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**Title:** Organ injury after coronary bypass grafting: a biomarker study towards optimal surgical strategy  
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Chapter 9 | Minimised closed circuit coronary artery bypass grafting in the elderly is associated with lower levels of organ-specific biomarkers: A prospective randomised study

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Abstract

Background: Restrictive fluid management may protect organ function and improve postoperative outcome in elderly coronary artery bypass grafting (CABG) patients.

Objective: We assessed organ-specific biomarker release to study the contribution of a fluid restrictive closed circuit concept to organ protection in elderly CABG patients. Cardiac, respiratory and abdominal organ injury was measured during and following minimal fluid coronary artery bypass grafting (mCABG), off-pump coronary artery bypass (opCAB) surgery and conventional CABG with high volume prime and cold crystalloid cardioplegia (cCABG). The results were related to differences in clinical outcome.

Design: Prospective randomised trial.

Setting: Dutch tertiary single centre study.

Patients: Sixty patients over 70 years of age (38 men and 22 women) were randomised to one of the three different techniques. Inclusion criteria were as follows: first time CABG, elective surgery, ejection fraction more than 30% and multivessel disease. Acetylsalicylic acid and clopidogrel administration or requiring less than three distal anastomoses were an exclusion.

Main outcome measures: Organ-specific markers of the heart – heart fatty acid binding protein (HFABP), troponin T, pro-brain natriuretic peptide (pro-BNP) and creatinine phosphokinase (CPK), lung Clara cell 16 protein, pneumoprotein (CC16), intestinal fatty acid binding protein (IFABP) and liver glutathione S-transferase ([alpha]-GST) – were measured perioperatively. Postoperative PaO$_2$ levels, ventilation time, blood product consumption and adverse events were noted.

Results: Myocardial organ-specific biomarker troponin T showed significantly lower median levels during mCABG compared with the cCABG and op CAB groups [troponin 0.25 mg l$^{-1}$ (interquartile range, IQR 0.18 to 0.40), 0.39 mg l$^{-1}$ (IQR 0.23 to 0.49) and 0.36 mg l$^{-1}$ (IQR 0.23 to 0.50), respectively (P <0.003)]. HFABP, IFABP and [alpha]-GST levels were significantly higher during cCABG compared with opCAB and mCABG [HFABP 38.6 mg l$^{-1}$ (IQR 29.6 to 47.1), 23.3 mg l$^{-1}$ (IQR 16.5 to 31.0) and 21.1 mg l$^{-1}$ (IQR 15.7 to 28.8; P < 0.001), IFABP 0.57 mg l$^{-1}$ (IQR 0.37 to 1.11), 0.44 mg l$^{-1}$ (IQR 0.16 to 0.74) and 0.37 mg l$^{-1}$ (IQR 0.13 to 1.05; P < 0.02) and [alpha]-GST 11.5 mg l$^{-1}$ (IQR 7.7 to 15.7), 7.0 mg l$^{-1}$ (IQR 4.5 to 13.8) and 7.3 mg l$^{-1}$ (IQR 6.2 to 11.2), respectively (P <0.009)]. There was a trend towards higher median CC16 levels in the cCABG group (P <0.07). CPK and pro-BNP were not significantly different. On the first postoperative day, PaO$_2$ levels and duration of mechanical ventilation were significantly improved, and there was lower use of blood products in the mCABG group than in the cCABG and opCAB groups (P <0.05).

Conclusion: Following mCABG with low volume myocardial preservation and restrictive fluid management, early respiratory performance was improved and consumption of blood products reduced compared with opCAB and cCABG.
Minimised closed circuit coronary artery bypass, associated with lower levels of organ-specific biomarkers
Introduction

Cardiopulmonary bypass (CPB) has facilitated precise suturing on a still heart during coronary artery bypass grafting (CABG) until the revival of off-pump coronary artery bypass (opCAB) surgery in the 1990s. Organ injury and coagulation disorders after cardiac surgery with CPB are caused by two related pathophysiological mechanisms: ischaemia–reperfusion injury and systemic inflammatory response syndrome (SIRS). Ischaemia–reperfusion injury may give rise to generation of so-called reactive oxygen species (ROS), which have a destructive effect on tissue cells. Ischaemia–reperfusion injury has been reported to occur in the heart, lungs, kidneys and intestines.1,2 Coagulation disorders that may cause significant blood loss, necessitating transfusion of allogenic blood products, often occur after CABG.3,4 CPB may give rise to multiple organ injury, with subsequent increase in postoperative morbidity, ventilation times and length of hospital stay.5,6

In the 1990s, a growing interest in safer alternatives to conventional CABG (cCABG) led to a revival of opCAB. Initial concerns regarding thoroughness and quality of revascularisation, particularly in the lateral and posterior walls, were addressed by use of modern stabilisers, heart-positioning devices and shunts.7 Several randomised trials including mixed-risk patient groups have been completed 8,9 and have proven that results are comparable between techniques, but depend largely on surgical skills.10 In most follow-up studies, no differences in late mortality were found.11–15 At present, however, only 20% of worldwide CABG are being performed off-pump. Many surgeons do not feel comfortable with this technique.16 Another new development is minimised closed extracorporeal circulation (minimal fluid coronary artery bypass grafting, mCABG), a technique developed in order to reduce haemodilution and to avoid a blood–air interface, while providing better flow dynamics.4,17 This concept offers a blood preservation effect, which can be attributed in part to the centrifugal pump, the heparin-coated tubing sets and to the presence of a closed circuit.18
Compared with conventional extracorporeal CABG, mCABG resulted in a lower inflammatory response.19 Also, during mCABG, there were fewer Doppler high-intensity transient signals (HITSs) than during cCABG.20 Doppler HITSs can be detected and counted by a transcranial Doppler probe positioned at an acoustic window on the skull where it can detect middle cerebral artery flow.21 In addition, a number of emboli can be effectively removed by venous bubble trap (VBT) removers.22,23 Finally, the use of blood cardioplegia, an integral part of mCABG, adds to the concept of avoidance of haemodilution.24

Although similar beneficial effects on the inflammatory response have been reported during opCAB,25 some indications require, and many surgeons request, on-pump CABG. An improvement in extracorporeal circuits (ECCs) in relation to conventional CPB might offer better outcomes.26 We hypothesised that the use of restrictive fluid management in mCABG might improve organ protection and subsequently improve outcome. To test this, we compared the impact of three different treatment modalities, mCABG, opCAB and cCABG on perioperative organ function by measuring various biomarkers associated with myocardial, lung and intestinal organ injury.

**Methods**

After obtaining Institutional Ethics Committee approval (Commissie Toetsing Medische Experimenten, TME. Koekoekslaan1, 3435CM Nieuwegein, The Netherlands. Protocol registration nr: TME/C-03.10. Date of approval: December 2003), all patients gave written informed consent. Between 2004 and 2007, we recruited patients aged over 70 years admitted for elective first time (ejection fraction >30%) multivessel CABG. Exclusion criteria were use of acetylsalicylic acid and/or clopidogrel, and requiring fewer than three distal anastomoses.

The surgeon recruited patients (inclusion/exclusion criteria) during daily heart-team meetings. The programmer, who was unaware of the study protocol, assigned patients to the study surgeon. The technique that was to be applied followed a strict predefined order (first procedure mCABG, second procedure, opCAB, next procedure, cCABG, next procedure mCABG, op, c, m, op, c and so on). The surgeon was blind to this predefined order, which was revealed only in the operating theatre.
Anaesthetic technique
Premedication with oral temazepam 10 mg was given 2 to 3 h before the surgical procedure. A central venous catheter and continuous radial artery blood pressure monitoring helped maintain haemodynamic balance. In accordance with a standard protocol, the balanced opioid-based general anaesthesia technique began at induction with fentanyl 5 to 10 μg kg⁻¹ together with midazolam 0.05 to 0.1 mg kg⁻¹ and propofol (1%) 1.5 to 2 mg kg⁻¹, tailored to the cardiovascular status of the patient. Before incision, before sternotomy, before aorta cannulation and before opening of the pericardium, as needed, additional fentanyl (200 to 500 μg bolus) was given. After induction, a low-dose remifentanil infusion 0.15 μg kg⁻¹ ideal body weight⁻¹ min⁻¹ was started. Cardiac filling pressures were maintained in the face of vasodilatation by means of vasoactive drugs.

In the cCABG group, fluid was not restricted. In the opCAB group, a fluid regimen and vasoactive drugs were used to achieve acceptable haemodynamic values during tilting. In the mCABG group, fluid management was restricted mainly by team awareness and retrograde autologous prime reduction (retrograde autologous priming, RAP). A strict protocol was used for extubation. Patients were rewarmed (37°C rectal temperature) awake, adequate and cooperative without signs of active bleeding. The nursing staff responsible for extubation were blind to the assessment. After extubation, 2 l min⁻¹ of nasal oxygen were given. Postoperative pain was controlled by intravenous morphine (0.5 mg kg⁻¹ h⁻¹) and paracetamol, 1000 mg administered rectally three times daily.

Perfusion technique conventional coronary artery bypass grafting
The ECC consisted of a roller pump (Sarns, Worcester, Massachusetts, USA), a hollow fibre polypropylene oxygenator with an incorporated collapsible softshell reservoir (Maquet AG, Rastatt, Germany) and an open cardiotomy reservoir, exclusively used as a cell saver (Cobe Optima XP; Cobe Cardiovascular Inc., Arvada, USA) and plasticised polyvinyl chloride tubing. The pump was primed with 1.5 to 2 l of 50% primer solution (Na 140 mmol l⁻¹, K 5 mmol l⁻¹, Mg 1.5 mmol l⁻¹, Cl 98 mmol l⁻¹, acetate 27 mmol l⁻¹, gluconate 23 mmol l⁻¹). The heart was protected with topical cooling, together with 1000 ml of cold cardioplegic solution (22°C) based on hydroxyethyl starch (HES: 60 g l⁻¹; mw. 450 kDa; Fresenius Kabi, Bad Homburg Germany) and containing 2 mmol l⁻¹ D,L-magnesium aspartate, 4 mmol l⁻¹ procaine hydrochloride, 0.5 mmol l⁻¹ calcium hydrochloride, 25 mmol l⁻¹ sodium chloride, 5 mmol l⁻¹ potassium chloride, 10 mmol l⁻¹ glucose, 200 mmol l⁻¹ mannitol and dexamethasone 20 mg l⁻¹ with an osmolarity of 320 mosmol l⁻¹, pH 7.4.27
Perfusion technique minimised closed circuit coronary artery bypass grafting
The minimal extracorporeal circuit (MECC) system consisted of a closed system, containing
a Rotaflow centrifugal pump (Maquet GmbH) and a Quadrox membrane oxygenator (Maquet
GmbH). The venous line was connected via short tubing directly to a centrifugal pump, which
passed through the oxygenator and returned the blood via the arterial line. All components
were completely Bioline coated (Maquet GmbH). The priming volume of the system was 500 ml
(this solution contains 500 ml Voluven Fresenius Kabi, mw. 130 kDa in 3 l NaCl 0.9% 308 mosmol
l\(^{-1}\) and pH 4 to 5.5). The reduction in crystalloid pump prime volume depended on baseline
fluid status and haemodynamic tolerance. Replacement of the standard prime solution by
blood taken retrogradely from the arterial line is called RAP. During bypass, the nasopharyngeal
temperature was maintained at 33 to 34°C. Acid–base status during mild hypothermia was
monitored and the cardiac index was kept between 2.0 and 2.4 l min\(^{-1}\) m\(^{-2}\). Preservation of the
heart was with a modified Calafiore technique (warm blood cardioplegia with 30 ml potassium
chloride 2 mol l\(^{-1}\) and 6 ml magnesium sulfoxide 1 mol l\(^{-1}\))\(^{28}\)

Surgical procedure
In all groups, median sternotomy and harvesting of the internal mammary artery were followed
by full exposure of the coronary artery branches to be revascularised. No arterial filters or
antifibrinolytic agents were used. All patients, in all groups, were placed in the Trendelenburg
position at less than 20° tilt.

In the opCAB group, revascularisation was carried out on the beating heart (temperature 35
to 36°C) using the Medtronic Octopus device (Medtronic, Minneapolis, Minnesota, USA).\(^7\)
Temporary coronary occlusion was achieved using Acland clamps (S&T Marketing Limited,
Neuhausen am Rheinfall, Switzerland); no shunts were used. Haemodynamic management
was based on fluid administration. In the mCABG and cCABG groups, standard cannulation
procedure involved a DLP (Medtronic) arterial cannula in the ascending aorta and a DLP 2
stage cannula in the right atrium. In all groups, blood was collected from the surgical field into
a cell-saving device (Cobe BRAT2) and re-infused after washing and centrifugation. Heparin
was administered at 300 IU kg\(^{-1}\) in the on-pump CAGB group, and 150 IU kg\(^{-1}\) in the opCAB and
mCABG groups. Activated clotting time (ACT) was monitored and adjusted periodically during
ECC, to keep at more than 300 s for opCAB and mCABG, and at more than 400 s for cCABG.
After completing all anastomoses, heparin was neutralised with protamine chloride 120 IU per
heparin 150 IU.

Sample collection and analyses
Samples were obtained from the radial artery, and collected in tubes containing EDTA or lithium
heparin before, during and after CABG.
All results were corrected for haemodilution using Beaumont’s method. Albumin remained constant at all time points (data not shown). Sample points are as follows: T0, postanaesthesia induction; T1, 10 min after cross-clamp removal; T2, arrival at ICU; T3, 6 h post-ICU arrival; T4, at 07:00 postoperative day 1; T5, at 07:00 postoperative day 2; T6, at 07:00 postoperative day 3.

Blood gases were routinely processed using a standard laboratory system.

The samples were immediately placed on ice and transported to the laboratory within 15 min of collection. Plasma was obtained by spinning (lithium heparin blood) at 2200g for 10 min and the samples were frozen at −80°C for later analysis. Routine analyses were performed within 1 h. Laboratory analysts were blind to the clinical data.

Biomarkers

**Myocardial injury biomarkers**

Plasma heart-type fatty acid binding protein (HFABP; a cytosolic protein released from injured myocytes) was tested with ELISA (HyCult Biotechnology B.V, Uden, The Netherlands); cardiac troponin T (cTnT: myofibril protein released from injured myocytes), creatine phosphokinase (CPK) and creatine kinase-MB (CK-MB) activity were measured on a Cobas 501/601 analyser (Hofmann-La Roche, Basel, Switzerland).

**Alveolar injury biomarker Clara cell 16 protein**

An important immunosuppressive and anti-inflammatory protein in the lung tested with ELISA (Bio Vendor, Heidelberg, Germany).

**Intestinal injury biomarkers**

Intestinal type fatty acid binding protein (IFABP; a cytosolic protein readily released into the circulation after enterocyte damage) tested with ELISA (HyCult Biotechnology B.V).

**Hepatic injury biomarkers**

[alpha]-Glutathione S-transferase ([alpha]-GST; enzyme released from centrilobular and periportal damaged hepatocytes) tested with ELISA (Biotrin International Ltd., Dublin, Ireland).

**Pulmonary function tests**

Preoperatively, standard lung function tests were performed in all patients. PaO₂ levels and ventilation times were monitored as clinical variables.
Local transfusion protocol

Intraoperative
During all surgical procedures, blood loss from the surgical field was collected in and processed by a cell-saving device (Cobe BRAT 2). All shed blood was given back as red blood cell (RBC) concentrate at the end of surgery.

Postoperative
RBC concentrates were given postoperatively in the ICU as indicated by haematocrit (hct) and the clinical circumstances:
1. hct less than 0.18 l l\(^{-1}\), acute blood loss.
2. hct less than 0.23 l l\(^{-1}\), uncomplicated CABG.
3. hct less than 0.25 l l\(^{-1}\), older than 80 years. 4. hct less than 0.28 l l\(^{-1}\), inability to increase cardiac output to compensate for haemodilution.
Platelet concentrates were given postoperatively in the ICU, if the platelet count was less than 50 × 10^9 l\(^{-1}\) and significant bleeding was present. Fresh frozen plasma (FFP) was administered depending on fibrinogen levels and severity of bleeding.

Collection of clinical data
ICU staff were instructed to follow strict guidelines with respect to the extubation protocol. All fluid inputs and outputs were calculated to maintain fluid balance. On the first postoperative day, oxygenation levels were measured by comparing all PaO\(_2\) values at similar levels of oxygen supply. Data were collected in a standard database set.

Statistical analysis
For data analyses, we used SPSS software version 15.0. Continuous data are expressed as mean ± SD. Non-parametric data are expressed as median with interquartile range. Continuous variables were tested using the Friedman test to compare statistical significance between the three groups.

Discrete variables were calculated using Fisher’s exact test or Pearson [chi]2 test as appropriate. Values of P < 0.05 were considered statistically significant.

Power analysis was performed using the biomarkers CC16 and HFABP with a difference of 3.5 and 10, respectively, between the time points T0 and T1 (P = 0.90, [alpha] = 0.05). This indicated that 12 to 15 per group were required.
Results

Clinical data
A total of 38 men and 22 women undergoing elective CABG were enrolled in this prospective randomised study. Of these, 20 were operated within the mCABG protocol (age 73.6 ± 3.6 years), 20 within the opCAB protocol (73.8 ± 2.6 years) and 20 within the cCABG protocol (74.6 ± 4.0 years). All survived the hospital stay. Preoperative clinical data were similar among the study groups with respect to smoking habits, age, severity of coronary disease, diabetes mellitus, New York Heart Association functional class, left ventricular function and extent of vessel disease. However, chronic obstructive pulmonary disease (COPD) was overrepresented in the mCABG group. Preoperative laboratory analysis Table 1 revealed no patients with renal dysfunction (plasma creatinine levels of >120 µmol l⁻¹).
Surgical data showed the groups were similar with respect to the number and distribution of distal anastomoses. Other preoperative clinical and surgical data are presented in Tables 1 and 2.
Table 1: Preoperative and peroperative clinical data

<table>
<thead>
<tr>
<th>Variable</th>
<th>CCABG</th>
<th>MCABG</th>
<th>OPCAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Male/Female</td>
<td>8/12</td>
<td>15/5</td>
<td>15/5</td>
</tr>
<tr>
<td>Age</td>
<td>74.6 ± 4.0</td>
<td>73.6 ± 3.6</td>
<td>73.8 ± 2.6</td>
</tr>
<tr>
<td>Diabetes I or II</td>
<td>2</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>COPD</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>BSA (m²) (range)</td>
<td>1.89 (1.55-2.45)</td>
<td>1.91 (1.49-2.28)</td>
<td>1.90 (1.51-2.41)</td>
</tr>
<tr>
<td>Mean length (M)</td>
<td>1.72 (1.42-1.89)</td>
<td>1.73 (1.47-1.88)</td>
<td>1.73 (1.72-1.85)</td>
</tr>
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<td>Mean weight Kg)</td>
<td>75 (62-92)</td>
<td>75.8 (55-105)</td>
<td>75.4 (59-96)</td>
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<td>Angina Class</td>
<td></td>
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<tr>
<td>I</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>1</td>
<td>2</td>
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<tr>
<td>I, II, or III-Vessel Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>17</td>
<td>16</td>
<td>18</td>
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<tr>
<td>Left ventricular function</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Good (EF 50-65%)</td>
<td>10</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Impaired (EF 30-50%)</td>
<td>10</td>
<td>7</td>
<td>8</td>
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<tr>
<td>ICU stay (days)</td>
<td>1.0 ± 0.0⁸</td>
<td>1.0 ± 0.0⁸</td>
<td>1.7 ± 2.3⁴</td>
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<td>Euro score</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>≤ 3</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3 - 5</td>
<td>15</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>CBP time (min)</td>
<td>82 ± 23</td>
<td>76 ± 14</td>
<td>-----</td>
</tr>
<tr>
<td>Cross-clamp time (min)</td>
<td>59 ± 19</td>
<td>50 ± 12</td>
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<tr>
<td>Grafts per patients</td>
<td>4.7 ± 1.2²</td>
<td>3.8 ± 0.6¹</td>
<td>3.8 ± 0.8³</td>
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<td>RBC concentrate (%)</td>
<td>65</td>
<td>20</td>
<td>30³</td>
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<tr>
<td>Platelets 6 donors (%)</td>
<td>15³</td>
<td>5³</td>
<td>5³</td>
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<tr>
<td>Fresh frozen plasma (%)</td>
<td>25</td>
<td>5³</td>
<td>10³</td>
</tr>
<tr>
<td>PaO₂ (kPa)</td>
<td>12.1 ± 2.2</td>
<td>16.4 ± 3.9⁹</td>
<td>11.5 ± 2.5</td>
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<tr>
<td>Fluid balance; per-operative (ml)</td>
<td>2549 ± 765</td>
<td>1524 ± 603⁹</td>
<td>2969 ± 1597</td>
</tr>
<tr>
<td>Mechanical ventilation times (hrs)</td>
<td>7.8 ± 3.6</td>
<td>5.7 ± 2.6⁹</td>
<td>8.0 ± 4.2</td>
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</table>
Table 2: Pre- and postoperative haematocrit (Ht)

<table>
<thead>
<tr>
<th>Moment</th>
<th>parameter</th>
<th>MCABC</th>
<th>OPCAB</th>
<th>CCABG</th>
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<tbody>
<tr>
<td>Inclusion</td>
<td>Hct (l1-3)</td>
<td>0.42 ± 0.04</td>
<td>0.41 ± 0.05</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>Arrival PACU</td>
<td>Hct (l1-3)</td>
<td>0.29 ± 0.04</td>
<td>0.30 ± 0.05</td>
<td>0.27 ± 0.04</td>
</tr>
</tbody>
</table>

PACU=post anaesthesia care unit

BSA: body surface area in square meters
EF: Ejection fraction

Data are presented as mean ± standard deviation
£ Denotes p<0.05; CCABG versus MCABG
* Denotes significance p < 0.001 between CCABG versus OPCAB and MCABG
# Denotes significance p < 0.01 between OPCAB and MCABG
$ Denotes significance p < 0.01 between CCABG versus OPCAB and MCABG
§ Denotes significance p = 0.01 between OPCAB and MCABG
Ө Significance between MCABG and CCABG versus OPCAB (p < 0.05)
Myocardial injury biomarkers

Significantly higher median HFABP levels were measured during cCABG than during mCABG and opCAB (P = 0.001; Table 3, Fig. 1a). Significantly lower median troponin T levels were measured during mCABG than during opCAB and cCABG (P = 0.003; Table 3, Fig. 1b). Median CPK levels, CK-MB levels and pro-brain natriuretic peptide (pro-BNP) levels were not significantly different among the treatment modalities studied (Table 3).

Table 3: Baseline to peak median rise of biomarker levels per treatment modality

<table>
<thead>
<tr>
<th></th>
<th>CK (U l⁻¹)</th>
<th>Trop (mg l⁻¹)</th>
<th>HFABP (mg l⁻¹)</th>
<th>IFABP (mg l⁻¹)</th>
<th>α-GST (mg l⁻¹)</th>
<th>CC16 (µg l⁻¹)</th>
<th>Pro-BNP (mg l⁻¹)</th>
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<tbody>
<tr>
<td>mCABG</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>76</td>
<td>0.01</td>
<td>2.31</td>
<td>0.12</td>
<td>3.2</td>
<td>15.5</td>
<td>24</td>
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<tr>
<td>IQR</td>
<td>47 - 123</td>
<td>0.01 - 0.01</td>
<td>0.56 - 3.78</td>
<td>0.00 - 0.38</td>
<td>2.4 - 5.7</td>
<td>12.1 - 19.05</td>
<td>10 - 90</td>
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<tr>
<td>Peak median</td>
<td>384</td>
<td>0.25</td>
<td>21.1</td>
<td>0.37</td>
<td>7.3</td>
<td>22.8</td>
<td>457</td>
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<tr>
<td>IQR</td>
<td>324 - 542</td>
<td>0.18 - 0.40</td>
<td>15.7 - 28.8</td>
<td>0.13 - 1.05</td>
<td>5.0 - 11.2</td>
<td>16.1 - 27.1</td>
<td>283 - 615</td>
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<table>
<thead>
<tr>
<th></th>
<th>CK (U l⁻¹)</th>
<th>Trop (mg l⁻¹)</th>
<th>HFABP (mg l⁻¹)</th>
<th>IFABP (mg l⁻¹)</th>
<th>α-GST (mg l⁻¹)</th>
<th>CC16 (µg l⁻¹)</th>
<th>Pro-BNP (mg l⁻¹)</th>
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<td>opCAB</td>
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<tr>
<td>Baseline</td>
<td>70</td>
<td>0.01</td>
<td>1.3</td>
<td>0.105</td>
<td>3.45</td>
<td>13.5</td>
<td>26</td>
</tr>
<tr>
<td>IQR</td>
<td>53 - 91</td>
<td>0.01 - 0.01</td>
<td>0.0 - 3.0</td>
<td>0.00 - 0.257</td>
<td>2.52 - 7.77</td>
<td>10.1 - 16.3</td>
<td>15 - 86</td>
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<tr>
<td>Peak median</td>
<td>433</td>
<td>0.39</td>
<td>23.3</td>
<td>0.44</td>
<td>7.0</td>
<td>19.9</td>
<td>648</td>
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<tr>
<td>IQR</td>
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<td>0.23 - 0.49</td>
<td>16.5 - 31.0</td>
<td>0.16 - 0.74</td>
<td>4.5 - 13.8</td>
<td>16.6 - 23.0</td>
<td>353 - 1173</td>
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<table>
<thead>
<tr>
<th></th>
<th>CK (U l⁻¹)</th>
<th>Trop (mg l⁻¹)</th>
<th>HFABP (mg l⁻¹)</th>
<th>IFABP (mg l⁻¹)</th>
<th>α-GST (mg l⁻¹)</th>
<th>CC16 (µg l⁻¹)</th>
<th>Pro-BNP (mg l⁻¹)</th>
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<td>cCABG</td>
<td></td>
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<td>Baseline</td>
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<td>0.01</td>
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<td>0.22</td>
<td>4.0</td>
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<tr>
<td>IQR</td>
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<td>0.01 - 0.01</td>
<td>0.66 - 2.70</td>
<td>0.07 - 0.40</td>
<td>2.5 - 18.1</td>
<td>8.6 - 16.1</td>
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<tr>
<td>Peak median</td>
<td>339</td>
<td>0.36</td>
<td>38.6</td>
<td>0.57</td>
<td>11.5</td>
<td>24.0</td>
<td>651</td>
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<tr>
<td>IQR</td>
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<td>0.23 - 0.50</td>
<td>29.6 - 47.1</td>
<td>0.37 - 1.11</td>
<td>7.7 - 15.7</td>
<td>16.1 - 27.5</td>
<td>302 - 814</td>
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</table>

IQR = interquartile range = 25 – 75 percentile
Clara cell 16 pneumoprotein
There were no significant differences among groups (Table 3).

Intestinal and hepatic injury biomarkers
Significantly lower median IFABP levels were measured in the opCAB and mCABG groups than in the cCABG group (P = 0.02; Table 3, Fig. 1c). Significantly higher median [alpha]-GST levels were measured during cCABG than during mCABG and opCAB (P = 0.009; Table 3; Fig. 1d).

Blood product consumption
RBC transfusion was necessary in 65% of cCABG, 30% of opCAB and in 20% of mCABG cases (Table 1). There was a significant difference between the opCAB and mCABG groups with respect to the cCABG group (P = 0.03). There was a trend towards lower consumption of platelets and FFP products in the mCABG study group (Table 1).

Clinical outcome data
On postoperative day 1, PaO₂ levels were significantly higher in the mCABG group than in the cCABG and opCAB groups (P = 0.03; Table 1). There was no significant difference in oxygen supply given to the patients in all study groups at that time point. Postoperative ventilation time was significantly more prolonged in the cCABG and opCAB groups than in the mCABG group (P = 0.01; Table 1).

Discussion
This study was based on the hypothesis that the use of restrictive fluid management as in minimised closed circuit CABG (mCABG) might preserve organ function better and improve outcome. We have shown that mCABG and opCAB were associated with significantly lower median levels of biomarker release associated with myocardial and abdominal injury. In addition, a trend towards lower median blood levels of lung-specific biomarkers associated with alveolar dysfunction was noted in the mCABG and in the opCAB study arms compared with cCABG. Fluid restriction during mCABG resulted in better maintenance of physiological balances and can be considered the reason for the improved ventilation times and blood product consumption. Restrictive fluid management may also have a positive impact on resources.

Anaesthesia related to organ injury
Patients undergoing cardiac surgery develop a severe inflammatory response. This may lead to increased mortality and morbidity. The extent of this inflammatory response depends on the surgical procedure and on genetic predisposition and comorbidities of the cardiac surgical patient. The type and duration of general anaesthesia are also thought to influence the extent of this perioperative inflammatory response, either by disturbing the functions of
immune cells or by modulating the stress response. However, these effects are transient and of minor importance in patients with normal immune systems, though some impact on high-risk patients cannot be ruled out, the extent depending on concentrations used. Propofol probably produces cell-mediated effects on non-specific immunity. Fentanyl has immunosuppressive effects, but there is no evidence that fentanyl attenuates the direct cell immune response by means of specific opioid receptors. Volatile anaesthetic agents may have dose- and time-dependent effects as well. All these effects seem transient. It is clear that the assessment of the potential effects on the inflammatory process of different anaesthetic agents, given alone or in combination, is difficult and that the exact clinical relevance of all findings remains unclear. In the present study, however, the anaesthesia protocol was standardised and effects would have been the same across the groups. However, the dose of heparin given differed among the groups. In the mCABG and in the opCAB groups, an initial dose of 150 IU kg\(^{-1}\) was administered. Heparin reduces reperfusion injury when added to the cardioplegic solution in animals. This would have advantaged only the mCABG and opCAB groups because heparinised blood circulates through the coronary branches in these study arms. Furthermore, in a study by Gedik et al., a minimal ACT level of 200 s was found to be protective against reperfusion injury in animals. In the present study, ACT levels were monitored during the procedures and kept within safe limits.

Global and myocardial oxidative stress and cell injury
In the mCABG group, significantly lower median troponin T levels were recorded. HFABP release was significantly higher during cCABG compared with the release during mCABG and opCAB. HFABP and troponin show early peak levels representing early myocardial injury. In an earlier study, we compared the impact of myocardial protection technique on myocardial oxidative stress and subsequent myocardial cell injury. Myocardial oxidative stress and reperfusion injury were overwhelmingly present in the cCABG study arm of that study. ROS generation, not neutralised by the antioxidant capacity of the circulating blood, can persist and its potentially destructive characteristics may induce injury to other vital organs, such as abdominal organs. Oxygenised myocardial preservation [provided by blood microcardioplegia (mCABG)] together with preservation of haemoglobin/hct levels (provided by restrictive fluid management) reduced organ injury, as demonstrated in the mCABG study arm of this study (Table 2). Substantially reduced generation and better neutralisation of ROS are the basic mechanisms that may explain low myocardial biomarker release during mCABG and opCAB. The relatively high myocardial troponin T enzyme peak levels during opCAB can be explained by the fact that coronary branches were temporarily occluded during suturing of distal anastomoses in this study. This manoeuvre might have induced some ischaemia. The non-significant different enzyme levels of CPK (MB) and pro-BNP can be explained as follows. CPK is not organ-specific. CK-MB is a specific myocardial enzyme, though less sensitive than troponin and HFABP.35 Pro-BNP is an enzyme more representative as an outpatient marker for chronic
Minimised closed circuit coronary artery bypass, associated with lower levels of organspecific biomarkers

heart failure. However, there is some evidence that BNP can act as a predictor of outcome after cardiac surgery.36 Little is known about the importance of the association between baseline antioxidant state and outcome after CABG in elderly patients.37 In future studies, calibration of baseline identification of individual antioxidant status may help to further distinguish between the protective capacities of treatment strategies.

Alveolar injury measured by pneumoprotein Clara cell 16 protein

Although pulmonary complications after cardiac surgery are quite common, lung-specific injury markers are seldom used for the assessment of lung function after CABG. In addition to pneumoprotein CC16, nitric oxide measurements in exhaled breath or in bronchoalveolar lavage have also been used. In 2005, we observed significantly lower CC16 blood levels during mCABG compared with cCABG in a pilot study.1 In the present study, CC16 measurements resulted in a trend towards lower CC16 levels in the mCABG and the opCAB study arms (P <0.07). Clinically we observed better early postoperative alveolar gas exchange (PO2 levels) leading to shorter ventilation times in the mCABG group. These findings are consistent with other reports (Table 1),38,39 and were despite the overrepresentation of COPD patients in the mCABG study arm (Table 1).

Unexpectedly, CC16 release was observed in the opCAB group in this study also. The reason for this is probably unrelated to the use of bypass.40 While trying to maintain haemodynamic performance during tilting of the heart, fluid balance became significantly positive in the opCAB study group (Table 1). Fluid overload might be the cause of the less favourable early postoperative gas exchange that was observed in the opCAB study arm.41 The operative fluid strategy in opCAB remains a point of discussion.42 The use of vasoactive drugs and less fluid might have a place here. In the cCABG study arm, in common with Morariu et al.,43 the administration of corticosteroids did not influence respiratory recovery time substantially. Less inflammation, preserved oxygen transport (hct levels) and reduced consumption of transfusion products explain the superior respiratory performance in the mCABG group. A short duration of ventilation is beneficial, especially to elderly patients, and reduces the impact on resources.44 Currently, use of alveolar biomarkers is not extensively developed within this field and further study is needed.
Specific biomarkers of intestine and liver injury
The lowest median IFABP and [alpha]-GST levels were observed in the mCABG and opCAB study arms. There is some evidence that a drop in hct to below 0.24 l l⁻¹ and the subsequent transfusion of RBCs is related to the release of injury markers from the kidneys and splanchnic organs (Table 2). 45 Holmes et al. 46 demonstrated the relationship between elevated IFABP levels in urine and abdominal gastrointestinal complications in high-risk cardiac surgical patients, validating this intestine-specific biomarker. This together with our results shows the importance for intestinal function of reducing perioperative ROS generation and maintaining near to physiological hct levels (Table 2). The elderly patients studied here are considered high-risk. Protection of their abdominal organs is important in order not to delay recovery time. In addition, in the early postoperative phase, the liver plays an important role in maintenance of coagulation and detoxification of pharmaceuticals. 47

Consumption of transfusion products
Red blood cell transfusion was administered to just 20% of the mCABG group. Similarly, low percentages of FFP and platelets were given during and after both mCABG and opCAB. Reducing the consumption of blood products is cost-effective and is associated with fewer side-effects and less mortality. The effect of reduced heparin dosage with improved coagulation in the mCABG group in the early postoperative period should also be taken into account. 17, 18 Although reduced consumption of blood products was also seen in opCAB, this treatment arm was not the best performer in this study. 3 Different intraoperative fluid management (non-restricted during opCAB and cCABG) may explain the difference in outcome in these study groups (Tables 1 and 2). Also the use of crystalloid cardioplegia (including hetastarch and procaine) in the cCABG control group may account for impaired platelet function and additional haemodilution in this study arm. 48 This, together with extensive prime volume (1.5 to 2 l), explains the significantly increased consumption of homologous blood products in the cCABG study arm. 49

Intraoperative fluid management
In the present study, intraoperative fluid balance was monitored. Significantly less fluid was administered in the mCABG study group. Several explanations may account for this finding. First, during cCABG, the operation team did not practise fluid restriction. 41, 50 In the opCAB group, a combination of fluid and sometimes vasoactive drugs was used to obtain adequate blood pressure during tilting of the heart. In the mCABG group, fluid restriction was practised. Second, besides restriction of fluid during induction of anaesthesia, special manoeuvres were made prior to the start of extracorporeal circulation in the mCABG group. Depending on the baseline fluid status and if well tolerated, the amount of crystalloid pump prime was reduced. Replacement of standard prime solution by the patient’s own blood, taken from the arterial line, is called RAP. 51 Third, the closed coated system reduces blood–air contact and
subsequently reduces perioperative inflammation. This may reduce perioperative capillary leak, which is known to induce third space fluid accumulation. No exact fluid data were collected during postoperative recovery, which was in a short stay low care level facility aiming at quick mobilisation and withdrawal of intravenous fluid administration.

**Limitations**
Our results reflect local circumstances and protocols, all defined in this study. The authors understand that results from a comparison study between complex composite treatment modalities like those here are difficult to explain. Applying our findings to other centres may not be straightforward. Minor differences between the techniques may account for the different outcomes. With respect to this, it is important to appreciate that teamwork between anaesthesiologists, perfusionists, surgeons and intensivists is necessary to realise the sustained positive effect of restrictive fluid management. The different number of distal anatomoses in the different study arms (4.7 in the cCABG group, 3.8 in the mCABG and the opCAB groups) was due to the freedom of the surgeons to graft small diameter coronary branches at their discretion. These differences are unlikely to have had an important effect because across the groups, cross-clamp times remained balanced. Finally, the present data reflect results of only a small group and studies involving larger cohorts are needed.

**Conclusion**
Reduced heart and intestinal organ-specific biomarker-associated organ injury was measured during mCABG and opCAB. Low volume myocardial preservation together with restrictive fluid management improved early respiratory performance and consumption of blood products after mCABG.

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Conflicts of interest: WJPvB was under contract as a consultant to Maquet AG Rastatt during the period of this study. WJPvB, WBG, AHD and EPvD worked at the St Antonius Hospital during the study.

Presentation: none.
References


Minimised closed circuit coronary artery bypass, associated with lower levels of organ-specific biomarkers.