Off-pump coronary artery bypass surgery versus standard linear or pulsatile cardiopulmonary bypass: endothelial activation and inflammatory response

Francesco Onoratia, Antonino S. Rubinob, Sergio Nuceraa, Daniela Fotib, Vincenzo Sicad, Francesco Santinic, Elio Gullettab, Attilio Renzullia

a Cardiac Surgery Unit, Magna Graecia University of Catanzaro, Catanzaro, Italy
b Pathology Unit, Magna Graecia University of Catanzaro, Catanzaro, Italy
c Cardiac Surgery Unit, University of Verona, Verona, Italy
d Institute of Pathology, Second University of Naples, Naples, Italy

Received 16 July 2009; received in revised form 3 November 2009; accepted 6 November 2009; Available online 16 December 2009

Abstract

Objective: Poor outcomes after coronary artery bypass grafting (CABG) have been linked to perioperative endothelial activation and systemic inflammatory responses. The use of pulsatile cardiopulmonary bypass (PCPB) or off-pump CABG (OPCABG) may minimise these phenomena. We compared biochemical and clinical outcomes among patients who underwent CABG with PCPB, CABG with linear CPB (LCPB) or OPCABG.

Methods: Sixty consecutive patients undergoing isolated elective CABG were prospectively randomised trial to receive pulsatile CPB (group A, 20 patients), linear CPB (group B, 20 patients) or OPCABG (group C, 20 patients). Levels of proinflammatory cytokines (interleukins-2, -6, and -8), anti-inflammatory cytokines (interleukin-10) and endothelial markers (vascular endothelial growth factor (VEGF), monocyte chemo-attractant protein (MCP)-1) were measured before, during and after surgery.

Results: VEGF and MCP-1 levels increased significantly during surgery in all groups, but they increased the least and were the lowest overall with OPCABG. They rose most and peaked overall with LCPB. Interleukin-2 levels remained stable during OPCABG but decreased equally during PCPB and LCPB. Interleukin-6 and -8 levels rose significantly during both types of CPB versus OPCABG. Interleukin-10 levels increased significantly in all groups during surgery, but they rose least and were the lowest overall with OPCABG and rose most and were the highest overall with PCPB. Intubation times, intensive care unit (ICU) stay and hospital stay were significantly longer in the LCPB group than the other two groups.

Conclusions: LCPB appears to promote endothelial activation and cytokine secretion, which may delay recovery. OPCABG was associated with slight endothelial activation and cytokine response. PCPB significantly attenuates endothelial/cytokine leakage, resulting in hospital outcomes comparable with those after OPCABG.

© 2009 European Association for Cardio-Thoracic Surgery. Published by Elsevier B.V. All rights reserved.

Keywords: Grafting; Cardiopulmonary bypass; Endothelium; Interleukins; Inflammation

1. Introduction

Systemic inflammatory response syndrome (SIRS) after cardioangiopulmonary bypass (CPB) is considered an important cause of postoperative morbidity and mortality in coronary artery bypass grafting (CABG) [1]. The vascular endothelium has been shown to actively participate in maintaining normal cardiovascular homeostasis by influencing the regulation of membrane permeability, vasomotor tone, coagulation, fibrinolysis and inflammation. Endothelial cell activation, endothelial production of soluble cytokines and the cell surface expression of adhesion molecules are considered to be crucial steps in the systemic inflammatory response to CPB [2].

CABG-related endothelial activation can result from ischaemia—reperfusion injury after cardioplegic arrest, from interaction of blood with the artificial surfaces of the extracorporeal circuit and from induction of non-physiologic, pulsatile perfusion [1]. Attempts to minimise the impact of SIRS therefore have included modifications of CPB circuits, components and surfaces, among other approaches [1]. Simultaneously, indications for off-pump CABG (OPCABG), which avoids both cardioplegic arrest and CPB use, have widened worldwide [3]. The recent literature has reported a beneficial impact of OPCABG on endothelial activation and SIRS [3,4]. Moreover, although non-pulsatile blood flow obtained with standard CPB circuits is considered an acceptable, non-physiologic compromise conferring few disadvantages (including lack of induction of the inflammatory response) [1,4], we have reported beneficial effects on splanchnic, renal, respiratory and haemostatic functions when intra-aortic balloon pump (IABP)-induced pulsatile perfusion has been employed in routine coronary surgery.
The relative effects of all of these options on endothelial activation, inflammatory responses and clinical outcomes are unknown, however.

The aim of this randomised study, then, was to compare markers of endothelial and inflammatory responses and clinical outcomes among patients undergoing OPCABG, CABG with standard linear CPB (LCPB) or CABG with IABP-induced pulsatile CPB (PCPB).

2. Methods

2.1. Patients

Consecutive patients, aged 30–70 years, undergoing elective CABG at our institution were eligible for enrollment. Patients were excluded if they had chronic renal insufficiency/failure, liver failure, chronic obstructive pulmonary disease, abdominal aortic aneurysm with abdominal atheroatherosclerosis, autoimmune disease, severe anaemia (haemoglobin <8 g dl\(^{-1}\)), known coagulation disorder or unstable angina.

Eligible patients were randomised to receive PCPB, LCPB or OPCABG by lottery, drawing from sealed envelopes containing the group assignment. The protocol was approved by the institution’s ethical committee. Informed consent was obtained from each patient enrolled.

2.2. Management

All patients were taking aspirin before surgery (150 mg per day). Aspirin was withdrawn 3 or more days before surgery and substituted by subcutaneous enoxaparin. All patients were to receive preoperative IABP if they had any of the following high-risk characteristics [5–7]: critical left main coronary artery disease (≥90% stenosis with or without an ejection fraction <40%, ≥80% left main stenosis with ≥90% right coronary artery stenosis or chronic occlusion of the three main coronary arteries with a poor angiographic bed).

Anaesthesia was induced by intravenous propofol infusion (3 mg kg\(^{-1}\)) with fentanyl (0.10 mg kg\(^{-1}\)). Neuromuscular blockade was achieved by pancuronium bromide (4 mg h\(^{-1}\)), and lungs were ventilated to normocapnia with air and oxygen (45–50%). Anaesthesia was maintained with propofol infusion (150–200 \(\mu\)g kg\(^{-1}\) min\(^{-1}\)) and isoflurane (0.5% inspired concentration). Arterial and central venous catheters were the standard.

Inotropic support was recorded and defined as low-dose (enoximone \(\leq 5 \mu\)g kg\(^{-1}\) min\(^{-1}\)), medium-dose (enoximone 6–10 \(\mu\)g kg\(^{-1}\) min\(^{-1}\) or dobutamine 5–10 \(\mu\)g kg\(^{-1}\) min\(^{-1}\)) or high-dose (enoximone or dobutamine infusion \(>10 \mu\)g kg\(^{-1}\) min\(^{-1}\) or any epinephrine dose).

All patients underwent surgery at 8:00 a.m. to minimise any time-dependent variation in mediator synthesis. Institutional policy called for percutaneous insertion of IABP (7.5 Fr, 34 or 40 ml according to body surface area; Dataspoke Corp., Fairfield, NJ, USA) with the sheathless technique [5,6] through the best femoral artery before induction of anaesthesia. The balloon was connected to a Dataspoke CS-300 pump (Dataspoke Corp., Fairfield, NJ, USA). The correct placement of IABP was verified by postoperative chest X-ray or trans-oesophageal echocardiography.

Pulsatile-CPB patients received preoperative IABP before induction of anaesthesia, with IABP switched to automatic 80-bpm mode during cardioplegic arrest to achieve pulsatile flow during aortic cross-clamping. IABP was restarted at 1:1 mode immediately after cross-clamp removal. Linear-CPB patients received the same preoperative IABP, but the pump was discontinued during cross-clamping and resumed at a 1:1 mode after cross-clamp removal [5–7]. OPCABG patients underwent CABG with 1:1 IABP assistance throughout surgery. All patients underwent preoperative echo-Doppler scanning of peripheral arteries and the abdominal aorta to minimise the risk of major IABP-related vascular complications.

Surgery (arterial or venous CABG) was performed through median sternotomy by the same senior surgeons in all cases. CPB was standardised with use of Dideco (Mirandola, Modena, Italy) tubing sets, a 40-\(\mu\)m filter, a Stockert roller pump (Stockert Instrumente, Munich, Germany) and a hollow-fibre membrane oxygenator (Monolyth, Sorin Biomedica, Saluggia, Italy). Heparin (300 IU kg\(^{-1}\)) was given to achieve a target activated clotting time exceeding 480 s. Body temperature was kept between 32 °C and 34 °C. Myocardial protection was achieved with intermittent antegrade and retrograde hyperkalaemic blood cardioplegia. Total CPB flow was maintained at 2.6 l min m\(^{-2}\). Blood was recovered intraoperatively with an autotransfusion device (Autotrans Dideco, Mirandola, Modena, Italy), and blood transfused for a haemoglobin level below 8 g dl\(^{-1}\).

In the OPCABG group, exposure and stabilisation were achieved with the Octopus-IV tissue stabiliser (Medtronic Inc., Minneapolis, MN, USA). The lateral wall vessels were exposed with the aid of the Starfish-2 system (Medtronic Inc., Minneapolis, MN, USA). Visualisation was enhanced with a surgical blower—humidifier device (model SSVW-002, Surgical Site Visualisation Wand, Research Medical, Midvale, UT, USA) connected to a regulated CO\(_2\) gas source. Intra-coronary shunts were routinely and successfully used in all coronary arteries except the obtuse marginal branches, in which the success rate was 90%. Coronary snaring was not used during OPCABG. During OPCABG, the first grafted vessel was always the left anterior descending coronary artery, followed by proximal and then distal anastomoses.

Protamine was given at the end of surgery to fully reverse heparin. Patients then received low-molecular-weight heparin, starting when postoperative bleeding had been controlled (usually within 6 h) and continuing until postoperative day 3, followed by aspirin 150 mg daily. IABP was withdrawn when haemodynamic stability was restored (cardiac index ≥2.5 l min\(^{-1}\) m\(^{-2}\) with only minimal pharmacologic inotropic support consisting of dobutamine or enoximone at 5 \(\mu\)g kg\(^{-1}\) min\(^{-1}\)). Anaesthesiologists and cardiologists providing post-surgical care were blinded towards the intra-operative group assignment.

2.3. Blood sampling for markers of endothelial activation, cytokines and troponin I

Blood was collected from the peripheral arterial line preoperatively (time 0), after construction of proximal anastomoses in pulsatile and linear groups or completion of the last distal anastomosis in OPCABG group (time 1), at
the end of surgery (time 2) and at 12 (time 3) and 24 h (time 4) postoperatively.

Levels of cytokines — interleukins (ILs)-2, -6, -8 and -10 and monocyte chemo-attractant protein (MCP)-1 — and vascular endothelial growth factor (VEGF) were simultaneously and quantitatively determined by sandwich chemiluminescent immunoassay (Biochip Array Technology; Randox, UK) according to manufacturer's instructions and the values are presented after correction for the haemodilution.

2.4. Endpoint definitions

The primary endpoint for endothelial response was perioperative changes in MCP-1 and VEGF levels and that for cytokine leakage was perioperative changes in IL-10 levels for evaluation of anti-inflammatory response, whereas perioperative changes in proinflammatory cytokines IL-2, IL-6 and IL-8 were the secondary endpoints of cytokine burst.

Hospital outcomes were also analysed as secondary endpoints. Mortality was defined as death occurring within or 30 days after surgery. In-hospital morbidity was any complication requiring specific therapy or prolonging hospital or intensive care unit (ICU) stay. Acute respiratory insufficiency needing non-invasive positive-pressure ventilation was diagnosed if any of the following occurred: respiratory acidosis (arterial pH ≤ 7.35 with partial arterial pressure of CO₂ ≥ 45 mmHg); arterial O₂ saturation by pulse oximetry <90% or partial arterial pressure of O₂ <60 mmHg at an inspired O₂ fraction ≥0.5; ≥35 respirations min⁻¹; agitation, diaphoresis or decreased consciousness; clinical signs suggesting respiratory muscle fatigue; and increased work in breathing such as the use of respiratory accessory muscles, paradoxical motion of the abdomen or retraction of the intercostal spaces [5]. Acute renal insufficiency was defined as a serum creatinine level >50% higher than the preoperative value; and acute renal failure, as acute renal insufficiency requiring renal replacement therapy.

Perioperative acute myocardial infarction (MI) was defined by any of the following: new Q waves >0.04 ms with a peak TnI level >3.7 μg l⁻¹ or TnI concentration >3.1 μg l⁻¹ at 12 h following surgery; >25% reduction in R waves in two or more electrocardiographic leads associated with the same TnI peaks; or new akinetik or dyskinetic segments on echocardiography [5]. Low output syndrome (LOS) was diagnosed in cases of haemodynamic compromise or cardiac index <2.2 l min⁻¹ m⁻² during ICU stay despite IABP assistance and inotropic support, after correction of electrolyte and blood—gas abnormalities and after adjusting the preload to its optimal value. IABP-related complications included aortic dissection or perforation, limb or mesenteric ischaemia or infection or haemorrhage at the balloon entry point.

2.5. Statistical analysis

Statistical analysis was performed with the SPSS program for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were summarised as means ± standard deviation (SD) and categorical variables, as absolute numbers and/or percentages. Normally distributed continuous variables were compared using one-way analysis of variance (ANOVA) with post hoc Bonferroni correction for multiple comparisons where appropriate. Categorical variables were analysed using either the chi-square (χ²) test or Fischer's exact test. Group, time and group × time interactions at two-way ANOVA for repeated measures of five-time cytokine measurements in 20 patients for each study arm gave a power (1 — β error probability) of 99% with an α-error probability of 0.05. Comparisons were considered significant if P < 0.05.

3. Results

Between February and November 2008, 96 patients were admitted for elective primary CABG. In all, 36 patients were excluded from enrollment because of renal failure (n = 2), chronic renal insufficiency (n = 4), liver failure (n = 1), chronic obstructive pulmonary disease (n = 12), abdominal aortic aneurysm with abdominal arteriopathy (n = 2), autoimmune disease (n = 1), severe anaemia (n = 1), platelet disorder (n = 1) or unstable angina (n = 12). Thus 60 patients were eligible for randomisation and analysis. The three groups had similar preoperative and intra-operative characteristics (Table 1).

Table 1
Baseline and intra-operative characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Pulsatile CPB (n = 20)</th>
<th>Linear CPB (n = 20)</th>
<th>OPCABG (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Across groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatile versus linear</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulsatile versus OPCABG</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear versus OPCABG</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Mean age (y)             | 72.0 ± 4.7             | 71.4 ± 3.9           | 71.6 ± 4.8      | 0.90 |
| Male sex (n, %)          | 17 (85)                | 15 (75)              | 16 (80)         | 0.73 |
| Diabetes mellitus (n, %) | 6 (30)                 | 7 (35)               | 6 (30)          | 0.92 |
| Hypertension (n, %)      | 12 (60)                | 14 (70)              | 13 (65)         | 0.80 |
| Hypercholesterolemia (n, %) | 7 (35)          | 9 (45)               | 10 (50)         | 0.62 |
| Recent myocardial infarction (n, %) | 9 (45)  | 4 (20)               | 8 (40)          | 0.30 |
| Mean ejection fraction (%) | 43.3 ± 4.7          | 43.1 ± 4.2           | 43.0 ± 5.9      | 0.99 |
| Mean bypass grafts (no.) | 3.5 ± 0.7              | 3.3 ± 0.5            | 3.4 ± 0.5       | 0.65 |
| Mean aortic cross-clamp time (min) | 49.8 ± 16.3 | 46.8 ± 20.7         | 0.79            |     |
| Mean CPB time (min)      | 76.3 ± 27.3            | 78.4 ± 23.4          | 0.86            |     |

CPB indicates cardiopulmonary bypass. Data are numbers (%) of patients or mean ± SD.
3.1. Endothelial response

The three groups showed markedly different release patterns for markers of endothelial response. Levels of MCP-1 increased in patients receiving CPB, peaking at 12 h postoperatively (Fig. 1). Increases were significantly greater during the first 12 h among patients who received LCPB versus PCPB. By contrast, patients undergoing OPCABG showed slight decreases in MCP-1 levels after surgery.

Both CPB groups showed significant increase in VEGF levels, which peaked for both at the time of aortic declamping (time 1). Patients receiving LCPB released significantly more VEGF between time 1 and 24 h following surgery (time 4) than did patients receiving PCPB. VEGF levels did not change significantly after surgery in patients undergoing OPCABG; thus, VEGF leakage was significantly lower from time 1 to time 4 in this group than in patients receiving LCPB, whereas leakage was lower with OPCABG only at times 1 and 2 compared with patients receiving PCPB (Fig. 1).

3.2. Cytokine release

Patients undergoing OPCABG showed very low secretion of cytokines, whereas a significant burst was triggered by CPB (Figs. 2 and 3). Levels of anti-inflammatory IL-10 showed only a limited rise in the OPCABG group, peaking at time 3. However, IL-10 levels rose significantly during CPB, peaking at time 2 in both groups and increasing more in the PCPB group (Fig. 2).

Similarly, the OPCABG group showed no significant changes in perioperative leakage of proinflammatory cytokine IL-2 (Fig. 2) and showed only slight increases in IL-6 and IL-8 levels (Fig. 3). By contrast, both CPB groups showed significant and similar changes in the levels of these cytokines. IL-2 rose initially in both groups, but returned to the preoperative levels at time 3. IL-6 and IL-8 rose progressively beginning at time 1 and peaking at time 3.

3.3. Hospital outcomes

Most peri- and postoperative outcomes did not differ significantly among the groups (Table 2). The only difference in terms of hospital outcome among the three groups was a significantly longer intubation time, ITU stay and hospital stay in patients undergoing standard linear CPB (group B), whereas these outcome variables were comparable between the pulsatile-CPB and linear-CPB groups (Table 3).

Neither major nor minor IABP-related complications were reported, and inotropic support did not differ significantly among groups.
4. Discussion

Since the earliest experience in cardiac surgery, CPB-induced systemic inflammatory response has been considered a main determinant of postoperative morbidity and mortality [1]. Moreover, the exponential growth of interventional cardiology has led to increased surgical referral of patients with extensive organ co-morbidities and advanced cardiac pathologies, who are even more prone to exacerbated inflammatory responses after CPB [1,5]. Complex interactions between the surgical trauma; the contact phase, when circulating blood comes into contact with the artificial surfaces of the tubing set, the oxygenator, the filters, etc.; induction of linear perfusion with vasoconstrictive reflexes; haemodilution; activation of the coagulation, fibrinolysis and complement cascades; drug-related enhancement of inflammation itself; and the genetic substrate all contribute to clinical manifestation of SIRS [1,6]. Modifications of CPB machinery ranging from more biocompatible tubes and membranes to pulsatile CPB to CPB avoidance (OPCABG) or limitation (mini-CPB) have been suggested to attenuate the risk of SIRS. We noted beneficial effects on biochemical outcomes with the use of IABP-induced PCPB in this study, such that outcomes were similar to those of patients undergoing OPCABG, which avoids CPB use altogether.

We have previously demonstrated that the flow during CPB and aortic cross-clamping with IABP on in the automatic mode is effectively pulsatile and not only modulated, as resulting from the increase of surplus haemodynamic energy [7].

As far as coronary perfusion is concerned, ischaemic myocardium would benefit from an increased coronary perfusion, as it could be achieved by IABP. Accordingly, we agree with Lim et al. who demonstrated that the pulsatile flow achieved by a linear pump with the aid of counterpulsation gave the most physiological coronary blood perfusion. In addiction, IABP-induced pulsatility resulted in a lower peak pressure of the left ventricle than with other pulsatile tools, aiding cardiac recovery by reducing the ventricular afterload [8]. Therefore, we believe that IABP would be the easiest way to reach effective pulsatile CPB in association with significant improvement to coronary perfusion [9].

Endothelial activation is critical to the development and maintenance of systemic inflammation and thus plays a pivotal role in post-CBP morbidity and mortality [1,4,10,11]. Accordingly, the clinical use of biochemical assays to detect and quantify circulating cytokines and chemokines — the main effectors of inflammatory response — may help increase our knowledge regarding SIRS [1,4]. Despite the extensive literature, however, the mechanisms underlying post-CBP SIRS are not definitely understood, primarily because of

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Pulsatile CPB (n = 20)</th>
<th>Linear CPB (n = 20)</th>
<th>OPCABG (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Paroxysmal atrial fibrillation</td>
<td>2 (10)</td>
<td>5 (25)</td>
<td>1 (5)</td>
<td>0.16</td>
</tr>
<tr>
<td>Acute respiratory insufficiency</td>
<td>4 (20)</td>
<td>7 (35)</td>
<td>2 (10)</td>
<td>0.16</td>
</tr>
<tr>
<td>Acute renal insufficiency</td>
<td>1 (5)</td>
<td>2 (10)</td>
<td>—</td>
<td>0.36</td>
</tr>
<tr>
<td>Acute renal failure</td>
<td>—</td>
<td>1 (5)</td>
<td>—</td>
<td>0.37</td>
</tr>
<tr>
<td>Low output syndrome</td>
<td>1 (5)</td>
<td>3 (15)</td>
<td>1 (5)</td>
<td>0.42</td>
</tr>
<tr>
<td>Inotropic support</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-dose</td>
<td>16 (80)</td>
<td>14 (70)</td>
<td>16 (80)</td>
<td>0.69</td>
</tr>
<tr>
<td>Medium-dose</td>
<td>3 (15)</td>
<td>4 (20)</td>
<td>3 (15)</td>
<td>0.89</td>
</tr>
<tr>
<td>High-dose</td>
<td>1 (5)</td>
<td>2 (10)</td>
<td>1 (5)</td>
<td>0.77</td>
</tr>
<tr>
<td>Deep sternal wound infection</td>
<td>—</td>
<td>1 (5)</td>
<td>—</td>
<td>0.37</td>
</tr>
<tr>
<td>Superficial sternal wound infection</td>
<td>—</td>
<td>1 (5)</td>
<td>—</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Data are numbers (%) of patients or mean ± SD.
extreme differences in inter-group settings in both animal and clinical studies [1–6,9–12]. The literature also lacks studies of endothelial response and cytokine leakage associated with PCPB versus OPCABG. We considered patients receiving LCPB the ‘control’ group, against which we measured endothelial activation and cytokine release after induction of pulsatile perfusion [5,6,12] or with avoidance of CPB, as in OPCABG.

A significant, systemic endothelial response — as measured by MCP-1 and VEGF release — occurred after all three types of surgery, but it was significantly more pronounced with CPB use. The interaction of leucocytes with endothelium, via specific receptors, may provide intracellular signals that activate the expression of surface receptors and chemokines [13,14]. Our findings agree with the previous reports of increased circulating endothelial cells and increased endothelial cell apoptosis with on-pump versus off-pump CABG. Lockowandt et al. also have reported that cardioplegic arrest is more harmful to the coronary endothelium than are off-pump strategies [15]. This can be a critical point for future studies, considering the primary role of myocardial ischaemia—reperfusion injury in cytokine secretion and the risk of SIRS [16]. Finally, both CPB—CABG and OPCABG elicit mobilisation of endothelial progenitor cells into peripheral blood, but CPB impairs their migratory function and viability, which are conversely preserved after OPCABG [3,17]. We likewise noted endothelial activation during OPCABG, although significantly less pronounced than during CPB procedures.

These data may explain the different cytokine release patterns observed for CPB versus OPCABG surgeries. However, we found significantly increased endothelial activation during LCPB than during PCPB.

MCP-1 has been poorly investigated in cardiac surgery, except as in a recent report from Castellheim et al. [4]. We noted higher MCP-1 levels with LCPB at all perioperative time points, suggesting significantly lesser endothelial activation beginning at aortic cross-clamping. We also noted significantly reduced VEGF secretion with PCPB. The peripheral vasodilatory effects of IABP might have reduced the need for circulating VEGF, whereas the systemic vasoconstriction associated with LCPB [1] might have induced a compensatory, greater VEGF release. However, our data support a previous report of reduced endothelial shear stress and lesser endothelial production of nitric oxide (NO) with non-pulsatile flow [11]. These phenomena may contribute to the detrimental physiological effects observed during prolonged non-pulsatile flow [11].

Accordingly we confirm the results described by Castellheim et al., who described, on the other hand, lower concentration of cytokines compared to our study [4]. However, the high-risk profile of the patients included in the presut study (e.g., old age, diabetes, low ejection fraction and the complexity of the lesions that resulted in prolonged CPB and aortic cross-clamping time) surely affected the inflammatory response to cardiac surgery and accounted for higher release of cytokine throughout the study period. Moreover, our results are not completely comparable with that described by Castellheim et al. [4], because all the patients in our study (i.e., linear CBP, pulsatile CPB and OPCABG) received preoperative IABP and were therefore exposed to the effects of IABP throughout the study period. Accordingly, it could be speculated that the diastolic augmentation of coronary perfusion and the reduction of the afterload induced by the IABP had been beneficial in terms of myocardial and global organ perfusion.

In a recent in vitro study, a continuous, regular pulsatile flow maintained steady shear stress on the endothelial layer, which is ‘quiescent’, elongated and in a hyperpolarised state [19]. Change into a linear pulsatile flow triggered endothelial cell activation, with its corresponding alteration to a cuboidal shape and depolarised state [19]. Sequential change from a pulsatile, then to a linear and then back to a pulsatile state during standard LCPB (corresponding to the time before aortic cross-clamping, the cross-clamping time and the declamping time, respectively, in cardiac surgery in vivo) might mimic this in vitro endothelial reaction, leading to different endothelial activation, as we noted in another study [13]. These data may also explain the contradictory results obtained with different types of pulsatile devices. A major problem with these devices is translation of a ‘pulsation within the device’ to a ‘pulsation within the body’, which depends on the tip of the cannula, oxygenators, temperature, concomitant medications, etc. [7]. IABP may overcome these limitations by achieving pulsation directly within the body.

Activated endothelium also releases IL-6 and IL-8, which promote hepatic production of acute-phase proteins, modulate neutrophil trafficking to sites of inflammation and participate in myocardial injury [1]. Moreover, it has been described that neutrophils are efficient releasers of IL-8 and are strongly activated by surgery in itself. Therefore, both endothelial and neutrophil activations may contribute to the measured circulating IL-8 [14,18].

Accordingly, both CPB groups showed similar bursts of proinflammatory cytokines, whereas the OPCABG group showed a significantly attenuated cytokine response. In particular, IL-6 and IL-8 levels increased only slightly in the OPCABG group, peaking at time 2 and time 3, respectively, and returning to baseline values by time 4, whereas the CPB
groups had >200-fold increases in these cytokines, both peaking at time 3 but remaining significantly increased from baseline at time 4. These data agree with the previous findings that OPCABG is associated with reduced oxidative stress and lesser release of inflammatory cytokines IL-8 and tumour necrosis factor (TNF)-α than with conventional CPB—CABG [19]. Our data also support the findings of Dybdahl et al., who reported significantly higher release of immunomodulating heat-shock protein (HSP)-70 into the circulation after conventional CPB versus OPCABG [20] and Ascione et al., who showed consistently lower levels of IL-8 after OPCABG versus the standard on-pump technique until 24 h following surgery [21].

IL-6 and IL-8 release patterns did not vary significantly between the CPB groups in our study. IL-8 differs from the other cytokines in that levels are substantially increased by interaction with tube surfaces, in a complement-dependent manner [18]. Increases in IL-8 levels with different types of CPB might reflect different interactions between the blood and the artificial surfaces of the tubing [18]. The role of pulsatility in the IL-8 augmentation that we observed might therefore be of only limited importance compared with the role of contact with artificial surfaces. Neuhof et al. have shown that IL-6 and IL-8 secretions to correlate directly with CPB duration (particularly >97 min) [22]. The shorter (90 min) and comparable CPB duration in our two groups may account for their similarities in IL-6 and IL-8 levels.

The literature is lacking regarding secretion of T-helper lymphocyte-derived cytokine after different perfusion strategies. In particular, IL-2 is one of the most specific T-helper lymphocyte-derived cytokine, which modulates T-helper function together with a multitude of cytokine network and cell-to-cell signalling pathways. A few studies have indicated that its synthesis is initially suppressed after intervention [23]. In our study, both CPB methods were indeed associated with temporary reductions in IL-2 levels, which returned to baseline levels at 12 and 24 h after surgery. Although pulsatile perfusion did not seem to play a major role in IL-2 secretion, the temporary reduction in IL-2 secretion might explain the immune system dysfunction observed early after CPB [23]. Avoidance of CPB in the OPCABG group appeared to have no relationship with helper T-cell function; IL-2 levels remained constant throughout the perioperative period.

The balance between anti-inflammatory and proinflammatory cytokines may be at least as important, if not more so, than cytokine release alone [1]. CPB is associated with increased release of IL-10 [1], which not only suppresses production of proinflammatory cytokines but also protects reperfused myocardium by limiting neutrophil recruitment [12]. We found significantly higher IL-10 secretion in the two CPB groups than in the OPCABG group, similar to a previous review of randomised and non-randomised studies [24] and a study of conventional CPB—CABG versus OPCABG [20]. Both the CPB groups showed peak secretion of IL-10 at time 2, although levels were significantly higher in the PCPB group versus the LCPB group at times 2 and 3. These data support previous reports of greater anti-inflammatory marker release with the use of pulsatile perfusion during CPB [10,12], albeit with different pathophysiological mechanisms in different experimental settings. Our data also confirm in vitro findings that maintenance of steady shear stress on endothelial cells preserves up-regulation of the genes controlling anti-inflammatory, antioxidant and anti-thrombotic functions [25]. The anti-inflammatory response involves several cytokines, such as IL-10, IL-4, IFN-γ and IL-6, all of which are part of an extremely complex cytokine network. It is interesting to observe that the same trend of cytokine expression and release could be found either in IL-10 or IL-6 samples. Obviously, our study did not address all the existing cytokines and our results therefore stem from the observation of a limited number of cytokines. Certainly, more studies addressing this topic and including higher number of patients and higher incidence of clinical endpoint are warranted to further explain the complex network of cytokines and their activation following CPB. However, in our opinion, our results could suggest that pulsatile perfusion tend to a better balance of inflammatory and anti-inflammatory stimuli, by means of a lower expression of inflammatory cytokines coupled with a higher expression of anti-inflammatory cytokines.

In conclusion, standard linear CPB appears to promote endothelial activation and cytokine secretion, which may delay recovery after CABG. OPCABG was associated with only slight endothelial activation and cytokine response. PCPB appears to significantly attenuate endothelial activation and cytokine leakage, resulting in biochemical findings comparable to those after OPCABG.

4.1. Study limitations

The main limitation of the study is its relatively small sample size. This reflects the single-centre study design, which, conversely, guaranteed uniformity in perioperative management throughout the trial. We did enroll patients with similar risk profiles, avoiding enrolment of patients with severe co-morbid conditions that could have skewed the results. Due to the complexity of the cytokine and chemokine networks, which are substantially modulated by many patient and external factors [1,2], we cannot extrapolate our results to different types of CPB conduction. Studies in larger samples and examining different CPB modalities are needed to better define the role of pulsation in endothelial activation and inflammation during cardiac operations.

Moreover, it should be kept in mind that all the patients included in the present study received preoperative IABP. Therefore, every patient could benefit from the coronary flow augmentation and afterload reduction induced by IABP, either in linear-CPB group or pulsatile-CPB group or even in OPCABG group. However, significant increase of coronary perfusion could be achieved only when functioning grafts circumvent coronary stenoses. Accordingly, every patient have the IABP turned on at aortic declamping. Therefore, less inflammatory response and endothelial activation in OPCABG group should be the results of no contact with CPB circuit, haemodilution and so on; however, the difference between pulsatile-CPB group and linear-CPB group should be the result of effective pulsatile flow maintained during aortic cross-camping by the IABP in the automatic mode. Moreover, the introduction of small catheters and the possibility of introduction without the sheath significantly reduced the incidence of vascular complications. Accordingly, we did not find any IABP-related complication in the present study.
Therefore, our experience prompted us to report our results with pulsatile CPB associated with very low complication rate of IABP-related complications.

References