Relation of inflammatory cytokines to atrial fibrillation after off-pump coronary artery bypass grafting

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Abstract

Objective: It has been observed that a systemic inflammatory response after on-pump coronary artery bypass grafting (CABG) participates in the pathogenesis of postoperative atrial fibrillation (AF). In patients undergoing off-pump CABG, it is plausible that inflammation is associated with the development of postoperative AF. The present study examined relation of proinflammatory cytokines, which play an important role in the upstream of inflammatory cascade, to the development of AF after off-pump CABG. Methods: The present study included 39 patients undergoing off-pump CABG. Tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-8, were measured by enzyme-linked immunosorbent assay, on anesthetic induction, after sternotomy before anastomoses, at the completion of anastomoses, 3 and 6 h thereafter, and on postoperative days (POD) 1–4. C-reactive protein (CRP) was also measured by turbidimetric immunoassay, preoperatively, and on POD 1, 2, 3, 6, 9, and 13. Results: Eleven patients (28%) developed postoperative AF. Patients with postoperative AF were older (70 ± 6.4 years vs 60 ± 8.8 years, P = 0.001); however, there was no difference in other pre- and perioperative variables. TNF-α level did not change during the study period. However, IL-8 and CRP levels significantly increased after the surgery, although there was no significant difference between the two groups. IL-6 level also increased after the surgery with its peak at 6 h after the completion of anastomoses. IL-6 levels of 3 and 6 h after anastomoses were significantly higher in patients with postoperative AF (360 ± 143 pg/ml vs 230 ± 94 pg/ml, P = 0.0047, 435 ± 175 pg/ml vs 247 ± 102 pg/ml, P = 0.0005, respectively). Logistic regression analysis indicated that the highest quartile of IL-6 level immediately after the surgery (odds ratio 7.63; 95% CI, 1.06—54.9; P = 0.0005, respectively). Furthermore, the maximum level of IL-6 immediately after the surgery significantly correlated to age and intraoperative blood loss (r = 0.47, P = 0.04, respectively). Conclusions: Advanced age was a major risk factor for postoperative AF. Furthermore, inflammatory response induced by surgical trauma was also associated with the development of AF after off-pump CABG.

Keywords: Arrhythmia; Coronary artery bypass grafts; Inflammation

1. Introduction

Atrial fibrillation (AF) is the most frequent arrhythmic complication after coronary artery bypass grafting (CABG), with the incidence ranging from 25 to 35% [1]. Although several risk factors for postoperative AF have been elucidated [1], the mechanism by which postoperative AF develops is not entirely clear. A systemic inflammatory response caused by cardiopulmonary bypass (CPB) is known to be associated with the pathogenesis of postoperative AF [2,3]. Avoidance of CPB may result in lowering the incidence of postoperative AF [4]; however, similar incidence of postoperative AF between patients with and without CPB has been also reported [5,6]. Moreover, surgical trauma in CABG contributes postoperative inflammatory response to a higher degree than CPB [7]. Thus, it is suggested that inflammation participates in the pathogenesis of postoperative AF in patients undergoing off-pump CABG.

Proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-6, and IL-8, play a pivotal role in the upstream of inflammatory cascade. TNF-α stimulates the secretion of other proinflammatory cytokines including IL-6 and IL-8 [8]. IL-6 is a multifunctional proinflammatory cytokine, which also plays an important role in ischemia—reperfusion injury [9]. IL-8 attributes neutrophil migration and activation, which results in neutrophil-mediated organ injury [8]. However, association between these proinflammatory cytokines, which are induced by cardiac surgery, and postoperative AF has not been well evaluated, especially in patients undergoing off-pump CABG. We evaluated inflammatory cytokines after off-pump CABG and examined its relation to the occurrence of postoperative AF.
2. Patients and methods

2.1. Patients

Thirty-nine consecutive patients with coronary artery disease undergoing elective CABG at the Chiba University Hospital were enrolled in this study. Off-pump technique was attempted initially in all cases scheduled for elective CABG. Patients with preoperative AF, those with chronic renal failure requiring hemodialysis, those receiving preoperative administration of steroids, and those who had to be converted to a standard on-pump procedure were excluded. The study was approved by the institutional ethics committee and written informed consent was obtained from all cases.

2.2. Anesthesia and surgical technique

Anesthesia was induced and maintained by intravenous infusion of sufentanil citrate and propofol, and inhalation of isoflurane. Muscle relaxation was achieved with vecuronium bromide. Heparin (150 U/kg) was administered as an anticoagulant, and was neutralized by protamine after completion of anastomoses. All patients underwent a median sternotomy. The internal thoracic artery was harvested by a skeletonized technique, using ultrasonic scalpel. In cases with severe left main trunk disease (≥ 90%) or severe left ventricle dysfunction (ejection fraction < 30%), intra-aortic balloon pump (IABP) was initiated before the procedure. If necessary, the radial artery, right gastroepiploic artery, or saphenous vein were harvested. Octopus 4 (Octopus tissue stabilization system, Medtronic Inc, Minneapolis, MN, USA) was used to stabilize the target coronary vessel in all cases. In the majority of the cases, the left anterior descending artery (LAD) was revascularized first. The target vessel was exposed and snared above the anastomotic site by using a 4-0 polypropylene suture with felt. The coronary artery was then opened, and anastomosis was performed with an 8-0 polypropylene suture. Further distal anastomoses were performed using the same myocardial coronary artery stabilizer system. Intracoronary shunt was used in anastomosis of the LAD or proximal right coronary artery. Visualization was enhanced by the use of a surgical blower-humidifier.

2.3. Measurements

Samples were collected in bottles containing EDTA from each patient, on anesthetic induction, after sternotomy before anastomosis, at the completion of anastomoses before protamine infusion, 3 and 6 h thereafter, and on postoperative days (POD) 1–4. Samples were immediately centrifuged (3000 × g for 5 min) and plasma was stored in aliquots at −80 °C until assay. Levels of TNF-α, IL-6 and IL-8 were determined by means of commercially available enzyme-linked immunosorbent assays (R & D Systems, Minneapolis, MN, USA) in accordance with the manufacturer’s instructions. Serum levels of CRP were also measured by turbidimetric immunonassay, preoperatively, and on POD 1, 2, 3, 6, 9, and 13. Sensitivities of TNF-α, IL-6, IL-8, and CRP were 0.12 pg/ml, 0.70 pg/ml, 3.5 pg/ml, and 0.1 mg/dl, respectively.

2.4. Definition of AF

Postoperatively, heart rate and rhythm were continuously monitored until POD 7. In the present study, postoperative AF was defined as the characteristic arrhythmia lasting for ≥ 5 min and confirmed by 12-leads ECG. Magnesium sulfate was administered in all cases, from the end of surgery to POD 3, as a prophylaxis against postoperative AF. However, β-blockers were not routinely used postoperatively.

2.5. Statistical analysis

Continuous variables of clinical parameters were reported as mean ± SD and compared using the Mann–Whitney U-test. Discrete variables were reported as frequencies and percentages and analyzed using the chi-square or Fisher’s exact test as appropriate. A value of P < 0.05 was considered to be statistically significant. The distribution of cytokines and CRP levels were highly skewed, and data were thus logarithmically transformed before analysis, although untransformed data are shown (presented as mean ± SD). Analysis of variance for repeated measures was used for comparison of cytokines and CRP levels between the two groups at each time. After using the Bonferroni correction, we defined P < 0.0056 as statistically significant. To assess the association of cytokine level and postoperative AF, logistic regression analysis was used including clinically significant factors (P < 0.1) and all factors with P < 0.05. Correlations between cytokine levels and other continuous variables were assessed using Spearman’s rank correlation. A value of P < 0.05 was considered to be statistically significant.

3. Results

Thirty-nine patients were included in this study, and 11 patients (28%) developed AF postoperatively (three patients on POD 1, one patient on POD 2, two patients on POD 3, two patients on POD 4, two patients on POD 6, and one patient on POD 7). All patients survived the procedure and had a normal recovery, although one patient with postoperative AF required surgical exploration for cardiac tamponade that developed on POD 2.

3.1. Patient characteristics and perioperative data

Patient characteristics and perioperative data are shown in Table 1. Patients who developed postoperative AF were significantly older, and tended to have triple vessel disease more frequently than those who did not; however, the number of distal anastomoses performed were similar between the groups. Although operative time and the number of distal anastomoses were similar between the groups, intraoperative blood loss was slightly higher in patients with postoperative AF than in those without. Time to extubation in patients with postoperative AF was longer than that in patients without; however, pulmonary dysfunction requiring ventilation support more than 24 h occurred in only one patient with sleep apnea syndrome, who developed postoperative AF.
3.2. Inflammatory markers

Fig. 1 shows inflammatory markers, including TNF-α, IL-6, IL-8, and CRP. TNF-α did not elevate during the study period, and there was no difference between the two groups. IL-6 increased after the completion of anastomoses, peaked at 6 h, and declined thereafter. Plasma levels of IL-6 were significantly higher in patients with postoperative AF than in those without, at 3 and 6 h after completion of anastomoses (360 ± 143 pg/ml vs 230 ± 94 pg/ml, \( P = 0.0047 \), 435 ± 175 pg/ml vs 247 ± 102 pg/ml, \( P = 0.0005 \), respectively). IL-8 was significantly elevated after completion of anastomosis with a peak at 3 h after the procedure, and declined thereafter. Patients with postoperative AF had higher levels of IL-8 after the surgery than those without, although no statistically significant difference was seen. CRP also significantly elevated after the surgery, and peaked on POD 2. However, no significant difference between the two groups was observed during the study period.

### 3.3. Association between IL-6 and postoperative AF

To assess association between IL-6 and postoperative AF, maximum level of IL-6 immediately after the surgery was categorized into quartiles, and the highest quartile (>401 pg/ml) was compared to the combination of the other quartiles. Logistic regression analysis in a model that
intraoperative blood loss. The IL-6 level positively correlated to age and other clinical variables were assessed. Among clinical immediately after the surgery (as a continuous value) and 3.4. Correlations of IL-6 and other variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-6 (&gt;-401 pg/ml)</td>
<td>7.63</td>
<td>1.06—54.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Age</td>
<td>1.18</td>
<td>1.01—1.39</td>
<td>0.04</td>
</tr>
<tr>
<td>Triple vessel disease</td>
<td>2.41</td>
<td>0.19—30.3</td>
<td>0.5</td>
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included age, triple vessel disease, and IL-6 demonstrated that the highest quartile of IL-6 level and age was significantly associated with the development of postoperative AF (Table 2).

3.4. Correlations of IL-6 and other variables

Furthermore, correlations of maximum level of IL-6 immediately after the surgery (as a continuous value) and other clinical variables were assessed. Among clinical variables shown in Table 1, Spearman’s analysis revealed that the IL-6 level positively correlated to age and intraoperative blood loss \((r = 0.04, P = 0.01,\) and \(r = 0.47, P = 0.04,\) respectively). Regarding to correlations of the IL-6 level to other inflammatory markers, IL-8 level at 6 h after the anastomoses positively correlated to the IL-6 level \((r = 0.32, P = 0.045);\) however, no relationship between CRP levels after the surgery and the IL-6 level was found.

4. Discussion

The incidence of AF after off-pump CABG reportedly ranges from 12.9 to 26.4% \([4—6]\). Our incidence of postoperative AF in patients undergoing off-pump CABG was 28%, which may be slightly higher than the previous results. This might be because the incidence of postoperative AF depends on its definition, and modality of diagnosis.

Advanced age has been consistently reported as a risk factor for postoperative AF \([10,11]\), possibly because older patients have atrial structural changes affecting susceptibility to postoperative AF \([12]\). In the present study, patients with postoperative AF were 10 years older than those without, and logistic regression analysis revealed that age was significantly associated with postoperative AF. Thus, in agreement with the previous results, it was considered that advanced age is a major risk factor for AF after off-pump CABG.

The present study evaluated proinflammatory cytokines in patients undergoing off-pump CABG. TNF-\(\alpha\) level did not change significantly during the study period, possibly because systemic level of TNF-\(\alpha\) may not reflect its local concentration in the myocardium, which is a major source of TNF-\(\alpha\) after reperfusion \([13]\). Plasma level of IL-8 significantly increased after the surgery and was higher after the surgery in patients with postoperative AF compared with those without, although there was no statistically significant difference. IL-8, which is induced after reperfusion of ischemic myocardium, attracts and activates neutrophils, resulting in lung injury associated with pulmonary leukocyte sequestration following CPB \([8]\). In the present study, time to extubation tended to be longer in patients with postoperative AF than those without. However, pulmonary dysfunction, which is reportedly associated with postoperative AF \([1]\), occurred in only one patient with sleep apnea syndrome; moreover, most of our patients extubated within 24 h after the surgery. Thus, other factors than pulmonary dysfunction, such as delayed awakening after the surgery, may have an influence on time to extubation.

Plasma levels of IL-6 elevated immediately after the surgery and were higher in patients with postoperative AF compared with those without. It has been revealed that the release of IL-6 is induced by reperfusion of ischemic myocardium, surgical trauma, and CPB \([7,9]\). In the present study, the number of distal bypass graft was similar between the groups, while patients with postoperative AF were more likely to have triple vessel disease than those without. However, our result did not reveal whether reperfusion of ischemic myocardium induced the release of IL-6, although its attribution to the release of IL-6 may be less than surgical trauma \([7]\). Our result that intraoperative blood loss positively correlated to the maximum level of IL-6 suggested that surgical trauma mainly induced the release of IL-6 after off-pump CABG. Furthermore, it has been reported that polymorphism of the promoter of the IL-6 gene have an influence on plasma level of IL-6 after CABG \([14,15]\). On the basis of the result that IL-6 level after the surgery also correlated positively to age, it was suggested that advanced age may modulate the elevation of IL-6, since aging affects inflammatory response following stimulation \([16]\).

It has been revealed that complement-CRP complex 2 days after CPB surgery is associated with the occurrence of postoperative AF, although CRP level was not \([2]\). In agreement with the previous result, we had no significant difference in CRP level between the groups, although CRP significantly increased after the surgery, 50-fold from preoperatively. IL-6 is known to induce the production of acute phase proteins, and was higher in patients with postoperative AF than those without; however, the present study revealed no correlation between IL-6 and CRP levels, which may have been caused by differences in clearance rates \([2]\).

In logistic regression analysis, higher level of IL-6 as well as age was significantly associated with postoperative AF. Our results are concordant with the previous result that a specific IL-6 promoter gene variant modulates postoperative IL-6 level and is related to the development of postoperative AF \([14]\). Administration of glucocorticoids such as methylprednisolone and dexamethasone, which significantly reduces plasma level of IL-6, can reduce incidence of AF after on-pump CABG \([17,18]\). Thus, it is suggested that IL-6 is associated with the development of postoperative AF. IL-6 regulates a large variety of cellular response in multiple organ systems, besides, endocrine responses and central nervous system \([19,20]\). IL-6, like TNF-\(\alpha\) and IL-1\(\beta\), has a stimulatory effect on the hypothalamo-pituitary-adrenal (HPA) axis, increasing hypothalamic secretion of corticotropin-releasing hormone (CRH) \([21]\). CRH subsequently increases secretion of adrenocorticotropic hormone (ACTH) and cortisol. Furthermore, CRH stimulates noradrenergic neurons of the central nervous system, which induces the release of norepinephrine. Subcutaneous administration of IL-6 induces significant increases in plasma levels of ACTH, cortisol, and norepinephrine \([22]\). Thus, IL-6 not only is a
marker of severity of inflammation but also regulates inflammatory response and activates the sympathetic nervous system, which is associated with occurrence of postoperative AF in vulnerable patients [23,24].

Postoperative AF usually occurs within the first post-operative week, and mostly on postoperative days 2–4 [1]. In the present study, postoperative AF occurred within the first four postoperative days in 8 of 11 patients. However, only three patients developed postoperative AF on POD 1, while IL-6 level peaked at 6 h after anastomoses. The discrepancy in the time when IL-6 level peaked and postoperative AF occurred may be explained by association between IL-6 and subsequent sympathetic nerve activation.

In conclusion, advanced age is a major risk factor for the development of postoperative AF, and moreover, a higher degree of systemic inflammatory response, which is induced by surgical trauma, is also associated with the development of AF after off-pump CABG. The mechanism by which systemic inflammatory response to surgical trauma causes postoperative AF remains to be further elucidated.

References