Continued mechanical ventilation during coronary artery bypass graft operation attenuates the systemic immune response

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Abstract

OBJECTIVES: Cardiopulmonary bypass (CPB) is known to induce a short pro- and long-lasting anti-inflammatory immune response. The anti-inflammatory protein soluble ST2 (sST2) may be involved in the pathogenesis of postoperative immune dysfunction. We investigated whether continued mechanical ventilation during CPB has an impact on postoperative serum sST2 and cytokine release.

METHODS: Thirty patients undergoing conventional coronary artery bypass graft (CABG) operation were randomized into a ventilated on CPB group (VG; \(n=15\)) and non-ventilated on CPB group (NVG; \(n=15\)). Blood samples were drawn at the beginning and at the end of surgery, and at the 5 consecutive days. sST2, IL-4, IL-10, IgM, IgG, IL-6 and endotoxin were measured by ELISA. Data are given as mean standard deviation (SD). A Mann-Whitney U-test was used for statistical analysis.

RESULTS: Serum levels of sST2 and IL-10 were significantly higher in the NVG when compared with the VG at the first postoperative day (POD-1) \([sST2\text{pg/ml: }1366.4 (433) (VG) vs 2296.3 (1795.5) (NVG) \(P=0.029\); IL-10 \text{pg/ml: }107.7 (40.0) (VG) vs 154.6 (68.8) (NVG) \(P=0.038\)]). In addition, the secretion of proinflammatory IL-6 was slightly reduced in the VG at POD-1 \([IL-6\text{pg/ml: }83.1 (52.5) (VG) vs 110.2 (42.3) (NVG) \(P=0.033\)]). IL-4, endotoxin, IgM and IgG showed no differences between groups.

CONCLUSION: These data suggest that continued mechanical ventilation during CABG attenuates inflammatory and anti-inflammatory immune responses after CPB. Continued mechanical ventilation may have beneficial effects in the attenuation of the CPB-induced immune activation.

Keywords: Cardiopulmonary bypass • Heart surgery • Mechanical ventilation • Soluble ST2 • Endotoxin • Systemic immune response

INTRODUCTION

Although cardiopulmonary bypass (CPB) utilized in coronary artery bypass graft (CABG) surgery has become a routine procedure, crude in-hospital mortality ranges from 3 to 5\% [1]. The CPB can induce a systemic pro- and anti-inflammatory response. The term ‘systemic inflammatory response syndrome’ (SIRS) has been established to describe the immunological reaction after CABG surgery [2-5]. SIRS is believed to play a key role in postoperative cardiovascular and pulmonary dysfunction.

It is currently accepted that a brief CPB-induced proinflammatory response is followed by a long-lasting second phase of immune suppression, causing an enhanced risk of infection. The secretion of anti-inflammatory IL-10 and soluble (s)ST2 is thought to play a pivotal role in the immune-suppressive phase. ST2 is a protein of the interleukin-1 receptor family. Through alternative splicing, ST2 exists in three forms, membrane bound (ST2 ligand: ST2L), a variant form (ST2V) present mainly in the gut and a soluble form (sST2). sST2 secretion seems to be triggered by inflammatory cytokines such as IL-1\(\alpha\), IL-1\(\beta\), IL-6 and TNF-\(\alpha\), which are in turn released in the presence of endotoxin [6]. Activation of the membrane-bound ST2-receptor by its recently discovered ligand IL-33 leads to a rapid activation of NFkB and ultimately, the expression of T-helper cell (Th2) associated cytokines IL-4, IL-5 and IL-13. Furthermore, IL-33 has been shown to increase activated endothelial cells, enhancing the production of proinflammatory IL-6 and IL-8 in those cells. In contrast, soluble ST2 has been shown to capture IL-33, thereby acting as a decoy receptor for IL-33, leading to a reduction of the Th2 inflammatory responses [7]. Furthermore, sST2 directly interacts with macrophages and suppresses proinflammatory proteins in these cells by inhibition of Toll-like receptor signalling and by sequestration of MyD88 and Mal adaptor.
proteins resulting in down-regulation of NFκB [4, 8-10]. Additionally, sST2 can diminish the formation of proinflammatory proteins [11, 12]. Elevated serum concentrations of sST2 have been reported in patients with sepsis and trauma, after CABG surgery, dengue virus infection, lung fibrosis and chronic obstructive pulmonary disease as well as acute liver failure. In recent study, we investigated which cell types produce and secrete sST2 and could show that pulmonary alveolar epithelial and cardiac myocytes are the major source of sST2 in humans [6].

With regard to the current practice in CABG surgery, ventilation of lung tissue during CPB plays a non-significant role. During CABG, continued airway ventilation is discontinued on CPB and a complete collapse of the lung structure is deemed acceptable. The current practice is that iatrogenic atelectasis is accepted in order to better visualize the operative field. The systemic anti-inflammatory effect of this ‘operational procedure’ is not investigated until recently. The dual oxygen supply of the lung is thus only maintained through the bronchial arteries with a potential rise of pulmonary malperfusion. During CABG operation, continued airway ventilation is discontinued and a complete collapse of the lung structure is deemed acceptable.

Increased release of proinflammatory cytokines was reported after pulmonary ischaemia–reperfusion injury [13]. There are reports that have indicated that the cessation of ventilation during CPB is responsible for postoperative atelectasis, lung damage and increased postoperative infections [14, 15]. In previous clinical trials, continued mechanical ventilation during CPB did significantly reduce extravascular lung water [16] extubation time, [16] and modulated postoperative inflammatory response compared with CPB without ventilation [17]. However, pertaining to the systemic immune response, no data are currently available on the effects of continuous airway pressure during CPB on the CPB-induced immune response in vivo.

We hypothesized that this simple procedure during CABG operation attenuates the systemic pro and anti-inflammatory response. Therefore, we measured the anti-inflammatory proteins sST2 and IL-10, the inflammatory protein IL-6, endotoxin concentrations and the Th2 associated proteins IL-4, IgG and IgM.

**MATERIALS AND METHODS**

**Patients and clinical features**

The study was approved by the Institutional Ethics Committee (No. 2894-2008), and is in accordance with the Helsinki Declaration of 1975 and the guidelines for Good Scientific Practice of the Medical University of Vienna. Every patient enrolled in the study gave written consent before surgery, following accurate preparative information about the planned operation and the study protocol. Inclusion criteria were; isolated CABG on CPB operation without valvular pathology, a 2- or 3-vessel disease suitable for CABG, elective or urgent surgery, patients aged 45–80 years. Exclusion criteria were emergent CABG, unstable angina, STEMI or NSTEMI in the last 3 months, any acute infection, fever, infection in the last 3 months, haematological disorder, autoimmune disease, immunodeficiency, immunosuppressive therapy and hepatic or heart failure with an LVEF ≤35%.

Thirty consecutive patients with multivessel coronary artery disease undergoing CABG surgery were included. Coronary revascularization was performed in all cases using CPB. The patients were randomized into two groups: 15 patients were ventilated during CPB (ventilated group = VG), while in 15, mechanical ventilation was discontinued (non-ventilated group = NVG).

All patients received a uniform anaesthetic regimen as described below. The premedication consisted of 7.5 mg oral midazolam, 50 mg ranitidine and 1500 mg cefuroxime 1 h before starting anaesthesia. Anaesthesia was induced with 0.1–0.2 mg/kg midazolam, 0.005–0.03 mg/kg fentanyl and 0.15 mg/kg cis-atracurium. Anaesthesia was maintained with 1–3 Vol% sevoflurane supplemented with a repetitive administration of 5 mg midazolam and 250 μg fentanyl boli every 30–60 min.

Volume-controlled ventilation was performed with a tidal volume of 7 ml/kg, at 10–12 breaths/min and a PEEP of 5 mbar with a Primus or Zeus respirator (Drägermedical GmbH, Lübeck, Germany). In VG patients, tidal volume was reduced to 3–4 ml/kg for the duration of the CPB. Respiratory rate and PEEP remained at the same level. In the NVG patients, the lungs were allowed to collapse after the initiation of complete CPB until weaning. The extracorporeal circuit consisted of a heart–lung machine (Stöckert, Munich, Germany) and a membrane oxygenator (Sorin Pumox, Modena, Italy). Before going on CPB, heparin was administered at a dose of 3 mg/kg (=300 IE/kg) to achieve an ACT of >480 s. ACT was controlled every half an hour. During CPB, mild hypothermia (31–33°C) and a pump flow of 2.4–2.5 l/min/m² were applied. The priming solution contained 1.250–1.750 ml crystalloid solution, 1000 IE Na-heparin, 100 ml mannitol (20%) and 150 ml Na-bicarbonate (4.2%). At the end of CPB, heparin was antagonized with an equivalent dose of protamine (3 mg/kg) to achieve an ACT of <150 s.

**Serum samples**

Blood samples were drawn at the beginning of surgery, at the end of surgery and once on each of the five following postoperative days (PODs) in the morning. Serum was aspirated after centrifugation with 2300 g for 15 min at 4°C and aliquots were stored at −80°C until further analysis.

**Laboratory data**

Serum concentrations of sST2, IL-4, IL-6 and IL-10 (R&D Systems, Minneapolis, MN, USA), IgM and IgG (Bethyl Laboratories, Montgomery, AL, USA) and endotoxin (Life Sciences Advanced Technologies, Saint Petersburg, AL, USA) were measured using commercially available enzyme immunoassay sets. All assays were performed according to the manufacturer's instructions.

**Statistical analysis**

Statistical analysis was performed using SPSS software (SPSS, Chicago, IL, USA). Serum parameters between the two groups were compared using the Mann–Whitney U-test for non-parametric variables. Due to the explorative character of this study, we did not correct for multiple testing. Data are given as the mean (SD) or median (range). For those data that were not normally distributed, results were displayed as box plots. For categorical data, χ² analysis was used. A two-sided P-value of <0.05 was deemed to be significant.

Based on a power analysis, detection of group differences in IL-10 with an 80% power and an α-levels (two-sided) of 0.05 would require a number of 15 patients per group [3].
RESULTS

Patient characteristics are given in Table 1. There were no differences between groups with regard to their preoperative characteristics. No significant differences in intraoperative data and 28-day mortality rate could be detected (Table 2). There were no in-hospital deaths in either group. One NVG patient needed intubation at POD 2 and haemodialysis due to renal failure. Another NVG patient was readmitted to intensive care unit (ICU) due to dyspnoea of unknown origin for 2 days. A patient of the VG was admitted to ICU for 4 days because of confusion. As a main result of this study, we observed a significantly reduced sST2 concentration in VG patients compared with the NVG patients at POD-1, POD-2, POD-3 and POD-5 (Fig. 1A). IL-10 and IL-6 concentrations were significantly lower in VG patients compared with non NVG patients (Fig. 1B, Fig. 2A).

We did not find any difference between the groups for endotoxin (Fig. 2B), IgG (Fig. 3A), IgM (Fig. 3B) and IL-4 (data not shown) at any time point.

DISCUSSION

We investigated the influence of continued mechanical ventilation during CPB for CABG surgery on the systemic immune activation markers. Our clinical study indicates that continuous mechanical ventilation attenuates systemic proinflammatory IL-6 concentration and protein levels of anti-inflammatory sST2 and IL-10. Furthermore, both patient groups evidenced no alterations in serum endotoxin levels. No differences were detected in IL-4, IgG and IgM serum concentrations.

We deduce from these results that the ventilation of the lungs during CPB attenuates the pro and the anti-inflammatory immune responses. According to the literature, severe systemic infections after CABG occur in 17–22% of cases within 30 days after surgery [13]. We have recently shown that patients undergoing CABG surgery present with a long-lasting increase of sST2, a protein related to immune suppression.

Primary TLR-mediated activation of the innate immune systems obviously induces a state of tolerance against secondary infections in patients undergoing CABG operation; hence, surgery creates a ‘state of anergy’ or an ‘immunocompromised state’ due to secretion of sST2 [4, 5]. TLRs function as mammalian pattern-recognition receptors that signal the presence of microbial components to the innate immune system. Contact of the innate immune system with the surface of the CPB system could mimic exposure to microbial products such as peptidoglycan, bacterial lipopolysaccharide (LPS)—an endotoxin—reduces sensitivity to a second challenge with LPS resulting in a diminished production of numerous cytokines in both rodents and humans [18]. The molecular mechanisms of TLR-induced tolerance may involve the down-regulation of the TLR4-MD2 complex, which may lead to diminished TLR signalling via ST2, negatively regulated TLR4 and IL-1R signalling via sequestration of myeloid differentiation factor 88 (MyD88) [10]. Published work evidenced that the recombinant sST2 fusion protein has a direct anti-inflammatory property by acting directly on macrophages via the ST2-TLR4 route [19].

### Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Ventilation group (n = 15)</th>
<th>Non-ventilation group (n = 15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>12/3</td>
<td>13/2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65 (46–80)</td>
<td>66 (47–76)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.0 ± 0.7</td>
<td>28.9 ± 0.9</td>
</tr>
<tr>
<td>COPD (n)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Arterial hypertension (n)</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>50 ± 5</td>
<td>53 ± 9</td>
</tr>
<tr>
<td>EuroSCORE</td>
<td>5 (2–8)</td>
<td>4 (1–12)</td>
</tr>
<tr>
<td>Indication               (elective/urgent)</td>
<td>11/4</td>
<td>9/6</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>84 (67–113)</td>
<td>75 (60–113)</td>
</tr>
<tr>
<td>Instable angina pectoris (n)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>NYHA III (n)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Preoperative stroke (n)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Status post-AMI (n)</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Preoperative PCI (n)</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD, median (range) or absolute numbers, respectively.

AM: acute myocardial infarction; BMI: body mass index; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; NYHA: New York Heart Association; PCI: percutaneous coronary intervention.

<table>
<thead>
<tr>
<th>Intraoperative data</th>
<th>Ventilation group (n = 15)</th>
<th>Non-ventilation group (n = 15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of grafts (n)</td>
<td>4 (2–5)</td>
<td>4 (2–5)</td>
<td>0.712</td>
</tr>
<tr>
<td>AoX, duration (min)</td>
<td>55 ± 11</td>
<td>58 ± 17</td>
<td>0.237</td>
</tr>
<tr>
<td>CPB, duration (min)</td>
<td>95 ± 19</td>
<td>100 ± 25</td>
<td>0.660</td>
</tr>
<tr>
<td>ICU stay (h)</td>
<td>22 (17–45)</td>
<td>50 (17–172)</td>
<td>0.819</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>6 (6–12)</td>
<td>7 (6–19)</td>
<td>0.310</td>
</tr>
<tr>
<td>Ventilation support (h)</td>
<td>9 (4.5–20)</td>
<td>8 (4.5–85)</td>
<td>0.699</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>700 ± 378</td>
<td>600 ± 466</td>
<td>0.250</td>
</tr>
<tr>
<td>Autotransfusion (ml)</td>
<td>350 ± 332</td>
<td>400 ± 377</td>
<td>0.527</td>
</tr>
<tr>
<td>Transfusions units (n)</td>
<td>1 (0–5)</td>
<td>2 (0–6)</td>
<td>0.588</td>
</tr>
<tr>
<td>Hb preoperative (g/dl)</td>
<td>13.6 ± 1.5</td>
<td>13.6 ± 1.6</td>
<td>0.725</td>
</tr>
<tr>
<td>Hb after surgery (g/dl)</td>
<td>9.8 ± 0.9</td>
<td>10.1 ± 1.2</td>
<td>0.362</td>
</tr>
<tr>
<td>Hb POD-1 (g/dl)</td>
<td>10.9 ± 0.9</td>
<td>10.7 ± 1.2</td>
<td>0.894</td>
</tr>
<tr>
<td>Reoperation because of bleeding (n)</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Atrial fibrillation postop (n)</td>
<td>4</td>
<td>2</td>
<td>0.316</td>
</tr>
<tr>
<td>Perioperative AMI (n)</td>
<td>1</td>
<td>0</td>
<td>0.309</td>
</tr>
<tr>
<td>Percuticular tamponade (n)</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>28-day mortality (n)</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD, median (range) or absolute numbers, respectively.

AMI: acute myocardial infarction; AoX: aortic cross clamp; PCI: percutaneous coronary intervention; ICU: intensive care unit; Hb: haemoglobin; POD: postoperative day.
Another group has shown that administration of sST2-Fc fusion protein down-regulated levels of IL-6, IL-12 and TNF-α and effectively suppressed symptoms after the onset of murine collagen-induced arthritis [9].

Recently, we published a report on primary immunological sources for sST2 secretion in humans. We have utilized sST2 and IL-33 gene expression and protein secretion in pooled organ cDNAs and in primary cell cultures, respectively, by real-time PCR and enzyme-linked immunosorbent assay (ELISA) technology. The strongest sST2 mRNA expression was detected in lung and heart tissues, with correlated with spontaneous secretion of sST2 protein in vitro. Of utmost importance was our finding that inflammatory cytokines IL-1α, IL-1β, and TNF-α as well as supernatants of LPS-stimulated peripheral blood mononuclear cells led to an increased secretion of sST2 in cultured lung alveolar epithelial cells and, to a lower extent, in cardiomyocytes (an average of 3.5-fold increase in alveolar epithelial cells vs cardiomyocytes, in vitro). These cytokines (i.e. IL-1, TNF-α) enhanced sST2 secretion via an NFκB-dependent mechanism [6]. These experimental data were further corroborated by experimental LPS stimulation in healthy humans in vivo. We have summarized these observations as follows: endotoxin stimulates white blood cells to secrete proinflammatory cytokines (e.g. IL-1α, IL-1β and TNF-α (IL-6) and consequently induces alveolar epithelial and cardiomyocytes to secrete sST2 in vitro and in vivo.

Those patients who were ventilated on CPB evidence a significantly decreased secretion of sST2/IL-10 and IL-6.

The lung is a highly fragile gaseous exchange surface, particularly with its enormous surface area of ~150 m² and poses a colossal challenge to the fine tuning of the immune system after endotoxin exposure [20]. In the setting of continued ventilation, one might speculate that the prevention of complete atelectasis during CPB prevents harmful immune activation by decreasing intrapulmonary shunt and minimizing systemic inflammatory contact with pneumoepithelial cells. These speculations are supported by clinical reports that linked CABG-induced postoperative lung dysfunction, including ARDS, with conventional CABG operation [21].

The experimental data of Ng et al. [17] suggest that continued mechanical ventilation alters the release of inflammatory parameters. This effect appears to be independent of the degree of oxygenation. In one study, ventilation was continued after the start of CPB. This technique failed to improve haemodynamic parameters as well as pulmonary function tests [22]. Two trials used a tidal volume comparable with that used in this study.

![Figure 1](image1.png)  
Figure 1: (A and B) Serum concentrations of soluble ST2 and IL-10 are presented as box plots for each group and time point. *P < 0.05 **P < 0.01. POD: postoperative day.

![Figure 2](image2.png)  
Figure 2: (A and B) Serum concentrations of IL-6 and endotoxin are presented as box plots for each group and time point. *P < 0.05 **P < 0.01. POD: postoperative day.
In conclusion, this study indicates that the continuation of ventilation during CPB leads to an attenuated inflammatory response and the secretion of anti-inflammatory mediators. This simple intervention might therefore increase patient safety and improve outcome.

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