Intra-aortic balloon pump induced pulsatile perfusion reduces endothelial activation and inflammatory response following cardiopulmonary bypass

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Abstract

Objective: Intra-aortic balloon pump (IABP)-induced pulsatile perfusion has demonstrated that it can preserve organ function during cardiopulmonary bypass (CPB). We evaluated the role of IABP pulsatile perfusion on endothelial response. Methods: Forty consecutive isolated CABG undergoing preoperative IABP were randomized to receive IABP pulsatile CPB during aortic cross-clamping (group A, 20 patients) or standard linear CPB (group B, 20 patients) during cross-clamp time. Hemodynamic results were analyzed by Swan-Ganz catheter [mean arterial pressure (MAP), cardiac index (CI), indexed systemic vascular resistances (ISVR), indexed pulmonary vascular resistances (IPVR), wedge pressure (PCWP)]. Inflammatory/endothelial response was analyzed by pro-inflammatory (IL-2, IL-6, IL-8), anti-inflammatory cytokines (IL-10), and endothelial markers [vascular endothelial growth factor (VEGF) and monocyte chemotactic protein-1 (MCP-1)]. All measurements were recorded preoperatively (T0), before aortic declamping (T1), at the end of surgery (T2), 12 h (T3) and 24 h (T4) postoperatively. ANOVA for repeated measures was used to evaluate the differences of means. Results: Hemodynamic response was comparable except for higher MAP (p = 0.01 at T1) and lower ISVR (p = 0.001 at T1, p = 0.003 at T2) in group A. No differences were found in perioperative leakage of IL-2, IL-6, and IL-8 between the two groups (within-group p = 0.0001 either in group A and group B; between-groups p = NS at 2-ANOVA). Group A showed significantly lower VEGF (between-groups p = 0.001 at 2-ANOVA, p = 0.001 at T1, T2) and MCP-1 (between-groups p = 0.001 at 2-ANOVA, p = 0.001 at T1, T2) with higher IL-10 secretion (between-groups p = 0.001 at 2-ANOVA, p = 0.01 at T1, T2, T3). Conclusions: IABP-induced pulsatile perfusion allows lower endothelial activation during CPB and higher anti-inflammatory cytokines secretion.

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Keywords: Intra-aortic balloon pump; Pulsatile perfusion; Endothelial response; Cytokine; Inflammation

1. Introduction

Systemic inflammatory response syndrome (SIRS) following cardiopulmonary bypass (CPB) has been indicated as the main cause for postoperative morbidity and mortality in cardiac surgery [1,2]. CPB-related inflammatory cascade results from non-pulsatile flow and blood interaction with extracorporeal circuit artificial surfaces [1,2]. Despite non-pulsatile blood flow obtained with standard CPB circuits considered an acceptable, non-physiologic compromise with few disadvantages (including the induction of such inflammatory response) [2], different investigators have tried to obtain a more physiologic pulsatile perfusion in the daily clinical practice [3,4]. However, pulsatile perfusion still represents an open question: some studies have reported beneficial effects on microcirculation, metabolism, and organ functions [3,4], and our group has previously demonstrated notable beneficial effects on splanchnic, renal, respiratory and hemo-coagulative functions when intra-aortic balloon pump (IABP)-induced pulsatile perfusion has been employed in routine elective coronary surgery [5–8]. On the other hand, others did not observe any superiority of pulsatile perfusion with different devices over the traditional non-pulsatile CPB [9–11].

Endothelial cells have been recognized to be the rotors of SIRS, triggering cytokine release and neutrophil activation, and being themselves further activated by cytokines and neutrophils in a self-maintained manner [1,2]. Again, studies on endothelial activation following pulsatile and non-pulsatile perfusion have given contradictory results in the current literature [11–14]; some studies reported beneficial effects of pulsatile perfusion on endothelial response to CPB [12–14], others did not find any superiority of pulsatile CPB over non-pulsatile perfusion [11]. Despite IABP-induced pulsatile perfusion being first suggested by Pappas and co-workers in...
1975 [15], literature lacks studies analyzing the endothelial response and the cytokine release following this type of perfusion or standard linear CPB. Therefore, according to our previously reported beneficial effects of IABP-induced pulsatile perfusion on organ function, it was the aim of this study to evaluate endothelial, inflammatory and consequent hemodynamic effects of these two types of perfusion during isolated elective CABG.

2. Materials and methods

2.1. Patients and study design

Between September 2007 and February 2008, 40 patients undergoing isolated primary elective CABG with the need for a preoperative intra-aortic balloon pump, aged between 30 and 70 years old, were enrolled in the study. All patients were scheduled for preoperative IABP, as already reported [5—8], being considered at risk for preoperative ischemic events, due to severe and diffuse coronary lesions [any of the following: critical left main disease >90% ± poor left ventricular ejection fraction (<40%); severe left main lesion >80% with severe right coronary stenosis >90%; chronic occlusion of the 3 coronaries with poor angiographic bed]. On admission the patients were randomized by lottery, drawing pre-prepared sealed envelopes containing the group assignment. Twenty patients (group A, treatment group) received a preoperative IABP before induction of anesthesia, with IABP switched to automatic 80 bpm mode during cardiopulmonary arrest so to achieve pulsatile flow during aortic cross-clamping and restarted with a 1:1 IABP mode immediately after cross-clamp removal. The other 20 (group B) received the above-mentioned preoperative IABP, which was turned off during cross-clamp time, and switched again to a 1:1 IABP mode after cross-clamp removal [5—8]. In all cases preoperative echo-Doppler scanning of peripheral arteries and abdominal aorta was accomplished, in order to avoid major IABP-related vascular complications.

The study protocol was approved by the institution’s ethical committee/institutional review board (September 2007). Informed consent was obtained from each patient enrolled in the study.

2.2. Exclusion criteria

In order to avoid misleading data, 28 patients admitted at our institution during the same time period were excluded from the study because of isolated or combined severe splanchnic organ comorbidities (renal failure in 3 patients, chronic renal insufficiency in 8, liver failure in 1, chronic obstructive pulmonary disease in 10, abdominal aortic aneurysm with abdominal arteries vasculopathy in 5, autoimmune diseases in 3) and pre-existing hematologic and coagulative disorders (severe anemia (<8.0 g/dl of hemoglobin) in 2, platelet disease in 1, congenital deficiency of coagulative proteins in 1). According to the altered endothelial function of patients with unstable angina, 10 other patients were not enrolled in the study because of ongoing unstable angina. However, all patients enrolled were on preoperative aspirin (150 mg/day), which was withdrawn at least 10 days before surgery, and substituted with subcutaneous enoxaparin.

2.3. Anesthesia

All patients underwent Swan-Ganz catheter insertion before anesthetic induction. Postoperative chest X-ray confirmed its exact positioning. Mean arterial blood pressure (MAP), cardiac index (CI), pulmonary capillary wedge pressure (PCWP), indexed pulmonary vascular resistance (IPVR) and indexed systemic vascular resistances (ISVR) were measured. Anesthetic technique was standardized: induction of anesthesia consisted of intravenous propofol infusion at 3 mg/kg combined with fentanyl administration at 0.10 mg/kg. Neumuscular blockade was achieved by 4 mg/h pancuronium bromide, and lungs were ventilated to normocapnia with air and oxygen (45—50%). Propofol infusion (150—200 μg/kg per min) and isoflurane (0.5% inspired concentration) maintained anesthesia. Arterial and central venous catheters were the standard.

Inotropic support was recorded and defined as low-dose when enoximone was administered at a dosage lower than or equal to 5 μg/kg/min; medium-dose when enoximone was employed at a dosage between 6 and 10 μg/kg/min, or dobutamine was added at a dosage between 5 and 10 μg/kg/min; or high-dose when enoximone or dobutamine infusion was >10 μg/kg/min or epinephrine was added at any dose.

2.4. Surgical technique and cardiopulmonary bypass

All patients underwent surgery at 8:00 a.m. in order to minimize any time-dependent variation of mediator synthesis. It was institutional policy to percutaneously insert with the ‘sheathless-technique’ [5—8] IABP (7.5 F, 34 or 40 cc according to the body surface area; balloon Datascope Corp., Fairfield, NJ) connected to a Datascope CS-300 pump (Datascope Corp, Fairfield, NJ), through the best femoral artery, before induction of anesthesia, in order to better support the perioperative hemodynamic of patients. The correct placement of IABP was always assessed by post-operative chest X-ray or transesophageal echocardiography.

CPB and surgical techniques were standardized and did not change during the study period. Surgery was performed by the same senior surgeons in all cases. In all patients CABG was performed through a median sternotomy. Both arterial and venous CABG were accomplished. Cardiopulmonary bypass (CPB) was standardized: a Dideco (Mirandola – Modena) tubing set, which included a 40-micron filter, a Stockert roller pump (Stockert Instrumente, Munich, Germany) and a hollow fibre membrane oxygenator (Monolyth, Sorin Biomedica, Saluggia, Italy). Heparin was given at a dose of 300 IU/kg to achieve a target activated clotting time over 480 s. Systemic temperature was kept between 32 and 34 °C. Myocardial protection was always achieved with intermittent antegrade and retrograde hyperkalemic blood cardioplegia. Total CPB flow was maintained at 2.6 l/min m². Protamine was administered at the end of the operation to fully reverse heparin. Blood recovery with an autotransfusion device (Autotrans Dideco, Mirandola, Modena, Italy) was performed intraoperatively in all cases. A level of hemoglobin lower than 8 g/dl suggested blood transfusion. Following surgery, patients received antici-
oagulation with enoxaparin, starting when the postoperative bleeding was controlled (usually within 6 h) until the 3rd postoperative day. One hundred and fifty mg acetylsalicylic acid were administered daily, starting from the 3rd postoperative day. IABP was withdrawn when hemodynamic stability was restored (i.e., a cardiac index $\geq 2.0$ l/m$^2$ per min with only minimal pharmacologic inotropic support, dobutamine or enoximone at 5 $\mu$g/kg per min).

2.5. Assays of cytokines and endothelial markers

Blood was collected from the peripheral arterial line preoperatively (T0), immediately before aortic declamping (T1), at the end of surgery (T2), 12 h (T3) and 24 h (T4) postoperatively.

Cytokines and growth factors (IL-2, IL-6, IL-8, IL-10, MCP-1, VEGF) were simultaneously and quantitatively determined by sandwich chemiluminescent immunoassay from Biochip Array Technology (Randox, UK). All assays were performed according to the manufacturer’s instructions.

2.6. Study design and endpoints

Anesthesiologists, cardiologists and cardiac surgeons caring for the patients during the postoperative course were blinded towards the intra-operative group assignment. Primary endpoint of endothelial response was perioperative changes of MCP-1 for the evaluation of the endothelial response, whereas perioperative changes of VEGF were the secondary end-point of endothelial cell activation. Between-within interactions of 5-time MCP-1 measurement in 20 patients for each study arm gave a power (1-$\beta$ error probability) of 98% with an $\alpha$-error probability of 0.05. Primary endpoint of cytokine leakage was perioperative changes of IL-10 for the evaluation of the anti-inflammatory response, whereas perioperative changes of pro-inflammatory cytokines IL-2, IL-6 and IL-8 were the secondary endpoints of cytokine burst. Between-within interactions of 5-time IL-10 measurement in 20 patients for each study arm gave a power (1-$\beta$ error probability) of 98% with an $\alpha$-error probability of 0.05. Finally, hemodynamic response with 5-time measurements of CI, MAP, ISVR, IPVR, and PCWP, as well as hospital outcome (mortality, morbidity, perioperative myocardial infarction, in-hospital and intensive therapy unit (ITU) stay, IABP-related complications) were analyzed as secondary endpoints.

In-hospital mortality was defined as any death occurring during hospital stay or in the first 30 postoperative days. Hospital morbidity was defined as any complication requiring specific therapy or causing a delay in hospital or ITU discharge. In particular, acute respiratory insufficiency needing non-invasive positive-pressure ventilation (NIV) was diagnosed if patients had at least one of the following parameters: respiratory acidosis (arterial pH $< 7.35$ with partial pressure of carbon dioxide, arterial $\left[\text{PaCO}_2\right] > 45$ mmHg); arterial $\text{O}_2$ saturation by pulse-oxymetry less than 90% or $\text{PaO}_2$ less than 60 mmHg at inspired $\text{O}_2$ fraction 0.5 or greater; respiratory frequency greater than 35 per min; decreased consciousness, agitation, or diaphoresis; clinical signs suggestive of respiratory muscle fatigue, and increased work of breathing such as the use of respiratory accessory muscles, paradoxical motion of the abdomen, or retraction of the intercostal spaces [7].

Acute renal insufficiency was defined as a greater than 50% increase over the preoperative serum creatinine value, acute renal failure as acute renal insufficiency requiring renal replacement therapy, as previously reported [6]. Perioperative acute myocardial infarction was defined by any of the following criteria: (a) new Q waves of $>0.04$ ms with a peak Troponin I (TnI) $>3.7$ $\mu$g/l or TnI concentration $>3.1$ $\mu$g/l at 12 h postoperatively; (b) $>25$% reduction in R waves in at least two leads on ECG associated to the above-mentioned TnI peaks; (c) new akineti c or dyskinetic segments at echocardiography, as previously demonstrated [5]. Low output state (LOS) was diagnosed if the patient demonstrated hemodynamic compromise or a cardiac index lower than 2.2 l/min m$^2$ during the ITU-stay despite IABP assistance and inotropic support, after correction of all electrolyte and blood gas abnormalities, and after adjusting the preload to its optimal value.

Determinations of blood concentration of cardiac TnI were conducted perioperatively before anesthetic induction, from the coronary sinus 10 min following aortic declamping, at ITU admission, and on 1st and 2nd postoperative days. The TnI assays were carried out using diagnostic kits provided by Beckman Coulter (Fullerton, California; AccuTnITM Access Immunoassay System). IABP-related complications were defined as any aortic dissection or perforation, limb or mesenteric ischemia, or infection or hemorrhage at the balloon entry point.

2.7. Statistical analysis

Statistical analysis was performed by the SPSS program for Windows, version 15.0 (SPSS Inc., Chicago, IL). Continuous variables are presented as mean ± standard deviation (SD), and categorical variables are presented as absolute numbers and/or percentages. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired t-test, whereas the Mann–Whitney U-test was used for those variables that were not normally distributed. Categorical variables were analyzed using either the chi-square test or Fischer’s exact test. Comparison between and within-groups was made using two-way analysis of variance for repeated measures. Comparisons were considered significant if $p < 0.05$.

3. Results

3.1. Hospital outcome

The two groups proved homogeneous in preoperative baseline characteristics as well as intraoperative variables (Table 1). There were no hospital deaths, or perioperative acute myocardial infarctions during the study period. Transient low output state requiring prolonged (78 h) IABP assistance developed in 1 patient in group B (5%), not in group A (0%; $p = 0.50$). No differences were recorded in perioperative inotropic support. Eighteen patients in group A (90%) and 15 patients in group B (75%; $p = 0.20$) required low-doses of inotropes, whereas the remaining two patients in group A (10%) and five patients in group B (25%; $p = 0.20$) required medium doses of inotropes. No patient required high inotropic support. Perioperative TnI leakage proved similar between the two groups (Table 2).
When morbidity was considered, a similar proportion of patients in the two groups developed postoperative paroxysmal atrial fibrillation, requiring i.v. amiodarone infusion (Table 3). Accordingly, no differences were recorded in perioperative acute respiratory insufficiency requiring NIV, perioperative acute renal insufficiency and failure, and IABP-related complications (Table 3). The only registered IABP-related complication accounted for a transient ileus, which completely recovered 24 h after prompt IABP withdrawal, rehydration, and i.v. fenolopam administration.

Accordingly, the two groups showed comparable ITU-stay (group A: 42.8 ± 3.4 h vs group B: 40.4 ± 5.2; p = 0.09) and hospital stay (group A: 6.3 ± 0.4 days vs group B: 6.4 ± 0.5; p = 0.52).

3.2. Hemodynamic response

No differences were recorded in CI, PCWP, IPVR between the two groups (Table 4). However, MAP proved significantly higher at T1 in patients undergoing IABP-induced pulsatile perfusion (Table 4). ISVR proved significantly lower either at T1 and T2 in patients undergoing pulsatile CPB (Table 4).

3.3. Endothelial response

When markers of endothelial response were considered, a completely different pattern was found in the two groups. In particular, patients undergoing linear CPB demonstrated a progressive increase of MCP-1, which reached the peak value at T2, and then showed a progressive falling until T4 (Fig. 1A). On the other hand, patients undergoing pulsatile CPB demonstrated an initial reduction of circulating MCP-1 at T1, followed by a progressive increase and reaching the peak value at T3 (Fig. 1A). However, MCP-1 levels were significantly lower at T1 and T2 in patients undergoing pulsatile perfusion, and proved to have a significantly lower leakage compared to patients undergoing linear perfusion (between-groups p = 0.001).

When VEGF was considered, it showed a biphasic kinetic with a first peak at T1 followed by a second peak at T4 in both groups (Fig. 1B). Again, T1 and T2 levels were significantly higher in patients undergoing linear perfusion (Fig. 1B), giving a significantly lower leakage in patients undergoing pulsatile CPB (between-groups p = 0.001).

3.4. Cytokine leakage

When anti-inflammatory IL-10 was considered, it showed first a progressive rise, reaching the peak value at T2 in both groups, then demonstrated a progressive decline until T4 (Fig. 2A). However, IL-10 reached significantly higher values in the pulsatile perfusion group, since T1 to T3, thus giving a significantly higher temporal leakage when pulsatile CPB was employed (between-groups p = 0.001) (Fig. 2A).

When pro-inflammatory cytokines were analyzed, no differences were detected between the 2 groups, in terms of either IL-2 (Fig. 2B), IL-6 (Fig. 2C), or IL-8 (Fig. 2D). When the temporal patterns were considered, IL-2 showed an initial decline of its serum levels reaching the preoperative values only at T3 in both groups (Fig. 2B), whereas IL-6 and IL-8 showed a progressive rise in their serum values since T1, peaking at T3 in both groups (Fig. 2C and D, respectively).

4. Discussion

CPB was introduced during the 1950s and has since then been used extensively in CABG. Despite its extensive use, CPB has been associated with a substantial inflammatory response
Fig. 2. Perioperative leakage of IL-10 (A), IL-2 (B), IL-6 (C) and IL-8 (D) in the two groups. Data are presented as mean, with the minimum and the maximum value detected at each time point.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>p^a</th>
<th>p^c</th>
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<tbody>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Group A</td>
<td>70.4 ± 5.0</td>
<td>54.7 ± 2.6</td>
<td>67.4 ± 3.8</td>
<td>68.1 ± 4.0</td>
<td>66.2 ± 2.5</td>
<td>0.0001</td>
<td>0.009</td>
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<tr>
<td>Group B</td>
<td>71.3 ± 4.7</td>
<td>51.5 ± 4.3</td>
<td>66.1 ± 3.4</td>
<td>67.1 ± 1.8</td>
<td>66.3 ± 2.6</td>
<td>0.0001</td>
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<tr>
<td>p^b</td>
<td>0.54</td>
<td>0.010</td>
<td>0.27</td>
<td>0.32</td>
<td>0.90</td>
<td></td>
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<tr>
<td>CI (l/min m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Group A</td>
<td>2.4 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>0.43</td>
<td>0.58</td>
</tr>
<tr>
<td>Group B</td>
<td>2.5 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>2.7 ± 0.1</td>
<td>2.7 ± 0.1</td>
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<tr>
<td>p^a</td>
<td>0.67</td>
<td>0.10</td>
<td>0.56</td>
<td>0.72</td>
<td>0.62</td>
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<tr>
<td>ISVR (dyne s/cm² m²)</td>
<td></td>
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<tr>
<td>Group A</td>
<td>2155 ± 272</td>
<td>2292 ± 375</td>
<td>2350 ± 378</td>
<td>2223 ± 237</td>
<td>2182 ± 224</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Group B</td>
<td>2107 ± 273</td>
<td>2622 ± 142</td>
<td>2635 ± 146</td>
<td>2260 ± 279</td>
<td>2220 ± 239</td>
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<tr>
<td>p^b</td>
<td>0.58</td>
<td>0.001</td>
<td>0.003</td>
<td>0.65</td>
<td>0.60</td>
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<tr>
<td>IPVR (dyne s/cm² m²)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Group A</td>
<td>250 ± 35</td>
<td>—</td>
<td>257 ± 29</td>
<td>254 ± 30</td>
<td>250 ± 47</td>
<td>0.92</td>
<td>0.34</td>
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<tr>
<td>Group B</td>
<td>259 ± 37</td>
<td>—</td>
<td>253 ± 32</td>
<td>263 ± 31</td>
<td>254 ± 47</td>
<td>0.86</td>
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<tr>
<td>p^a</td>
<td>0.47</td>
<td>—</td>
<td>0.65</td>
<td>0.36</td>
<td>0.78</td>
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<td>PCWP (mmHg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Group A</td>
<td>10.2 ± 1.7</td>
<td>—</td>
<td>14.0 ± 1.7</td>
<td>14.0 ± 1.8</td>
<td>14.6 ± 1.9</td>
<td>0.62</td>
<td>0.50</td>
</tr>
<tr>
<td>Group B</td>
<td>10.3 ± 1.8</td>
<td>—</td>
<td>14.5 ± 1.8</td>
<td>14.4 ± 1.7</td>
<td>14.3 ± 1.9</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>p^a</td>
<td>0.93</td>
<td>—</td>
<td>0.34</td>
<td>0.48</td>
<td>0.56</td>
<td></td>
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</tbody>
</table>

MAP: mean arterial pressure; CI: cardiac index; ISVR: indexed systemic vascular resistances; IPVR: indexed pulmonary vascular resistances; PCWP: pulmonary capillary wedge pressure; p^a: p-value at each time-point; p^b: p-value within-group; p^c: p-value between-groups. Values indicated in italics are significant.
including activation of endothelium, leukocytes, platelets, and the complement system, as well as activation of the coagulation system [1,2,16]. Inflammation is suggested to be of critical importance in the pathogenesis of organ dysfunction after CPB [1,2], and CPB has been recognized as a major cause of systemic inflammatory response, contributing to postoperative complications [1,2,5—7,15]. During CPB the blood comes into contact with a vast artificial surface and pulsatile flow is converted to non-pulsatile. Both mechanisms are responsible for activation of blood circulating elements and endothelial cells [1,2,11—14]. In particular, the critical role of endothelial activation in determining and maintaining systemic inflammation has been clearly demonstrated, thus playing a pivotal role in post-CPB morbidity and mortality [1—3,11—14]. Despite extensive literature data, mechanisms underlying post-CPB systemic inflammatory response are not definitely understood, first of all because of extreme inter-group differences of both experimental and human settings [1—4,9—16]. In particular, CPB-induced shift from a physiologic pulsatile perfusion to a non-physiologic linear perfusion is considered a main trigger for endothelial activation [1,3,11—14], leading to changes in nitric oxide (NO) secretion, which may act as a primary trigger for peripheral vasoconstriction and organ hypoperfusion [12]. On the other hand, different authors considered linear perfusion during CPB an acceptable compromise with few (and temporary) disadvantages, well tolerated in the clinical practice [2,9,10]. Therefore, controversies still arise on the beneficial role of pulsatile perfusion during CPB. Our group has recently demonstrated a beneficial impact of IABP-induced pulsatile perfusion during elective coronary surgery on pulmonary, splanchnic, renal and hemo-coagulative clinical outcome, compared to traditional linear CPB perfusion [5—8]. However, no studies on endothelial activation and cytokine release following IABP-induced pulsatile perfusion are reported in the literature. Accordingly, the present study indicates less endothelial activation together with a preferential anti-inflammatory shift of the cytokine network with pulsatile CPB, thus suggesting that the better clinical outcome of our previous studies can be ascribed to an attenuated endothelial and inflammatory response to CPB.

In particular, VEGF and MCP-1 are considered biochemical markers of endothelial activation [16,17]. MCP-1 is a chemotactic protein preferentially released by endothelial cells; although it is synthesized to some extent in adipose tissue and it is likely it can be produced by most cell types and participates in the recruitment of monocytes and granulocytes to sites of inflammation [18]. MCP-1 has been shown to be a strong predictor for coronary events [18]. However, this chemokine has been poorly investigated in heart surgery, except for an in vitro model of CPB [18]. Our study demonstrated a completely different pattern of MCP-1 release with the two types of perfusion. When standard linear perfusion was employed, a progressive rise in plasma MCP-1 was detected (peaking at T2) followed by a progressive fall; on the other hand, when IABP-induced pulsatile perfusion was employed, MCP-1 showed a slight fall at T1 (i.e. before aortic declamping), thus suggesting a significant reduction of endothelial activation during the cross-clamp time, followed by a progressive increase in the following time-course. Moreover, MCP-1 leakage proved to be significantly reduced during the entire time-course, which followed IABP-induced pulsatile perfusion.

Accordingly, VEGF is an endothelial-derived growth factor with mitogen and vasorelaxing/vasodilatory properties, both in vitro and in vivo [19]. Again, this study showed a significantly reduced VEGF secretion at T1 and T2 with pulsatile perfusion. Apart from being a marker of less endothelial activation during pulsatile CPB, that data correlated with a significantly reduced ISVR in the pulsatile group at the same time points. It may be argued that the peripheral vasodilative effects of aortic counterpulsation reduced the need for circulating VEGF, whereas the systemic vasoconstriction associated with linear CPB [3] induced a compensatory higher VEGF release. However, our data confirm Macha et al. who demonstrated that non-pulsatile flow is associated with diminished endothelial shear stress with reduction in endothelial NO production, which may contribute to the detrimental physiologic effects observed in prolonged non-pulsatile flow states [12]. Accordingly Orime et al. found less endothelial damage with beneficial effects on the microcirculation with pulsatile Jostra system [14], as well as Sezai and co-workers, who demonstrated reduced endothelin-1 leakage and better peripheral circulation when pulsatile perfusion was employed [13]. Finally, Vedrine et al. found better preserved fetal/maternal endothelial nitric oxide biosynthesis and decreased activation of the fetal rennin—angiotensin pathway with pulsatile perfusion [20].

When the pathophysiology of these results are considered at a "cellular level", it has to be noted that a recent experimental study has demonstrated that the maintenance of a steady pulsatile flow (in vitro) induces a steady shear stress on the endothelial cell, which is in an elongated shape, an hyperpolarized state, and in a "quiescent" function; on the other hand, the modification of a pulsatile flow to a linear flow triggers endothelial cell activation, with its modification to a cuboidal shape, depolarized state and activated function [21]. It can be suggested that the sequential transition from a pulsatile to a linear, to a pulsatile state (corresponding to the pre-cross-clamping time, to the aortic cross-clamping, to the aortic declamping in cardiac surgery, in vivo), may mimic such endothelial in-vitro reaction, leading to a different endothelial activation, as we have found in the present study.

The vascular endothelium has a pivotal role in the systemic injury that follows CPB [1]. Activated endothelial cells release cytokines and express proteins on their surface that promote inflammatory reactions and thrombosis [1]. It has been demonstrated that one of the earliest responses to CPB is the secretion and synthesis by activated endothelial cell of pro-inflammatory cytokines, such as IL-6, IL-8, and MCP-1, which contribute to enhance inflammatory response [2]. In particular IL-8 and MCP-1 are powerful chemoattractants for neutrophils and macrophages, respectively, whereas IL-6 mainly regulates production of acute-phase proteins from the liver [2]. Accordingly, we found a significant burst of both IL-6 and IL-8 in the 2 groups, both peaking at T3, without differences at all time points between the 2 groups. It has been recently demonstrated that IL-8 differ principally from the other cytokines in that it is substantially increased by the PVC surface, in a completely complement-dependent manner, and that its increase in different settings of CPB may be induced through interaction between blood and the artificial surface of
the tubing [18]. It can be argued that the role of pulsation on IL-8 augmentation, as found in our study, is only of limited importance when compared to the role of contact phase with artificial surfaces. Moreover, Neuhof et al. demonstrated IL-6 and IL-8 secretion to be directly related with prolonged (>97 min) CPB times [22]; therefore the shorter (90 min) and comparable CPB time of our two study groups may account for the absence of differences between in IL-6 and IL-8.

When the major lymphocyte T-helper derived cytokine (i.e., IL-2) was considered, several studies have shown that T-helper cell function is altered after cardiac surgery with cardiopulmonary bypass (CPB) [23,24]. In particular, the synthesis of pro-inflammatory T-helper 1 cytokines (IFN-gamma and IL-2) is initially suppressed [23]. Our data confirm those of Franke and co-workers [23], showing an initial reduction of IL-2 secretion at T1 in both groups, which turned back to the preoperative values only at 12–24 h following surgery, and possibly accounting for a reduced competence of the specific and non-specific immune system in the early phases post-CPB [23,24]. Again, literature lacks studies on IL-2 secretion following different perfusion strategies, and our data indicate that pulsatile perfusion did not seem to play a major role in determining IL-2 secretion.

Finally IL-10 is considered the major anti-inflammatory cytokine involved during CPB. IL-10 inhibits synthesis of pro-inflammatory cytokines by monocytes and macrophages and induces production of IL-1 receptor antagonist, which downgrades the response to IL-1 [2]. When IL-10 secretion was considered, both groups showed a peak secretion at T2, although IL-10 leakage proved significantly higher in pulsatile perfusion group since T1 to T3. Our data confirm in a clinical scenario the findings of Gimbrone et al. who showed that the maintenance of a steady shear stress on endothelial cells in vitro up-regulated the genes for anti-inflammatory, anti-oxidant and anti-thrombotic functions [25]. Moreover, it is well known that it is necessary to evaluate both pro-inflammatory and anti-inflammatory cytokines to evaluate the balanced inflammatory load in patients undergoing CPB [1,2]. Our data therefore justify a significantly less inflammatory balance, through a significant reduction of endothelial cell activation, with IABP-induced pulsatile perfusion. These data conceptually confirm previous studies, such as those of Sezai et al. [13] and Orime et al. [14], who similarly found a reduced inflammation, although with different pathophysiological mechanisms in different experimental settings, when pulsatile perfusion during CPB was employed.

4.1. Limitations of the study

The main limitation of the study is related to the relatively small sample size of patients enrolled in the study. This is a result of the single-center design of the study itself, which, on the other hand, guarantees uniformity of the perioperative management of the patient population throughout the experimentation. Moreover, on an intention-to-treat basis, we enrolled patients with the most similar risk profile, avoiding severe organ comorbidities, which may mislead the results.

Moreover, according to the complexity of the cytokine and chemokine network, which are greatly influenced by duration of CPB, perfusion temperatures, perfusion equipment, aortic cross-clamp times, methods of myocardial protection, and perhaps exogenous factors such as priming solutions, anesthesia, intravascular drugs, age, left ventricular function, genetic factors and so on [2], we cannot extrapolate our results to different types of CPB conduction. Therefore, more studies on this topic with different modalities of CPB may be welcome, to better define the role of pulsation in endothelial activation and inflammation during cardiac operations.

References

Appendix A. Conference discussion

Dr D. Birnbaum (Bad Nauheim, Germany): Thank you to the research group in Catanzaro who provided us with these data giving us the chance to discuss and reconsider the topics which are rather critical in such a clinical setup. As I must say, the one point is the question to the pulsatility which we had the chance to discuss before.

If we talk about pulsatility, we need to define whether this is a pressure pulse or whether this is a flow pulse, and we need to define where we detect this pulsatility. There is a big difference whether we regard microcirculation, whether we regard arterial vessels, and whether we regard arterial vessels in a diseased status.

So in a clinical setting, it is very critical to use analysis to the term of pulsatility unless it is clearly defined, which is really difficult to do.

We have to admit that pulsatility application has a long history. And we learned today and in conclusion we have to teach, in my opinion, physiologists, that pulsatility is a phenomenon which is of no meaning in the daily experience of cardiac surgeons. We learn today that ventricular assist systems with a linear flow maintains life of human without any morbidity over years, five, six even more years nowadays, so we have to acknowledge, that pulsatility in the arterial system is a matter of academic questions in my opinion.

The next point you raise in your presentation is inflammatory response. So what is inflammatory response?, a question which as well is in the literature answering highly complex questions which we are confronted with in daily work. In detecting molecules of these kind we would need larger time frame observations, and I recommend in these cases to use analysis of area under curve of an individual. Only this allows us to compare the area under curve constitution of one individual with another, which would mean that under a control of cardiopulmonary bypass, you would have to do plenty of these analyses to expect data for true conclusions.

The other difficult problem to solve is whether an intra-aortic balloon pump would ameliorate the outcome of these patients. The authors meet controversies in the literature extensively. Therefore, the manuscript contains four pages of discussion in comparison to only one page of results.

The study is well done within the framework of the small setup of patients and within the limits for any conclusion drawn from these findings. Therefore, I have almost no questions to you because we would not be able to change the design of this study. We have to respect what you have found.

Maybe you would like to comment on a few details: Why you have not defined the health status of the patients preoperatively? Why did you use as a format of results the stay of the patient in an intensive care unit if only 15 patients were in focus? We know in questions of discharge of patients about the subjectivity of our decisions, for example on weekends and under the pressure of the patients wishes.

Maybe you would like, secondarily, to comment on your mentioning that you have controlled the correct position of the Swan-Ganz catheter and/or of the intra-aortic balloon pump system postoperatively. What have you done when the postoperatively obtained catheter was not in the right position? Did you omit the patient from the study or did you leave him in the group?

Dr Onorati: Okay. I thank you for your comments because they are the hot topics of these type of papers.

About pulsatility, I have just to comment that the main limitation of all studies regarding a pulsatile perfusion pump is the induction of a pulsation external to the body with great difficulties to transmit this pulsation to the body itself.

When we measure the surplus hemodynamic energy with this type of perfusion, according to our co-workers, and this is a matter of a forthcoming paper, we found that with the balloon we had a 15%, sometimes also 20% surplus hemodynamic energy. Therefore, we can conclude that we have a very high physiologic pulsation with this type of perfusion.

However, obviously, as all kinds of clinical papers, there are some limitations. Certainly the small number is a limitation for a clinical outcome study. However, the study was set to define the differences between the biochemical markers, and from a statistical point of view, measuring continuing values with 5-time point measurements gives us nearly 98% efficacy in defining the results.

However, on the other side, we did not have sufficient power, statistical power to prove a clinical outcome. However, our previous paper on a huge number of patients demonstrated that in terms of renal outcome and in terms of respiratory response to cardiopulmonary bypass.

When we look at the inflammatory response at the same time, it is very difficult to choose which is the best marker and what means inflammatory in the clinical practice. We tried to choose the cytokines that were released from the endothelium more than from monocytes because we know that the contact phase with the tube setting, which cannot be avoided with this type of perfusion, mainly influences the release of TNF-alpha and IL-1, whereas IL-6 and IL-8 depend almost always on the endothelial cells as well as the whole endothelial response. It is very difficult to say which is the best way to measure endothelial response.

Again, we choose monocyte chemotactic protein because it is the chemokine which is mainly released from the endothelial cells compared to other types of serum markers. We know that your group used the apoptotic activity of the serum, and mainly different results are reported in the literature with this type of measurement.

Therefore, it is very difficult to say which is the best marker in clinical practice. On the other hand, we know that a lot of components may influence endothelial and inflammatory response, from filters to tubing to oxygenators to tip of the cannula.

Therefore, obviously, the main limitation of this study is that these results can be applied to our mode, our modality of cardiopulmonary bypass.