times, hospital stay and patient morbidity. Perfusion (2003) 18, 23–31.
Introduction

Pulmonary dysfunction commonly develops following open-heart surgery.\textsuperscript{1-6} This complication leads to costly morbidity, manifested as protracted ventilator times, increased stay in the intensive care unit (ICU) and overall duration of hospitalization. More severe cases of pulmonary dysfunction present as respiratory failure. Expenses associated with the management of respiratory failure make it the second most costly complication of cardiopulmonary bypass (CPB). It is not surprising that, in the prevailing healthcare environment of economic constraint, the focus on reducing costly morbidity is significant.

The role of the leukocyte in mediating postpump pulmonary dysfunction is well recognized\textsuperscript{7,8} and the benefits of leukocyte reduction during CPB have been reviewed extensively.\textsuperscript{9-13} Reperfusion injury is due, at least in part, to neutrophil activation followed by the presentation of activated neutrophils in close proximity to target tissues. The damage is inflicted at the time of pulmonary vasculature reperfusion. In the case of open-heart surgery, pulmonary reperfusion injury most likely coincides with the resumption of cardiac rhythm.

Through an appreciation of both neutrophil activation and tissue proximity as requirements for the development of reperfusion injury, many interventional approaches to averting this injury become easily understood. The intervention may prevent neutrophil activation by neutralizing toxic substances elaborated by activated neutrophils, or by restricting the activated neutrophils from the target tissue. Interventions currently under investigation include heparin-bonded CPB circuits designed to prevent complement and, in turn, neutrophil activation. Antioxidants and/or anti-inflammatory drugs have been examined, with the serine protease inhibitor activity of aprotinin currently receiving considerable investigative attention. Finally, the use of leukocyte-reducing filters, commercially available since 1992, is another viable approach to averting reperfusion injury to the lungs.

This study was designed to answer the question of whether the combination of two leukocyte-attenuating processes could synergistically lessen the impact of CPB on pulmonary micro- and macrovascular pressures, thereby improving clinical outcomes assessed from length of ventilation, hospital stay and hospital charges.

Methods

This study was approved by the institutional review board of the University of North Texas Health Science Center and was conducted in accordance with US National Institutes for Health guidelines. The study design was prospective, randomized and controlled. Authors NT and RTM were blinded as to which group was strategically leukodepleted.

Clinical study

The strategic leukoreduction study group (\(n = 115\)) received only leukoreduced transfused (allogeneic) blood (packed cells, platelets and fresh frozen plasma) and leukoreduced reinfused salvaged (autologous) blood (RSI; Pall Biomedical Products, East Hills, NY, USA). In this group, total leukoreduction and strategically implemented filtration were accomplished by passing blood through a leukoreducing arterial line filter (LGB; Pall Biomedical Products, East Hills, NY, USA) 30 minutes before pulmonary vasculature reperfusion. This technique and the CPB circuit were described recently.\textsuperscript{32} A leukodepleting cardioplegia line filter was also used. In addition, the study group received standard Hammersmith dosing of aprotinin. The control group received full-dose aprotinin but no leukocyte depletion. Intraoperative and postoperative variables were kept constant for both groups.

The study enrolled 225 patients who were prospectively randomized to control (\(n = 110\)) and study (\(n = 115\)) groups. Study inclusion criteria required that participants be adult patients undergoing cardiac surgery with the assistance of an extracorporeal CPB circuit. The study was also limited to patients undergoing isolated primary coronary artery revascularization surgery with the use of blood cardioplegia as a technique for myocardial preservation. All participants had a pulmonary artery catheter (Abbott Laboratories, Abbott Park, IL, USA) and radial arterial line prior to inclusion in the study. Pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), pulmonary microvascular pressure (PMVP) and shunt fraction were measured to monitor pulmonary perfusion status. These variables were measured prior to bypass and at 1, 3, 6, 12, 18 and 24 hours postbypass. Shunt fractions were determined at 0, 2, 4, 6 and 8 hours postbypass.

Postoperative total ventilator time, hospital stay and total inpatient charges were tabulated to extend
the study findings to tangible economic and patient benefits. The incidence of pulmonary dysfunction, defined as either oxygen requirements of FiO₂ > 45% for more than 24 hours postoperatively or ventilator time greater than 18 hours, was also determined.

**Anesthesia, operation, perfusion and postoperative monitoring**

All patients received standard anesthesia induction and maintenance of general anesthesia. Radial arterial and jugular venous lines and Swan-Ganz catheters (Abbott Laboratories, Abbott Park, IL, USA) were placed in all patients. Full Hammersmith dose aprotinin (Bayer, West Haven, CT, USA) was administered during the procedure.

All patients were maintained on moderately hypothermic (34°C) CPB at a minimum flow of 2.2 L/m per m². Postoperative measurements in the ICU were recorded and direct pulmonary measurements were obtained from the Swan-Ganz catheter. PMVP was calculated from the following formula from Ermann, as modified by Mathru:

\[
\text{PMVP} = \text{PCWP} + 0.4 \text{(PAP}_{\text{mean}} - \text{PCWP})
\]

PMVP relates to the third zone of the lung, PCWP reflects left atrial pressure and 0.4 is the fraction of total pulmonary vascular resistance that is downstream from the microvascular exchange surface. The normal PMVP was considered to be 10–15 mmHg. Shunt fraction was calculated from a standard formula with the normal value accepted as 4–8%.

**Patient demographics**

The control (nonleukocyte-depleted) group consisted of 60 men and 50 women averaging 64 years of age (56–74 years). Comorbid factors included diabetes (35%) and pulmonary disease (15%), primarily chronic obstructive lung disease (COLD). The study (leukocyte filtration) group, consisting of 62 men and 53 women, averaged 63 years of age (54–72 years). This group also had two major comorbid factors, diabetes (35%) and COLD (16%).

**Statistical analysis**

Data are expressed as mean ± SD, except where noted otherwise. Functional data were compared by two-way repeated measures analysis of variance (MANOVA). Other data were compared by independent two-way MANOVA. When MANOVA detected statistically significant differences (p < 0.05), Student–Newman–Keuls multiple comparison tests were conducted post hoc to identify the specific differences. A Mann–Whitney U-test was also utilized. P values less than 0.05 were taken to indicate statistical significance.

**Results**

Two hundred and twenty-five patients completed the study (control group 110, study group 115). No patients were excluded from data analysis and there were no operative deaths. CPB and crossclamp times were similar between groups. The control group averaged 96 ± 14 minutes of bypass with a crossclamp time of 61 ± 12 minutes. In the study group, similar times were recorded with a bypass time of 98 ± 16 minutes and a crossclamp time of 59 ± 14 minutes.

PAP in the control group was 18 ± 2 mmHg pre-bypass, increased to 22 ± 2 mmHg at one hour postbypass, and then stabilized at 20 ± 2 mmHg up until 24 hours postbypass. The study group, however, started at 16 ± 2 mmHg, but declined with a reduction to 14 ± 2 mmHg at 16 through to 24 hours postbypass (p < 0.001) (Figure 1).

In the control group, PCWP rose slightly from 14 ± 1 to 16 ± 1 mmHg during the first three hours postbypass, then subsided and stabilized at 14 ± 1 mmHg for six to 24 hours postbypass. In contrast, PCWP fell by 4 ± 2 mmHg over the first 12 hours postbypass in the leukocyte-depleted group before stabilizing at 11 ± 1 mmHg; the decline in PCWP began immediately after bypass, unlike the control group (p = 0.061 versus control group at 24 hours; Figure 2).

PMVP increased during the first hour postbypass in both groups. PMVP was persistently elevated in the control group. In the study group, however, PMVP fell by 4 ± 2 mmHg between one and three hours, and continued to decline throughout the 24 hour postbypass period (p = 0.028 versus control group at 24 hours; Figure 3).

The shunt fraction (Qs/Qt%) was calculated using a standard formula. In the control group, Qs/Qt% increased sharply during the first two hours postbypass, then gradually fell over the next six hours (Figure 4). The postbypass increase in Qs/Qt% was markedly attenuated in the study group (p = 0.002 versus control group at two hours), and Qs/Qt% remained well below the respective control values at four and six hours postbypass. Indeed, the increased in Qs/Qt% during the first six hours postbypass in the leukofiltration group was less than half of that of the control group (Figure 4).

The average postoperative ventilator time was 13.5 ± 4 hours in the control group. The leukodepleted study group averaged a ventilator time of 7.1 ± 3 hours, a 47% reduction from the control value. It should be noted that anesthesia and postoperative use of sedation were identical in both groups (Figure 5).
The length of stay was reduced by an average of two days in the filtration (study) group (Figure 6). This reduction in length of stay, combined with the marked reductions in ventilator time and respiratory treatments, resulted in a cost savings of US$9632 per patient (Figure 7).
Discussion

The clinical utility of leukocyte reduction filtration technology remains equivocal. Some investigators report a small but statistically significant reduction in the white cell count in the early postbypass period.\textsuperscript{14,15} In several studies, a significant attenuation of postbypass leukocytosis was associated with
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Figure 5  Average postoperative ventilator time

Figure 6  Length of stay

Figure 7  Average hospital charges
improvement in pulmonary function. However, some studies failed to demonstrate either leukocyte reduction or clinical benefit. Thus, results of leukocyte filtration vary considerably, ranging from no observable therapeutic effect to a transient improvement in lung function, or even a more dramatic, clinically relevant improvement in patient outcome, resulting in a full day reduction in length of hospital stay and an average per patient saving of US$3000. The latter observation amounts to savings of US$3 million in patient charges, and perhaps half that in actual costs in an aggressive open-heart programme performing 1000 procedures annually.

The current randomized investigation demonstrates synergistic benefits of combining mechanical (leukocyte filtration) with pharmacological (aprotinin) treatment strategies. The overall improvement in pulmonary function permitted reductions in ventilator time, length of stay and hospital charges. A large, prospective, randomized trial compared the standard of practice using anti-inflammatory corticosteroids with three anti-inflammatory interventions: aprotinin, heparin-bonded circuits, and four types of leukocyte-reducing filters, representing a programme of treatment now referred to as total leukocyte control (TLC). When patients are stratified based upon preoperative risk scores, over 70% fall into the low-risk category and these patients are provided significant benefit only with TLC and no other intervention. In the TLC group, hospital length of stay was reduced from 6.8 to 5.4 days and per-patient charges fell by US$6000.

Recently, leukocyte counts in patients in whom conventional arterial line filters were used were compared with those of patients receiving leukocyte-reducing arterial line filters. Both groups displayed an initial leukopenia followed by leukocytosis. The levels of leukocytes were lower in the leukocyte-reduced group at 60 and 75 minutes of bypass, but after 90 minutes the leukocyte counts began to converge so that significant differences could not be discerned between controls and treated patients. These results suggest that leukoreduction with filter treatment is transitory and requires 30-45 minutes to achieve the nadir. This time course has been confirmed in vitro. Of equal importance, leukoreduction eventually fails, as both treated and control patients develop profound leukocytosis. Higher risk patients spend more time on bypass, and leukocytosis may become more evident with longer pump runs.

Clinical leukocyte reduction can more effectively inhibit reperfusion injury to the lung when employed strategically, as represented in the current study. The strategy considers the delay required to achieve maximal leukocyte reduction and attempts to co-ordinate the period of maximal leukopenia with the clinically relevant time of pulmonary vascular reperfusion. Thus, there is reason to believe that strategic timing of arterial line leukofiltration results in a more substantial improvement in pulmonary function postbypass. Coupling the leukocyte filtration timing strategy with full-dose aprotinin also decreased the incidence of atrial fibrillation.

The present investigation corroborates previous studies demonstrating the clinical utility of measuring PMVP. The original work by Erdmann et al. and Mathru et al.’s refinements indicated that the calculated PMVP appears to report the status of the pulmonary vascular bed more accurately than PCWP. We have also demonstrated the clinical usefulness of PMVP in a randomized trial examining the effects of dobutamine after mitral valve replacement. In that study, the calculated PMVP proved useful in guiding the treatment of interstitial pulmonary edema.

There are many studies supporting the use of aprotinin during CPB as a means of blunting the systemic inflammatory response. The molecular and cellular events associated with this response and the effects of aprotinin in limiting proinflammatory mediators were recently reviewed. Hall et al. reviewed current treatment modalities to ameliorate the inflammatory response, including anti-inflammatory steroids, protease inhibitors and leukofiltration. However, none of the studies reviewed in these articles examined the effects of a dual-modality approach, such as we advocate based on our current experience.

We have achieved a marked decline in the incidence of pulmonary dysfunction post-CPB (Figure 8). Our results compare favorably with the Society of Thoracic Surgeons regional and nationwide cardiac surgery databases reporting incidences of pulmonary dysfunction, defined as the requirement for greater than 45% supplemental oxygen for more than 18 hours and/or ventilator time exceeding 24 hours. Implementation of the timed leukodepletion-aprotinin treatment regimen progressively reduced the incidence of pulmonary dysfunction from 12% in 1999 to about 8% in 2001, well below the regional pulmonary dysfunction incidence. It is, therefore, evident that reducing PMVP by combining mechanical (leukofiltration) and pharmacological (aprotinin) interventions can appreciably improve patient outcomes and, thus, sharply lower hospital charges.
References


Figure 8 Incidence of pulmonary dysfunction


