Chapter 3

Organ Perfusion During Cardiopulmonary Bypass: Hematocrit, Blood Transfusion and Temperature Effect on Organ Viability.

Combined Strategy to Limit Perioperative Myocardial, Renal and Intestinal Tissue Injury in Patients Undergoing On–pump Coronary Artery Bypass Grafting.

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Abstract

Study Objectives

An experimental operative protocol was developed aiming to eliminate several potential stress factors that might lead to injury and dysfunction during coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB): (1) cold–crystalloid cardioplegia and atrial intracavitary cooling, (2) autologous priming and partial recovery of the cardioplegic fluid; (3) corporeal normothermia.

Methods


Results

Hematocrit values during, and immediately postoperative (experimental group 27.8±0.6%; standard group 24.4±0.7%) were significantly different (p=0.002). Postoperative CK–MB, NAG and I–FABP were significantly lower in the experimental group. In the standard hypothermic group of patients, the rectal temperatures measured after 60 min of CPB correlated negatively with postoperative I–FABP. The hematocrit explained 38% and 37% in the variability of postoperative NAG and I–FABP, respectively. An extra 21.5% of the NAG variability and 10% of the I–FABP variability, that could not be explained by the variation in hematocrit, were explained by the blood–transfusion requirements.

Conclusions

The addition of an intracavitary cooling system to the standard cold crystalloid cardioplegia during on–pump CABG offered a better protection of the myocardium, with decreased postoperative plasma CK–MB. Partial recovery of intra–atrial cardioplegic fluid and autologous–blood priming limited the extent of intra–operative hemodilution and blood transfusion requirements. An important attenuation of the transient renal and intestinal postoperative injury was observed. Additional protection on renal and intestinal injury was achieved by corporeal normothermia.
3.1 Introduction

The available scientific data concerning the effectiveness and safety of cardiopulmonary bypass (CPB) for patients undergoing coronary artery bypass grafting (CABG) brings to attention several key principles to serve as basis for practical guidelines. Encouraged and generally accepted techniques are cold crystalloid cardioplegia, mild corporeal hypothermia and hemodilution to a hematocrit of 23%.

*Myocardial protection against ischemic injury* during heart arrest and aorta cross clamping is achieved by hypothermia, which reduces oxygen demands and prolongs tolerable ischemic arrest time. Hypothermia can be induced using various techniques, such as cold saline/ice flush in the pericardial sac, cold crystalloid cardioplegia (4°C, high potassium, cold fluid perfused through coronary arteries), cooling jackets (cold fluid perfused through a very small cooling mattress that is pressed directly against the outside of the heart) and intracavitary cooling (intern cooling of the heart chambers).

*Systemic protection against global ischemic injury* during extracorporeal circulation: contrary to conventional thinking about the benefits of corporeal hypothermia, an increasing number of clinical studies support corporeal normothermia. It was demonstrated that hypothermic CPB is responsible for a greater platelet activation and endothelial dysfunction than normothermic CPB, leading to more profound changes in the hemostatic and inflammatory systems\(^1\). Furthermore, a significant positive influence of normothermic CPB temperature was registered on perfusion management, postoperative hemodynamics and blood loss\(^2\).

Normothermic cerebral protection during CPB was also confirmed by studies showing similar patterns of S-100\(_\beta\) release\(^3\) and less pronounced subclinical impairment of cognitive brain function in patients undergoing normothermic CPB as compared with mildly hypothermic CPB\(^4,5\).

*Hemodilution* is encouraged by the current CPB management guidelines, with the rationale based on the reduction of blood viscosity by hemodilution, and thus an improved regional blood flow in the setting of hypoperfusion and hypothermia\(^6\). However, excessive hemodilution may lead to organ ischemia via a reduction of oxygen–carrying capacity uncompensated by autoregulatory and/or rheologic increase in organ blood flow. In animal models, magnetic resonance and near–infrared spectroscopy suggested that brain injury might be caused by hypoxic–ischemic injury as a result of currently recommended protocols for hemodilution during CPB\(^7\). In clinical studies, a significant independent association was found between the lowest hematocrit during bypass and acute renal injury\(^8\), with significant benefits on renal function after reduction of the bypass prime volume\(^9\).

Besides lowering the oxygen carrying capacity, hemodilution was shown to be responsible of endothelial cell activation during CPB. By decreasing red blood cell aggregation and plasma viscosity, hemodilution is expected to modify homeo–rheological variables responsible for a constant shear stress at the endothelial wall, inducing mechanical endothelial activation\(^10\).
Our therapeutic strategy aimed to explore, advance and optimize simultaneously more than one method of protection by eliminating several potential stress factors that might lead to injury and dysfunction during CPB: inadequate hypothermic cardioplegia, excessive hemodilution with subsequent need for perioperative blood transfusion, and corporeal hypothermia.

An experimental operative protocol was developed to meet multiple objectives: (1) homogeneous cooling of the myocardium by combining cold crystalloid cardioplegia technique with intracavitary cooling of the heart; (2) prevention of excessive hemodilution by autologous priming of the extracorporeal circuit and partial recovery of the cardioplegic fluid; (3) corporeal normothermia, possible on the account of a more efficient topical cooling of the heart. The consequences on the postoperative organ injury and clinical outcome of the patients were investigated.

3.2 Patients, Materials and Methods

The clinical study was performed at the VU University Medical Center Amsterdam, The Netherlands.

The study was designed as a prospective, pseudo–double blind (blinding of the patient and lab investigator), randomized clinical trial, investigating the clinical benefits of a new experimental intraoperative protocol that combined cold crystalloid cardioplegia with intracavitary cooling, autologous priming and corporeal normothermia.

After approval by the hospital ethics committee, patients scheduled for elective first time CABG were prospectively screened according to entry criteria. All patients included (n=40) in the study were 45 to 70 years of age, had coronary artery disease with normal renal function (as assessed by a serum creatinine lower than 150 µmol.liter$^{-1}$ and normal urinalysis), normal hepatic, cerebral and cardiac function (ejection fraction > 45%). Written informed consent forms were obtained in all cases.

Patients with preoperative immunosuppressive therapy, preoperative use of NSAID, intra aortic balloon support, requiring aneurysmectomy, insulin–dependent diabetes mellitus, recent myocardial infarction, unstable angina, or recent use of radiocontrast were excluded.

**Anaesthetic management**

On the day of operation, patients received their usual early morning dose of antianginal medication, 5 mg of lorazepam, but no diuretics were administered. Anesthesia was induced using 3–7 µg.kg$^{-1}$ intravenous sufentanil forte, 0.1 mg.kg$^{-1}$ pancuroniumumbromide and 0.1 mg.kg$^{-1}$ midazolam. General anesthesia was maintained by a continuous infusion of propofol 5–15 mL.h$^{-1}$ (20 mg.mL$^{-1}$). After endotracheal intubation, patients were ventilated using an inspiratory mixture of 50% oxygen and 50% of air at a frequency of 14–18 breaths.min$^{-1}$ and 5 cm H$_2$O of PEEP.

After induction of anesthesia patients received 1 mg.kg$^{-1}$ dexamethasone and 1500 mg
cefuroxime. Radial artery and thermodilution pulmonary artery catheters were inserted for hemodynamic monitoring and blood sampling. A continuous positive airway pressure of 5 cm H₂O and a FiO₂ of 21% was maintained in the lungs during CPB. Nitroglycerin infusion of 1 mg·kg⁻¹·min⁻¹ was started in the rewarming period. In case of hemodynamic instability, fluid replacement and inotropic support with dopamine (4 mg·ml⁻¹) and/or nitroglycerin were the first steps taken to stabilize the patient. The mean arterial blood pressure was maintained between 65 and 80 mmHg, pulmonary artery wedge pressure between 8 and 12 mmHg. Transfusion of packed cells were given at a hemoglobin < 4.5 mmol.L⁻¹.

**Extracorporeal circuit**

In both groups we used a S3 heart–lung machine (Stöckert Instrumente GmbH, Munich, Germany) with a centrifugal pump (Delphin, Terumo Europe NV, Leuven, Belgium), a heat exchange device (Stöckert Instruments GmbH), polyvinyl tubing system (Medtronic Inc., Minneapolis, MN, USA), a hollow fiber oxygenator (Affinity, Medtronic), a soft shell collapsible venous reservoir (MVR 1600, Medtronic), an arterial line filter (Affinity, 38 mic, Medtronic), and a cardiotomy reservoir (Intercept cardiotomy, Medtronic).

In the control group, the total priming volume consisted of 1400 ml: 1000 ml modified fluid gelatin (Gelofusin, Braun, Melsungen, Germany), 50 ml lactated Ringer’s solution, 200 ml of aprotinin, 100 ml mannitol and 50 ml sodium bicarbonate (8.4%) containing 1500 mg cefuroxime and 5000 IU bovine heparin.

In the experimental group, retrograde autologous priming was performed using 500 ml autologous blood, collected in a transfer bag during the bicaval cannulation procedure. The priming was completed using the priming solution described for the control group. Coronary sinus drainage was achieved by cold crystalloid antegrade cardioplegia (St. Thomas solution) delivery through the aortic root. Use of the experimental cannula allowed partial recovery of the cardioplegic solution. In the middle of the cannula’s balloon, on the cardiac site, a side hole is positioned. This specific side hole is connected to a suction line. During and after cardioplegia infusion, the cardioplegia solution (and/or residual systemic blood) from the right atrium was recovered via the suction tubing.

**Cardiopulmonary bypass**

*Standard group:* standard cannulation technique using a two stage venous cannula placed in the inferior vena cava and right atrium.

*Experimental group:* bicaval cannulation through the superior caval vein using a dual–stage bicaval atrial cooling cannula. This cannula is a cardiopulmonary dual stage venous catheter with an integrated cooling balloon. The system is designed to circulate cold saline (0.9% NaCl) in the right atrium enabling homogeneous cooling of
the atrium. The cannula is a polyvinyl chloride (PVC) fashioned into an “L” shape. An opening at the elbow of the L curve collects venous blood from the superior vena cava. The distal end of the cannula has a cone shape with openings to collect venous blood from the inferior vena cava. Between these two collection areas, a 9 cm long medical grade silicon balloon has been attached. The balloon has a maximum volume capacity of 40 ml. Inflation and deflation of the balloon depends on the saline volume added in the attached closed circuit. Immediately prior to aortic cross-clamping the balloon was filled with saline solution, and thereafter total CPB was initiated. A heat-exchanger (Bentley HE-30) cooled the saline to 4°C and a small roller pump maintained the saline circulation at a flow rate of 300 ml.min⁻¹. CPB was initiated after systemic heparinization (300 I.U. kg⁻¹), with a celite activated clotting time higher than 480 seconds. The target hematocrit during CPB was higher than 23%. The non-pulsatile blood flow rate was maintained between 2.2–3.0 L.min⁻¹.m⁻² with mild hypothermia in the standard group (30–33°C), and normothermia (35–36°C) in the experimental group. Recovered cardioplegia fluid and suctioned blood were discarded. Electrocardiograms were obtained preoperatively, on intensive care unit admission, and on postoperative days 1 and 2.

**Organ injury biomarkers**

*Myocardial injury biomarker:* creatine kinase MB (CK–MB) activity – Vitros analyzer (Ortho Clinical Diagnostics; Beerse, Belgium).

*Kidney injury biomarker:* urine N-acetyl-glucosaminidase (NAG) release in urine signifies renal damage and ischemia localized at the proximal tubules level¹¹. The method of detection was a modified enzyme assay according to Lockwood¹² at pH 4.5 and corrected for non-specific conversion (HaemoScan, Groningen, The Netherlands).

*Intestinal injury biomarkers:* intestinal fatty acid binding protein I–FABP, ELISA kit (HyCult Biotechnology BV, Uden, The Netherlands) is a cytosolic protein readily released into the circulation following enterocytes damage, reported as sensitive and specific urine markers for the detection of intestinal injury and prediction of gastrointestinal complications after cardiopulmonary bypass¹³,¹⁴. Urine NAG and I–FABP concentrations were measured preoperative (baseline) and postoperative, in the urine collected during the first 2 hours postoperative, as previous investigations showed peak values of both markers coming during the first 2 hours after the end of the surgical procedure¹⁵. Urinary excretions of NAG and I–FABP were calculated as ratio to urine urea concentration in order to correct for urine dilution. In addition to these organ injury biomarkers, standard laboratory investigation were performed every 24 hours until hospital discharge. Standard laboratory investigations included: hemoglobin, serum glucose, HCO₃⁻, sodium ions, potassium ions, urea, serum creatinine, lactate, ASAT, ALAT, C Reactive Protein, total proteins, albumin,
total creatine kinase. 

_Lactate concentrations in the venous whole blood_ (Rapidlab 865; Chiron Diagnostics Corp., East Walpole, MA) – end product of anaerobe glycolysis in skeletal muscle, brain and erythrocytes; lactate was measured to give an indication on systemic exposure to hypoxia.

**Statistical Analysis**

A power analysis based on previous studies in this population on peak postoperative CK–MB plasma activity suggested that at least 40 patients have to be studied in order to detect a 1 SD difference between the two groups, with a reliability of 5% and a power of 80%

Before analysis, the data was tested for distribution according to Kolmogorov–Smirnov goodness of fit test. Continuous variables were compared by means of parametric (Student T Test) or nonparametric tests (Mann–Whitney). Fisher’s exact test was used to compare discrete variables. Correlation between variables was tested using Spearman correlation test. Linear regression analysis was used to detect predictors in the model. Results are presented as mean±SEM (unless stated otherwise). Independent predictors were tested using linear regression analysis. Multivariate analysis was used to find significant predictor models.

### 3.3 Results

Twenty patients were randomized to each group. Patients’ demographic and clinical characteristics, operating times, perioperative fluid management, hemodynamics, coagulation variables and blood loss are presented in Table 3.1. Phenylephrine was administered intraoperatively to three patients in the experimental group (0.83±0.16) and ten patients in the standard group (0.85±0.31), (Fisher’s exact test 1–sided p=0.02).

The following complications were diagnosed: re–thoracotomie (n=2, 1 of surgical and 1 of non–surgical etiology) and postoperative atrial fibrillations (n=8). The distribution of complications was similar in both groups. No clinical diagnose of postoperative myocardial infarction, cerebral vascular accidents/transitory cerebral ischemic accidents, acute renal injury or gastrointestinal complications was established. In–hospital mortality was 0% for both groups.

**Temperature**

The minimum corporeal (intrarectal) temperatures measured during CPB were 32.9±0.2° C in the standard hypothermic group and 35.9±0.1° C in the experimental normothermic group.
In the standard hypothermic group of patients, the rectal temperatures measured after 60 min of CPB correlated negatively with postoperative I-FABP urine concentrations (Spearman’s corr. –0.501, p=0.029). In other words, lower rectal temperatures during CPB were associated with higher intestinal damage.

<table>
<thead>
<tr>
<th>Standard group Mean±SEM</th>
<th>Experimental group Mean±SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Male/Female</td>
<td>18/2</td>
<td>5/5</td>
</tr>
<tr>
<td>Age (y)</td>
<td>60.9±1.8</td>
<td>59.4±1.7</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>2±0.03</td>
<td>2.03±0.03</td>
</tr>
<tr>
<td>Distal anastomoses per patient</td>
<td>3.7±0.2</td>
<td>4.2±0.1</td>
</tr>
<tr>
<td>Priming volume (ml)</td>
<td>1377±38</td>
<td>857±44</td>
</tr>
<tr>
<td>Cardioplegic volume (ml)</td>
<td>1382±84</td>
<td>1373±49</td>
</tr>
<tr>
<td>Recovered cardioplegic volume (ml)</td>
<td>0</td>
<td>576±46</td>
</tr>
<tr>
<td>Fluid balance during CPB</td>
<td>1742±117</td>
<td>771±48</td>
</tr>
<tr>
<td>Fluid balance 1st POD</td>
<td>1286±240</td>
<td>1091±296</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>100±5</td>
<td>97±5</td>
</tr>
<tr>
<td>Aorta cross-clamping time (min)</td>
<td>67±5</td>
<td>69±4</td>
</tr>
<tr>
<td>lowest MAP during CPB (mmHg)</td>
<td>53.8±1.4</td>
<td>53.1±1</td>
</tr>
<tr>
<td>CI preoperative (L/min/m²)</td>
<td>2.43±1.14</td>
<td>2.22±0.13</td>
</tr>
<tr>
<td>CI postoperative (L/min/m²)</td>
<td>3.2±0.15</td>
<td>3.15±0.19</td>
</tr>
<tr>
<td>CRP preoperative (mg/l)</td>
<td>8.6±4</td>
<td>6.1±1.1</td>
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<tr>
<td>CRP 2nd POD (mg/l)</td>
<td>60.6±13.3</td>
<td>58.2±8.4</td>
</tr>
<tr>
<td>CRP 3rd POD (mg/l)</td>
<td>94.4±14.2</td>
<td>80.6±13.3</td>
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<tr>
<td>max ICU APTT (sec)</td>
<td>46.7±1.7</td>
<td>43.8±1.3</td>
</tr>
<tr>
<td>max ICU INR 1h</td>
<td>1.55±0.03</td>
<td>1.58±0.03</td>
</tr>
<tr>
<td>Total blood loss postoperative (ml)</td>
<td>828±89</td>
<td>822±76</td>
</tr>
<tr>
<td>Time to tracheal extubation (h)</td>
<td>10.8±1.11</td>
<td>10.2±0.9</td>
</tr>
<tr>
<td>ICU stay (days)</td>
<td>1.1±0.1</td>
<td>1.05±0.05</td>
</tr>
</tbody>
</table>

Table 3.1: Patient characteristics and operative data (mean±standard error of the mean). CPB=cardiopulmonary bypass, ICU=intensive care unit, MAP=Mean arterial pressure, CI=cardiac index, APTT=activated partial thromboplastin time, INR=international normalized ration. Statistics: Continuous variables where compared by means of parametric (Student T Test) or nonparametric tests (Mann–Whitney). Fisher’s exact test was used to compare discrete variables.

**Hematocrit**

Hematocrit values were measured systematically for 24 h, starting with a preoperative baseline time point (Fig. 3.1). The values measured in the beginning of CPB (“1st
value during CPB”: standard group 24.5±0.7% versus experimental group 28.3±0.7%)
and at the end of CPB (“post-CPB”: standard group 24.4±0.7% versus experimental
group 27.8±0.6%) were significantly different (Mann–Whitney p=0.002 both time
points).

**Transfusion requirements**

Seven patients in the standard group (1.6±0.2 units/patient) and two patients in
the experimental group (1 unit/patient) received packed cell (PC) transfusion in the
operating room (Fisher’s exact test 1–sided p=0.064). In the ICU, twelve patients in
the standard group (1.6±0.3 units/patient) and six patients in the experimental group
(1.5±0.5 units/patient) received PC transfusion (Fisher’s exact test 1–sided p=0.055).
No other blood products (single donor plasma or platelets concentrates) were admin-
istered in the operation room or ICU to the patients included in this study.

**Organ injury biomarkers (Fig. 3.2)**

**Myocardial injury**

Electrocardiographic modifications: ECGs were obtained preoperatively, on intensive
care unit admission, and on postoperative days 1 and 2. No postoperative develop-
ment of new Q waves was registered at any time point.

CK–MB (Fig. 3.2a) increased abruptly during the operation in both groups, with
significantly different postoperative CK–MB activity between groups (4 h ICU: Mann–
Whitney p=0.021, 8 h ICU: Mann–Whitney p=0.024). The peak values of CK–MB
measured after the patients were transferred in the ICU were significantly higher in
the standard protocol group than in the experimental protocol group (22.6±2.3 U/l
versus, 15.5±1.3 U/l respectively; Mann–Whitney p=0.026).

**Renal injury**

Serum creatinine values stayed within normal ranges for the entire investigated pe-
riod, with no differences between groups. The peak values of serum creatinine mea-
sured during the investigated period were 91.3±2.1 µmol/l in the standard group, and
93.5±2.3 µmol/l in the experimental group.

Postoperative urine N–acetyl–glucosaminidase (NAG, Fig. 3.2b) concentrations reached
significantly higher values in the standard protocol group than in the experimental
protocol group (213.6±54.5 mU/mmol urea versus 51.2±11.8 mU/mmol urea, Mann–
Whitney p<0.001).
Intestinal injury

Postoperative urine concentrations of intestinal-type fatty acid binding protein (I–FABP, Fig. 3.2c) was significantly higher in the standard protocol operated patients as compared with values of the patients in the experimental protocol group (325.5±34 versus 181.4±24, Mann–Whitney p=0.003).

Lactate concentrations in the venous whole blood stayed at all time points within normal ranges with no differences between the groups. The lactate values measured during CPB (experimental group 1.07±0.04 mmol/l, standard group 1.16±0.1 mmol/l) correlated positively with the postoperative I–FABP values (Spearman’s corr. 0.566, p=0.003) and postoperative NAG values (Spearman’s corr. 0.391, p=0.05).
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Figure 3.2: a) Creatine kinase–MB (CK–MB) activity in the plasma of 40 patients undergoing on–pump CABG according to an experimental CPB protocol (n=20) or the standard CPB protocol (n=20). The values are represented as mean (symbols) and standard error of the mean (bars).

* p<0.05. 

b) N–acetyl–glucosaminidase (NAG) excretion in the urine of 40 patients undergoing on–pump CABG according to an experimental CPB protocol (n=20) or the standard CPB protocol (n=20). Urinary excretions of NAG was calculated as ratio to urine urea concentration in order to correct for urine dilution. The values are represented as mean (symbols) and standard error of the mean (bars). ** p<0.01.

c) Intestinal–type fatty acid binding protein (I–FABP) in the urine of 40 patients undergoing on–pump CABG according to an experimental CPB protocol (n=20) or the standard CPB protocol (n=20). Urinary excretions of I–FABP was calculated as ratio to urine urea concentration in order to correct for urine dilution. The values are represented as mean (symbols) and standard error of the mean (bars). ** p<0.01.
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**Multivariate regression analysis**

The following parameters were examined as potential explanatory variables for the values of postoperative CK–MB, urine NAG and urine I–FABP production: patient age, gender, body surface area, co–morbidities (diabetes type II, preoperative TIA/CVA), perfusion time, cross–clamping time, lowest intraoperative mean arterial pressure, cardiac output, pre/postoperative hematocrit, PC units transfused in the operating room, priming volume, cardioplegic volume (total volume minus recovered volume), lowest core temperature during CPB, administration of peripheral vasoconstrictors. 

*The postoperative peak of CK–MB* correlated at a significance level < 0.2 with the following variables: gender, body surface area, perfusion time, cross–clamping time, postoperative hematocrit, and PC units transfused in the operating room. These variables were introduced in a backward multivariate regression analysis. The best predicting model (R²=24%, Sig=0.002) included the BSA (B=260.4, Sig.=0.019) and total perfusion time (B=2.001, Sig=0.011).

*The postoperative NAG* correlated at a significance level < 0.2 with the following variables: gender, body surface area, lowest intraoperative MAP, lowest core temperature during CPB, postoperative hematocrit, PC units transfused in the operating room, administration of peripheral vasoconstrictors, cardioplegic volume and priming volume. These variables were introduced in a backward multivariate regression analysis. The best predicting model (R²=56.9%, Sig<0.001) included the gender (higher NAG concentrations in women, B=181.1, Sig.=0.009), the postoperative hematocrit (B=–15.19., Sig.=0.054) and PC units transfused in the operating room (B=82.7, Sig=0.065).

*The postoperative I–FABP* correlated at a significance level < 0.2 with the following variables: age, gender, body surface area, lowest intraoperative MAP, lowest core temperature during CPB, postoperative hematocrit, PC units transfused in the operating room, diabetes, administration of peripheral vasoconstrictors, and priming volume. These variables were introduced in a backward multivariate regression analysis. The best predicting model (R²=70%, Sig<0.001) included the gender (higher I–FABP concentrations in men, B=–93.4, Sig.=0.048), the postoperative hematocrit (B=–16.19, Sig.=0.022), PC units transfused in the operating room (B=92.18, Sig=0.002), and priming volume (B=0.147, Sig.=0.027).

**Univariate regression analysis**

No predictors were detected for the variability in postoperative peak CK–MB concentrations in a univariate regression model.

The post–CPB Htc values were significant independent predictors for the variability in postoperative NAG concentrations (adjusted R²=38.5%, B=–34.87, p<0.001) and I–FABP concentrations (adjusted R²=37.4%, B=–27.01, p<0.001). Low hematocrit values were significantly associated with high levels of NAG and I–FABP.
Intraoperative PC transfusion was found as significant independent predictor of NAG release (adjusted $R^2=44.4\%$, $B=308.2$, $p<0.001$), and I–FABP release (adjusted $R^2=31.8\%$, $B=207.1$, $p<0.001$). The patients who received PC transfusion had significantly higher NAG and I–FABP levels.

Priming volume predicted significantly NAG variability (adjusted $R^2=13.2\%$, $B=0.236$, $p=0.012$) and I–FABP variability (adjusted $R^2=29.3\%$, $B=0.263$, $p<0.001$). A higher priming volume was independently associated with higher urine NAG and I–FABP values.

### 3.4 Discussion

The data presented in this study documents decreased postoperative myocardial, renal and intestinal tissue injury in patients undergoing on–pump CABG when using a modified operative protocol combining cold crystalloid cardioplegia and intracavitary cooling of the heart, autologous priming, and corporeal normothermia.

**Myocardial protection**

The protection of the heart during the ischemic arrest time was performed by combining the standard cold crystalloid cardioplegia with an intracavitary cooling system. A cold, sterile saline–filled balloon attached to a dual stage bicaval cannula enabled an additional local, homogeneous cooling of the right atrium. As a consequence, postoperative myocardial damage, as quantified by plasma levels of creatine kinase MB (CK–MB), was significantly lower in the patients in the experimental group.

Increased postoperative peak CK–MB values were demonstrated to be strong predictors of adverse outcomes, indicating increased risk of severe postoperative left ventricular dysfunction and mortality within 30 days of coronary artery bypass grafting\(^{16}\).

**Renal protection**

Transient proximal tubules injury was significantly attenuated in the patients benefiting from the experimental operative protocol, as shown by the urine concentrations of N–acetyl–beta–D glucosaminidase (NAG). NAG is a lysosomal enzyme of 130 kDa molecular mass, normally excreted in low amounts in urine as a consequence of the normal exocytosis process\(^{17}\). NAG was proposed as a valuable marker of tubulo–interstitial damage in various human glomerular diseases including diabetic nephropathy\(^{18}\), primary and toxic glomerulonephritis\(^{17,19}\). In patients undergoing CPB, measurement of urinary NAG additional to standard clinical tests was demonstrated to be useful in recognizing early and differentiated changes in renal function\(^{20,21}\).

In our study, NAG levels were in average 4 folds higher in the patients undergoing CPB according to the standard protocol, as compared to the experimental protocol.
Intestinal protection

Transient intestinal damage, as quantified by the urinary excretion of intestinal–type fatty acid binding protein (I–FABP), was significantly decreased in patients undergoing on pump CABG according to the experimental protocol. I–FABP is a $15\text{kDa}$ cytosolic protein uniquely located in the mature epithelium of the villi, which are most susceptible to ischemic injury. Clinical studies demonstrated that I–FABP is released into the blood stream and excreted by kidneys early in the course of intestinal ischemia. Elevated I–FABP urine levels predict the development of gastrointestinal complications after CPB, and correlate with the clinical development of the systemic inflammatory response syndrome in critically ill patients. In our study, the urinary excretion of I–FABP in the standard protocol group was in average 2 folds higher than in the experimental protocol group.

Postoperative renal and intestinal injury were independently predicted by three variables: postoperative hematocrit, transfusion requirements during operation, and priming volume of the extracorporeal circuit.

Effect of hematocrit

Analysis of variance for linear regression showed that variation in Htc independently explained 38% and 37% in the variability of postoperative NAG and I–FABP, respectively. A decrease with one unit (1%) in hematocrit predicted significantly an increase with a quarter of the peak postoperative NAG values. The same decrease with one unit (1%) in hematocrit predicted significantly an increase of a tenth of the peak postoperative I–FABP values. In addition, hypoxia during CPB, as quantified by lactate values, correlated positively with intestinal and renal injury postoperative. This association could represent an additional indication that a decrease in hematocrit, with a subsequent decrease in oxygen–carry capacity, might be responsible of intestinal and renal injury post CPB. With regards to the effect of hemodilution on post operative myocardial injury, our study showed no significant predictor values for the post–operative hematocrit values on the post–operative CK–MB peak concentrations.

These findings quantify for the first time, to our knowledge, the effect of hemodilution and subsequent hematocrit values on postoperative renal and intestinal injury in the clinical setting of on–pump CABG. These data bring additional information to explain previously reported conclusions of large observational studies reporting a significant greater incidence of postoperative complications as hematocrit values decrease: renal failure (~ four–fold), multiorgan failure (~ seven–fold), and septicemia (~ three–fold). The combined operative strategy presented in this study allowed higher intra– and post–operative hematocrit values as a consequence of the partial recovery of the car-
dioplegic solution and autologous blood priming of the extracorporeal circuit.

**Effect of transfusion requirements**

The differences between groups in PC transfusion requirements, both intraoperatively and in the ICU, were significant at a 10% level. Packed cell transfusion in the operating room predicted independently a variation of 44% in NAG values and 32% of I–FABP values. However, in order to subtract from this estimation the effect of low hematocrit of the patients that received transfusion, we introduced in the same regression model both hematocrit values and transfusion requirements. By analyzing the proportions of residuals we could conclude that an extra 21.5% of the variability in NAG and 10% of the variability in I–FABP that could not be explained by the variation in hematocrit values were explained by the transfusion requirements. In other words, intraoperative packed cells transfusion to the patients was correlated significantly with postoperative renal and intestinal injury, independently of the hematocrit value.

The effect described by the present data support the previous raised concerns regarding transfusion–associated morbidity such as hemolytic or allergic reactions, infections (human immunodeficiency virus, cytomegalovirus, hepatitis)\(^{27}\), graft–versus–host disease\(^ {28}\) and an increased incidence of postoperative infections in patients undergoing coronary artery bypass surgery\(^ {29}\).

**Effect of priming volume**

The volume of the solution used to prime the extracorporeal circuit was an independent predictor of renal injury (13% of NAG variation) and intestinal injury (29% I–FABP variation). However, this effect proved to reflect only the subsequent hemodilution effect on organ injury.

As a consequence of partial cardioplegic volume recovery, the fluid balance was significantly lower in the experimental group than in the standard group.

**Effect of corporeal temperature**

The “cold heart, warm body” principle applied in this study proved to be safe and effective in preserving myocardial integrity and, in the same time, in attenuating postoperative intestinal injury. In the hypothermic group, lower intrarectal temperatures measured at 60 min of CPB correlated significantly with higher intestinal damage postoperatively. The association between lower corporeal temperatures with higher postoperative intestinal injury reflects the findings of Ohri and all.\(^ {30}\), showing significantly reduced gastric mucosal blood flow during cross clamping in patients undergoing hypothermic CPB as compared to patients undergoing normothermic CPB. Regarding the validity of normothermic cardiopulmonary bypass, the data presented
in this study support the previous studies evaluating the normothermic CPB associated with topical heart hypothermia, that reported it to be safe\textsuperscript{1–5}, to simplify surgical procedures, and to facilitate postoperative management\textsuperscript{31}. Additional to the description of the independent predictors for renal and intestinal injury, a multivariate analysis revealed the importance of patients demographics, perioperative variables, medication and co-morbidity for the variability of post operative cardiac, renal and intestinal damage. High postoperative myocardial damage was predicted by a large body surface area and long perfusion durations. Post operative transient renal injury was higher in women, in patients with a low postoperative hematocrit and those who received packed units intraoperatively. Post operative transient intestinal injury was higher in men, in patients with a low postoperative hematocrit, in those patients who had high priming volumes and who received packed units intraoperatively.

3.5 Conclusions

In conclusion, this chapter describes the experimental infrastructure and clinical application of a comprehensive operative strategy that limits postoperative myocardial, renal and intestinal tissue injury in patients undergoing heart–lung machine assisted coronary artery bypass grafting. The addition of an intracavitary cooling system to the standard cold crystalloid cardioplegia offered a better protection of the myocardium, with decreased postoperative plasma levels of creatine kinase MB. Partial recovery of intra-atrial cardioplegic fluid and autologous blood priming limited the extent of intra-operative hemodilution and blood transfusion requirements (10% significance level), and resulted in an important attenuation of the transient renal and intestinal postoperative injury.

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Chapter 3


