Off-Pump Coronary Artery Bypass Surgery Is Associated with Reduced Neutrophil Activation as Measured by the Expression of CD11b: A Prospective Randomized Study

(#2002-10208 . . . January 16, 2003)

Sharif Al-Ruzzeh, FRCS,[1] Ginette Hoare, BSc,[1] Nandor Marczin, PhD,[1] George Asimakopoulos, FRCS,[2] Shane George, FRCA,[1] Kenneth Taylor, FRCS,[2] Mohamed Amrani, FRCS[1]

[1]National Heart and Lung Institute, Imperial College of Science, Technology and Medicine, Harefield Hospital, Middlesex; [2]Hammersmith Hospital, London, United Kingdom

Dr. Al-Ruzzeh

ABSTRACT

Background: Coronary artery bypass grafting (CABG) surgery is associated with systemic inflammation. Activation of neutrophils is a crucial step in inflammation and results in neutrophil sequestration within the tissues. One of the potential advantages of performing off-pump coronary artery bypass (OPCAB) surgery is the attenuation of the systemic inflammatory response. This prospective randomized study compares neutrophil activation in patients undergoing OPCAB versus those undergoing CABG with cardiopulmonary bypass (CPB).

Methods: Twenty patients undergoing primary isolated CABG were randomly divided prospectively into 2 groups: 1 group underwent CABG with CPB, and the other group underwent OPCAB. Central venous blood samples were obtained before skin incision and at 15 minutes, 60 minutes, 2 hours, 5 hours, and 24 hours following the initiation of CPB or application of the stabilization device. Differential white cell counts were measured with routine laboratory techniques. CD11b surface expression on neutrophils was measured by flow cytometry. Interleukin 8 levels in the plasma were measured by enzyme-linked immunosorbent assays.

Results: The 2 groups were matched with respect to preoperative and operative characteristics. White cell and neutrophil counts rose in both groups following the operation but were significantly higher in the OPCAB group at 5 hours ($P < .001$ and $P = .002$, respectively). Interleukin 8 concentrations were significantly higher in the CPB group at 5 hours following the initiation of CPB ($P = .034$). CD11b levels were significantly higher in the CPB group at 60 minutes ($P = .002$).

Conclusion: This prospective randomized study demonstrates that the activation of circulating neutrophils as measured by CD11b expression is lower following OPCAB than in CPB. Although OPCAB is associated with significantly higher neutrophil counts, these neutrophils exhibit fewer activation markers. The lower postoperative neutrophil counts occurring in the CPB group may be explained by the activation and consequent sequestration of the neutrophils in the CPB circuit and tissues.

INTRODUCTION

The systemic inflammatory response is an important complication of coronary artery bypass (CABG) surgery. It consists of many components that represent the pathophysiological responses initiated by cardiopulmonary bypass (CPB), ischemia/reperfusion of the heart and lung, and operative trauma [Asimakopoulos 1999].

Activation of humoral inflammatory mediators such as complement and factor XII results in activation of leukocytes and endothelial cells. This process is followed by the systemic secretion of cytokines and other inflammatory mediators. The neutrophil is a crucial participant in the inflammatory process because of its rapid activation and response to chemotactic stimuli. The induced expression of adhesion molecules on activated neutrophils and endothelial cells results in the sequestration of neutrophils within the tissues. Activated neutrophils secrete such enzymes as elastase, myeloperoxidase, and lactoferrin that promote endothelial damage and neutrophil extravasation [Weiss 1989, Boyle 1997].

CPB is often associated with an initial neutropenia followed by postoperative neutrophilia [Van Eeden 1995]. The leukocyte integrin CD11b is an adhesion molecule that promotes firm adhesion of neutrophils to endothelial cells and also serves as a reliable early indicator of neutrophil activation. Its expression is increased during CPB [Le Deist 1995, Mathew 1995, Asimakopoulos 1998].

Interleukin 8 (IL-8) belongs to the group of chemokines that are cytokines of small molecular weight acting as chemoattractants for leukocytes. Raised plasma levels of IL-8 have been detected following the use of CPB [Frering 1994, John 1998].

One of the potential advantages of performing off-pump coronary artery bypass (OPCAB) surgery is the reduction in the systemic inflammatory response. Previous studies have demonstrated that OPCAB is associated with reductions in the plasma concentrations of certain inflammatory markers, compared with plasma concentrations in patients who have undergone CPB [Ascione 2000, Matata 2000]. The present prospective randomized study compares neutrophil activation in patients who have undergone OPCAB with that in patients who have undergone CPB.

MATERIALS AND METHODS

Patient Groups

An ethics committee approval was obtained. Twenty patients who were to undergo primary isolated CABG were randomly divided prospectively into 2 groups. Randomization was carried out with a computer program that uses minimization and block randomization on the basis of patient age, sex, diabetes occurrence, and left ventricular function. One group (n = 10) underwent CABG with CPB, and the other group (n = 10) underwent CABG with OPCAB. Patients were excluded from the study if they met any of the following criteria: severe left ventricular dysfunction (ejection fraction ≤30%), renal failure (creatinine level ≥180 μmol/L or active renal replacement therapy), concomitant cardiac surgery requiring CPB, or emergency surgery following angiographic intervention.

Anesthetic and Operative Techniques

Anesthetic premedication included morphine 10 mg, metoclopramide 10 mg, and temazepam 10 mg administered the morning of the operation. The general anesthetic technique comprised a low-intermediate dose of opioid (usually fentanyl, 8-15 μg/kg) and a propofol infusion (3 mg/kg per hour). Additional monitoring by transesophageal echocardiography was undertaken when necessary.

Off-Pump (OPCAB). Anticoagulation was achieved with 150 U/kg of heparin. The activated clotting time was maintained above 250 seconds. Heparin effects were reversed with protamine at the end of the procedure. The operation was performed through a median sternotomy with the Octopus 3 (Medtronic, Minneapolis, MN, USA) stabilization device. Mean arterial blood pressure was maintained between 50 and 70 mm Hg during the procedure by means of repositioning the heart and the selective use of vasoconstrictors. Other measures to improve cardiac output, such as elevating the feet and increasing the heart rate, were used as appropriate. A standby perfusionist with a primed bypass circuit was available for all cases.

On-Pump (CPB). Anticoagulation was achieved with 300 U/kg of heparin. The activated clotting time was maintained above 480 seconds. Heparin effects were reversed with protamine at the end of the procedure. The operation was performed through a median sternotomy, and CPB was instituted with a single right atrial cannula and an ascending aorta perfusion cannula. Standard bypass management included membrane oxygenators and nonpulsatile flow of 2.4 L/min per m^2 with a mean arterial blood pressure of greater than 50 mm Hg. Myocardial protection was achieved with intermittent cold blood cardioplegia (4:1 blood-crystalloid ratio).

Collection and Processing of Samples

Time Points. Central venous blood samples were obtained at the following time points:
- t-1, before the skin incision to obtain the preoperative baseline;
- t-2, 15 minutes after initiation of CPB or application of Octopus 3;
- t-3, 60 minutes after initiation of CPB or application of Octopus 3;
- t-4, 2 hours after initiation of CPB or application of Octopus 3;
- t-5, 5 hours after initiation of CPB or application of Octopus 3;
- t-6, 24 hours after initiation of CPB or application of Octopus 3;
- t-7, 4 days after initiation of CPB or application of Octopus 3.

White Cell Counts. Blood samples were placed immediately into EDTA-containing tubes. Differential white cell counts were measured with routine hematology laboratory techniques at time points t-1, t-5, t-6, and t-7.

CD11b. Blood samples (5 mL) were obtained from patients and placed immediately into heparin-containing tubes at time points t-1, t-2, t-3, and t-4. The blood sample tubes were immediately placed on ice and directly processed for flow cytometric analysis. The technique our group uses for the flow cytometric analysis of neutrophils has been described [Asimakopoulos 2000]. Briefly, blood samples were placed in 12 ± 75 mm polystyrene tubes (Falcon; Becton Dickinson UK, Cowley, UK) with, per condition, 90 μL whole blood and 10 μL primary antibody (100 μg/mL) directed against the CD11b component of integrin Mac-1. Primary incubations were carried out on ice for 15 minutes. Each antibody was matched at each time point to an irrelevant isotypic control, which is an antibody that is produced by the same host as the main antibody and displays nonspecific binding characteristics typical of the host. After 2 washes with phosphate-buffered saline, fluorescein isothiocyanate–conjugated secondary antibodies were added at the manufacturer's recommended concentrations (Sigma Chemical Company, Dorset, UK), and incubation was continued for an additional 15 minutes. Erythrocytes were lysed for 60 seconds by the addition of 1 mL Coulter whole blood lysing reagent and fixed in 250 μL fixative solution (Beckman Coulter Electronics, Luton, UK). Lysed samples were read on a flow cytometer (EPICS XL, Beckman Coulter Electronics) immediately afterwards. Neutrophil populations were identified by their characteristic forward-and-side-scatter profiles. Fluorescent intensities of experimental versus isotypic control antibodies were presented as the relative fluorescence intensity (ratio of experimental mean fluorescence intensity to the irrelevant isotypic control mean fluorescence intensity, where a relative fluorescence intensity of 1.00 indicates no expression).

Preoperative Characteristics and Postoperative Outcome*

Variable	CPB (n = 10)	OPCAB (n = 10)	P
Preoperative characteristics			
Age, y†	63.9 ± 10.9	63.8 ± 8.4	NS
Female sex, n	1	2	NS
Family history, n	6	5	NS
Hypercholesterolemia, n	8	9	NS
Hypertension, n	6	5	NS
Smoker, n	7	7	NS
Previous MI, n	5	6	NS
Angina class III or IV, n	6	6	NS
Dyspnea class III or IV, n	2	3	NS
Ejection fraction >50%, n	7	7	NS
Operative characteristics			
No. of grafts†	3.4 ± 0.3	3.5 ± 0.2	NS

*CPB indicates cardiopulmonary bypass; OPCAB, off-pump coronary artery bypass; NS, not significant; MI, myocardial infarction.

†Data are presented as the mean ± SD.

Interleukin 8. Blood samples were placed into heparin-containing tubes, at time points t-1, t-3, t-4, t-5, and t-6. The tubes containing blood were placed on ice and immediately centrifuged at 3600 rpm (3000g) for 15 minutes to obtain plasma exclusive of platelets and blood cells. Samples were then stored at –80°C until analysis. Enzyme-linked immunosorbent assays for IL-8 were performed in a 96-well microplate format. The microplates and reagents were supplied by the manufacturer. All reagents, including samples, were brought to room temperature before use, and assays were carried out according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Aspirations and washes were performed manually. Samples were read at the recommended wavelengths (450 and 540 nm) with a microplate reader (Multiskan EX; Labsystems, Beverly, MA, USA). All plasma and standard samples were assayed in duplicate, and concentrations were calculated from a standard curve.

Statistical Analysis

Numerical variables are presented as the mean ± SD for both patient groups and compared via the Student t test or the Mann-Whitney test when appropriate. Patient characteristics are compared with the Fisher exact test or the chi-square test where appropriate. Values of $P < .05$ indicate a statistically significant difference.

RESULTS

Clinical Data

The clinical data regarding preoperative and operative characteristics are listed in the Table. The 2 groups were matched with respect to preoperative and operative characteristics.

White Cell Counts

Comparison of the patient groups revealed significantly higher total white cell and neutrophil counts in the OPCAB group at 5 hours (t-5); $P < .001$ (Figures 1 and 2).

CD11b

Relative fluorescence intensities of neutrophils for CD11b were significantly higher in the CPB group at 60 minutes (t-3) than in the OPCAB group; $P = .002$ (Figure 3).

Interleukin 8

IL-8 was undetectable preoperatively and at 15 minutes in both groups. IL-8 levels were significantly higher in the CPB group at 5 hours (t-5) at $P = .034$, but no significant differences were apparent between the 2 groups at any other time point (Figure 4).

DISCUSSION

As shown by the plasma levels of IL-8 and the surface expression of CD11b on peripheral blood neutrophils, this prospective randomized study demonstrates that OPCAB is associated with a reduced systemic inflammatory response. This result suggests that OPCAB may be a means of reducing
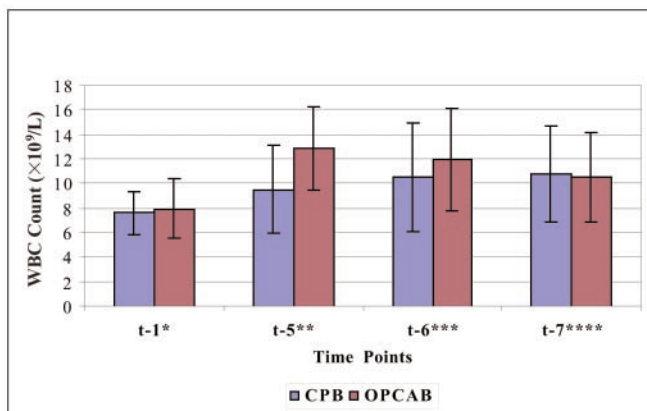

Figure 1. White blood cell (WBC) count. CPB indicates cardiopulmonary bypass; OPCAB, off-pump coronary artery bypass. Indicated P values are .528 (*), <.001 (**), .216 (***), and .708 (****).
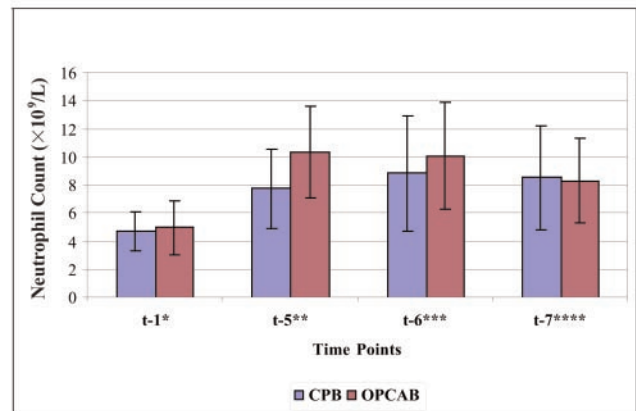

Figure 2. Neutrophil count. CPB indicates cardiopulmonary bypass; OPCAB, off-pump coronary artery bypass. Indicated P values are .564 (*), .002 (**), .087 (***), and .957 (****).
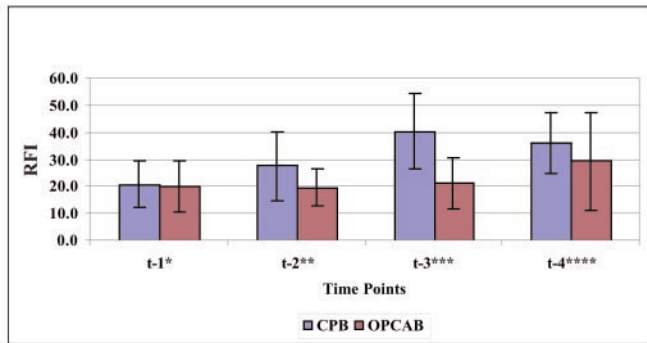
Figure 3. CD11b expression on neutrophils. RFI indicates relative fluorescence intensity; CPB, cardiopulmonary bypass; OPCAB, off-pump coronary artery bypass. Indicated *P* values are .835 (*), .098 (**), .002 (***), and .334 (****).

the neutrophil-led pathway of the systemic inflammatory response to CABG surgery.

Neutrophil mobilization from the bone marrow, neutrophil activation, and adhesion to endothelium and extravasation are decisive steps in the systemic inflammatory response. Mobilization into the circulation and activation are promoted by certain cytokines. After exiting the bone marrow, neutrophils assume their role in host defense and have a half-life of 7 to 10 hours in the circulation. Subsequently, neutrophils are drawn to inflammatory sites by specific chemotactic factors. The stimulation of neutrophil surface receptors by these factors leads to neutrophil movement via the modification of intracellular proteins. Components of the complement cascade system and IL-8 are some of the most potent neutrophil chemoattractants [Jandl 1996].

In addition to stimulating chemotaxis, the binding of inflammatory cytokines to their neutrophil surface receptors triggers enzymatic reactions that lead to the so-called respiratory burst that places the neutrophil in an activated state. Activated neutrophils interact intensively with endothelial cells in an adhesion cascade that precedes the entrance of the neutrophils into tissue. Adhesion molecules play an important role during inflammation on both sides of the endothelial barrier, first by helping to recruit leukocytes from the vascular compartment to the vessel wall and subsequently by modulating their responsiveness within the tissues by transducing signals from the subendothelial matrix. The integrins constitute a family of adhesion molecules that participate in leukocyte–endothelial cell adhesion and extravasation. The integrin CD11b binds to ligands such as complement factor iC3b and fibrinogen [Albelda 1994]. Although integrins exist on the surface of leukocytes in a relatively inactive form under normal conditions, inflammatory insults result in increased expression and changes in their activation state [Springer 1994]. Up-regulation of CD11b indicates a neutrophil activation that is associated with increased ability of the neutrophil to adhere to endothelium, which is a prerequisite for neutrophil-induced tissue damage.

After extravasation, neutrophils release toxic enzymes through degranulation. Enzymes promoting production of highly toxic oxygen-derived free radicals and proteases destroy

normal cells and dissolve the extracellular matrix [Weiss 1989]. This exaggerated form of inflammatory response may result in major organ dysfunction and, occasionally, death.

In investigating the mechanisms of the systemic inflammatory response to CPB, we have focused on different aspects of neutrophil activation. We measured total blood white cell and neutrophil cell counts as markers of neutrophil mobilization. We also measured plasma levels of IL-8, a neutrophil chemoattractant that is produced by various cell types, including activated neutrophils. Surface activation of CD11b was chosen as a specific indicator of neutrophil activation.

In contrast with the results of previous reports [Ascione 2000], total white cell counts and neutrophil counts were higher in the OPCAB group in our study. A potential explanation for these different findings is that activated neutrophils become "trapped" within tissues during CPB. Previous early work carried out by our group demonstrated that during CABG surgery with CPB and after the administration of protamine the neutrophil count in the pulmonary artery exceeds the count in the systemic arterial blood, suggesting that neutrophils are sequestered in the lungs [Braude 1986].

Previous studies demonstrated increased plasma levels of IL-8 in CPB patients compared with OPCAB patient groups [Diegeler 2000, Matata 2000]. In our study, detectable levels of IL-8 were measured only in the CPB group. Although these data demonstrate a temporal relationship between IL-8 and neutrophil numbers after CPB, there is a dissociation of IL-8 and leukocytosis in the OPCAB group. This result suggests that although IL-8 can be considered a stimulus for neutrophil mobilization during CPB, it may not be responsible for neutrophilia in the OPCAB patients.

The reason for the difference in IL-8 levels between the 2 groups is not entirely clear. The inflammatory injury is presumably reduced in OPCAB because of an absence of contact between blood components and the CPB circuit and also because of less myocardial ischemia. Indeed, myocardial injury as assessed by the postoperative release of troponin I and myocardial muscle creatine kinase isoenzyme appears to be lower after OPCAB than after CPB [Ascione 1999,
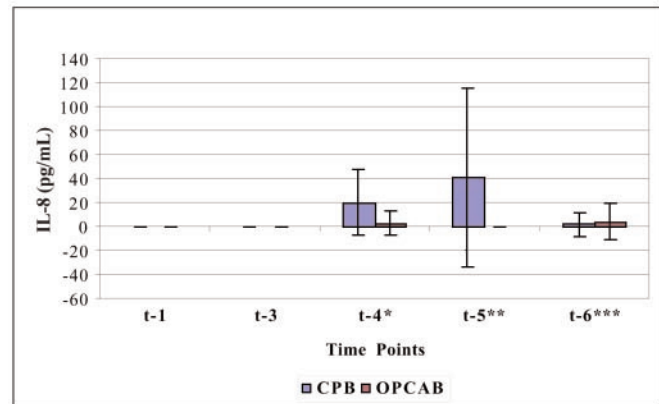

Figure 4. Interleukin 8 (IL-8) concentration. CPB indicates cardiopulmonary bypass; OPCAB, off-pump coronary artery bypass. Indicated *P* values are .104 (*), .034 (**), and .911 (***).

Czerny 2000, Wildhirst 2000]. However, the difference in the degree of myocardial injury between the 2 groups does not fully explain the difference in IL-8 levels. Although IL-8 is produced by the ischemic myocardium, the heart is not the main source of IL-8 after coronary reperfusion [Karube 1996, Lango 2001]. IL-8 gene transcription occurs in activated monocytes and neutrophils in inflammatory conditions, and, therefore, it is likely that most of the IL-8 detectable in plasma as a result of CPB originates from endothelial cells [John 1998].

It has previously been demonstrated that CD11b is up-regulated at an early postoperative stage on the surface of neutrophils in patients undergoing cardiac surgery with CPB [Asimakopoulos 1998]. Work carried out previously by our group has shown increased CD11b expression on neutrophils at 15 minutes of CPB, a result that is consistent with the main body of literature on this subject [Asimakopoulos 2000]. To our knowledge, the present article is the first literature report showing an absence of CD11b up-regulation on the surface of neutrophils in patients undergoing OPCAB. However, it is important to emphasize that up-regulation of CD11b is not a necessary factor for neutrophil–endothelial cell interaction. It can also be achieved through the action of other adhesion molecules, such as CD11a. However, our data should be interpreted as evidence of a significant difference between CPB and OPCAB in the activation status of neutrophils.

Leukocyte integrins such as CD11b and CD11a are implicated to play an important role in the pathophysiological mechanisms associated with the inflammatory response [Plow 1997]. Their role in neutrophil adhesion and transmigration are regarded as complementary, although in the CD11b-deficient mouse model CD11b has been shown to play a critical role in neutrophil binding to fibrinogen and in neutrophil degranulation, whereas neutrophil transmigration has been shown to be more dependent on CD11a [Lu 1997]. Following leukocyte activation, CD11b is rapidly mobilized from intracellular secretory granules to the neutrophil surface via chemoattractant-stimulated granule–plasma membrane fusion.

The findings of this study suggest strongly that OPCAB causes less neutrophil activation. This result may have significant clinical implications in view of the leading role that neutrophils play in the systemic inflammatory response induced by CABG surgery.

REFERENCES

Albelda SM, Smith CW, Ward PA. 1994. Adhesion molecules and inflammatory injury. FASEB J 8:504-12.

Ascione R, Lloyd CT, Gomes WJ, Caputo M, Bryan AJ, Angelini GD. 1999. Beating versus arrested heart revascularization: evaluation of myocardial function in a prospective randomized study. Eur J Cardiothorac Surg 15:685-90.

Ascione R, Lloyd C, Underwood M, Lotto A, Pitsis A, Angelini G. 2000. Inflammatory response after coronary revascularization with or without cardiopulmonary bypass. Ann Thorac Surg 69:1198-204.

Asimakopoulos G, Kohn A, Stefanou D, Haskard D, Landis RC, Taylor KM. 2000. Leukocyte integrin expression in patients undergoing cardiopulmonary bypass. Ann Thorac Surg 69:1192-7.

Asimakopoulos G, Taylor K. 1998. Effects of cardiopulmonary bypass on leukocytes and endothelial adhesion molecules. Ann Thorac Surg 66:2135-44.

Asimakopoulos G. 1999. Mechanisms of the systemic inflammatory response syndrome. Perfusion 14:269-77.

Boyle J, Pohlman T, Johnson M, Verrier E. 1997. The systemic inflammatory response. Ann Thorac Surg 64:S31-7.

Braude S, Nolop KB, Fleming JS, Krausz T, Taylor KM, Royston D. 1986. Increased pulmonary transvascular protein flux after canine cardiopulmonary bypass. Am Rev Respir Dis 134:867-72.

Czerny M, Baumer H, Kilo J, et al. 2000. Inflammatory response and myocardial injury following coronary artery bypass grafting with or without cardiopulmonary bypass. Eur J Cardiothorac Surg 17:737-42.

Diegeler A, Doll N, Rauch T, et al. 2000. Humoral immune response during coronary artery bypass grafting: a comparison of limited approach, "off-pump" technique and conventional cardiopulmonary bypass. Circulation 102(suppl 3):III95-100.

Frering B, Phillip I, Dehoux M, Rolland C, Langlois J, Desmonts J. 1994. Circulating cytokines in patients undergoing normothermic cardiopulmonary bypass. J Thorac Cardiovasc Surg 108:636-41.

Jandl JH. 1996. Blood: textbook of hematology. 2nd ed. Boston, Ma: Lippincott Williams & Wilkins. p 615-49.

John A, Galea J, Francis SE, Holt CM, Finn A. 1998. Interleukin-8 mRNA expression in circulating leukocytes during cardiopulmonary bypass. Perfusion 13:409-17.

Karube N, Adachi R, Ichikawa Y, Kosuge T, Yamazaki I, Soma T. 1996. Measurement of cytokine levels by coronary sinus blood sampling during cardiac surgery with cardiopulmonary bypass. ASAIO J 42:M787-91.

Lango R, Anisimowicz L, Siebert J, et al. 2001. IL-8 concentration in coronary sinus blood during early coronary reperfusion after ischemic arrest. Eur J Cardiothorac Surg 20:550-4.

Le Deist F, Menasche P, Kucharski C, Bel A, Piwnica A, Bloch G. 1995. Hypothermia during cardiopulmonary bypass delays but does not prevent neutrophil–endothelial cell adhesion: a clinical study. Circulation 92(suppl 2):354-8.

Lu H, Smith CW, Perrard J, et al. 1997. LFA-1 is sufficient in mediating neutrophil emigration in Mac-1–deficient mice. J Clin Invest 99:1340-50.

Matata B, Sosnowski A, Galinanes M. 2000. Off-pump bypass graft operation significantly reduces oxidative stress and inflammation. Ann Thorac Surg 69:785-91.

Mathew J, Rinder C, Tracey J. 1995. Acadesine inhibits neutrophil CD11b up-regulation in vitro and during in vivo cardiopulmonary bypass. J Thorac Cardiovasc Surg 109:448-56.

Plow EF, Zhang L. 1997. A MAC-1 attack: integrin functions directly challenged in knockout mice. J Clin Invest 99:1145-6.

Springer TA. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 76:301-14.

Van Eeden S, Miyagashima R, Haley L, Hogg J. 1995. L-selectin expression increases on peripheral blood polymorphonuclear leukocytes during active marrow release. Am J Respir Crit Care Med 151:500-7.

Weiss SJ. 1989. Tissue destruction by neutrophils. N Engl J Med 320:365-76.

Wildhirst S, Schulze C, Conrad N, et al. 2000. Reduced myocardial cellular damage and lipid peroxidation in off-pump versus conventional coronary artery bypass grafting. Eur J Med Res 5:222-8.