Leukocyte-Aprotinin Atrial Fibrillation Study (LAFFS): Impact of Aprotinin and Leukofiltration on Atrial Fibrillation, Renal Insufficiency and Encephalopathy Post-Cardiopulmonary Bypass

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Abstract

Purpose: Atrial fibrillation remains the leading postoperative complication following cardiopulmonary bypass. A randomized trial was undertaken to evaluate the effectiveness of leukocyte filtration and aprotinin, applied separately and in combination, on the incidence of post-operative atrial fibrillation. A secondary component of the study was the impact of these adjunct interventions on post-surgical renal and neurological dysfunction.

Methods: A total of 1,220 patients undergoing primary isolated coronary artery bypass grafting were randomly assigned to one of four treatment groups. The control group (305 patients) received standard cardiopulmonary bypass with moderately hypothermic (34°C) cardioplegic arrest. In the filtration group (310 patients) leukocyte reducing filters were incorporated into the bypass circuit and deployed strategically. The aprotinin group (285 patients) received full Hammersmith dose aprotinin. The combination therapy group (320 patients) received both aprotinin and leukocyte filtration.

Results: The incidences of atrial fibrillation were 25% in the control group, 16% in the filtration group, 19% in the aprotinin group and 10% in the combination therapy group (P < 0.001). Renal dysfunction was detected in 3% of the control group, 2% of the filtration group, 8% of the aprotinin group, and 5% of the combination group (P < 0.005). Neurological dysfunction occurred in 2% of the control group, 2% of the filtration group, 1% of the aprotinin group, and 2% of the combination group (P = n.s.).

Conclusions: Combination therapy with aprotinin and leukocyte filtration markedly reduced atrial fibrillation post-cardiopulmonary bypass, and was more effective than the individual treatments. Aprotinin treatment increased the incidence of renal dysfunction, and the addition of leukocyte filtration partially mitigated this detrimental effect of aprotinin. Thus, strategic leukocyte filtration augments aprotinin’s anti-arrhythmic effects while suppressing its nephrotoxic sequelae. Key words: aprotinin, atrial fibrillation, cardiopulmonary bypass, encephalopathy, leukofiltration, renal failure

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Introduction

Atrial fibrillation ranks among the most frequent and potentially deleterious complications associated with cardiopulmonary bypass. This arrhythmia has been reported in up to 50% of cases, and its incidence remains high despite advances in surgical and anesthetic techniques. The arrhythmia occurs approximately 48-72 hours after surgery and can be manifested by persistent tachycardia, hypotension, and in many cases cardiac failure and cerebrovascular events.

Different strategies have been advocated to prevent atrial fibrillation. Pharmacological agents such as anti-arrhythmics have been used in some studies with variable results. Another approach is the use of leukocyte filters, as there seems to be a connection between atrial fibrillation and inflammation. Indeed, systemic inflammation can elicit atrial fibrillation by activating the complement system and stimulating release of activated cytokines.

To minimize the incidence of atrial fibrillation, we advocate therapy combining a pharmacological strategy, in which the serine protease inhibitor aprotinin is included in the cardioplegia, with a mechanical approach involving the placement at multiple positions within the bypass circuit of filters to selectively remove activated leukocytes. In a recent, randomized trial, this type of combination therapy produced a marked, 67% decrease in atrial fibrillation.

In addition to atrial fibrillation, renal and neurological dysfunction rank among the leading co-morbidities of cardiopulmonary bypass surgery. Cardiopulmonary bypass causes numerous complex and interactive deleterious effects to the kidney, including inflammatory injury to the organ inflicted by activated neutrophils. Cardiopulmonary bypass also may induce neurological encephalopathy; however, the precise mechanisms of encephalopathy are poorly understood, and its reported incidence is variable, criteria-dependent and subject to clinical judgement.

This study was undertaken to evaluate pharmacological (aprotinin) and mechanical (leukocyte filtration) anti-inflammatory measures, applied singly or in combination during cardiopulmonary bypass, as strategies to minimize the occurrence of atrial fibrillation, renal dysfunction and neurological impairment following coronary artery bypass grafting (CABG) on cardiopulmonary bypass.

Methods

This study was approved by the Institutional Review Board at the University of North Texas Health Science Center, and included two of the major teaching hospitals associated with the Science. The study was constructed as a randomized comparative trial and was designated the Leukocyte-Aprotinin Atrial Fibrillation Study (LAAFS). Only patients undergoing primary CABG were included; all patients undergoing re-do or adjunct procedures, including valve repair/replacement, were excluded. Echocardiograms were performed on all patients to measure pre-operative ejection fraction.

Surgical Preparation and Cardiopulmonary bypass Procedure

All patients were intravenously pre-medicated with 0.7 mg/kg of ranitidine, 2-4 mg/kg of glycopyrrolate, 0.7 mg/kg of diphenhydramine, and 20-40 mg/kg of midazolam. Monitoring catheters were placed in all patients including a radial arterial catheter, a central venous jugular introducer with a continuous cardiac output pulmonary artery catheter (Abbott Laboratories, Abbott Park, IL), and one or two large bore peripheral intravenous cannulae. General anesthesia was induced by 1-2 mg/kg of propofol, 0.7-2.0 mg/kg of sufentanil, and 0.1 mg/kg of vecuronium. After endotracheal intubation, anesthesia was maintained with sevoflurane on supplemental O2. Additional narcotic and relaxants were administered to maintain sedation throughout the surgery.

Patients were systemically heparinized to effect an ACT of > 700 s and were given protamine sulfate to restore clotting after separation from bypass. All patients had direct ascending aorta and right atrial cannulation and were maintained on moderately hypothermic (34°C) cardiopulmonary bypass. Cold blood-crystalloid potassium based cardioplegia (4 vol. blood:1 vol. crystalloid solution) was administered to produce cardiac arrest. Initial induction of cardiac arrest was accomplished by administering 500 ml cardioplegia antegrade via the aortic root, followed by retrograde infusion of an additional
500 ml via the coronary sinus at a pressure of 45 mmHg. Supplemental cardioplegia was administered through the vein grafts at a pressure of 14 mmHg, for 2 min per infusion, after completion of each distal anastomosis.

**Treatment groups**

From January 2004 - March 2006, a total of 1,220 patients undergoing isolated coronary artery revascularization were enrolled and assigned by a sealed envelope approach to one of the four treatment groups described below. There were no statistically significant differences between the groups regarding demographics, co-morbid conditions, pre-operative laboratory data, durations of cardiopulmonary bypass and cross clamp, or number of grafts completed (Table 1).

**Control Group**

(n = 305): Standard cardiopulmonary bypass was instituted as described above and cold potassium based cardioplegia was utilized for arrest.

**Leukocyte Filtration Group**

(n = 310): These patients received standard cardioplegia as in the control group; in addition, leukocyte depleting filters were incorporated into the bypass circuit, including a Pall LGB and a BC1B filter (Pall Biomedical Products, East Hills, NY, USA). The circuit prime and configuration are described in a previous report. These patients were strategically leukodepleted by use of filtration instituted 30 min before crossclamp release, and all arterial, cardioplegia and cell saver blood was leukocyte filtered.

**Aprotinin Group**

(n = 285): This group received standard cardiopulmonary bypass with cold potassium based cardioplegia, and a standard extracorporeal circuit was employed. In addition, full Hammersmith, regimen A dosing of aprotinin (Bayer Pharmaceuticals, West Haven, CT, USA) was utilized. After a test dose the pump was primed with 200 ml of aprotinin fortified priming solution, then aprotinin solution was infused at 50 ml/h.

**Combination Group**

(n = 320): This group received the combination of aprotinin and total leukocyte filtration.

**Evaluation of Brain, renal and Electrocardiographic Function**

All subjects had pre-operative echocardiography

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient Demographics</th>
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<tbody>
<tr>
<td></td>
<td>CONTROL</td>
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<tr>
<td>N</td>
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<tr>
<td>Mean age (years)</td>
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<td>Females</td>
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<tr>
<td>Mean Wt. (kg)</td>
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<tr>
<td>Mean X Clamp (min)</td>
<td>56</td>
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<tr>
<td>Mean #grafts</td>
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ns: No statistically significant differences were detected between the treatment groups.
and carotid duplex scanning. Patients exhibiting post-operative encephalopathy, defined as post-operative confusion, agitation or delirium within 24 h after bypass,\textsuperscript{17} or frank stroke were evaluated by a neurologist and underwent cerebral CT scanning. Post-operative renal function was categorized by peak post-surgical serum creatinine concentration according to National Kidney Foundation guidelines [normal: creatinine < 1.6 mg/dl; renal dysfunction: creatinine 1.6-2.5 mg/dl; acute renal failure: creatinine > 2.5 mg/dl].\textsuperscript{18} All patients exhibiting renal dysfunction or frank renal failure were evaluated by a nephrologist, who determined the need for hemodialysis. All patients had continuous ECG monitoring throughout the hospital stay. Arrhythmias were detected by Hewlett-Packard 78220 arrhythmia monitoring system (Hewlett-Packard, Palo Alto CA, USA) and validated by the cardiology service.

**Statistical Analysis**

Values for continuous variables, e.g. ejection fraction or serum creatinine, are expressed as mean ± standard deviation. Between-group comparisons of these variables were accomplished by single-factor analyses of variance (ANOVA), with treatment as the factor. None of these ANOVAs revealed statistically significant differences, so post-hoc tests to identify specific differences were not employed. Incidences of discrete events among the treatment groups were compared by chi-square tests.\textsuperscript{19} Yates correction for continuity was applied to chi-square comparisons of two groups.\textsuperscript{19} P values < 0.05 were taken to indicate statistically significant treatment effects.

**Results**

Table 1 summarizes the demographic characteristics of the four treatment groups. Age, gender, and body mass were typical for patients requiring CABG. Occurrences of comorbidities, including diabetes mellitus and hypertension, were also as expected. There were no statistically significant

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**Figure 1:** Impact of aprotinin and leukocyte filtration, applied separately and in combination, on incidence of atrial fibrillation after cardiopulmonary bypass surgery.
differences among the treatment groups for any of these characteristics. Moreover, operative cardiopulmonary bypass and cross clamp times, and number of grafts did not differ among the groups.

Atrial fibrillation (Figure 1) occurred in 76 (25%) of the 305 control patients, 49 (16%) of the 310 leukocyte filtered patients, 55 (19%) of the 285 aprotinin patients and 32 (10%) of the 320 combined treatment patients (P < 0.001). Leukofiltration (P < 0.01) and the leukofiltration:aprotinin combination (P < 0.001) lowered incidence of atrial fibrillation vs. control. The combination treatment also decreased atrial fibrillation incidence vs. aprotinin (P < 0.005) and leukofiltration (P < 0.05) alone.

The combination group and, especially, the aprotinin group had higher incidences of renal insufficiency (Figure 2A) and renal failure requiring hemodialysis (Figure 2B). Renal insufficiency occurred in 9 (3%) of control patients, 6 (2%) of leukocyte filtration patients, 22 (8%) of the aprotinin group, and 16 (5%) of the combination group (P < 0.005). Acute renal failure requiring hemodialysis (Figure 2B) was observed in 1 control patient, 3 leukocyte filtered patients,
In a retrospective review of off-pump CABG patients, Edgerton et al.\textsuperscript{22} reported an atrial fibrillation incidence between 10.6 and 18.5%, with the lower atrial fibrillation rate occurring in patients who were extubated immediately in the operating room.

Previously we reported incidence of atrial fibrillation in patients undergoing isolated elective CABG.\textsuperscript{11} We also observed a tendency of atrial fibrillation to occur within 48-72 hours after surgery. This non-blinded, randomized study enrolled 120 patients (55 control, 65 treatment), with the treatment arm receiving strategic total leukocyte filtration and full Hammersmith dose of aprotinin. The atrial fibrillation rate was lowered from 27% in the control group to 7.6% in the aprotinin group, a reduction of 72%.\textsuperscript{11} A follow-up study compared rates of atrial fibrillation in on-pump vs. off-pump procedures.\textsuperscript{12} In that study the incidence of atrial fibrillation in the on-pump leukocyte filtered-aprotinin group was 7.8% compared to 23.3% in the non-treatment group. In the off-pump cohort of 90 patients, atrial fibrillation rate was 17.8%.\textsuperscript{12}

Encephalopathy (Figure 3) was documented in 6 (2%) of the control patients, 6 (2%) of the leukocyte filtered patients, 3 (1%) of the aprotinin patients and 6 (2%) of the combined therapy patients (P = n.s.). Acute cerebral vascular events evidenced by focal neurological signs and CT findings were not detected in the leukocyte filtered and aprotinin groups. Three (1%) of the control patients and one (0.3%) combined treatment patient had neurologically significant findings.

Discussion

Atrial fibrillation remains a very common complication post cardiopulmonary bypass with a reported incidence of 25-60\%\textsuperscript{1,20}. Peak incidence of the arrhythmia occurs between 48-72 post bypass.\textsuperscript{21}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Incidence of encephalopathy post-cardiopulmonary bypass}
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The current LAFFS trial also demonstrated a reduction in atrial fibrillation by the combination of aprotinin and leukocyte filtration. Atrial fibrillation occurred in 10% of the combination treatment group, as compared to 25% in the control group. The combined application of aprotinin and leukocyte filtration appears to produce a synergistic anti-inflammatory effect in the clinical setting, by impacting both complimentary and distinct pathways of the inflammatory cascade. Indeed, Lamm et al reported a strong correlation between elevated post-operative white blood cell counts and atrial fibrillation. In their study of 253 patients, consisting of both primary CABG and valve cases, the atrial fibrillation rate was 39.1%. Collectively, these results are concordant with the concept that reduction in activated neutrophils either by mechanical (filtration) or pharmacologic (aprotinin) means, could directly impact the development of atrial fibrillation post cardiopulmonary bypass.

In the present study, the aprotinin group experienced increased incidence of renal insufficiency and acute renal failure requiring hemodialysis. Interestingly, the leukocyte-filtered group was observed to have a slightly reduced incidence (2%) of renal insufficiency and acute renal failure vs. respective control values, although these trends did not reach statistical significance. Mangano et al. utilizing a propensity-adjusted, multivariate logistic regression analysis of an observational study of 4,474 patients, found a 5.5% vs. 1.8% (P < 0.001) incidence of renal dysfunction in aprotinin-treated vs. untreated patients, an apparent tripling of the risk of renal failure requiring dialysis. In contrast, the Bayer pharmaceutical aprotinin package insert from a pooled data base of 2,003 aprotinin and 1,084 control patients reported a 1% incidence of acute renal failure in the aprotinin treatment arm and 0.6% in the placebo arm. A meta-analysis by Sedrakyan et al. showed that the risk ratio of developing renal failure from aprotinin was 1.01 (95% CI 0.55-1.83) vs. placebo. Sedrakyan et al.’s analysis examined studies drawn from MEDLINE, EMBASE and PHARMLINE from 1988-2001, and included data from 35 CABG trials.

Cardiopulmonary bypass has also been associated with a spectrum of neurological impairments from transient encephalopathy to frank stroke (CVA). In a retrospective analysis of 1,524 patients undergoing CABG, Frumento et al. reported a reduced incidence of cerebral events in aprotinin-treated patients. The study consisted of control, half-dose and full dose aprotinin groups. The overall incidence of CVA was 16% in the control versus 0% in the full dose aprotinin group; interestingly, the half-dose cohort had a CVA incidence of 22%. There were no acute strokes in either the filtration or aprotinin group, and the incidence of encephalopathy did not differ significantly among the groups.

Limitations

A limitation to the LAFFS study may be a bias in patient selection as only isolated primary CABG subjects were enrolled, as well as a lack of risk-stratification. Nevertheless, the reduction in atrial fibrillation with leukocyte filtration and or aprotinin is striking. In comparison Gunaydin et al. also found a reduction in post-operative atrial fibrillation in patients who had leukocyte filtration. In their study patients were assigned to three distinct groups based on Euroscore risk stratification: low risk Euroscore 0-2, medium risk Euroscore 3-5, high risk Euroscore 6+. These groups were further subdivided to conventional, non-filtered extracorporeal circuit (control), phosphorylcholine inert surface coated circuit, or leukocyte filtered. Leukocyte filtration lowered incidence of atrial fibrillation in the medium and high Euroscore groups. The non-coated, non-filter group medium to high risk Euroscore had a 13% incidence of atrial fibrillation versus the leukodepleted medium to high risk Euroscore group, which had a 3% incidence of atrial fibrillation (p<0.05).

A second limitation of this trial is that other pre-existing factors that could possibly affect the incidence of post-operative atrial fibrillation, including hypertension, valve disease, hyperlipidemia, pre-operative atrial fibrillation, medications or nutraceuticals, were not recorded. However, the size and randomized design of the trial make it likely that these factors were evenly distributed among the four groups and therefore were not independent contributors to the different outcomes. In addition, post-operative factors, in-
cluding post-pericardiotomy syndrome and the use of anti-inflammatory agents, were not documented.

Summary

Leukocyte filtration and aprotinin were both effective in reducing the incidence of atrial fibrillation after cardiopulmonary bypass surgery for coronary revascularization. The synergistic combination of both treatment modalities was the most effective. However, aprotinin treatment was associated with increased post-surgical incidence of renal insufficiency and acute renal failure. The addition of leukocyte filtration lessened the incidence of renal dysfunction in aprotinin-treated patients. There are two important components to leukocyte filtration: leukocyte filters must be deployed strategically (30 minutes prior to cross-clamp release), and total leukocyte filtration must also be employed. Further research is necessary to define the mechanisms and pathophysiology involved in the generation of atrial fibrillation post-cardiopulmonary bypass, and to develop newer molecular strategies to address this major complication of cardiopulmonary bypass surgery. With the recent withdrawal of aprotinin from the U.S. market, implementation and refinement of strategic leukocyte filtration, including the use of novel absorptive materials and carbon-based nanotechnology, seem warranted.

Acknowledgements

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