Original Article

Cytokine Responses to Myocardial Revascularization on Cardiopulmonary Bypass: Intermittent Crossclamping versus Blood Cardioplegic Arrest

Song Wan, MD, PhD, FRCS, Ahmed A. Arifi, MD, FRCS, Innes Y. P. Wan, FRCS, Calvin S. H. Ng, MB, BS (Hons.), Tak Wai Lee, FRCS, and Anthony P. C. Yim, MD, FRCS

Background: The inflammatory responses to the different myocardial protection approaches have not been thoroughly investigated. We sought to study the cytokine responses to cardiopulmonary bypass (CPB) using the intermittent aortic crossclamping with ventricular fibrillation (IAC) versus blood cardioplegic arrest (BC) techniques.

Methods: Perioperative plasma levels of tumor necrosis factor α (TNF-α), interleukins (IL) 6, 8, 10, and cardiac troponin-I (cTnI) were measured serially before surgery, at the end of surgery, and 2, 24, and 48 hours after elective coronary revascularization in 31 patients (IAC: n=15; BC: n=16).

Results: Demographics, preoperative status, and number of grafts (IAC: 2.7±0.6; BC: 3.0±0.4) were similar between groups. No major complications occurred in either group. The total ischemic time and duration of CPB were shorter in group IAC (17±5 and 58±10 min vs 45±14 and 81±21 min; both $p<0.01$). Although the intergroup difference in postoperative cTnI levels was not statistically significant, the release of both TNF-α and IL-8 were higher in group IAC than in group BC. However, IL-6 and IL-10 levels were lower after surgery in group IAC.

Conclusion: Despite the duration of ischemia and CPB being shorter, intermittent aortic crossclamping is associated with an enhanced pro-inflammatory but a reduced anti-inflammatory response compared to the cardioplegic arrest technique. Its clinical relevance needs to be further defined. (Ann Thorac Cardiovasc Surg 2002; 8: 12–17)

Key words: cardiac troponin, cardioplegia, cardiopulmonary bypass, cytokine, inflammatory response, myocardial protection

Introduction

The past two decades have witnessed a growing demand in treating ischemic heart disease worldwide, as well as a greater success achieved by coronary artery bypass grafting (CABG) surgery. While different intra-operative cardioplegic arrest protocols have been widely applied in clinical myocardial protection,[1] the technique of intermittent aortic crossclamping with ventricular fibrillation has also been proven to be safe and effective.[2-6] It is evident, however, that a complex systemic inflammatory cascade following the use of cardiopulmonary bypass (CPB) contributes to the development of postoperative morbidity including cardiac dysfunction.[7] Some recent clinical investigations supported the concept that improved myocardial preservation could be accomplished by the avoidance of CPB during CABG,[8,9] although the exact underlying mechanisms are yet to be fully understood.

Any clinical study of CABG with or without CPB is in fact a comparison of two entirely different surgical approaches, of which CPB is only a part. Hence, it is interesting to investigate the inflammatory response to

Frome Division of Cardiothoracic Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong

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Address reprint requests to Ahmed A. Arifi, MD, FRCS: Division of Cardiothoracic Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, People's Republic of China.

Table. Clinical data

	BC group	IAC group
No. of patients	16	15
Male/Female	11 / 5	11 / 4
Age (yrs)	65 ± 11	64 ± 9
Body surface area (m^2)	1.72 ± 0.16	1.71 ± 0.12
Old myocardial infarction	5	4
Unstable angina	4	2
Left main coronary stenosis	3	1
Cardiopulmonary bypass time (min)	81 ± 21	58 ± 10*
Aortic crossclamping time (min)	45 ± 14	17 ± 5*
No. of grafts	3.0 ± 0.4	2.7 ± 0.6

BC: blood cardioplegia; IAC: intermittent aortic crossclamping; Data are mean ± standard deviation; *p<0.01 between groups.

CPB with only one of the following conditions being changed: temperature, the use of cardioplegia, other pharmacological management (i.e., the heparin-protamine dosage), and the nature of ischemia during surgery (i.e., regional versus global). In the present study, we compared the release of cardiac troponin-I (cTnI) as well as cytokines, including tumor necrosis factor α (TNF-α), interleukin (IL)-6, IL-8, and IL-10, in patients undergoing CABG using an identical CPB protocol with or without the use of cardioplegia.

Methods

Following university ethical committee approval, a total of 31 consecutive patients with ischemic heart disease undergoing elective CABG were prospectively recruited in the current study. Those undergoing redo CABG, had a history of myocardial infarction within six weeks, with left ventricular ejection fraction less than 30%, with preoperative renal failure or chronic liver dysfunction, were excluded from the study. Informed written consent was obtained from all participating patients. Patients were divided into two groups. Two surgeons from the same surgical team performed operations for each group, respectively. One surgeon used the intermittent aortic crossclamping (IAC) technique, while the other used the blood cardioplegic arrest (BC) method. There were no clinical differences between the two groups with respect to age, gender, or symptoms (Table).

All patients received a similar balanced anesthetic regimen including fentanil and midazolam. Muscle relaxation was achieved with pancuronium. Anesthesia was maintained by continuous infusion of propofol (4-6 $mg \cdot kg^{-1} \cdot h^{-1}$). Cefuroxime (Zinacef, GlaxoWellcome, Greenford, Middlesex, UK) was given intravenously for antimicrobial prophylaxis at a dose of 1.5 eight at induction of anesthesia, followed by 0.75 g every 8 hours for 48 hours. No patients received corticosteroids or aprotinin before, during, or after surgery. Intraoperative transesophageal echocardiography monitoring was applied in every case.

The extracorporeal circuit consisted of a roller pump (Sarns 9000, 3M Health Care, Ann Arbor, Michigan) and a membrane oxygenator (Turbo, 3M Health Care, Ann Arbor, Michigan). Standard systemic heparinization (3 mg/kg) was performed and an activated clotting time of greater than 480 seconds was maintained during CPB. The pump flow was set at 2.4 L/min/m^2. No patients were cooled during CPB.

During the IAC procedures, ventricular fibrillation was induced prior to aortic crossclamping by an electrical fibrillator (Fibrillator Fi10M, Stockert Instrumente, Munich, Germany). The fibrillation was maintained over the crossclamping periods by attaching the electrode to the ventricle. Two silicone elastomer surgical tapes (Quest Medical Inc., Allen, Texas) were routinely used to temporarily occlude the coronary artery on either side of the anastomosis site. Each distal anastomosis was completed with a single running 7-0 prolene suture during a brief period of aortic crossclamping. The aortic clamp was removed at the end of each distal anastomosis and the heart was defibrillated. Construction of the corresponding proximal end was performed immediately after the distal anastomosis was completed, allowing a similar duration of myocardial reperfusion. The proximal anastomosis of the venous graft was always performed with the aid of an aortic side-biting clamp. On the other hand, patients in the BC group received intermittent antegrade normothermic (35°C) blood cardioplegia every 20 minutes, which was a mixture of 400 to 600 mL of oxygenated blood with graduated doses of potassium-magnesium solution as reported before.[10] The technique for anastomosis was identical between groups. Heparin was routinely neutralized with protamine sulfate on discontinuation of CPB.

Blood samples were collected from each patient before surgery, at the end of surgery, and 2, 24, 48 hours after surgery, using the tubes with ethylenediaminetetraacetic acid. All samples were immediately cooled down to 4°C and centrifuged (3000 g for 10 minutes at 4°C). Plasma was stored at –70°C until assay.

Data were stored and analyzed using standard computer software (StatView Software, Brainpower Inc., Calabasas, California). An unpaired t test was used for comparing clinical variables between groups. A two-way analysis of variance for repeated measures was used for comparison of cytokines and cTnI levels between the groups at each time point. Values of the biochemical parameters are presented as mean ± standard error of the mean. Clinical data are shown as mean ± standard deviation. P-values less than 0.05 were considered to indicate statistical significance.

Results

Fifteen patients underwent intermittent aortic crossclamping with ventricular fibrillation for myocardial protection during CABG, while the other 16 patients experienced blood cardioplegic arrest. Their clinical details are summarized in the Table. Duration of CPB and the total cumulative ischemic time were both shorter in the IAC group, but the number of grafts was similar between the two groups. Complete revascularization was achieved in all patients. Each patient received an internal mammary artery graft on the left anterior descending coronary artery. Only one patient in the BC group required re-exploration for bleeding after surgery.

There was no hospital mortality or major complications in either group. No patient needed excessive inotropic support in the postoperative period. No intergroup differences were detected regarding the time on ventilator and the stay in the intensive care unit or in hospital. No postoperative stroke was noted in either patient group. In addition, no new Q-wave on the electrocardiogram was observed after surgery in any patients and none of the patients received perioperative intra-aortic balloon pumping.

TNF-α levels were transiently elevated after surgery in the IAC group, but not in the BC group. Meanwhile, the release of IL-8 was also significantly higher in the IAC groups two hours after surgery. However, the values of these two cytokines were equally low in both groups on postoperative days 1 and 2 (Fig. 1).

In contrast, IL-6 levels both at the end of operation and 24 hours after surgery were significantly lower in the IAC group than in the BC group. IL-10 production at the end of surgery remained relatively low in the IAC group but was markedly enhanced in the BC group (Fig. 1).

No statistical significant differences were noted between the two groups regarding serial cTnI measurements (Fig. 2).

Discussion

For the purpose of myocardial protection during CABG, both the intermittent aortic crossclamping with ventricular fibrillation and the cardioplegic arrest techniques have been successfully employed in the clinical setting. Although the former method might not be as popular nowadays as blood cardioplegic arrest, its clinical safety and efficiency has been repeatedly proven even under high-risk circumstances.[2,11,12] In earlier studies, few significant differences between the two techniques were demonstrated when using hemodynamic data, biochemical parameters, and clinical measurements such as hospital mortality as the end points.[2-6] As far as myocardial injury is concerned, the activity of oxygen free radicals during CABG following both types of myocardial protection has been shown to be similar.[13] The postoperative cardiac troponin-T levels were not higher in patients receiving intermittent crossclamping and fibrillation in several studies,[5,13-15] except in one,[16] when compared with those after crystalloid or blood cardioplegic arrest. However, an increased use of the intra-aortic balloon pumping was discovered in patients following the intermittent aortic crossclamping with fibrillation in a recent retrospective analysis.[12]

Meanwhile, previous experimental observations remain controversial. The UCLA group reported that blood cardioplegia may be superior to intermittent ischemia in terms of preserving left ventricular contractility and retaining myocardial adenosine triphosphate.[17] It has also been noted that the myocardium distal to a critical stenosis suffers a progressive reduction in endocardial and epicardial blood flow during ventricular fibrillation, and such a phenomenon was suggested to play a role in the pathogenesis of intra-operative myocardial injury.[18] In another earlier study in a canine model, intermittent crossclamping was associated with a higher degree of myocardial edema and poorer diastolic compliance than continuous aortic crossclamping with multidose hypothermic cardioplegia.[19] Nevertheless, it was recently suggested that intrinsic myocardial protection could be achieved with intermittent crossclamping and fibrillation in the rat heart, which was even more effective than single-dose cardioplegic arrest with respect to left ventricular function recovery.[20]

The present study evaluated the effects of cardioplegia on the production of cytokines following on-pump CABG. Surprisingly, the intermittent crossclamping and ventricular fibrillation approach was associated with an

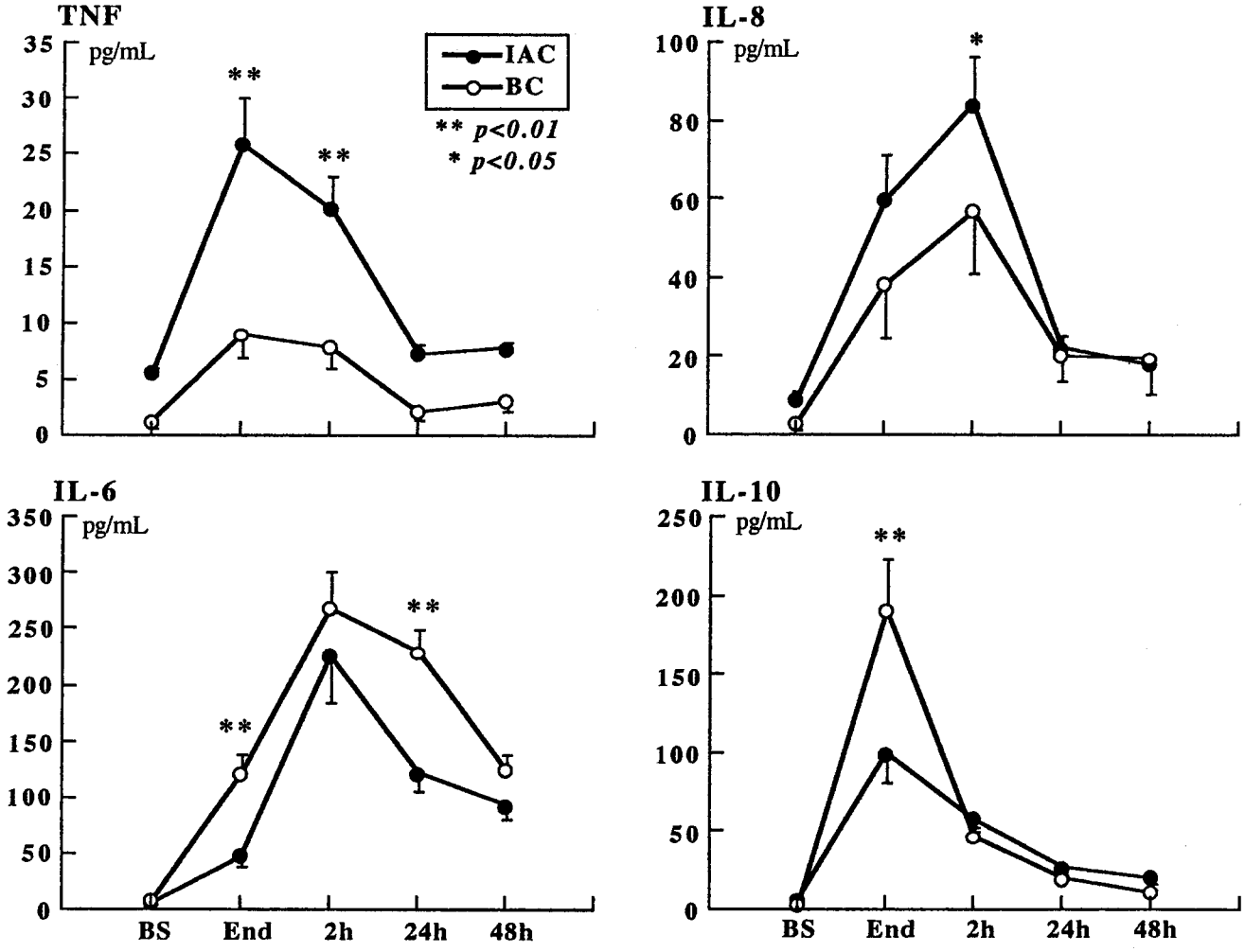

Fig. 1. Plasma levels of tumor necrosis factor α (TNF), interleukin (IL)-6, IL-8, and IL-10 in patients undergoing cardiopulmonary bypass for myocardial revascularization, using the intermittent aortic crossclamping with ventricular fibrillation (IAC, n=15) versus blood cardioplegic arrest (BC, n=16) techniques.
Data are mean ± SEM. BS: before surgery; End: end of surgery; 2 h, 24 h, and 48 h: time points after surgery.

enhanced pro-inflammatory (TNF-α and IL-8) but a reduced anti-inflammatory (IL-6 and IL-10) response compared with the blood cardioplegic arrest technique. As expected, the CPB time and the cumulative ischemic duration were shorter in the former group. These differences are inherent in each technique and therefore almost unavoidable.

The mechanism behind these observations is still unclear and deserves further evaluation, since myocardial ischemia-reperfusion injury is currently believed to be an acute inflammatory process in which the cytokine network plays a crucial role. In patients undergoing CPB for CABG, the ischemic-reperfused myocardium has been shown to release some pro-inflammatory cytokines, such as TNF-α and IL-8.[21] Pro-inflammatory cytokines can activate leukocytes and endothelial cells and then initiate a detrimental chain of events, whereas at least theoretically anti-inflammatory cytokines may limit these harmful effects.[9] Indeed, accumulating evidence indicates that the balance between pro- and anti-inflammatory mediators is very important in determining the extent of inflammatory injury.[7-9,22] Despite the determined imbalance of pro- and anti-inflammatory cytokine responses in the current study, no statistical significant differences on the postoperative release of cardiac troponin-I were apparent. The trend of the cardiac troponin-I levels appeared even to be lower in the intermittent crossclamping and ventricular fibrillation group. This may be due to the short ischemic duration in our intermittent crossclamping group and, more likely, due to the

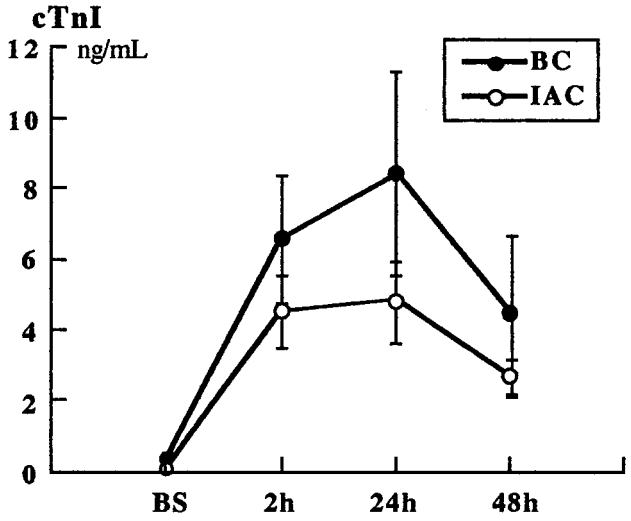

Fig. 2. Plasma levels of cardiac troponin-I (cTnI) in patients undergoing cardiopulmonary bypass for myocardial revascularization, using the intermittent aortic crossclamping with ventricular fibrillation (IAC, n=15) versus blood cardioplegic arrest (BC, n=16) techniques.
Data are mean ± SEM. BS: before surgery; 2 h, 24 h, and 48 h: time points after surgery.

fact that cytokines play only a partial role in the complex inflammatory cascade.[7] Hence, it is difficult to advocate either of the techniques based solely on the present observation.

We acknowledge that the current study is limited by the non-randomized nature. It is understandable that the use of intermittent crossclamping and ventricular fibrillation is often an individualized choice, based on a surgeon's experience. It has been emphasized that the intermittent crossclamping and ventricular fibrillation method requires careful logistical organization with special technical demand.[23] The fundamental issue, which has been recognized for more than two decades,[24] is that a prolonged ischemic duration may be particularly harmful under ventricular fibrillation. Moreover, the time ratio of ischemic duration to reperfusion duration has also been proposed to be an important factor in determining the recovery of ventricular function.[20] The prolonged duration of ischemia together with shorter periods of reperfusion may explain the less adequate myocardial protection following the application of intermittent crossclamping and fibrillation in those earlier experimental studies.[17,19] It has also been recently noted that one-fourth of deaths within 30 days following CABG may be due to myocardial ischemic-reperfusion injury and, importantly, the severity of such injury correlated with aortic crossclamping time.[25] If ventricular fibrillation can be limited within a short duration, however, it appears safe as regards to myocardial metabolism.[26] Whether the cardioprotective effect of intermittent crossclamping during clinical CABG is similar to that of "ischemic preconditioning" is still a subject of debate and to be clearly defined in future studies.

Our observations of an enhanced pro-inflammatory and a reduced anti-inflammatory response associated with the intermittent crossclamping and ventricular fibrillation technique during CABG may provide insight into an improved understanding of the underlying mechanisms in such type of myocardial protection. Its clinical relevance certainly needs to be further investigated.

References

1. Buckberg GD. Update on current techniques of myocardial protection. *Ann Thorac Surg* 1995; **60**: 805–14.
2. Bonchek LI, Burlingame MW, Vazales BE, Lundy EF, Gassmann CJ. Applicability of noncardioplegic coronary bypass to high-risk patients: selection of patients, technique, and clinical experience in 3000 patients. *J Thorac Cardiovasc Surg* 1992; **103**: 230–7.
3. Pepper JR, Lockey E, Cankovic-Darracott S, Braimbridge MV. Cardioplegia versus intermittent ischaemic arrest in coronary bypass surgery. *Thorax* 1982; **37**: 887–92.
4. Gerola LR, Oliveira SA, Moreira LFP, Dallan LAO, da Luz PL, Jatene AD. Blood cardioplegia with warm reperfusion versus intermittent aortic crossclamping in myocardial revascularization: randomized controlled trial. *J Thorac Cardiovasc Surg* 1993; **106**: 491–6.
5. Anderson JR, Hossein-Nia M, Kallis P, et al. Comparison of two strategies for myocardial management during coronary artery operations. *Ann Thorac Surg* 1994; **58**: 768–73.
6. Alhan HC, Karabulut H, Tosun R, et al. Intermittent aortic cross-clamping and cold crystalloid cardioplegia for low-risk coronary patients. *Ann Thorac Surg* 1996; **61**: 834–9.
7. Wan S, LeClerc JL, Vincent JL. Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. *Chest* 1997; **112**: 676–92.
8. Wan S, Izzat MB, Lee TW, Wan IYP, Tang NLS, Yim APC. Avoiding cardiopulmonary bypass in multivessel-CABG reduces cytokine response and myocardial injury. *Ann Thorac Surg* 1999; **68**: 52–7.
9. Wan S, Yim APC. Cytokines in myocardial injury: impact on cardiac surgical approach. *Eur J Cardiothorac Surg* 1999; **16** (suppl 1): 107–11.
10. Calafiore AM, Teodori G, Mezzetti A, et al. Intermittent antegrade warm blood cardioplegia. *Ann Thorac*

Surg 1995; **59**: 398–402.
11. Taggart DP, Aratari C, Wong P, Paul EA, Wright JE. Applicability of intermittent global ischemia for repeat coronary artery operations. *J Thorac Cardiovasc Surg* 1996; **112**: 501–7.
12. Liu Z, Valencia O, Treasure T, Murday JA. Cold blood cardioplegia or intermittent cross-clamping in coronary artery bypass grafting? *Ann Thorac Surg* 1998; **66**: 462–5.
13. Cohen AS, Hadjinikolaou L, McColl A, Richmond W, Sapsford RA, Glenville BE. Lipid peroxidation, antioxidant status and troponin-T following cardiopulmonary bypass: a comparison between intermittent crossclamp with fibrillation and crystalloid cardioplegia. *Eur J Cardiothorac Surg* 1997; **12**: 248–53.
14. Taggart DP, Bhusari S, Hopper J, et al. Intermittent ischaemic arrest and cardioplegia in coronary artery surgery: coming full circle? *Br Heart J* 1994; **72**: 136–9.
15. Musumeci F, Feccia M, MacCarthy PA, et al. Prospective randomised trial of single clamp technique versus intermittent ischaemic arrest: myocardial and neurological outcome. *Eur J Cardiothorac Surg* 1998; **13**: 702–9.
16. Krejca M, Skiba J, Szmagala P, Gburek T, Bochenek A. Cardiac troponin T release during coronary surgery using intermittent cross-clamp with fibrillation, on-pump and off-pump beating heart. *Eur J Cardiothorac Surg* 1999; **16**: 337–41.
17. Follette MD, Mulder DG, Maloney JV, Buckberg GD. Advantages of blood cardioplegia over continuous coronary perfusion or intermittent ischemia: experimental and clinical study. *J Thorac Cardiovasc Surg* 1978; **76**: 604–19.
18. Ciardullo RC, Schaff HV, Flaherty JT, Gott VL. Myocardial ischemia during cardiopulmonary bypass: the hazards of ventricular fibrillation in the presence of a critical coronary stenosis. *J Thorac Cardiovasc Surg* 1977; **73**: 746–57.
19. Roberts AJ, Abel RM, Alonso DR, Subramanian VA, Paul JS, Gay WA Jr. Advantages of hypothermic potassium cardioplegia and superiority of continuous versus intermittent aortic cross-clamping. *J Thorac Cardiovasc Surg* 1980; 79: 44–58.
20. Bessho R, Chambers DJ. Experimental study of intermittent crossclamping with fibrillation and myocardial protection: reduced injury from shorter cumulative ischemia or intrinsic protective effect? *J Thorac Cardiovasc Surg* 2000; **120**: 528–37.
21. Wan S, DeSmet JM, Barvais L, Goldstein M, Vincent JL, LeClerc JL. Myocardium is a major source of proinflammatory cytokines in patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1996; **112**: 806–11.
22. Wan S, LeClerc JL, Antoine M, DeSmet JM, Yim APC, Vincent JL. Heparin-coated circuits reduce myocardial injury in heart or heart-lung transplantation: a prospective, randomized study. *Ann Thorac Surg* 1999; **68**: 1230–5.
23. Bonchek LI. Technical considerations for coronary artery bypass without cardioplegia. *J Card Surg* 1992; **7**: 333–41.
24. Buckberg GD, Hottenrott CE. Ventricular fibrillation: its effect on myocardial flow, distribution, and performance. *Ann Thorac Surg* 1975; **20**: 76–85.
25. Weman SM, Karhunen PJ, Penttilä A, Järvinen AA, Salminen US. Reperfusion injury associated with one-fourth of deaths after coronary artery bypass grafting. *Ann Thorac Surg* 2000; **70**: 807–12.
26. Runsio M, Bergfeldt L, Rosenqvist M, Owall A, Jorfeldt L. Changes in human coronary sinus blood flow and myocardial metabolism induced by ventricular fibrillation and defibrillation. *J Cardiothorac Vasc Anesth* 1998; **12**: 45–50.