Off-Pump Coronary Surgery causes Immediate Release of Myocardial Damage Markers

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ABSTRACT
Off-pump coronary surgery does not eliminate the risks of ischemia-reperfusion injury. The main objective of this study was to describe the extent and time course of changes in myocardial metabolism and development of myocardial injury associated with revascularization. Coronary sinus and arterial blood samples for measurement of troponin I, creatine kinase MB, lactate, glutathione, and interleukin-6 were taken from 23 patients prior to grafting, after completion of each anastomosis, and up to the 1st postoperative morning. The results were evaluated together with parameters of cardiac function. Release of lactate, creatinine kinase MB, and troponin I into the coronary sinus was evident after completion of the 1st graft, and increased over time. During the procedure, only trace amounts of oxidized and reduced glutathione were detected in coronary sinus and arterial blood. Significant increases in interleukin-6 were found in coronary sinus samples after 5 and 20 min of reperfusion. Surgical trauma during off-pump coronary surgery is sufficient to activate an inflammatory response in the myocardium, together with unfavorable metabolic conditions to cause myocardial necrosis.

KEYWORDS: Coronary Artery Bypass, Off-Pump, Glutathione, Interleukin-6, Myocardial Reperfusion Injury, Troponin I

INTRODUCTION
Off-pump coronary artery bypass (OPCAB) is gaining wide acceptance because it is associated with decreased myocardial enzyme release up to 24 postoperative hours, early neurocognitive dysfunction, renal insufficiency, blood loss, and the need for transfusion, compared to conventional coronary artery bypass grafting (CABG)¹. Despite these advantages, OPCAB does not eliminate the risks completely, and even short periods of ischemia during the grafting procedure on a beating heart possess...
the possibility of developing ischemia-reperfusion injury distinguished by myocardial necrosis, stunning, and vascular endothelial injury.²–⁴

The extent of myocardial injury after cardiac surgery is usually evaluated by the levels of cardiac biomarkers, most often during the early postoperative hours or days. Whether a release of cardiac biomarkers occurs during the grafting procedure is not so well described. The main objective of this study was to describe the extent and time course of OPCAB-associated myocardial injury. For detailed characterization of ischemia-reperfusion injury, markers of necrosis, oxidative stress, and inflammation in coronary sinus blood were assessed together with parameters of cardiac function.

PATIENTS AND METHODS
The study design was approved by the institutional Ethics Review Committee on Human Research, and written informed consent was signed by all patients. Adult patients scheduled for primary elective OPCAB with at least 3 distal anastomoses were included. Exclusion criteria were: preoperative ejection fraction <40% (evaluated by echocardiography); unstable angina; elevated cardiac troponin I (cTnI), troponin T, or creatine kinase MB-isoenzyme (CK-MB); type I or II diabetes mellitus; and hepatic, renal, or pulmonary disease. All medications except salicylates were allowed up to the morning of the operation. Twenty-two men and 1 woman were included. Their demographic and surgical data are presented in Table 1.

A standardized anesthetic technique with fentanyl, midazolam, and pancuronium was used in all cases. To avoid vasospasm of the radial artery graft (used in all patients) intravenous nitroglycerin 1–2 mg·min⁻¹ was infused from the beginning of the procedure. To correct hemodynamic responses during the procedure, metoprolol was administered or the dosage of nitroglycerin was adjusted, as needed. After midline sternotomy and opening of the pericardium, a manually inflatable 15F coronary sinus cannula (Medtronic, Inc., Minneapolis, MN, USA) was introduced through the right atrial wall into the coronary sinus for blood sampling. Percardial traction sutures and elevating gauze pads were used to facilitate visibility and access to the left and right sides of the heart. The Octopus heart stabilizer (Medtronic, Minneapolis, MN, USA) was inserted immediately after opening the coronary artery in all cases. The left anterior descending artery was always grafted first, using left internal mammary artery. Additional bypasses were constructed using radial artery and saphenous vein grafts in a sequence decided by the surgeon. In case of hemodynamic deterioration, the right pericardium was opened to the pleural cavity, and intravenous boluses of phenylephrine together with infusions of crystalloid or colloids were given. Proximal anastomoses were performed to the ascending aorta. Two experienced surgeons carried out all operations. At the end of the operation, graft patency was verified by flowmetry (Transit Time & Doppler Flowmeter; CardioMed A/S, Oslo, Norway). No conversion to cardiopulmonary bypass was needed. Total grafting time was counted from opening the first coronary artery for grafting (left anterior descending artery in all cases) until release of the partial aortic crossclamp after completion of the last proximal anastomosis.

Blood samples for measurement of lactate, cTnI, CK-MB mass, and oxidized (GSSG) and reduced glutathione (GSH) were collected serially from the coronary sinus and radial artery immediately after insertion of the coronary sinus cannula (baseline), 1 min after restoration of blood flow to each grafted artery, and 5, 10, and 20 min after completion of the last anastomosis. Blood for interleukin-6 (IL-6) determination was sampled by the same methodology at baseline and after 5 and 20 min of reperfusion. The balloon of the coronary sinus cannula was manually inflated at these times to obtain blood exclusively from the coronary sinus. Additional arterial samples for all markers were drawn 60 min after completion of the last anastomosis and in the morning of the 1st postoperative day. Blood was centrifuged immediately after sampling, and the serum was stored at −80°C until analyses. To remove protein for glutathione measurements, plasma was mixed with metaphosphoric acid in equal proportions, stored at 4°C for 15 min, and centrifuged again. Protein-free supernatant was stored at −80°C until further analysis. Lactate was measured photometrically using a Konelab

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Patients</th>
</tr>
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<tr>
<td>Age (years)</td>
<td>64 ± 7</td>
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<tr>
<td>Sex (male/female)</td>
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</tr>
<tr>
<td>Triple-vessel disease</td>
<td>20</td>
</tr>
<tr>
<td>Left main stem stenosis &gt; 50%</td>
<td>3</td>
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<tr>
<td>Preoperative ejection fraction</td>
<td>57% ± 9%</td>
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<tr>
<td>Preoperative medications</td>
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<tr>
<td>Ca-channel blockers</td>
<td>9</td>
</tr>
<tr>
<td>Nitrates</td>
<td>16</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>17</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>7</td>
</tr>
<tr>
<td>Statins</td>
<td>10</td>
</tr>
<tr>
<td>Total grafting time (min)</td>
<td>137 ± 36</td>
</tr>
<tr>
<td>Vessels bypassed</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Need for inotropic support</td>
<td>1</td>
</tr>
</tbody>
</table>

ACE = angiotensin-converting enzyme grafting.

Table 1. Patient characteristics and surgical data
60i (Thermo Electron Corp., Vantaa, Finland). CK-MB and cTnI were determined by chemiluminescent immunoassay in a Bayer ACS:180 analyzer (Bayer Corp., Tarrytown, NY, USA). For measurement of total glutathione and GSSG, the sample was divided into 2 parts and a previously described enzymatic method was used. The concentration of GSH was calculated as the difference between total glutathione and GSSG, and the glutathione redox ratio (GSSG/GSH) as μmol GSSG/μmol GSH. IL-6 was measured by the quantitative sandwich enzyme immunoassay technique (Human IL-6 Immunoassay kit; R&D Systems, Inc., Minneapolis, MN, USA).

Myocardial release of biochemical markers is expressed as the difference between arterial and coronary sinus concentrations; thus negative values indicate myocardial release of these substances.

Mean arterial, pulmonary artery, and pulmonary capillary wedge pressures, heart rate, and cardiac output were recorded with a thermodilution pulmonary artery catheter at baseline (after intubation of the trachea and before sternotomy) and at 15 min, 1, 2, 4, 6, 9, and 12 h after completion of the grafting procedure. Cardiac index, right and left ventricular stroke work indices, and pulmonary vascular resistance index were calculated using standard formulas. Hemodynamic measurements were performed only when the pulmonary capillary wedge pressure was above 8 mm Hg. Otherwise, the patient was given infusions of crystalloid or colloid solutions, and hemodynamic parameters were measured after reaching the accepted filling pressures.

Patient data are presented as mean ± standard deviation. To analyze the biochemical data from baseline up to 20 min of reperfusion, multiple comparisons were made with the Mann-Whitney U test and corrected according to the Bonferroni method. These data are presented as medians with upper and lower quartiles. As the hemodynamic data showed normal distribution, multiple comparisons between baseline and subsequent time points were made with the t test for dependent samples, corrected according to the Bonferroni method, and presented as the mean ± standard deviation. Correlation is expressed as Pearson correlation coefficient. Significance was assumed at p < 0.05.

**RESULTS**
**DEMOGRAPHIC DATA**
The time between declamping the aorta and taking arterial blood samples on the 1st postoperative morning was 20 ± 0.5 h. Twelve patients had 4 distal anastomoses; in all other cases, 3 coronary arteries were bypassed. One patient needed inotropic support with dobutamine 5 μg·kg⁻¹·min⁻¹ and norepinephrine 5 μg·kg⁻¹·min⁻¹ during the first postoperative night. None of the patients developed criteria indicative of CABG-related myocardial infarction.

**CINI AND CK-MB MASS**
The baseline levels of cTnI and CK-MB mass were within normal limits (<0.2 ng·mL⁻¹ and <4.5 ng·mL⁻¹, respectively) in all patients. Both markers appeared in the coronary sinus blood after completion of the 1st distal anastomosis, and increased gradually during the grafting procedure (Figure 1). Sixty minutes after completion of the grafts, cTnI in the arterial blood was 0.9 (0.3; 1.3) ng·mL⁻¹ and rose to 2.7 (1.77; 5.1) by the 1st postoperative morning. The respective arterial levels of CK-MB were 7.9 (6.2; 11.1) ng·mL⁻¹ and 18.2 (13.8; 39.2) ng·mL⁻¹. The values of cTnI and CK-MB were not dependent on the number of anastomoses performed.

**LACTATE**
Arterial levels of lactate remained within the normal range (0.6–2.4 mmol·L⁻¹) throughout the study. Figure 2 illustrates the release of lactate from the myocardium during the grafting procedure, with

![Figure 1. Myocardial release of (A) troponin I (TnI) and (B) creatine kinase MB-isoenzyme (CK-MB) during the grafting procedure and early reperfusion. Values are given as medians with upper and lower quartiles. *p < 0.01 compared to baseline.](ASIANCARDIOVASCULAR&THORACICANNALS)
maximal values after the 3rd anastomosis. The release diminished quickly when all anastomoses were completed and the heart was no longer being handled.

**OXIDIZED AND REDUCED GLUTATHIONE**

Neither arterial and coronary sinus values of GSSG and GSH nor the glutathione redox ratio indicated glutathione-associated oxidative stress during the grafting procedure. The maximal concentration of GSSG in the coronary sinus was 0.10 (0; 0.19) μmol·L⁻¹ after completion of the 2nd graft. For GSH, the maximal value was 1.50 (1.27; 1.62) μmol·L⁻¹ (after 20 min of reperfusion). The glutathione redox ratio in coronary sinus blood, a sensitive index of oxidative stress, remained in the borderline zone (0.1–0.3) of oxidative stress; the values were <0.15 at all time points.

**INTERLEUKIN-6**

The arterial levels of IL-6 were within reference limits (<3.4 pg·mL⁻¹) before grafting. The levels of IL-6 in coronary sinus blood exceeded the arterial values at baseline and after 5 and 20 min of reperfusion (Figure 3A). In arterial blood, IL-6 was markedly increased already by the 5th postgrafting minute and reached its maximal value by the 1st postoperative morning (Figure 3B). The duration of the grafting procedure influenced both arterial and coronary sinus blood concentrations of IL-6. Significant relationships were observed during the reperfusion period, but not in the 1st postoperative morning. The relationship was most clear-cut in the coronary sinus blood after 5 min ($r = 0.59$, $p = 0.006$) and 20 min ($r = 0.58$, $p = 0.01$) of reperfusion.

**HEMODYNAMIC MEASUREMENTS**

All patients had a cardiac index $>2.2$ L·m⁻²·min⁻¹ at baseline. Right ventricular stroke work index was $6.0 \pm 2.8$ g·m⁻²·min⁻¹ at baseline, and did not change significantly after the grafting procedure. Conversely, left ventricular stroke work index was markedly depressed up to 6 h postoperatively (Figure 4B).

**DISCUSSION**

This study demonstrates that OPCAB is associated with an immediate release of IL-6 from the heart. The unfavorable metabolic conditions, shown by the release of lactate into the coronary sinus blood, were associated with a minor release of cTnI and CK-MB mass during the grafting procedure.

In the heart, rapid upregulation and production of proinflammatory cytokines represents an intrinsic or innate stress response against myocardial injury. Proinflammatory cytokines are not constitutively expressed in the normal heart. mRNA for proinflammatory cytokines is only expressed early during reperfusion. During the acute inflammatory response, IL-6 is involved in the induction of acute phase reactions, and controls the level of acute inflammatory responses by downregulation of the expression of proinflammatory molecules and upregulation of antiinflammatory molecules. IL-6 levels during reperfusion have been shown to correlate with the severity of injury,
as assessed by left ventricular wall motion abnormalities and a negative inotropic effect. \(^\text{11,12}\) Furthermore, blockade of proinflammatory cytokines has been reported to reduce neutrophil chemotaxis and sequestration, and to attenuate ischemia-reperfusion injury. \(^\text{13}\) Transmyocardial release of IL-6 has been described after cardiac surgery with cardiopulmonary bypass and coronary stenting for stable angina. \(^\text{14,15}\) OPCAB surgery can now be added, indicating that even minor myocardial injury during the procedure is sufficient to activate the proinflammatory reaction. Increases in arterial IL-6 most probably reflect a nonspecific whole-body inflammatory response to the surgery.

The working heart has limited tolerance to ischemia during normothermic conditions, and that poses a risk of myocardial damage even with short ischemic times. Lifting and turning the heart for optimal operating conditions may lead to further temporary and unfavorable blood flow redistribution. Although single episodes of disturbed coronary flow are of a very short duration as a rule, the total ischemic burden may have a significant effect. Thus it has been reported that prolonged target vessel occlusion during OPCAB is associated with increased arterial cTnI and CK-MB levels, and contractile dysfunction. \(^\text{3}\) Therefore, the use of intracoronary shunts has been advocated. \(^\text{16}\) Despite using shunts in all cases, we observed unfavorable metabolic conditions in the myocardium (release of lactate into the coronary sinus blood) after completion of each graft. No glutathione-associated oxidative stress occurred, but some degree of myocardial cell injury was evidenced by the release of cTnI and CK-MB after completion of the 1st graft, even before the heart was extensively handled. Combining this with increased IL-6 release into the coronary sinus blood in cases of prolonged grafting procedures, the data support the intuitive belief that procedures of shorter duration cause less harm. However, the extent of release of damage markers was negligible, and most importantly, less than we previously observed during conventional on-pump CABG in a similar patient population. \(^\text{17}\)

Whether the troponin rises detected in the present study immediately after the procedure were of cytosolic origin and reflect a surgery-induced increase in cell permeability \(^\text{18}\) or irreversible myocardial cell injury \(^\text{19}\) is controversial. Fellahi and colleagues \(^\text{20}\) suggested that the best cutoff value to predict death over a 2-year period after coronary surgery was between 12.1 and 13.4 µg·mL\(^{-1}\), considerably higher than the levels in our study. Observed depression of left ventricular function in our patients was most probably of a temporary nature, i.e. myocardial stunning, as it gradually reversed by the 1st postoperative morning. Taken together, the results of our current and previous \(^\text{17}\) studies further confirm the superiority of the off-pump technique over conventional CABG. This is despite the immediate release of biomarkers during manipulation of the heart, because the grafting procedure per se appears to cause only minor myocardial injury.

**ACKNOWLEDGMENTS**

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**REFERENCES**


**Figure 4.** Changes in cardiac function before grafting and in the early postoperative period: (A) cardiac index (CI) and (B) left ventricular stroke work index (LVSWI). Values are given as the mean ± standard deviation. *\(p < 0.05\) compared to baseline.


