

Activation of the receptor activator of the nuclear factor- κ B ligand pathway during coronary bypass surgery: comparison between on- and off-pump coronary artery bypass surgery procedures

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Abstract

OBJECTIVES: The receptor activator of the nuclear factor kappa-B (NF- κ B) ligand (RANKL), its membrane receptor RANK and its decoy receptor osteoprotegerin (OPG) are all members of the tumour necrosis factor family involved in bone metabolism and immune response. We evaluated the activation of the OPG/RANKL/RANK pathway in patients undergoing cardiac surgery with and without cardiopulmonary bypass (CPB).

METHODS: Twenty consecutive patients undergoing elective coronary artery surgery were enrolled in the study and assigned either to the on-pump or to the off-pump group. Pre- and postoperative serum levels of OPG and RANKL were evaluated by enzyme-linked immunosorbent assay; gene expression of OPG, RANKL, RANK and NF- κ B p50 subunits were determined by real-time polymerase chain reaction in peripheral blood T-cells and monocytes.

RESULTS: Serum levels of OPG significantly increased after surgery in both groups, whereas serum levels of RANKL did not differ over time. T-cells from the on-pump group showed increased gene expression of OPG, RANKL and RANK after the intervention, whereas no mRNA variation for these genes was detected in T-cells from off-pump patients. Gene expression of p50 subunit increased in T-cells and monocytes from both groups.

CONCLUSIONS: Cardiac surgery induces the activation of the OPG/RANKL/RANK pathway; both on- and off-pump procedures are associated with increased postoperative OPG serum levels and up-regulation of the NF- κ B p50 subunit.

Keywords: Cardiopulmonary bypass • Inflammatory mediators

INTRODUCTION

Cardiac surgery with cardiopulmonary bypass (CPB) is associated with a systemic inflammatory response syndrome (SIRS) that may lead to postoperative complications, such as myocardial, pulmonary, neurological, liver and renal dysfunction, as well as postoperative bleeding [1]. Contact of blood components with the artificial surface of the CPB circuit, ischaemia-reperfusion injury, endotoxemia and surgical trauma itself all lead to the activation of the inflammatory, coagulation and fibrinolytic systems [1]. The avoidance of CPB can reduce the inflammatory response [2]; however, some studies suggest that activation of inflammation still occurs in off-pump coronary artery bypass surgery (OPCAB), but is slightly delayed with respect to on-pump bypass [3, 4] and persists several days after surgery [5]. Nuclear factor kappa-B (NF- κ B) is a ubiquitous inducible transcription factor involved in the

regulation of the transcription of many proinflammatory genes [6]; recently, NF- κ B involvement in inflammatory diseases, such as atherosclerosis, unstable angina, heart failure and myocardial ischaemia-reperfusion, has been detected. Its activation during cardiac surgery with CPB has been demonstrated, while its involvement in OPCAB has been undetected [7–9]. The receptor activator of the NF- κ B ligand (RANKL), its membrane receptor RANK and its decoy receptor osteoprotegerin (OPG) are all members of the tumour necrosis factor (TNF) family involved in bone metabolism, extracellular matrix regulation and immune response [10]. OPG/RANKL/RANK pathway activation has been detected in inflammatory diseases such as atherosclerosis [11–13]; elevated OPG serum levels are associated with coronary artery disease (CAD) [14, 15] and represent a risk factor for disease progression [16]. In women, elevated OPG serum levels are associated with higher cardiovascular mortality [17]. In patients with unstable angina, T-cells expression of RANKL and monocytes expression of RANK are enhanced and promote matrix metalloproteinase

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activity in smooth muscle cells (SMCs), leading to plaque destabilization [18]. The role of the OPG/RANKL/RANK pathway in an acute inflammatory state, such as CPB-induced SIRS, has not been established. The aim of this study was to evaluate the activation of NF- κ B in on-pump and OPCAB, with the hypothesis being that both surgical techniques should induce some degree of inflammatory cellular reaction. Moreover, the activation of the OPG/RANKL/RANK pathway has been studied for the first time in cardiac surgical patients, considering its linkage with NF- κ B.

PATIENTS AND METHODS

Patients

The study was conducted according to the ethical guidelines at our hospital and the Helsinki declaration and was approved by the hospital's authorized representative. After informed consent, 20 consecutive patients requiring elective coronary artery surgery were prospectively enrolled in the study and assigned either to the on-pump or to the off-pump group, based on surgeon preference and clinical judgement. The following exclusion criteria were applied: infections, body temperature $>37.5^{\circ}\text{C}$, white blood cells count $>12\,000/\mu\text{l}$, current neoplasia, chronic inflammatory or autoimmune disease, corticosteroid and/or immunosuppressive treatment, emergency.

Perioperative management

After premedication with lorazepam, anaesthesia was induced with a combination of fentanyl, midazolam and sodium thiopental and maintained with propofol. Heparin (300 U/kg) was given to achieve systemic anticoagulation in both groups. Intraoperative heparin monitoring was performed by standard activated clotting time (ACT) (HEMOCHRON Jr. ACT+, ITC, Edison, NJ, USA). Additional heparin bolus (5000 U) was given if the activated clotting time was <400 s. Protamine was administered to reverse heparin (1 mg of protamine/100 U of heparin) in both groups. In the on-pump group, CPB was established with a two-stage venous cannula and aortic return. Moderate hypothermia (34°C) was maintained during the operation. Cardiac arrest was induced and maintained with antegrade cold-blood cardioplegia. Cardiomyotomy suction was used in all patients of the on-pump group. A heart stabilizer (Octopus; Medtronic, Inc., Minneapolis, MN, USA) has been used in all OPCAB, and intracoronary shunts have been used to allow bloodless anastomoses. Cell-saving devices were used in all patients. Prophylactic antibiotics were administered according to institutional protocols. Postoperatively, patients received aspirin (100 mg orally or intravenously) and low molecular-weight heparin (enoxaparin, 4000 anti-Xa IU daily subcutaneously) starting 12 h after the end of the operation in the absence of significant mediastinal bleeding.

Samples

Peripheral blood (PB) samples from the on- and off-pump group patients were collected into Vacutainer[®] tubes containing 3.8% sodium citrate or gel for serum separation (Becton Dickinson, USA) at four different times before, during and after the operation:

(i) T0: before the beginning of the operation;

- (ii) T1: 2 h after the end of CPB (on-pump group) or distal anastomoses (off-pump group);
- (iii) T2: first postoperative day (POD);
- (iv) T3: sixth POD.

Serum samples were centrifuged for 30 min at 3500 rpm and stored at -80°C until assayed.

Enzyme-linked immunosorbent assay

Serum levels of OPG and RANKL were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Biomedica, GmbH, Wien, Austria); the absorption was determined with an ELISA reader at 450 nm (550 Microplate Reader; Bio-Rad). All ELISA assays were doubled tested, and the mean value was used for analysis.

Cell isolation

T-cells and monocytes were purified from PB mononuclear cells (PBMCs) of the on- and off-pump group patients. First, PBMCs were isolated by centrifugation over Histopaque 1077 density gradient (Sigma Chemical, St Louis, MO, USA) and after diluted at 10×10^6 cells/ml in phosphate buffer saline supplemented with 2% bovine serum albumin (Sigma). The T-cell and monocytes isolation were performed using anti-CD2 antibody or anti-CD14 (Ab)-coated immunomagnetic Dynabeads (Dyna, Lake Success, NY, USA), respectively. In particular, CD2⁺ or CD14⁺ cells were captured from the PB buffy coats, incubating appropriate 2×10^7 beads/ml with 10×10^6 cells/ml for 30 min at 4°C on an apparatus that allows both gentle tilting and rotation.

RNA isolation and real-time polymerase chain reaction amplification

T-cells and monocytes were subjected to RNA extraction using spin columns (RNeasy; Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA (1 μg) was reverse-transcribed, using the Super Script First-Strand Synthesis System kit for real-time polymerase chain reaction (RT-PCR; Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA was amplified with the iTaq SYBR Green supermix with ROX kit (Bio-Rad Laboratories, Bio-Rad Laboratories, Inc., Hercules, CA, USA), and the PCR amplification was performed using the Chromo4 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.). The running conditions were: incubation at 95°C for 3 min, and 40 cycles of incubation at 95°C for 15 s and 60°C for 30 s. After the last cycle, the melting curve analysis was performed into 55 – 95°C interval by incrementing the temperature of 0.5°C . The fold change values were calculated by the Pfaffl method [19]. The sense and antisense primers sequences of OPG, RANKL, RANK, p50 and housekeeping gene cyclophilin are reported in Table 1. Amplification reactions specific for the OPG, RANKL, RANK and p50 were carried out on T-cells, whereas RANK and p50 reactions were performed on CD14⁺ monocytes.

Statistical analyses

Data are given as mean values \pm standard deviation (SD), and categorical variables as frequencies and percentage. Statistical

Table 1: Sense and antisense primer sequences

Gene	Sense primer	Antisense primer
RT-hOPG	GACCACTACTACAGACAG	AAGCAGAACTATCTCAAGG
RT-h-RANKL	ATCACAGCACATCAGAGCAGAG	GGACAGACTCACTTATGGGAACC
RT-h-RANK	CAGGATGCTCTCATTGGTCAG	AGAAAGGAGGTGTGGATTTC
RT-h-p50	CTGCAGTTTGATGATGAAGA	GTGCAGAGGAAACGTCAGAA
RT-h-cyclophilin	CAGGTCCTGGCATCTTGCC	TTGCTGGTCTTGCCATTCT

OPG: osteoprotegerin; RANKL: receptor activator of nuclear factor κ B ligand; RANK: receptor activator of nuclear factor κ B.

analyses were performed using two factor repeated-measures analysis of variance (ANOVAs) with one repeated factor (time from T0 to T3) and one grouping factor (on-pump vs off-pump group) with Bonferroni correction for multiple comparisons. After a normality test (Shapiro-Wilk), OPG values were natural log transformed. Because of the skewed distribution, RANKL was compared over time using a Friedman test. Patient's characteristics were compared with a non-parametric Mann-Whitney *U*-test or Fisher's exact test for frequencies. Because of the exploratory nature of the study, sample size was not formally computed but decided *a priori* to be 20 patients. The results were considered statistically significant for *P*-values <0.05. The analyses were made using STATA software, version 12 (StataCorp, College Station, TX, USA).

RESULTS

The characteristics of the on- and off-pump group patients are summarized in Table 2. Age, sex, cardiovascular risk factors, associated diseases and ejection fraction were similar in both groups, and there were no statistically significant differences in the post-operative outcome between groups.

OPG and RANKL serum levels

The trends over time of serum levels of OPG and RANKL in the on- and off-pump groups are illustrated in Figs 1 and 2. At ANOVA of OPG values, no overall difference was detected according to the study group (*P* = 0.51). A significant effect of time (*P* < 0.0001) and an interaction between group and time (*P* = 0.008) were observed. Baseline serum levels of OPG were 7.60 ± 3.08 pmol/l (range 3.57–13.28 pmol/l) in the on-pump group and 8.14 ± 5.88 pmol/l (range 3.31–17.42 pmol/l) in the off-pump group. At T1, serum levels of OPG were significantly higher compared with baseline in the on-pump group (13.92 ± 3.53 pmol/l; *P* < 0.0001); conversely in the off-pump group, serum levels of OPG start to rise but were not significantly higher compared with baseline (10.07 ± 8.00 pmol/l). At T2, serum levels of OPG reached the peak in both groups (on-pump group: 15.16 ± 5.41 pmol/l, *P* < 0.0001 compared with T0; off-pump group: 13.01 ± 7.09 pmol/l, *P* < 0.0001 compared with T0). At T3, serum levels of OPG declined in both groups, but were still significantly higher compared with baseline only in the off-pump group (on-pump group: 10.71 ± 5.67 pmol/l, *P* = 0.26 compared with T0; off-pump group: 11.90 ± 7.56 pmol/l, *P* = 0.018 compared with T0). There were no statistically significant differences in OPG serum levels between

Table 2: Pre-, intra- and postoperative characteristics of the on- and off-pump group patients

	On-pump group (N = 10)	Off-pump group (N = 10)	P-value
Age (years)	71 ± 9	72 ± 7	>0.99
Male	7 (70%)	7 (70%)	>0.99
BMI (kg/m ²)	29 ± 3	28 ± 4	>0.99
Systemic hypertension	9 (90%)	9 (90%)	>0.99
Diabetes mellitus	5 (50%)	8 (80%)	0.35
Hypercholesterolaemia	3 (30%)	5 (50%)	0.65
Obesity	1 (10%)	2 (20%)	>0.99
Smoking history	1 (10%)	1 (10%)	>0.99
Previous AMI	3 (30%)	6 (60%)	0.37
Unstable angina	1 (10%)	2 (20%)	>0.99
EF (%)	50 ± 9	51 ± 12	0.69
Chronic AF	1 (10%)	1 (10%)	>0.99
Previous CVA	1 (10%)	1 (10%)	>0.99
PVD	1 (10%)	3 (30%)	0.58
CRF	1 (10%)	3 (30%)	0.58
COPD	4 (40%)	3 (30%)	0.67
EuroSCORE	5.4 ± 1.3	6.1 ± 3.2	0.95
Operation time (h)	4.4 ± 0.7	4.1 ± 0.6	0.36
CPB (min)	105.1 ± 25.5	-	-
Cross-clamping (min)	63.3 ± 6.9	-	-
Number of distal anastomosis	3.2 ± 0.8	2.7 ± 0.8	0.56
ICU stay (h)	38.7 ± 8.2	33 ± 2.3	0.47
Overall stay (days)	7.7 ± 0.8	7.3 ± 1.2	0.35
Mechanical ventilation (h)	8.6 ± 5.1	8.4 ± 3.1	0.74
Inotropic drugs	3 (30%)	2 (20%)	>0.99
IABP	0 (0%)	0 (0%)	-
AF	2 (20%)	3 (30%)	>0.99
CVA	0 (0%)	0 (0%)	-
ARF	0 (0%)	0 (0%)	-
Mediastinal blood loss (ml)	310 ± 110	395 ± 120	0.37
Pleural blood loss (ml)	345 ± 388	357 ± 273	0.53
Re-exploration for bleeding	0 (0%)	0 (0%)	-
RBC transfusions (units)	1.4 ± 1.4	0.7 ± 1.2	0.31
FFP transfusions (units)	0.1 ± 0.4	0.3 ± 0.9	0.96
PLT transfusions (units)	0 (0%)	0 (0%)	-
Sepsis	0 (0%)	0 (0%)	-
Mortality	0 (0%)	0 (0%)	-

BMI: body mass index; AMI: acute myocardial infarction; EF: ejection fraction; AF: atrial fibrillation; CVA: cerebro-vascular accident; PVD: peripheral vascular disease; CRF: chronic renal failure; COPD: chronic obstructive pulmonary disease; CPB: cardiopulmonary bypass; ICU: intensive care unit; IABP: intra-aortic balloon pump; ARF: acute renal failure; RBC: red blood cell; FFP: fresh frozen plasma; PLT: platelets.

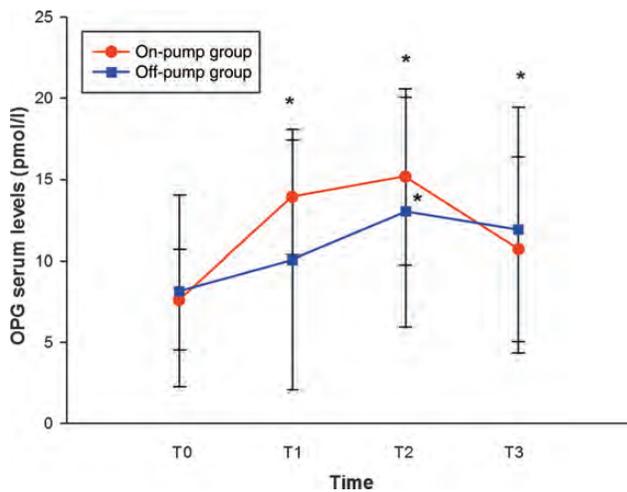


Figure 1: Serum levels of OPG in patients from the on- and off-pump groups; values are expressed as mean \pm SD; * $P < 0.05$ vs T0.

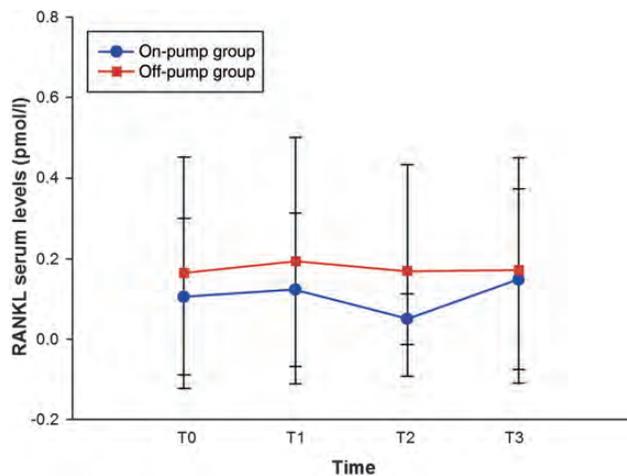


Figure 2: Serum levels of RANKL in patients from the on- and off-pump groups; values are expressed as mean \pm SD.

the on- and off-pump groups at each sample time with the exception of T1, which showed values significantly lower in the off- than in the on-pump group ($P < 0.0001$). Serum levels of RANKL did not differ over time in both groups.

OPG/RANKL/RANK expression by T-cells

OPG/RANKL/RANK gene expression was studied by RT-PCR in T-cells isolated from the on- and the off-pump group patients at all above-mentioned sample times. T-cells from the on-pump group showed increased gene expression of OPG, RANKL and RANK after the intervention, whereas no mRNA variation for these genes was detected in T-cells from off-pump patients at T1, T2 and T3 (Fig. 3A–C). In particular, OPG mRNA levels resulted up-regulated in the on-pump group at T1, T2 and T3 respect to T0 (fold change 1.76, 1.81 and 1.85, respectively), although the difference was not statistically significant. Similarly, RANKL mRNA levels increased postoperatively in T-cells from the on-pump group at T1, T2 and T3 respect to T0 (fold change 1.62, 2.57 and 2.05, respectively), even if the difference was not statistically significant.

Moreover, RANK expression was up-regulated at T1, T2 and T3 (fold increase 1.62, 1.96 and 1.43, respectively) in T-cells from the on-pump group without reaching a statistical significance.

RANK expression by monocytes

RANK gene expression was also evaluated by RT-PCR in CD14⁺ monocytes isolated from the on- and the off-pump groups. In particular, in CD14⁺ monocytes from the on-pump group, the increase in RANK expression was stronger than that observed in T-cells with an early peak at T1 (fold increase 2.6), even if it was not statistically significant compared with T0 (Fig. 3E); RANK mRNA levels continued to be up-regulated at T2 (fold increase 2.23). Interestingly, the expression of RANK also resulted up-regulated in CD14⁺ monocytes isolated from off-pump group patients at T1 (fold change 2.15), even if it was not statistically significant.

NF- κ B p50 subunit expression

We studied by RT-PCR, the expression of p50 subunits in T-cells and CD14⁺ monocytes isolated from the on- and the off-pump groups. The expression of p50 subunits increased in T-cells and CD14⁺ monocytes isolated from both groups without reaching a statistical significance and with a different pattern (Fig. 3D and F). In particular, in the on-pump group T-cells, p50 mRNA levels reached the peak at T1 (fold change 3.09), while in CD14⁺ monocytes p50 expression increased at T2 and T3 (fold change 2.39 and 2.62, respectively); it was thus later with respect to T-cells. In the off-pump group, p50 increase reached the peak at T2 in both T-cells and CD14⁺ monocytes (fold change 3.82 and 2.75, respectively).

DISCUSSION

Our study first demonstrates that cardiac surgery is associated with the activation of the OPG/RANKL/RANK pathway. Both on- and off-pump procedures are associated with a significant increase in OPG serum levels in the postoperative period. We found similar baseline serum levels of OPG in the two groups, so one can speculate that the postoperative increase was mainly due to the intervention; additionally, the surgical technique influenced the pattern of release of OPG. In the on-pump group, we observed a prompt and statistically significant increase in serum levels of OPG at T1, whereas in the off-pump group, the increase in serum levels of OPG was statistically significant later at T2. Serum levels of OPG reached the peak in both groups at T2 and were still significantly elevated at T3, compared with T0 only in the off-pump group. It has been well established that cardiac surgery with CPB induces an acute systemic inflammatory response characterized by the activation of the coagulation, fibrinolytic, kallikrein and complement cascades resulting in possible postoperative morbidity [1]. However, our data showed that both surgical techniques are associated with the activation of the OPG/RANKL/RANK system, as we found no statistically significant differences in OPG serum levels between on- and off-pump groups at each sample time with the exception of T1. The inflammatory response in the off-pump, compared with the on-pump procedures, is delayed because of the lack of CPB and aortic cross-clamping, which represent potent

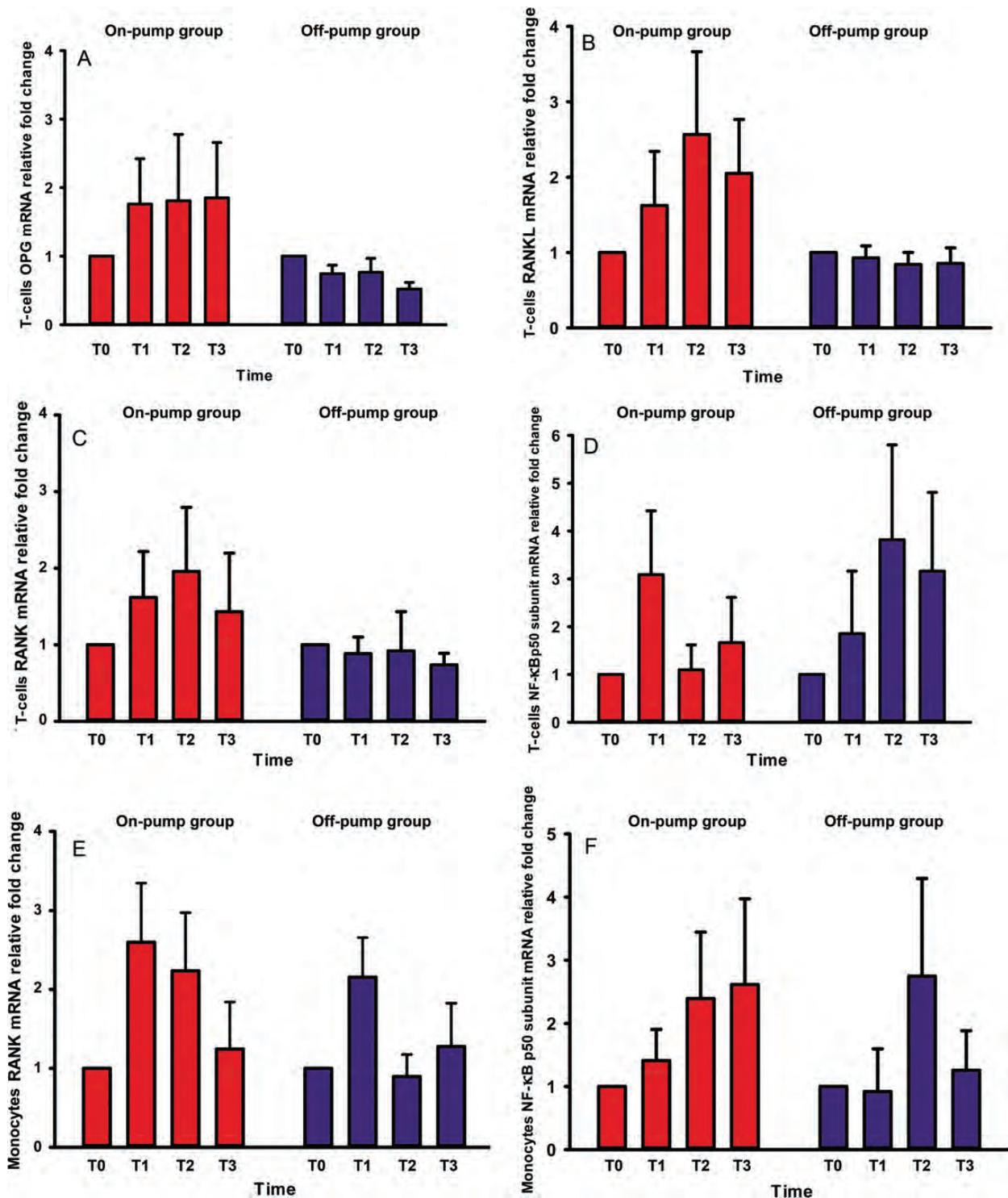


Figure 3: Gene expression of OPG (A), RANKL (B), RANK (C) and NF- κ B p50 subunit (D) in T-cells and of RANK (E) and NF- κ B p50 subunit (F) in monocytes from the on- and off-pump groups; mRNA levels were quantified using RT-PCR, and data are presented relative to the gene expression of cyclophilin (mean \pm SEM).

inflammatory stimuli. The difference in the release pattern of inflammatory cytokines between on- and off-pump procedures has already been described by previous reports, which found that inflammation in OPCAB is slightly delayed compared with the on-pump procedure [3, 4] and persists several days after surgery [5]. The OPG/RANKL/RANK pathway was initially described in the

context of bone mass regulation [20]. Now, its prominent role not only in bone metabolism, but also in cardiovascular disease, is emerging. The activation of the OPG/RANKL/RANK pathway has already been described in several acute and chronic inflammatory diseases. In patients with unstable angina, higher expressions of RANKL in T-cells and of RANK in monocytes have been reported

in comparison with stable angina [18]. Clinical studies have observed higher circulating OPG levels in patients with unstable angina and with acute myocardial infarction (AMI), but not in those with stable angina or stable CAD [18, 21, 22]. Experimental studies report the activation of the OPG/RANKL/RANK system in acute inflammatory diseases and reveal a potential protective role of RANKL [23]. In this study, we demonstrated that the OPG/RANKL/RANK pathway can be acutely activated by an inflammatory stimulus such as cardiac surgery, either earlier as in the on-pump procedure or later as in the OPCAB procedure. We analysed the gene expression of OPG, RANKL and RANK in T-cells and CD14⁺ monocytes in order to find the source of the increased serum levels of OPG. We found that T-cells from patients undergoing coronary surgery with CPB, but not from those undergoing OPCAB, showed increased gene expression of OPG and RANKL. Additionally, we found that the up-regulation of RANKL in T-cells was associated with that of RANK in both T-cells and CD14⁺ monocytes. We think that T-cells are less activated in off-pump, compared with on-pump, patients because of the lack of CPB and aortic cross-clamping; additionally, OPG is expressed by several cells other than T-cells, essentially endothelial cells (ECs), and the latter could be responsible for the increased OPG serum levels in off-pump patients. OPG is expressed in the vascular system, including ECs and vascular SMCs, and is modulated in these cells by proinflammatory cytokines, such as interleukin (IL)-1 and TNF- α . Within ECs, OPG is associated with the von Willebrand factor in secretory granules called Weibel-Palade bodies [24]. Upon stimulation with TNF- α or IL-1 β *in vitro*, the OPG-von Willebrand factor complex is secreted into the surrounding growth medium. This complex is also noted in human serum, indicating ECs activation by proinflammatory cytokines as one of the possible sources of circulating OPG in patients with active atherosclerosis. These cells are probably activated following coronary surgery and may contribute to the increase in serum levels of OPG in the acute phase in both on- and off-pump procedures. Conversely, serum levels of RANKL were unchanged over time in both groups compared with baseline.

The main transcription factor activated by the RANKL-RANK interaction is NF- κ B, which is also clearly one of the most important regulators of proinflammatory gene expression; synthesis of cytokines such as TNF- α , IL-1 β , IL-6 and IL-8 as well as adhesion molecules such as E-selectin, VCAM-1 and ICAM-1 is mediated by NF- κ B [2]. The most abundant form of NF- κ B is a p50/p65 heterodimer in which p65 contains the transcriptional activation domain. NF- κ B activation has been previously demonstrated only in myocardial biopsies from patients undergoing coronary surgery with CPB [9] and never in those receiving off-pump procedures. We found a relevant up-regulation in the expression of NF- κ B p50 subunit in T-cells from patients undergoing both on- and off-procedures; in particular, we showed that the up-regulation of the T-cells gene expression of NF- κ B p50 subunit reached a peak at T1 in the on-pump group and at T2 in the off-pump group. This further demonstrates that cardiac surgery induces the activation of the inflammatory system that appears earlier in the on-pump group likely because CPB represents a potent stimulus to the activation of the inflammatory system and later in the OPCAB group, confirming our initial hypothesis.

It is well established that surgical trauma is a potent activator of the inflammatory system. Proinflammatory cytokines are elevated following major surgery, and the degree of activation increases with the intensity of surgical insult [25]. The inflammatory response and the resultant stress response are significantly

attenuated when surgical exposure is reduced during laparoscopy compared with open major abdominal surgery [26]. Our study suggests that surgical trauma is mainly responsible for the activation of the inflammatory system during cardiac surgery, independently of the surgical technique; however, in off-pump procedures the inflammatory response is delayed compared with on-pump because of the lack of CPB and aortic cross-clamping.

Limitations of the study are mainly represented by the small sample size. This is a small study demonstrating the activation of the OPG/RANKL/RANK pathway after coronary surgery. Currently, no data exist regarding its involvement in any end-organ morbidity following cardiac surgery; larger studies are needed to evaluate the involvement of the OPG/RANKL/RANK pathway in the inflammatory response and postoperative morbidity of patients undergoing cardiac surgery. Moreover, this study has been conducted on patients affected by coronary atherosclerosis, which is known to be associated with the activation of the OPG/RANKL/RANK pathway. Future studies conducted on patients undergoing valvular surgery (i.e. degenerative mitral insufficiency) could better clarify the role of the pathway in the postoperative inflammatory response in the absence of atherosclerosis.

CONCLUSIONS

Our study demonstrates that cardiac surgery induces the activation of the OPG/RANKL/RANK pathway as suggested by the increased postoperative OPG serum levels in both on- and off-pump procedures and is associated with an increased expression of the NF- κ B p50 subunit. Larger studies are needed to better evaluate the involvement of the OPG/RANKL/RANK pathway in the postoperative morbidity of patients undergoing cardiac surgery.

Conflict of interest: none declared.

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EDITORIAL COMMENT

The end of all our exploring ...

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For over half a century, only a handful of subjects in the field of cardiovascular surgery have consistently attracted intense research interests, cardiopulmonary bypass (CPB) being one of them. Few would argue that the numerous investigations to date focusing on the occurrence of systemic inflammatory response syndrome (SIRS) after open-heart surgery have had positive impacts on the healthy development of CPB technology and techniques [1]. However, a number of surgical approaches that do not involve CPB have also been in widespread practice over the past two decades. A prominent example is off-pump coronary artery bypass grafting (CABG). Because it avoids CPB, and thus cardioplegic arrest, many believe off-pump CABG to be 'minimally invasive' (and thus advocate the procedure), as SIRS appears to no longer be a major burden. In the current era of evidence-based medicine, however, this belief is yet to be convincingly proven.

In this issue of the journal, Galeone *et al.* [2] demonstrate that both the on-pump and off-pump CABG approaches are associated with similar degrees of inflammatory response, as reflected

by the activation of the receptor activator of the nuclear factor- κ B ligand pathway and enhanced postoperative cellular expression of the nuclear factor- κ B p50 subunit. Conversely, the blood osteoprotegerin concentration reached peak levels slightly later in their off-pump group than in the on-pump group, but remained higher than the baseline until postoperative day 6. As far as SIRS is concerned, their study reveals little meaningful intergroup difference. Their lack of exploration of participants' anti-inflammatory response simultaneously, however, means that it is difficult to form a definitive view [3]. Although the puzzle remains unresolved and the jury is still out, Galeone and colleagues' observation does indeed provide us with some food for thought. In particular, their finding may stimulate readers to reappraise the ongoing debate on whether CABG should be performed with or without the pump (even though the real issue involved is far more complex than the pump alone [4]).

CABG is arguably the most thoroughly investigated operation in the history of surgery. Although much is yet to be learned despite the multiple 'off-pump vs on-pump' comparisons made

over the past decade, at least one thing is clearer now: it is no longer appropriate to simply label the off-pump approach 'minimally invasive.' In fact, there is emerging evidence to suggest that, in this off-pump vs on-pump competition, SIRS may actually make little difference in major clinical outcome parameters. In a single-center study involving 6665 consecutive patients undergoing isolated CABG over an 8-year period, the off-pump group ($n = 3266$) did indeed experience a reduced incidence of postoperative atrial fibrillation, less need for blood transfusions and a shorter duration on mechanical ventilation [5]. However, of the 97.6% of the study participants who were followed up for an average of 4.5 years, those in the off-pump group had significantly higher rates of repeat revascularization and major vascular events, as well as more rehospitalization owing to cardiovascular causes (which significantly increased the costs for this group) [5]. More importantly, a recent systematic review [6] of 86 randomized clinical trials (involving 10716 patients) of off-pump vs on-pump CABG failed to confirm any significant benefits of the off-pump approach with regard to postoperative mortality, stroke or myocardial infarction. On the contrary, better long-term survival was observed in patients who underwent on-pump CABG [6]. Møller *et al.* [6] therefore concluded that 'based on the current evidence, on-pump CABG should continue to be the standard surgical treatment,' although 'off-pump CABG may be acceptable' when there are contraindications for cannulation of the aorta and CPB. Along the same lines, two additional multicenter randomized trials [7, 8] were published after these researchers' meta-analysis. In the first, the CORONARY Investigators [7] reported that fewer grafts were performed, and more repeat revascularization occurred within just 30 days of CABG, in the off-pump group ($n = 2375$). In the second, the latest GOPCABE trial [8] of 2539 patients aged 75 and above (i.e. patients with one of the oft-mentioned high-risk factors), more repeat revascularization was again required following off-pump CABG relative to conventional CABG during the first postoperative month.

To conclude, we could not agree more with T.S. Eliot's famous lines from the *Four Quartets*: 'We must not cease from exploration

and the end of all our exploring will be to arrive where we started and know the place for the first time.' For many (if not all) surgeons, it is unlikely that more randomized clinical trials on this particular subject are desirable in the foreseeable future, as the old question no longer seems relevant. We should be proud that CPB and its related management are far more advanced than they were half a century ago [1, 9]. To tailor a procedure to a patient, and not *vice versa*, we are definitely more confident today in modifying the definition of 'minimally invasive' to embrace 'on a better pump' rather than simply 'off pump.'

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