Effects of Off-Pump Versus On-Pump Coronary Artery Bypass Grafting: Apoptosis, Inflammation, and Oxidative Stress

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ABSTRACT

Background: It has been suggested that off-pump coronary artery bypass grafting (CABG) surgery reduces myocardial ischemia-reperfusion injury, postoperative systemic inflammatory response, and oxidative stress. The aim of this study was to measure serum malondialdehyde (MDA), high-sensitivity C-reactive protein (hs-CRP), M30, and M65 levels and to investigate the relationship between M30 levels and oxidative stress and inflammation in patients undergoing on- and off-pump CABG surgery.

Methods: Fifty patients were randomly assigned to on-pump or off-pump CABG surgery (25 patients off-pump and 25 on-pump CABG surgery), and blood samples were collected prior to surgery, and 30 minutes, 60 minutes, 6 hours, and 24 hours after CABG surgery.

Results: Compared to the on-pump group, serum MDA levels at 30 minutes, 60 minutes, 6 hours, and 24 hours after the CABG surgery were significantly lower in the off-pump group ($P = .001$, $P = .001$, $P = .001$, and $P = .001$, respectively). Serum M30 levels were found to be elevated in both groups, returning to baseline at 24 hours. When compared to baseline, the hs-CRP level reached its peak at 24 hours at 13.28 ± 5.32 mg/dL in the on-pump group, and 15.44 ± 4.02 mg/dL in the off-pump group.

Conclusion: CABG surgery is associated with an increase in inflammatory markers and serum M30 levels, indicating epithelial/endothelial apoptosis in the early period.

INTRODUCTION

Coronary artery bypass grafting (CABG) surgery with cardiopulmonary bypass (CPB) (on-pump technique) has been an effective and safe treatment alternative for patients with coronary artery disease [Matata 2000; Misoph 1997]. Despite the highly positive outcomes obtained with on-pump coronary artery bypass surgery, this method is associated with systemic inflammatory activation and leads to an acute phase response and increased postoperative morbidity. These results led to the development of the less invasive off-pump technique, which allows performing coronary artery bypass surgery on the beating heart without using CPB [Filsoufi 2001; Filsoufi 2002].

Ischemia-reperfusion injury is a clinical problem associated with procedures such as thrombolytic treatment, angioplasty, and CABG surgery. Reactive oxygen species, including superoxide anions, hydroxyl radicals, hydrogen peroxide, and hypochlorous acid are formed in tissues following ischemia-reperfusion injury [Kato 1998]. The contact of the circulating blood with non-physiological surfaces during CPB has been suggested as the major source of oxidative stress. Extracorporeal circulation has been demonstrated to increase free radical production in dialysis patients [Eiselt 2001]. Oxidative stress in patients undergoing on- and off-pump techniques have been compared using markers such as malondialdehyde (MDA), thiobarbituric acid, and superoxide dismutase in several studies. Akila et al showed that myocardial reperfusion in patients undergoing CABG surgery with the on-pump technique led to a significant increase in oxidative stress, whereas oxidative stress was less in patients undergoing the off-pump technique [Akila d'souza 2007].

Inflammatory activation following CABG surgery is an important issue. This condition is closely associated with oxidative stress and significantly affects postoperative mortality and morbidity. It has been shown that serum reactive protein (CRP) levels are increased following CABG surgery, with higher levels reported in patients undergoing the on-pump technique [Schulze 2000]. The contact of blood with artificial surfaces in the heart-lung machine leads to activation of leukocytes, platelets, plasma cascade systems, and endothelial cells [Elgebaly 1994].

Apoptosis has been considered one of the main mechanisms responsible for myocyte loss during ischemia reperfusion [Gottlieb 2004]. Aebert et al showed that, in humans, CPB strongly affected apoptosis in endothelial cells and reported that this might have contributed to postoperative vascular dysfunction [Aebert 2000]. Cytokeratin 18 has been detected in the endothelial cells of the cardiac microvascular structure, and high titers of antibodies against this

molecule have been found in individuals with ischemic heart disease [Mattey 2004]. The fragment called cytokeratin 18 (M30-antigen) is cleaved by caspases in the early period of apoptosis and cannot be detected in viable and necrotic cells [Linder 2004]. A monoclonal antibody, M30, specifically recognizes this caspase-cleaved cytokeratin 18. Szerafin et al were the first to demonstrate that M30 levels, as an apoptotic marker, were increased following CABG surgery [Szerafin 2006].

Oxidative stress and inflammation are known as triggering factors for apoptosis. It has been shown that CABG surgery using on- and off-pump techniques leads to different levels of inflammation and oxidative stress. In the current study, we used plasma MDA levels to assess oxidative stress, high-sensitivity C-reactive protein levels to assess inflammation, M30 antigen levels as a specific marker of apoptosis, and M65 antigen levels as a marker of both apoptosis and necrosis (total cell death). Thus, the relationship of apoptosis/necrosis with oxidative stress and inflammation was investigated in patients undergoing CABG surgery.

MATERIALS AND METHODS

Fifty patients undergoing CABG surgery in the Department of Cardiovascular Surgery of University Faculty of Medicine were included in the study. Patients with multivessel coronary disease were included if they were deemed equally treatable with on-pump and off-pump CABG surgery by means of consensus of a cardiac surgeon base of coronary angiographic analysis. Fifty patients were eligible for both on-pump and off-pump CABG surgery and were prospectively randomized to on-pump (n = 25) or off-pump (n = 25) CABG surgery.

Patients were excluded from the study for the following reasons: presence of an infection, redo surgery or emergency surgery, malignancies, immunological disorders, history of myocardial infarction within the last month, steroid use, New York Heart Association (NYHA) Class IV heart failure, cardiogenic shock, severe valvular disease, renal failure, and hepatic failure.

Cases included in this study were patients undergoing elective surgery. The operative risk scoring system developed for cardiac surgery, the European System for Cardiac Operative Risk Evaluation (EuroSCORE), was used before surgery to determine the risk of death following cardiac surgery. Left internal mammary artery (LIMA) and saphenous vein grafts were used as bypass conduits in all patients. Factors that might affect perioperative and postoperative mortality and morbidity (e.g., age, gender, diabetes mellitus, hypertension, cigarette smoking, EuroSCORE, number of grafted vessels, ejection fraction, and the presence of chronic obstructive pulmonary disease) were assessed. All of the surgical procedures were performed by the same surgical team, who were highly experienced in both on-pump and off-pump CABG surgery.

The study was approved by the local ethics committee of Uludağ University, and all patients gave written informed consent.

Off-Pump Technique

Following median sternotomy, the LIMA was harvested. The pericardium was opened longitudinally, and its edges were suspended in all patients. Heparin was given intravenously at a dose of 150 IU/kg to maintain an activated clotting time (ACT) of at least 300 seconds during anastomosis construction. The saphenous vein was used in addition to the LIMA as the conduit in multiple bypass patients. Deep pericardial suspension sutures with 0-silk were placed between the inferior vena cava and left superior pulmonary vein and snared. After myocardial stabilization was ensured using the Octopus-4 tissue stabilizer [Medtronic Inc, Minneapolis, MN, USA], distal anastomoses were performed. To improve the surgical visualization of coronary arteries in the inferior and posterior sides and increase the incoming blood volume to the right heart, the patients were put in a 20 degrees Trendelenburg position. In patients with critical left anterior descending artery (LAD) lesions, anastomosis of the LIMA to the LAD was performed first, followed by anastomosis of the diagonal and right coronary arteries. After all grafts were opened to blood flow, heparin was neutralized by a half-dose of protamine sulfate.

On-Pump Technique

Patients were heparinized (300-400 U/kg) to maintain an ACT of at least 400 seconds. After cannulation of the aorta and right atrium, CPB was initiated. A roller pump [Sarns 9000 perfusion system, 3 M, Ann Arbor, Michigan, USA] and membrane oxygenators [Sechrist 3500/3500 HL Series, Anaheim, California, USA] were used. The pump priming consisted of 1500 mL of Ringer's lactate solution, 150 mL of 20% mannitol, and 60 mL of 8.4% sodium bicarbonate. For myocardial protection, moderate hypothermia (30-32°C), topical cooling, and intermittent (every 15-20 minutes) antegrade and retrograde cold blood cardioplegia beginning with crystalloid maintenance was performed. Distal anastomoses were constructed first. After the cross-clamp was removed, proximal anastomoses were completed by placing a side-clamp on the ascending aorta.

Ongoing cardiac medications and medical treatment regimens used in the intensive care unit were recorded.

BLOOD SAMPLING

For serum hs-CRP, MDA, M30, and M65 measurements, 10 mL of venous blood samples were drawn at baseline (before CABG surgery), and 30 minutes, 60 minutes, and 6 and 24 hours after the CABG surgery. In addition, serum creatine kinase (CK), CK-MB, and troponin I levels were measured immediately from blood samples obtained before surgery and at 1, 6, and 24 hours after the CABG surgery.

M30 and M65 Measurements

Blood samples were obtained from all patients and centrifuged for 10 minutes at 2500 g. Serum samples were frozen and stored at -80 °C. M30 and M65 levels were measured using ELISA kits (a solid phase sandwich enzyme-linked immunosorbent assay; M30-Apoptosense ELISA kit and M65 ELISA kit, Peviva AB, Bromma, Sweden). In brief,

Table 1. Demographic Characteristics*

	Off-Pump (n = 25)	On-Pump (n = 25)	P
Age, y	57.68 ± 8.36	56.84 ± 10.65	.058
BMI, kg/m2	27.6 ± 3.90	26.81 ± 4.55	.525
Sex, female/male	3/22	3/22	.667
DM, n (%)	6 (24%)	3 (12%)	.463
Hypertension, n (%)	17 (68%)	17 (68%)	.619
COPD, n (%)	1 (4%)	2 (8%)	.552
Cigarette smoking, n (%)	17 (68%)	17 (68%)	.619
Preoperative EF, %	53.76 ± 15.07	52.31 ± 10.8	.301
Family history, n (%)	8 (32%)	10 (40%)	.769
Number of grafted vessels	2.53 ± 1.04	2.57 ± 0.83	.06
CPB time, min	—	94.2 ± 14.1	—
Aortic cross-clamp time, min	—	52.6 ± 10.3	—
Hemoglobin, g/dL	12.4 ± 6.3	12.8 ± 7.1	.421
Preoperative inotropic drug use, n (%)	2 (8%)	5 (20%)	.417
Postoperative inotropic drug use, n (%)	1 (4%)	5 (20%)	.189
Prolonged mechanical ventilation, n (%)	1 (4%)	3 (12%)	.609
MI, n (%)	1 (4%)	3 (12%)	.609
Neurological complications, n (%)	—	3 (12%)	.235
Mediastinitis, n (%)	—	3 (12%)	.235
Mortality, n (%)	—	1 (4%)	.1

*Data are presented as the mean ± SD where indicated. BMI indicates body mass index; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; EF, ejection fraction; CBP, cardiopulmonary bypass; MI, myocardial infarction.
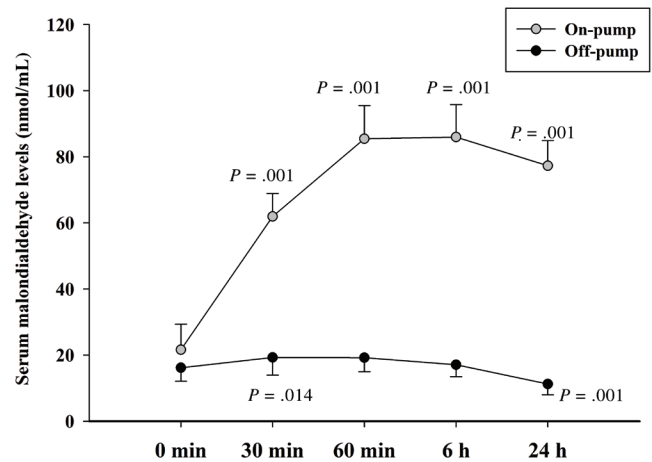

Figure 1. Malondialdehyde levels in patients undergoing CABG surgery using off- and on-pump techniques. Data are expressed as mean ± standard deviation.

samples were placed in mouse monoclonal antibody-coated wells. A horseradish peroxidase-conjugated antibody (M30 or M65) was used for detection. After sandwich formation, unconjugated compound was washed, and tetramethylbenzidine substrate was added to the medium. Finally, absorbance at 450 nm was measured using a microplate reader. The amounts of M65 and M30 antigen in the samples were calculated by plotting a standard curve from known concentrations versus measured absorbance. The concentrations of the antigens were expressed as units per liter (U/L) in accordance with the manufacturer's instructions. These measurements were performed in the Department of Biochemistry of University Faculty of Medicine.

Malondialdehyde Measurement

High performance liquid chromatography (HPLC) was used (nmol/mL plasma).

High-sensitivity C-reactive Protein Measurement

An immunonephelometric method was used, and measurements were made using N Latex CRP mono reagent (Dade Behring, Marburg, Germany).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software, version 16.0 (SPSS Inc, Chicago, Illinois USA). The Kolmogorov-Smirnov test was used to test for normality. Continuous variables are presented as the mean ± standard deviation. Categorical variables are reported as frequencies and group percentages. Two group comparisons were performed using an unpaired t test for parametric variables, Mann-Whitney U test for non-parametric variables, and a Fisher's exact test for categorical variables. Intra-group comparisons were performed using a paired t test for parametric and the Wilcoxon test for non-parametric variables. Correlation analysis was performed using Pearson's correlation. A P value of <.05 was considered statistically significant.

RESULTS

General characteristics of the patients are presented in Table 1. There were no significant differences between the two groups in terms of age, ejection fraction, or body mass index, and the majority of the patients in both groups were male. Moreover, preoperative inotropic drug use, complication rates, and the mortality rate were also similar in both groups. Mean cardiopulmonary bypass and aortic cross-clamp times in on-pump patients were 94.2 ± 14.1 minutes and 52.6 ± 10.3 minutes, respectively.

There was no significant difference in the number of grafted vessels between the off-pump group and on-pump group (respectively, 2.53 ± 1.04 versus 2.57 ± 0.83, P = .06).

Serum MDA levels of the patients undergoing CABG surgery are shown in Figure 1. Serum MDA levels at 30 minutes,
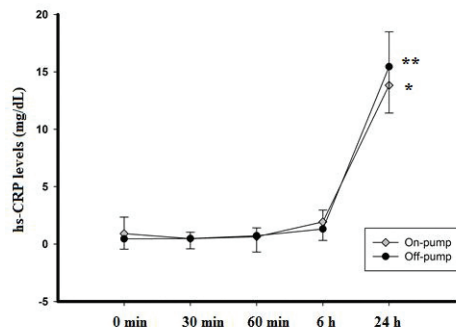
Figure 2. High-sensitivity C-reactive protein levels in patients undergoing CABG surgery using off- and on-pump techniques. Data are expressed as mean ± standard deviation. *P = .001 in on-pump group compared with baseline; **P = .001 in off-pump group compared with baseline.
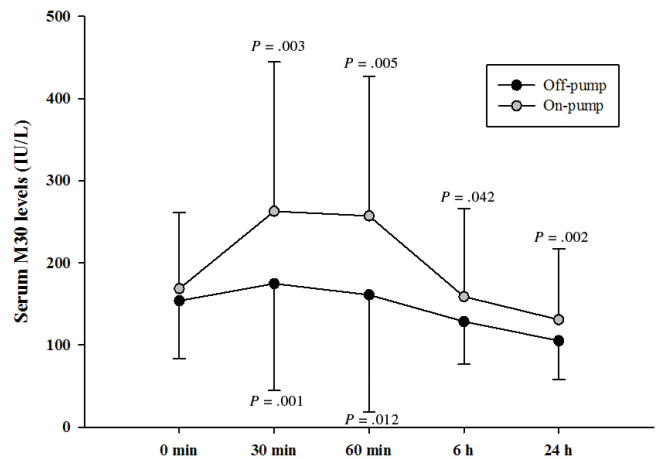

Figure 3. Serum M30 levels in patients undergoing CABG surgery using off- and on-pump techniques. Data are expressed as mean ± standard deviation.
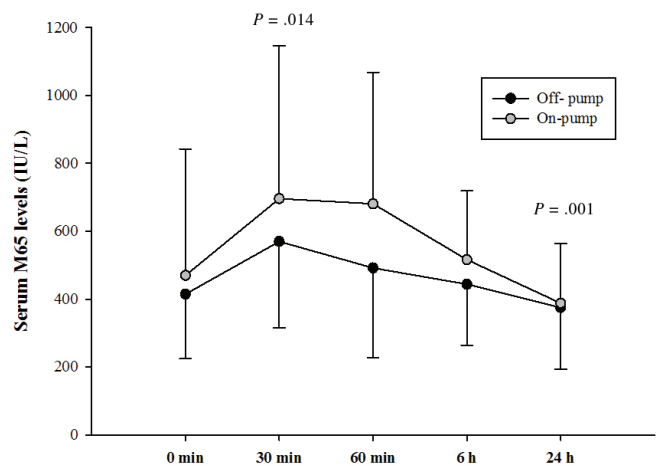

Figure 4. Serum M65 levels in patients undergoing CABG surgery using off- and on-pump techniques. Data are expressed as mean ± standard deviation.

60 minutes, 6 hours, and 24 hours after the surgery were significantly lower in the off-pump group compared to the on-pump group (P = .001, P = .001, P = .001, and P = .001, respectively). Moreover, serum MDA levels were significantly increased in the on-pump group at 30 minutes, 60 minutes, 6 hours, and 24 hours after the surgery compared to baseline (P = .001, P = .001, P = .001, and P = .001, respectively). Conversely, in the off-pump group, there was a significant increase in MDA levels at 30 minutes (P = .014), while a significant decrease was found at 24 hours after the surgery compared to baseline MDA levels (P = .001).

Serum hs-CRP levels of the patients undergoing CABG surgery are shown in Figure 2. There were no significant differences between the two groups at baseline (P = .33) or at 30 minutes, 60 minutes, 6 hours, or 24 hours after the surgery (P = .89, P = .56, P = .84, and P = .06, respectively). However, there was a significant increase in hs-CRP levels at 6 and 24 hours after the surgery in both groups, compared to baseline values (P = .001 and P = .001, respectively).

Serum M30 and M65 levels in patients undergoing CABG surgery are presented in Figure 3 and Figure 4, respectively. There was a measurable but not statistically significant difference at baseline serum M30 levels between groups. A similar pattern of release was observed in serial measures of serum M30 levels, with no difference between both study groups at 30 minutes, at 60 minutes, and at 24 hours. Intra-group comparisons revealed that serum M30 levels were significantly increased at 30 minutes (P = .003) and 60 minutes (P = .005) compared to baseline, and significantly decreased at 6 hours (P = .042) and 24 hours (P = .002) in the on-pump group. Serum M30 levels were significantly increased at 30 minutes and 60 minutes (P = .001 and P = .012) in the off-pump group.

Serum M65 levels were similar in the on-pump group compared to the off-pump group at baseline. No significant differences were noted between the two groups at other time points. Compared to baseline, a significant increase in the serum M65 levels was noted at 30 minutes after the surgery (P = .014), and a significant decrease was noted at 24 hours after the surgery in the on-pump group (P = .001)(Figure 4).

No significant correlation was noted between serum M30 levels, hs-CRP, and MDA levels in any of the groups (P > .05).

DISCUSSION

In the current study, the relationship of apoptosis with oxidative stress and inflammation in patients undergoing on- or off-pump CABG surgery was evaluated. Inflammation was found to be increased at 24 hours compared to baseline in both of the groups, and oxidative stress was significantly lower in the off-pump group. In addition, the data from this study indicate that off-pump CABG surgery, like on-pump CABG surgery, increases circulating cytokeratin 18 levels, indicating endothelial or epithelial cell apoptosis.

Oxidative stress is generally associated with the increased production of free oxygen radicals, and it modifies

phospholipids and proteins, leading to lipid peroxidation and the oxidation of thiol groups [Biglioli 2003]. Lipids and thiol groups are closely associated with the inflammatory response including complement activation, cytokine release, and activation of several adhesion molecules. Several studies have shown increased production of free oxygen radicals during CPB [Davies 1993]. MDA, which is used to assess oxidative stress, is formed as a result of lipid peroxidation [Gerritsen 2001]. Several studies have shown that MDA levels are lower in patients undergoing off-pump bypass surgery [Gerritsen 2001; Gerritsen 2006]. Consistent with previous studies, we found that MDA level, an oxidative stress marker, was lower in the group of patients undergoing CABG surgery with the off-pump technique at all time-points. Our aim was to investigate the relationship of apoptosis with oxidative stress and inflammation in this group of patients.

Many studies have shown that on-pump procedures significantly increase systemic inflammation and oxidative stress compared to off-pump procedures [Gerritsen 2001]. This has been explained by several material-dependent (the contact of blood with non-physiological surfaces during extracorporeal circulation) or material-independent causes [Elgebaly 1994]. Cytokine release, leukocyte activation, and the expression of adhesion molecules during these procedures lead to significant inflammation [Biglioli 2003]. Some studies have demonstrated the formation of free oxygen radicals and their time course during CBP [Davies 1993]. Oxidative events lead to the depletion of plasma antioxidants, lipid peroxidation, and the formation of other damaging metabolites [Pyles 1995; Toivonen 1994]. In the current study, we noted a significant increase in inflammation at 6 hours and particularly 24 hours after the initiation of surgery compared to baseline in both of the groups; however, oxidative stress levels were similar in both groups during the postoperative period. No significant correlation was found between oxidative stress and inflammation in either the off- or on-pump groups.

It has been emphasized that several mediators released during CPB are responsible for the induction of the systemic inflammatory response [Levy 1993]. One of the mechanisms blamed is the apoptosis of endothelial and epithelial cells in patients undergoing CABG surgery. Scarabelli et al showed that in the very early stages of reperfusion, apoptosis is first seen in the endothelial cells from small coronary vessels [Scarabelli 2001]. Also, reperfusion induces the release of soluble pro-apoptotic mediators from endothelial cells that promote myocyte apoptosis. Cytokeratin 18 is the type I intermediate-filament protein found in cells. It is cleaved in cells undergoing apoptosis by caspases 3, 6, and 7. The monoclonal antibody M30, which recognizes the caspase-cleaved cytokeratin 18 released from endothelial/epithelial cells undergoing apoptosis, has been demonstrated. Schmid et al showed that circulating endothelial cells and apoptotic endothelial cells were elevated in both groups of patients, but the increase was more pronounced in the on-pump group compared to the off-pump group [Schmid 2006]. They studied the apoptosis-inducing activity of serum samples on endothelial cells using a tissue culture assay system, and used TUNEL stain to analyze the numbers of cells with DNA damage. This

increase reached its peak in the initial 6-12 hours and lasted for 48 hours. In our study, both groups showed an increase in apoptotic activity, but there were no differences in serum M30 levels between both study groups at 30 minutes, at 60 minutes, and at 24 hours. It has been proposed that endothelial cell activation may be a part of the systemic inflammatory response, especially in patients undergoing on-pump bypass surgery, and that it may be responsible for organ dysfunction that occurs during CPB. It has been demonstrated that off-pump CABG may provide better clinical outcomes due to lower levels of endothelial cell activation [Kalawski 1998; Assaly 2001]. Szerafin et al was the first to show that cytokeratin 18 levels were increased in patients undergoing on- and off-pump CABG surgery and then returned to baseline at 24 hours [Szerafin 2006]. They concluded that CABG surgery may lead to the release of products derived from epithelial or endothelial cells undergoing apoptosis. We found that serum M30 levels were increased at 30 and 60 minutes after the initiation of bypass surgery compared to baseline in both groups; however, there was no significant difference between the two groups. This contrasts with the findings of Szerafin et al, who reported that the rise from baseline to 30 minutes was significant in only the on-pump group [Szerafin 2006]. The results of the present study did not show a consistent association between the inflammatory biomarker and oxidative stress, while prior cross-sectional studies have shown such association. Moreover, there was no significant correlation between inflammation and serum M30 levels.

Limitations

There are several important limitations to this study. First, this study is limited in that it is a single-center study of a relatively small number of patients. This may limit the generalizability of our findings and, therefore, the results need to be confirmed with a larger number of patients. Second, because the preoperative patient characteristics and intraoperative data were balanced between the 2 groups, it remains possible that unknown confounders affected the results. Third, because off-pump CABG surgery was performed in normothermia, while on-pump CABG surgery was performed in moderate hypothermia, we speculate that the use of crystalloid cardioplegic solution during CBP may influence inflammatory responses.

Conclusion

In the current study, levels of M30 antigen, indicating endothelial and epithelial cell apoptosis, were found to be increased after CABG surgery, regardless of the surgical technique used; and levels of MDA, indicating oxidative stress, were found to be lower in patients undergoing CABG surgery with the off-pump technique. Moreover, levels of hs-CRP, indicating inflammation, were significantly increased 24 hours after the initiation of surgery in both groups. Serum M30 levels can be used as a marker indicating endothelial cell activation and/or damage following CABG surgery. Studies investigating the mechanisms of endothelial cell damage may aid our understanding of the dysfunction that may develop following CABG surgery.

REFERENCES

Aebert H, Kirchner S, Keyser A, et al. 2000. Endothelial apoptosis is induced by serum of patients after cardiopulmonary bypass. Eur J Cardiothorac Surg 18:589-93.

Akila d'souza B, Vishwanath P, D'souza V. 2007. Oxidative injury and antioxidants in coronary artery bypass graft surgery: Off-pump CABG significantly reduces oxidative stress. Clinica Chimica Acta 375:147-52.

Assaly R, Olson D, Hammersley J, et al. 2001. Initial evidence of endothelial cell apoptosis as a mechanism of systemic capillary leak syndrome. Chest 120:1301-8.

Biglioli P, Cannata A, Alamanni F, et al. 2003. Biological effects of off pump vs. on-pump coronary artery surgery: focus on inflammation, hemostasis and oxidative stress. Eur J Cardiothorac Surg 24:260-9.

Davies SW, Duffy JP, Wickens DG, et al. 1993. Time-course of free radical activity during coronary artery operations with cardiopulmonary bypass. J Thorac Cardiovasc Surg 105:979-87.

Eiselt J, Racek, J, Opatrny KJ. 2001. Paired filtration dialysis and free radicals. Cas Lek Cesk 140:238-41.

Elgebaly SA, Houser SL, el Kerm AF, Doyle K, Gillies C, Dalecki K. 1994. Evidence of cardiac inflammation after open heart operations. Ann Thorac Surg 57:391-6.

Filsoufi F, Aklog L, Adams DH. 2001. Minimally invasive CABG. Curr Opin Cardiol 16:306-9.

Filsoufi F, Adams DH. 2002. Surgical approaches to coronary artery disease. Curr Treat Options Cardiovasc Med 4:55-63.

Gerritsen WB, Boven WJ, Driessen, AH, Haas JF, Aarts LP. 2001. Off-pump versus on pump coronary artery bypass grafting: oxidative stress and renal function. Eur J Cardiothorac 20:923-9.

Gerritsen WB, Van Boven WJ, Boss DS, Haas FJ, van Dongen EP, Aarts LP. 2006. Malondialdehyde in plasma, a biomarker of global oxidative stress during mini-CABG compared to on- and off-pump CABG surgery: a pilot study. Interact Cardiovasc Thorac Surg 5:27-31.

Gottlieb RA, Burleson KO, Kloner RA, Babior BM, Engler RL. 2004. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 94:1621-8.

Kalawski R, Bugajski P, Smielecki J, et al. 1998. Soluble adhesion molecules in reperfusion during coronary bypass grafting. Eur J Cardiothorac Surg 14:290-5.

Kato K, Shao Q, Elimban V, Lukas A. Dhalla NS. 1998. Mechanism of depression in cardiac sarcolemmal Na+-K+ ATPase by hypochlorous acid. Am J Physiol 275:826-31.

Levy JH, Kelly AB. 1993. Inflammation and cardiopulmonary bypass. Can J Anaesth 40:1009-15.

Linder S, Havelka AM, Ueno T, Shoshan MC. 2004. Determining tumor apoptosis and necrosis in patient serum using cytokeratin 18 as a biomarker. Cancer Lett 214:1-9.

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

Mattey DL, Dawes PT, Nixon NB, Goh L, Banks MJ, Kitas GD. 2004. Increased levels of antibodies to cytokeratin 18 in patients with rheumatoid arthritis and ischaemic heart disease. Ann Rheum Dis 63:420-5.

Misoph M, Babin-Ebell J. 1997. Inter individual variations in cytokine levels following cardiopulmonary bypass. Heart Vessels 12:119-27.

Pyles LA, Fortney JE, Kudlak JJ, Gustafson RA, Einzig S. 1995. Plasma antioxidants depletion after cardiopulmonary bypass in operations for congenital heart disease. J Thorac Cardiovasc Surg 110:165-71.

Scarabelli T, Stephanou A, Rayment N, et al. 2001. Apoptosis of endothelial cells precedes myocyte cell apoptosis in ischemia/reperfusion injury. Circulation 104:253-6.

Schmid FX, Vudattu N, Floerchinger B, et al. 2006. Endothelial apoptosis and circulating endothelial cells after bypass grafting with and without cardiopulmonary bypass. Eur J Cardiothorac Surg 29:496-500.

Schulze C, Conrad N, Schütz A, et al. 2000. Reduced expression of systemic proinflammatory cytokines after off-pump versus conventional coronary artery bypass grafting. Thorac Cardiovasc Surg 48:364-9.

Szerafin T, Horvath A, Moser B, et al. 2006. Apoptosis-specific activation markers in on- versus off-pump coronary artery bypass graft (CABG) patients. Clin Lab 52:255-61.

Toivonen HJ, Ahotupa M. 1994. Free radical reaction products and antioxidant capacity in arterial plasma during coronary artery bypass grafting. J Thorac Cardiovasc Surg 108:140-7.