# High-sensitivity C-reactive protein (hs-CRP) and tumor necrotizing factor-alpha (TNF-α) after on- and off- pump coronary artery bypass grafting

H. Javadzadegan[1], N. Nezami[2], K. Ghobadi[2], A. Sadighi[3], A.A. Abolfathi[4], N.D. Nader[5]

*[1]Department of [1]Cardiology, [2]Drug Applied Research, [3]Tuberculosis and Lung Disease Research Center,*
*[4]Biochemistry, Tabriz University (Medical Sciences), Tabriz, Iran,*
*[5]Department of Anesthesiology, University at Buffalo, Buffalo, New York*

*HSR Proceedings in Intensive Care and Cardiovascular Anesthesia 2010; 2: 27-33*

## ABSTRACT

**Introduction:** Coronary artery bypass grafting (CABG) is one of the most frequently performed operations around the world. The aim of this study is to evaluate high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor-alpha (TNFα) differences between on-pump and off-pump coronary surgery.
**Methods:** In this prospective study, 90 patients with coronary artery disease referred for CABG were enrolled from July 2006-November 2007. Levels of hs-CRP and TNFα were measured by ELISA using commercial kits.
**Results:** hs-CRP levels significantly ($p < 0.001$) increase after CABG. But no difference between off-pump and on-pump groups was noted for hs-CRP and TNF-a levels ($p = 0.4$, $p = 0.8$).
**Conclusions:** There was no difference in high-sensitivity C-reactive protein (hs-CRP) and TNFα between on-pump and off-pump CABG surgery.

**Keywords:** *coronary artery bypass grafting, C-reactive protein (CRP), tumor necrotizing factor-alpha (TNF-alpha), on-pump, off-pump, cardiopulmonary bypass.*

## INTRODUCTION

Coronary artery bypass grafting (CABG) is commonly performed worldwide and any improvement in the safety and efficacy of the procedure would have an impact on outcome in absolute terms (1, 2).
It is well accepted that cardiopulmonary bypass (CPB) during on-pump CABG and pulmonary-myocardial reperfusion activates the immune system. The extent of this immune reaction has been correlated to clinical and post-operative complications. (3,4) It has been proposed that avoiding CPB and myocardial ischemia-reperfusion significantly reduces the postoperative systemic complications which negatively affect the perioperative course after surgical myocardial revascularization (5-7).
The aim of this study is to evaluate differences in high-sensitivity C-reactive protein (hs-CRP) and tumor necrotizing factor-alpha (TNF-α) between on-pump and off-pump coronary surgery.

*Corresponding author:*
Hassan Javadzadegan, MD,
C/O Nader D. Nader, MD, PhD, Professor of Anesthesiology,
University at Buffalo,
Rm 202C, 3495 Bailey Ave,
Buffalo, NY 14215
e.mail: nnader@buffalo.edu

We hypothesize that serum concentrations of both C-reactive protein and TNFα are lower in patients who undergo CABG without using CPB.

## METHODS

This prospective case-matched study was conducted in Tabriz Shahid Maddani Hospital from July 2006-November 2007. The study protocol was approved by the Tabriz University of Medical Sciences (TUMS) ethics committee, which was in compliance with the Declaration of Helsinki. Ninety patients with coronary artery disease who were referred for CABG were enrolled in the study: 45 were enrolled in the on-pump group and 45 were enrolled in the off-pump group.

All patients signed a consent form before being included in the study. Inclusion criteria were: age between 35-55 years; no sign of current/previous neoplasm, including benign and malignant cancers; no history of steroidal or non-steroidal anti-inflammatory drug (NSAID) therapy during the past 2 weeks; no history of respiratory system failure (including asthma, adult respiratory distress syndrome, prolonged mechanical ventilation, acute lung injury); no history of rheumatologic diseases (including gout, lupus and related subtypes dermatomyositis, polymyositis, scleroderma, rheumatoid arthritis, osteoarthritis); no autoimmune neurologic diseases (such as myasthenia gravis, multiple sclerosis); no chronic hemathologic and oncologic diseases (such as hemolytic anemia, idiopathic and thrombotic thrombocytopenic purpura); no insulin dependent diabetes, acute/chronic renal failure, or active infectious diseases during the past month.

Presence of any complication during 24 hours after surgery (including acute tubular necrosis, cardiac arrest), inability to obtain serum samples and prolonged operation time were considered exclusion criteria.

Anesthesia was induced with etomidate (200–300 mg/kg) and fentanyl (20–30 ug/kg). After muscle relaxation with cisatrocurium (0.15 mg/kg) and endotracheal intubation, anesthesia was maintained using fentanyl, midazolam, and isoflurane (0.4–1.5 %).

On-pump patients were managed as follows: CPB equipment included nonpulsatile roller pumps (Stoeckert, Munich, Germany) and membrane oxygenators (Affinity, Avecor Cardiovascular, Plymouth, USA).

The pump was primed with a standard electrolyte solution containing 5000 IU heparin, 1000 mL Ringer's lactate, 500 mL NaCl 0.9 %, and 250 mL of a 15 % mannitol solution (Osmofundin 15 % w, Braun Melsungen, Melsungen, Germany). Heparin (300 IU/kg) was administered immediately before vascular cannulation. After the institution of CPB at a flow rate of 2.4–3 L/m2 per min, the aorta was cross-clamped and a bloody warm cardioplegic solution was injected.

Off-pump patients were managed as follows: once the pericardium was opened, an initial heparin dose of 2 mg/kg was administered. Intravenous heparin then was used to maintain an activated clotting time (ACT) of more than 350 s until the anastomoses were created. An Octopus stabilizer system (Medtronic Inc., Minneapolis, MN) was used during the operation. After incision of the coronary artery, an intracoronary shunt was inserted and an anastomosis was performed.

Following revascularization, the heparin effect was reversed with protamine sulphate (at a ratio of 1.5:1) in all patients. Cefazoline was given for perioperative antibiotic prophylaxis.

Venous blood samples were taken immediately before surgery and 24 hours post-

surgery. Samples were collected in sterile tubes, centrifuged at 3000 rpm for 10 minutes at 4°C, and then stored at -79°C until assayed.

Demographic data included age, sex, weight, height, body mass index (BMI), history of hypertension, diabetes, and hyperlipidemia, history of drug use, smoking, and alcohol, family history, number of coronary arteries involved, graft type, and ejection fraction (EF).

Serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), creatinine (Cr), blood urea nitrogen (BUN) and uric acid (UA) levels were measured using an automated chemical analyzer (Abbott analyzer, Abbott laboratories, Abbott Park, North Chicago.IL) with commercial reagents obtained from Pars Azmoon Laboratories Ltd. (Tehran, Iran). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (8).

Levels of hs-CRP and TNF-α were measured by ELISA using commercial kits (hs-CRP by Monobind Inc., 100 North Point Drive, Lakeforest, California (92630), USA, Lot No: EIA-1K2L7, and TNF-α by Human INF-alfa ELISA, Bender MedSystems, GmbH, Campus Vienna Biocenter, 1030 Vienna, Austria) as directed by the manufacturer. Light absorbance was read at 450 nm.

### *Statistical analyses*

Statistical analyses were performed using the SPSS Statistical Package version 13.0 (SPSS Inc, Chicago, IL). The results are presented as mean ± standard deviation (SD).

The Kolmogorov-Smirnov test was used for checking the normality. Independent sample *t*-test, paired *t*-test and Wilcoxon signed rank tests were used to assess the differences between stages, as appropriate. Comparison of qualitative data was done by chi-square test. The null hypothesis was rejected if the p value was less than 0.05.

### *Results*

The groups were well matched for demographic characteristics and comorbidities (*Table 1*), concomitant medications (*Table 2*) and preoperatively blood tests (*Table 3*).

In both off-pump and on-pump groups, hs-CRP level after CABG surgery increased significantly ($p < 0.001$, $p < 0.001$), but comparison of hs-CRP level increase (Δh-sCRP = hsCRP after CABG-hsCRP before CABG) between off-pump and on-pump

**Table 1** - *Demographics and surgical characteristics.*

| | Type of CABG surgery | | P value |
|---|---|---|---|
| | Off-pump (n = 45) | On-pump (n = 45) | |
| Age (years) | 48.1 ± 5.1 | 49.2 ± 4.2 | 0.3 |
| Ejection fraction (%) | 43.9 ± 9.3 | 45.5 ± 8.3 | 0.4 |
| Smoking | 28 | 27 | 0.5 |
| Uncontrolled hypertension | 11 | 15 | 0.14 |
| Diabetes mellitus | 9 | 9 | 0.9 |
| Hyperlipidemia | 10 | 15 | 0.4 |
| Number of grafts One/Two/Three vessels | 2/15/28 | 0/14/31 | 0.2 |
| Type of graft LIMA/SVG/Both | 10/3/32 | 6/0/39 | 0.13 |
| LIMA: left internal mammary artery; SVG: saphenous vein graft | | | |

groups revealed no significant difference (*Table 4*).

TNFα levels after CABG, in both off-pump and on-pump groups, were not increased significantly ($p = 0.2$, $p = 0.7$), and comparison of TNFα levels increase between off-pump and on-pump groups revealed no significant difference (*Table 4*).

### *Discussion*

In this study, we aimed to compare TNFα and hs-CRP levels in off-pump and on-pump

**Table 2** - *Medication history of the participants according to their surgical groups.*

| *Drug type* | *Type of CABG Surgery* | | *P Value* |
|---|---|---|---|
| | *Off-pump* | *On-pump* | |
| ACE-I/ARBs | 15 | 18 | 0.2 |
| Beta blocker | 36 | 39 | 0.2 |
| Ca channel blocker Dihydropyrodin/ Non-Dihydropyrodin | 6/2 | 9/2 | 0.7 |
| Digoxin | 2 | 4 | 0.7 |
| Anticoagulant ASA/ Enoxaparin | 30/1 | 32/1 | 0.9 |
| Diuretic | 1 | 4 | 0.5 |
| Statins | 38 | 30 | 0.1 |
| Nitrates | 28 | 29 | 0.8 |

**Table 3** - *Comparison of biochemical parameters between On-pump and off-pump.*

| | **Type of CABG surgery** | | |
|---|---|---|---|
| | **Off-pump** | **On-pump** | **P value** |
| Triglyceride (mg/dl) | 196.6 ± 114.0 | 188.8 ± 127.4 | 0.8 |
| Total cholesterol (mg/dl) | 170.9 ± 51.9 | 188.2 ± 63.0 | 0.3 |
| HDL (mg/dl) | 33.2 ± 6.1 | 34.9 ± 8.2 | 0.4 |
| LDL (mg/dl) | 97.6 ± 45.9 | 115.5 ± 53.6 | 0.2 |
| Creatinine (mg/dl) | 1.0 ± 0.3 | 1.0 ± 0.2 | 0.9 |
| Blood urea nitrogen (mg/dl) | 16.1 ± 6.7 | 15.3 ± 4.4 | 0.6 |
| Uric acid level (mg/dl) | 5.6 ± 1.8 | 5.8 ± 1.6 | 0.6 |

HDL: high density lipoprotein; LDL low density lipoprotein

**Table 4** - *Comparison of hsCRP and TNF-α levels between study groups.*

| | **Type of CABG surgery** | | |
|---|---|---|---|
| | **ON-pump** | **Off-pump** | **P value** |
| hsCRP before surgery (mg/dL) | 10.8 ± 14.8 | 10.9 ± 14.9 | 0.9 |
| hsCRP after surgery (mg/dL) | 116.1 ± 93.0 | 136.1 ± 88.8 | 0.3 |
| TNF-α before surgery (pg/mL) | 12.7 ± 11.8 | 12.6 ± 12.1 | 0.8 |
| TNF-α after surgery (pg/mL) | 14.3 ± 11.3 | 14.5 ± 12.5 | 0.9 |
| ΔhsCRP (mg/dl) | 125.3 ± 91.9 | 105.2 ± 95.6 | 0.4 |
| ΔTNF-α (pg/mL) | 1.6 ± 6.6 | 0.9 ± 5.2 | 0.8 |

hsCRP: high sensitivity C-reactive protein; TNF: Tumor necrosis factor

groups before and after CABG. Although hs-CRP and TNFα levels after CABG were increased in both off-pump and on-pump groups, this increase was statistically significant only in hs-CRP level. Comparison of the increase in hs-CRP and TNF-α levels between off-pump and on-pump groups revealed no significant difference.

The incidence of life threatening morbidity and mortality after CPB has decreased significantly in recent years; however, the inflammatory effects still are commonly recognized. The systemic inflammatory response, with an increased release of cytokines and adhesion molecules after conventional CABG with CPB, has been shown to provoke postoperative organ dysfunction (1, 2, 4). Systemic inflammation now is known to result in activation of coagulation, downregulation of physiological anticoagulant mechanisms, and inhibition of fibrinolysis. For instance, IL-6 affects the coagulation cascade at different levels, increasing tissue factor and factor VIII mRNA levels in monocytes and liver cell lines, enhancing platelet production, and increasing the transcription of fibrinogen gene (4). Some of these effects also may be mediated by other inflammatory markers such as C-reactive protein (3).

It is known that CABG elicits a normal inflammatory reaction in the early hours after surgery. Interestingly, some inflammatory markers (IL-1, IL-6, some leukocyte subsets) show similar behavior in both on-pump and off-pump techniques, whereas others (TNF-α, IL-8, IL-10, and elastase) show earliest and highest peak levels in CABG during the time span between the final steps and the very early hours after the surgical procedure. Afterwards, the differences in the inflammatory profile progressively fade and finally cancel out (7).

The strong relationship between postoperative changes in the levels of IL-6, but not of TNFα, with that of C-reactive protein and fibrinogen, similar in both techniques, suggests a link between these variables. Because these variables were unaffected by the use of CPB, it suggests a possible link between inflammatory and haemostatic pathway activation. Indeed, fibrinogen, besides being an acute phase protein and a risk factor for future cardiovascular events, strongly affects hemostasis, blood microcirculation, platelet aggregation, and endothelial function (9). In conclusion, this study shows that there is a protracted postoperative activation of the inflammatory pathways after coronary bypass surgery performed both on- and off-pump. These findings may be of pathophysiological and therapeutic interest in the treatment of patients undergoing coronary bypass surgery, whichever revascularization strategy (on- or off-pump) is chosen.

Off-pump coronary artery bypass is a surgical technique under clinical evaluation for its role and indications in ischemic heart disease. The avoidance of CPB may protect from perioperative systemic activation of inflammation and reduce hemostasis changes. In retrospective studies this has been shown to be associated with a varying degree of improvement of the early morbidity and mortality in the off-pump group when compared to the on-pump group (5, 6).

Consistent advantages of off-pump over on-pump are limited to some inflammatory markers and to the time span between the final steps and the very early hours after the surgical procedure. Some inflammatory markers (activated complement factors, TNFα, IL-8, IL-10 and elastase) increase compared to baseline in both on-pump and off-pump, but the peak levels are highest in on-pump. Afterwards, the difference in the inflammatory profile fades progressively and finally cancels out (10). In the early phase, CPB is the major determinant of the onset of the inflammatory reaction, but in

the late phases, the surgical trauma itself likely plays a predominant role. Evidence about other markers (IL-1, IL-6, some leukocyte subsets) is less consistent, and in some cases, even contradictory (10). Overall, it can be hypothesized that CPB itself only minimally influences the circulating levels of these markers.

The trauma resulting from the surgical procedure likely may be the major determinant of inflammatory reaction.

The activation of the inflammatory system, although less marked than those of CPB cases, during vascular and non-cardiac thoracic surgery further supports this hypothesis (11, 12).

In summary, the analysis of the available data shows that, in terms of biological impact, there are non-significant differences between off-pump and on-pump, and the general surgical trauma may play a role more significant than CPB. We have to recognize that in most of the studies quoted in the present discussion, the CPB was structured and conducted in a standard way, i.e. using mild or moderate systemic hypothermia, cold cardioplegia, uncoated circuits, without the administration of anti-fibrinolytic drugs or of any other inflammation modulating drug and using the cardiotomy suction devices. However, during the last decade, the practice of CPB has undergone major refinements and improvements in the field of biocompatibility, such as the introduction of warm heart surgery, coated circuits, antifibrinolytic drugs and the elimination of cardiotomy suction devices. Warm cardioplegia seems to reduce the oxidative stress (13-18) and inflammatory response (19, 20).

In summary, the analysis of the available data shows that, in terms of biological impact, there are subtle differences between off-pump and on-pump, and the general surgical trauma may play a role more significant than CPB.

**REFERENCES**

1. Howson CP, Fineberg HV, Bloom BR. The pursuit of global health: the relevance of engagement for developed countries. Lancet 1998; 35: 586-590.
2. Yacoub M. Off-pump coronary bypass surgery: in search of an identity. Circulation 2001; 104: 1743-1745.
3. Franke A, Lante W, Fackeldey V, et al. Pro-inflammatory cytokines after different kinds of cardiothoracic surgical procedures: is what we see what we know? Eur J Cardiothorac Surg 2005; 28: 569-575.
4. Levy JH, Tanaka KA. Inflammatory response to cardiopulmonary bypass. Ann Thorac Surg 2003; 75: 715-720.
5. Cleveland JC Jr., Shroyer AL, Chen AY, et al. Off-pump coronary artery bypass grafting decreases risk-adjusted mortality and morbidity. Ann Thorac Surg 2001; 72: 1282-1288.
6. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
7. Sabik JF, Gillinov AM, Blackstone EH, et al. Does off-pump coronary surgery reduce morbidity and mortality? J Thorac Cardiovasc Surg 2002; 124: 698-707.
8. Asimakopoulos G, Taylor KM. Effects of cardiopulmonary bypass on leukocyte and endothelial adhesion molecules. Ann Thorac Surg 1998; 66: 2135-2144.
9. Parolari A, Camera M, Alamanni F, et al. Systemic inflammation after on-pump and off-pump coronary bypass surgery: a one-month follow-up. Ann Thorac Surg 2007; 84: 823-828.
10. Biglioli P, Cannata A, Alamanni F, et al. Biological effects of off-pump vs. on-pump coronary artery surgery: focus on inflammation, hemostasis and oxidative stress. Eur J Cardiothorac Surg 2003; 24: 260-269.
11. Fosse E, Mollnes TE, Ingvaldsen B. Complement activation during major operations with or without cardiopulmonary bypass. J Thorac Cardiovasc Surg 1987; 93: 860-866.
12. Hiesmayr MJ, Spittler A, Lassnigg A, et al. Alterations in the number of circulating leucocytes, phenotype of monocyte and cytokine production in patients undergoing cardiothoracic surgery. Clin Exp Immunol 1999; 115: 315-323.
13. Cermak J, Key NS, Bach RR, et al. C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. Blood 1993; 82: 513-520.
14. Kerr R, Stirling D, Ludlam CA. Interleukin 6 and haemostasis. Br J Haematol 2001; 115: 3-12.
15. Westaby S. Organ dysfunction after cardiopulmo-

nary bypass. A systemic inflammatory reaction initiated by the extracorporeal circuit. Intensive Care Med 1987; 13: 89-95.
16. Biagioli B, Borrelli E, Maccherini M, et al. Reduction of oxidative stress does not affect recovery of myocardial function: warm continuous versus cold intermittent blood cardioplegia. Heart 1997; 77: 465-473.
17. Mezzetti A, Calafiore AM, Lapenna D, et al. Intermittent antegrade warm cardioplegia reduces oxidative stress and improves metabolism of the ischemic-reperfused human myocardium. J Thorac Cardiovasc Surg 1995; 109: 787-795.
18. Morishige N, Tashiro T, Yamada T, Kimura M. Retrograde continuous warm blood cardioplegia reduces oxidative stress during coronary artery bypass grafting. Ann Thorac Cardiovasc Surg 2002; 8: 31-37.
19. Ohata T, Sawa Y, Kadoba K, et al. Normothermia has beneficial effects in cardiopulmonary bypass attenuating inflammatory reactions. ASAIO J 1995; 41: 288-291.
20. Wan S, Yim AP, Arifi AA, et al. Can cardioplegia management influence cytokine responses during clinical cardiopulmonary bypass? Ann Thorac Cardiovasc Surg 1999; 5: 81-85.