Endotoxemia in coronary artery bypass surgery: A comparison of the off-pump technique and conventional cardiopulmonary bypass

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Objectives: The endotoxemia associated with cardiac surgery is thought to be dominantly influenced by the use of cardiopulmonary bypass. The objectives of this study were to assess the relative contribution of cardiopulmonary bypass on endotoxemia apart from cardiac surgical access and to improve our understanding of the potential benefits of off-pump procedures.

Methods: Thirty patients undergoing coronary artery bypass grafting were followed up prospectively. The patients were divided into 2 equal groups: those who underwent bypass grafting through a sternotomy incision without cardiopulmonary bypass (off-pump group) and those who underwent bypass grafting through a sternotomy incision with cardiopulmonary bypass (CPB group). Blood sampling for endotoxin, lactate, and cardiac index measurements were performed during the following time points: (1) after sternotomy; (2) during the coronary occlusion period in the off-pump group and during aortic clamping in the CPB group; (3) after removal of the coronary occlusion sutures in the off-pump group and after removal of the aortic clamp in the CPB group; (4) 30 minutes after the completion of all distal anastomoses in the off-pump group and immediately after weaning from cardiopulmonary bypass in the CPB group; (5) 1 hour postoperatively; and (6) 12 hours postoperatively.

Results: Endotoxin and lactate levels were significantly ($P < .05$) lower in the off-pump group at all sampling time points, except after sternotomy.

Conclusions: In conclusion, this study has shown that endotoxemia during coronary artery bypass surgery seems mainly to be associated with cardiopulmonary bypass procedure. The relatively lower endotoxin levels observed in off-pump surgery might contribute to improved postoperative recovery.

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Endotoxin is a lipopolysaccharide from the cell wall of gram-negative bacteria and is recognized to be a major stimulus for the development of the systemic inflammatory response syndrome.[1]

Transient endotoxemia in patients undergoing surgery involving cardiopulmonary bypass (CPB) has been widely recognized.[2-11] The endotoxemia associated with cardiac surgery is thought to be dominantly influenced by the use of CPB. Gut translocation has traditionally been

perceived as the primary source of endotoxemia. However, the pathogenesis involved in this phenomenon is not entirely clear.

In an attempt to avoid the deleterious effects of CPB, off-pump coronary artery bypass grafting (CABG) has recently been rediscovered. Because the data for the clinical benefits of beating-heart surgery are being substantiated at midterm and now at longer-term follow-up, the basic science behind these differences is still lacking. Many studies have been conducted to compare different inflammatory responses after CABG with or without CPB.[12-17] To our knowledge, there is no literature available about the comparison of blood endotoxin levels between off-pump and conventional coronary artery bypass surgery with CPB.

The objectives of this study were to assess the relative contribution of CPB on endotoxemia apart from cardiac surgical access and to improve our understanding of the potential benefits of off-pump procedures.

Patients and Methods

Patients

Thirty patients undergoing CABG were followed prospectively from September through November 2001. The study was approved by the Institutional Committee on Human Research, and all patients provided informed consent. The patients were divided into 2 groups: those who underwent bypass grafting through a sternotomy incision without CPB (the off-pump group) and those who underwent bypass grafting through a sternotomy incision with CPB (CPB group). Exclusion criteria were as follows: redo or emergency operations, active or prior history of autoimmune disorders, medication with immunomodulating agents (eg, steroids), increased white blood cell counts, increased C-reactive protein levels, high sedimentation rates, any history or signs of infectious disease before surgical intervention, previous major abdominal operations, disorders involving the intestines, connective tissue disorders, and abdominal aorta and mesenteric artery diseases. All patients had multivessel coronary artery disease on the basis of preoperative cardiac catheterization and ejection fractions of greater than 35%. The decision regarding which procedure was to be performed on an individual basis was made by the attending surgeon in consultation with the patient. Patients with diseases involving the posterior descending or circumflex coronary arteries were considered candidates for either procedure.

Preoperative urine and blood cultures were obtained from each patient. Patients with positive blood or urine culture results were also excluded from the study.

Anesthesia

Before the operation, the radial artery was cannulated, and a flow-directed thermodilution catheter (Abbott Critical Care Systems) was inserted into the pulmonary artery percutaneously through the internal jugular vein. All patients received a similar balanced anesthetic regimen, including fentanyl, propofol, and sevorein. Curarization was achieved with pancuronium bromide. Cefazolin was administered intravenously for antimicrobial prophylaxis at a dose of 1 g at the induction of anesthesia and followed by 1 g every 6 hours for 24 hours. Postoperatively, both groups of patients were admitted to the intensive care unit. The patients were extubated as soon as clinically indicated.

Off-pump Technique

After median sternotomy, all patients were heparinized (100 U/kg) to achieve an activated clotting time of greater than 250 seconds. The distal anastomoses were completed with the use of mechanical stabilizers for immobilization of the myocardial surface at the site of the target coronary artery (Octopus, Medtronic). Two 4-0 polypropylene sutures were used to temporarily occlude the coronary artery on either side of the anastomosis site to obtain a bloodless field. Revascularization of the left anterior descending coronary artery with the left internal thoracic artery was performed first, which was followed by the revascularization of the right coronary artery and circumflex distributions. The proximal anastomoses were performed before the distal anastomoses with the assistance of a partial occlusion aortic clamp. The total coronary occlusion times required for the completion of all distal anastomoses were recorded. Heparin was not reversed with protamine sulfate.

CPB Technique

After median sternotomy, all patients were given 300 to 400 U/kg heparin, and after standard cannulation, patients were placed on CPB. Nonpulsatile pump (Delphin 7850 centrifugal pump, 3M) and membrane oxygenators (Dideco D 703 Compactflo System) were used in all operations. The pump priming consisted of Ringer lactate (1500 mL), 20% mannitol (150 mL), and 8.4% sodium bicarbonate (60 mL). Activated clotting times were maintained at a minimum of 400 seconds, hematocrit levels were maintained at approximately 20% to 25%, and the pump flow was set at 2.2 to 2.4 $L/m^2/min$. Patients were cooled to 28°C. For myocardial protection, potassium-based cold blood cardioplegia was intermittently administered, and cold (4°C) Ringer solution was topically applied to the heart. In every instance the left internal thoracic artery was used as a left anterior descending coronary artery graft. On discontinuation of CPB, heparin was neutralized with protamine sulfate.

Time Points of Blood Sampling for Endotoxin, Lactate, and Cardiac Index Measurement

Blood sampling for endotoxin, lactate, and cardiac index measurements (assessed by means of thermodilution) was performed during the following time points: (1) after sternotomy in both study groups; (2) during the coronary occlusion period of the last distal anastomosis in the off-pump group and during aortic crossclamping in the CPB group; (3) after removal of the coronary occlusion sutures in the off-pump group and after removal of the aortic crossclamp in the CPB group; (4) 30 minutes after the completion of all distal anastomoses in the off-pump group and immediately after weaning from CPB in the CPB group; (5) 1 hour postoperatively in both study groups; and (6) 12 hours postoperatively in both study groups. Rectal temperatures and electrocardiographic changes were recorded at all points during the study.

All blood samples were obtained from the radial artery, except during the CPB period in the CPB group, when samples were taken from the arterial blood outlet of the oxygenator. Samples were

TABLE 1. Clinical data

	Off-Pump group (n = 15)	CPB group (n = 15)	*P* value
Preoperative data			
Age (y)	60.3 ± 10	62.1 ± 7	.5
Male (No.)	11 (73%)	14 (93%)	.3
BSA (m^2)	1.8 ± 0.11	1.8 ± 0.9	.7
EF (%)	46 ± 5	44 ± 5	.2
Parsonnet score	3.3 ± 3	2.7 ± 1.8	.9
Perioperative data			
No. of graft	1.5 ± 0.7	2.4 ± 0.5	.0009
TCO time*/crossclamp time† (min)	33.7 ± 17	60 ± 16	.0002
CPB time (min)		93.2 ± 17	—
OR time (min)	182 ± 52	251 ± 23	.0002
Postoperative data			
Transfusion of blood products (mL)	580 ± 174	1226 ± 406	.0001
Total chest drainage (mL)	830 ± 156	1022 ± 196	.8
Time on ventilator (h)	7 ± 3	10 ± 4	.055
ICU stay (h)	24 ± 13	24 ± 12	.8
Stay in hospital (d)	6 ± 2	6.9 ± 2	.3

BSA, Body surface area; *EF,* ejection fraction; *TCO,* total coronary occlusion; *OR,* operating room; *ICU,* intensive care unit.
*For the off-pump group.
†For the CPB group.

obtained from the prime solutions in the CPB group and from transfused blood and blood products in both study groups for endotoxin measurement.

All blood samples were collected in an aseptic fashion into sterile, endotoxin-free, silicon-coated tubes (Terumo Venoject, Europe N.V. 3001) and kept at room temperature for 30 minutes before centrifugation. Samples were stored at −70°C.

At the first postoperative day, blood, urine, and catheter cultures were obtained from all patients.

Measurement of Endotoxins

All glassware was covered with aluminum foil and heated in a hot-air oven at 200°C for 4 hours.

Endotoxin levels were measured by using the quantitative chromogenic *Limulus* amebocyte lysate (LAL) assay with the QCL 1000 kit (product number E50-640, lot number L3330, Biowhittaker, Inc). The kit contained lyophilized LAL, lyophilized endotoxin (*Escherichia coli* 0111:B4), and chromogenic substrate. Lyophilized endotoxin was resuspended in pyrogen-free water as stock solution. Serial endotoxin dilutions were made with pyrogen-free water to cover the concentration range of 0.1 to 1.0 EU/mL.

Samples were diluted 1:10 with sterile endotoxin-free water and heat treated for 10 minutes at 70°C to remove inhibitors. Then they were kept at room temperature for 15 minutes. Samples were processed in microtiter plates. Fifty microliters of sample was incubated for 30 minutes at 37°C with 50 μL of LAL. In the second step 100 μL of chromogenic substrate was added, and the mixture was incubated for 6 minutes at 37°C. The reaction was terminated with 100 μL of 10% sodium dodecylsulfate solution. Absorbance of each sample was read at 405 nm in an enzyme-linked immunosorbent assay plate reader. The concentrations of endotoxins in the samples were calculated from standard endotoxin measurements.

Statistical Analysis

Data are expressed as the means ± SD. The significance of difference between groups was assessed by using the Student *t* test, the Mann-Whitney *U* test, the χ^2 test, or the Fischer exact test, as appropriate.

Results

Patients

The patients' clinical data are listed in Table 1. No great variation was observed among patients' demographic data. None of the patients in both study groups required perioperative pharmacologic support, including inotropes and vasopressors. All of the patients recovered uneventfully, except for 2 patients in the CPB group, who required surgical re-exploration for bleeding. All blood, urine, and catheter culture results were negative.

Endotoxin Concentrations in Samples

Endotoxins were not found in the prime solutions in the CPB group or in samples obtained from transfused blood and blood products in both study groups.

Figure 1 shows blood endotoxin levels. Endotoxin concentrations at different time points were as follows: (1) 0.38 ± 0.2 EU/mL after sternotomy in the off-pump group and 0.42 ± 0.2 EU/mL in the CPB group ($P < .6$); (2) 1.96 ± 0.7 EU/mL during the coronary occlusion period of the last distal anastomosis in the off-pump group and 2.7 ± 0.4 EU/mL during aortic crossclamping in the CPB group ($P < .0011$); (3) 1.93 ± 0.7 EU/mL after removal of the coronary occlusion sutures in the off-pump group and

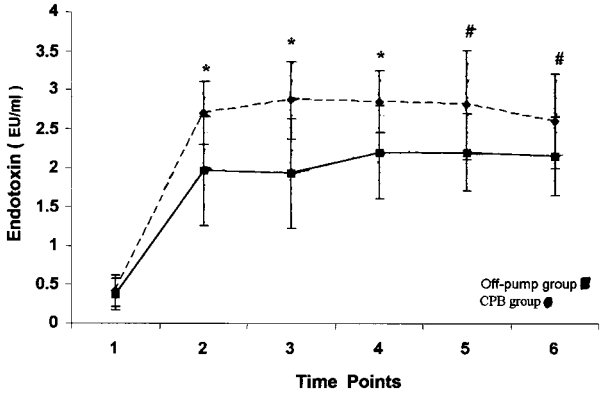

Figure 1. Endotoxin levels. Data are presented as means, and *error bars* represent SDs. For sampling time points, see text. *$P <$ .005; #$P <$ .05.

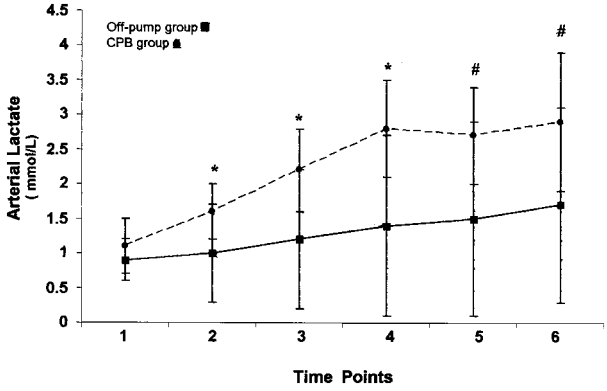

Figure 3. Arterial lactate levels. Data are presented as means, and *error bars* represent SDs. For sampling time points, see text. *$P <$ .001; #$P <$ .05.

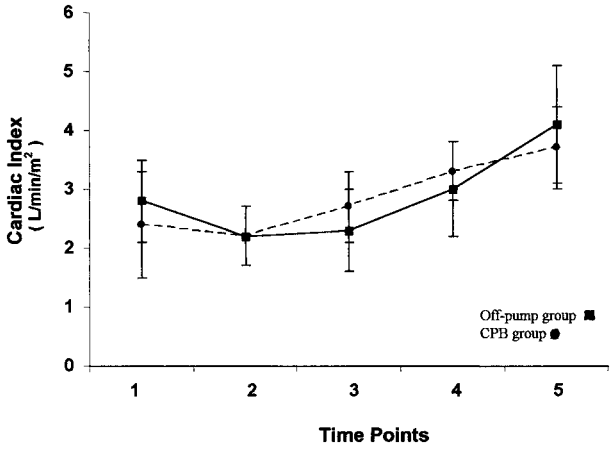

Figure 2. Cardiac indices assesed by means of thermodilution. Data are presented as means, and *error bars* represent SDs. For measurement time points, see text.

2.87 ± 0.5 EU/mL after removal of the aortic crossclamp in the CPB group ($P <$ .0008); (4) 2.2 ± 0.6 EU/mL 30 minutes after the completion of all distal anastomoses in the off-pump group and 2.85 ± 0.4 EU/mL immediately after weaning from CPB in the CPB group ($P <$ .0062); (5) 2.2 ± 0.5 EU/mL 1 hour postoperatively in the off-pump group and 2.81 ± 0.7 EU/mL in the CPB group ($P <$ .0014); and (6) 2.15 ± 0.5 EU/mL 12 hours postoperatively in the off-pump group and 2.6 ± 0.6 EU/mL in the CPB group ($P <$ .04).

Cardiac Index, Arterial Lactate, and Rectal Temperature Measurements

Cardiac index and arterial lactate measurements are shown in Figures 2 and 3, respectively.

The maximum rectal temperature of the patients within 12 hours postoperatively was 37.9°C ± 0.7°C in the off-pump group and 38.8°C ± 0.7°C in the CPB group, which showed a significant difference ($P <$ .009).

Discussion

Endotoxemia is known to occur in most patients undergoing operations involving CPB, although the origin of the endotoxin remains a subject of great debate.[2-11]

Two possible factors, endogenous and exogenous, have been incriminated.[18] Normally, intestinal flora contain a large amount of endotoxin from gram-negative microorganisms.[19] Many of the bacteria in the intestine are dead, and thus endotoxin can also enter the blood stream contained within cell-membrane fragments of dead bacteria.[19] Previous studies have shown that CPB causes splanchnic ischemia by means of capillary closure and shunting.[20] Intestinal ischemia impairs intestinal wall permeability and causes the endotoxin to pass into circulation.[21] Jansen and associates[10] proposed that endotoxin levels might be related to the degree of initial vasoconstriction and the degree of the hypo-oncotic state during CPB. The type of flow during CPB has been postulated to play a role in inducing endotoxin release.[3,7,11]

It has been shown that endotoxin levels increase when hypothermia deepens, probably because of intestinal ischemia, decreasing intestinal motility, lower enzyme activity, and impaired function of Kuppfer cells.[22]

Endotoxins are ubiquitously present in the environment and are stable and difficult to eliminate. Endotoxin has been detected in intraoperatively administered fluids.[2] Apart from the infusion solution components of the extracorporeal setup, several types of pharmaceutical and medical technical materials, such as dialysis membranes, filters, and surgical material, such as instruments and gloves, must also be considered.[8] Better procedures in making fluids and utensils more pyrogen free might be of great importance, together with optimal aseptic procedures in the operating room.[2]

In our study comparisons for time points 2, 3, and 4 were not identical in both groups. Despite these artificial comparisons in terms of time periods, the differences were significant at all times during both operations, except after sternotomy. Although we detected relatively lower endotoxin levels in the off-pump surgery group compared with those in the conventional CPB group, endotoxemia occurred during off-pump surgery. This might be explained by exogenous factors. On the other hand, with the hemodynamic changes frequently observed during off-pump surgery, it is not unlikely that endogenous release of endotoxins from the intestines might have contributed to the endotoxemia. The gut is one of the most susceptible organs to hypoperfusion during conditions of trauma or stress.[23-25] Studies have shown that during periods of hypovolemia, the gut vasoconstricts, thus shunting blood toward "more vital organs," such as the heart and brain.[23-25] In addition to hypovolemic administration of vasoconstrictors, such as phenylephrine, to increase blood pressure, endogenously released vasoconstrictors (eg, angiotensin II, thromboxane A2, and vasopressin), low central venous pressures, and lower fluid balance were associated with intestinal hypoperfusion.[19] Hemodynamics change during different stages of the operation; in other words, cardiac index decreases during elevation of the heart, and temporary occlusion of the coronary arteries might likely lead to a reduction of gut perfusion. However, in this study we did not collect simultaneous blood samples from the inferior and superior vena cavae, and we compared the endotoxin concentrations to support this hypothesis.

The results of the study have shown that blood lactate levels were significantly higher in the CPB group. This might be explained by numerous pathophysiologic effects of CPB and hypothermia. However, an anaerobic metabolism is not the only source of lactate. Evidence suggests that the lactate accumulation in sepsis is not the result of oxygen deprivation.[26] The culprit might be endotoxin, which blocks the actions of pyruvate dehydrogenase enzyme that moves pyruvate into mitochondria. Pyruvate then accumulates in the cell cytoplasm and is converted to lactate. Endotoxin infusion to animals has been associated with a progressive increase in blood lactate levels.[27] The relatively high endotoxin levels in the CPB group might have been a contributing factor to the high lactate levels seen in this group compared with those in the off-pump group. However, whether this effect of endotoxin could be evident at the observed endotoxin concentrations in the present study is questionable.

Endotoxins have been recognized to have profound biologic effects with the ability to activate different cascade systems, such as complement, coagulation, and white blood cells, and might produce a range of clinical symptoms from headache, chills and myalgia over fever, hypotension, and metabolic acidosis to septic shock and disseminated intravascular coagulation.[28-30] Despite the relatively high endotoxin levels in the CPB group, no intraoperative or postoperative complications were found. There are, however, good reasons to believe that endotoxemia results are negative for the postoperative condition of the patient and should therefore be minimized.[8] Some of the endotoxin-related symptoms might be easily masked and overlooked in the early postoperative course.

In conclusion, this study has shown that endotoxemia during CABG surgery seems mainly to be associated with CPB procedure. Although the initiation of endotoxemia is coincident with CPB, the mechanisms remain to be elucidated. The relatively lower endotoxin levels observed in off-pump surgery might contribute to improved recovery from surgical revascularization procedures, particularly in critically ill patients.

CSP

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