

## ORIGINAL ARTICLE

# Apoptosis-Specific Activation Markers in On- versus Off-Pump Coronary Artery Bypass Graft (CABG) Patients

TAMÁS SZERAFIN<sup>1</sup>, AMBRUS HORVATH<sup>1</sup>, BERNHARD MOSER<sup>2,3</sup>,  
STEFAN HACKER<sup>2</sup>, KONRAD HOETZENECKER<sup>2</sup>, BARBARA STEINLECHNER<sup>4</sup>, MARKUS  
BRUNNER<sup>2</sup>, GEORG ROTH<sup>2,3</sup>, GEORGE BOLTZ-NITULESCU<sup>5</sup>,  
ARPAT PETERFFY<sup>1</sup>, ERNST WOLNER<sup>2</sup>, HENDRIK JAN ANKERSMIT<sup>2</sup>

<sup>1</sup>Department of Cardiac Surgery, University of Debrecen, Debrecen, Hungary

<sup>2</sup>Department of Cardiothoracic Surgery, General Hospital Vienna, Medical University Vienna, Austria

<sup>3</sup>Department of Surgery, Columbia University, New York, USA

<sup>4</sup>Department of Anesthesiology, General Hospital Vienna, Medical University Vienna, Austria

<sup>5</sup>Department of Pathophysiology, General Hospital Vienna, Medical University Vienna, Austria

### SUMMARY

**Background:** The relation of epithelial/endothelial apoptosis and secretion of death-inducing receptors (DIR) in comparison to vascular adhesion molecules is not known in patients undergoing the On- versus Off-pump coronary artery bypass graft (CABG) procedure.

**Methods:** 30 patients were prospectively included in the study (On- vs. Off-pump CABG, each n=15). Serum samples were obtained prior to, and 30 minutes, 60 minutes and 24 hours after CABG operation. ELISA was utilized to detect caspase-cleaved cytokeratin-18 (CK18) by means of M30 antibody, soluble VCAM-1, soluble ICAM-1, and soluble DIR TNFR-1 and CD95.

**Results:** Soluble caspase-cleaved CK18 was increased and leveled to initial values at 24 hrs. sICAM-1 showed a significant decrease at 30 minutes and 60 minutes in comparison to preoperative values. sTNFR-1/sCD95 showed a rise that was not significant to preoperative values.

**Conclusion:** These results indicate for the first time that epithelial/endothelial apoptosis is occurring in patients undergoing bypass operation, irrespective of the CABG procedure selected. (Clin. Lab. 2006;52:255-261)

### KEY WORDS

Apoptosis, endothelium, caspase-cleaved cytokeratin-18, coronary artery bypass, cell adhesion molecules

### INTRODUCTION

Cardiopulmonary bypass (CPB), as used in the On-pump CABG procedure, induces a well-described systemic inflammatory response syndrome (SIRS). A number of patients have organ dysfunction, which may delay postoperative recovery and may influence morbidity and mortality (1,2). SIRS may lead to adult respiratory distress syndrome and multi-organ failure by means of the same mechanisms that occur in septic conditions.

The release of a variety of inflammatory mediators has been implicated in the pathogenesis of SIRS during CPB (3,4,5,6). It has been hypothesized that the capillary leak syndrome is induced by the inflammatory reaction described above, but no verified evidence for a speculative culprit mechanism such as epithelial/endothelial cell apoptosis was demonstrated in patients undergoing CABG operation. However, it was shown that leukocyte-endothelial interactions are pivotal steps in mediating inflammatory responses. In addition to the activation-induced transendothelial migration of blood leukocytes, endothelial cell (EC) programmed cell death (apoptosis) may indirectly contribute to an increased efflux of immune effector cells because of the leakiness of the vessel wall (7,8). In order to detect epithelial/endothelial apoptosis in patients undergoing On- vs. Off-pump CABG procedure, we utilized the

**Table 1: Patient Demographics and Laboratory Data**

	<b>CABG (n=15)</b>	<b>OPCAB (n=15)</b>	<b>p-value</b>
Age (years)	59.9±2.2	58.6±2.6	0.712
Sex (%male)	53.3	56.7	0.456
Body mass index	26.8±1.1	27.7±0.86	0.495
Mean CV risk factors	1.667±0.2	1.667±0.2	1
Vessel disease	2.9±0.1	2.7±0.1	0.285
NYHA (class)	3.2±0.1	3.1±0.1	0.481
EF (%)	50.1±3.4	50.3±1.5	0.947
Euroscore	4.3±0.5	3.9±0.6	0.696
Average number of grafts	2.2±0.1	2.3±0.2	0.577
Graft type			
Single	1	2	
Double	10	6	
Triple	4	7	
Aortic clamping time (min)	61.7±3.0		
Coronary occlusion time (min)		98.2±5.5	
OP time (min)	223.9±8.7	218±11	0.686
Creatine kinase after 24 hours (U/L)	761.2±72.8	984.6±302.3	0.480
Creatine kinase-MB after 24 hours (U/L)	46.9±5.2	72.1±23.6	0.307
Creatine kinase-MB % after 24 hours	8.4±2.1	7.42±1.2	0.727
Hemoglobin preoperative (g/dl)	12.6±8	12.8±0.5	0.325
Hemoglobin postoperative (g/dl)	16.9±6.9	16.8±5.9	0.986
Hemoglobin one day after operation (g/dl)	17.3±6.9	10.9±0.3	0.403
Blood loss (ml)	607.3±54	440±66.8	0.071
Transfused units	2.3±0.1	1.7±0.1	0.211
ICU stay (hours)	22.4±1.6	32.22±9.8	0.162
Hospital stay (days)	6.6±0.3	10.3±3	0.223
Fluid balance (ml) at 60 mins	2700±91.7	575±42.2	0.001
Fluid balance (ml) at 24 hours	2187±157.3	1537±137.5	0.003

Values are mean ± SEM (Standard error of the mean) or percent.

CABG = coronary artery bypass grafting, OPCAB = off-pump coronary artery bypass, EF = ejection fraction, ICU = intensive care unit, NYHA = New York Heart Association

detection of cytokeratin-18 (CK18), a type I intermediate filament protein, in serum samples of our study cohorts. Cytokeratin-18 is a major component of single layer and glandular epithelial cells. If apoptosis is taking place in these cells, CK18 is cleaved by the effector caspases 3, 6 and 7. It has been previously shown that the monoclonal antibody M30, directed to a CK18 neo-epitope, recognizes apoptotic epithelial/endothelial cells. This assay makes it possible to detect apoptotic products derived from epithelial/endothelial cells which have undergone programmed cell death triggered by immune activation (9-12). Moreover, M30, an antibody detecting caspase-cleaved CK18, was utilized to demonstrate that aortic endothelium underwent apoptosis in a septic rat model (13). In septic patients, serum levels of M30 were significantly increased (14). In order to adjust our caspase-cleaved CK18 measurements we concomitantly evaluated the secreted adhesion mole-

cules VCAM-1 (CD106) and ICAM-1 (CD154). The vascular cell adhesion molecule (VCAM) is a member of the immunoglobulin supergene family and is expressed by synovial lining, germinal center dendritic cells, bone marrow derived fibroblasts, and macrophages. Unstimulated EC express very little cell surface VCAM-1. During exposure to proinflammatory agents, however, this adhesion molecule is prominently expressed by the latter (15,16). The intracellular adhesion molecule-1 (ICAM-1, CD154) is responsible for cell-cell adhesion interactions with the endothelium. ICAM-1 is weakly expressed on leukocytes and resting endothelial cells as well as some other cell types, but its expression is increased following the exposure of EC to IL-1 $\beta$ , TNF $\alpha$  and lipopolysaccharide (LPS). Interactions between ICAM and integrins, such as leukocyte function-associated-antigen-1 (LFA-1, CD11a/CD18) or macrophage-1 antigen (Mac-1, CD11b/CD18) play an important role

in the margination and extravasation of leukocytes into inflammatory sites (17).

In a further attempt we sought to investigate the presence of death-inducing receptors such as Fas/CD95 and TNFR-1 during the CABG procedure. Fas (CD95), like TNFR-1, is a glycoprotein of the TNF receptor family and after immune activation leads to apoptosis of vascular endothelium and is deemed an integral part of the systemic inflammatory response syndrome (18,19). In conclusion we investigated three issues in this non-randomized and non-blinded prospective cohort study including On- vs. Off-pump CABG patients with no a priori hypothesis:

- (1) to detect soluble cytokeratin-18, indicating specific epithelial/endothelial apoptosis;
- (2) to observe whether shedding of soluble VCAM and ICAM is correlated with cytokeratin-18 and
- (3) to investigate whether death inducing receptors sCD95 and sTNFR-1, as markers with potential of inducing epithelial/endothelial apoptosis, become detectable after initiation of the CABG procedure.

## MATERIALS AND METHODS

The study protocol was approved by the "Ethics commission of the Medical University of Vienna and the General Hospital of Vienna". All study and control subjects or their legal designees signed a written informed consent. 30 consecutive patients with multivessel coronary artery disease undergoing the CABG procedure were studied. Patients were selected for Off-pump CABG only when complete revascularization was technically feasible. There were no differences between the two groups of patients with respect to age, sex, symptoms, or functional class (Table 1).

All patients received a similar balanced anesthetic regimen as described below:

Premedication (morph. sulph.: 0.1 mg/kg im., midazolam: 0.05-0.1 mg/kg, atropin sulph.: 0.005 mg/kg); anesthesia (midazolam: 0.1-0.15 mg/kg, fentanyl 0.005 mg/kg);

muscle relaxant (pipecuronium bromide 0.08 mg/kg or atracurium: 0.5 mg/kg); Maintenance of anesthesia (continuous iv. propofol infusion 0.07-0.14 mg/kg/min + inhalation of isoflurane narcotic gas 0.2-1.5 vol% + repetitive administration of fentanyl bolus 0.0025mg/kg for pain relief).

In order to attain constant muscle relaxation pipecuronium bromide (0.02 mg/kg) or continuous iv. infusion of atracurium (0.5 mg/kg/hr) were administered.

Heparinization: On-pump group – 3 mg/kg Na/heparin (300 IE/kg), ACT – was determined to be above 600 sec, mild hypothermia (32-34 °C), minute volume 2.5 l/min, Composition of priming solution: 1200-1750 ml crystalloid solution + 1000 IE Na-heparin + 100 ml mannite (20% solution) + 150 ml Na-bicarbonate solution (4.2%), neutralization with protamine sulph.

Heparinization: Off-pump: half of the On-pump dosis = 1.5 mg/kg (150 IE/kg) was administered. ACT was 300 sec during operation. At the end of the operation a ACT was always performed and was below 200 sec. – no protamine was administered at the end of the operation.

### Exclusion criteria

Criteria such as infections, redo or emergency operation, malignancies, verified immunological disorders, acute myocardial infarction less than 2 weeks ago and medication with immune-modulating agents such as steroids or antiphlogistics were causes for exclusion from the study.

### Cardiopulmonary Bypass Technique for On-Pump CABG

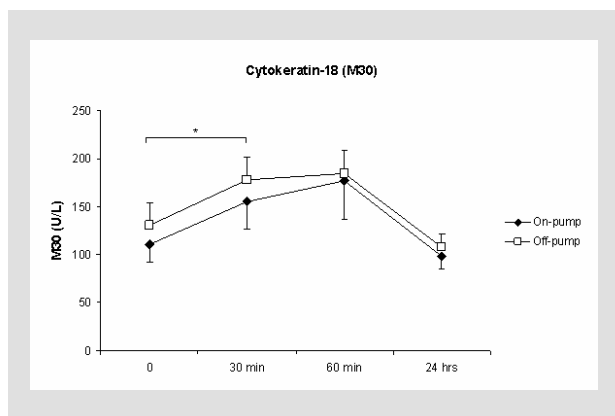
The extra-corporal circuit consisted of a roller pump (Pemco Inc., Cleveland, OH, USA; Stöckert, Munich, Germany) and a membrane oxygenator (Dideco D-703). Standard systemic heparinization with target levels of activated clotting time of greater than 480 seconds was maintained during CPB. The pump flow was set at 2.4 l/min/m<sup>2</sup>. Patients were cooled to 32°C during CPB, and they received intermittent antegrade normothermic crystalloid cardioplegia, which was a mixture of graduated doses of potassium-magnesium solution. At the end of CPB the heparin effect was neutralized with equivalent doses of protamine.

### Off-Pump Technique

Traction sutures were applied to the pericardial edges, displacing the heart anteriorly. For exposure of the left anterior descending coronary artery or its diagonal branches, additional pericardial traction sutures were inserted anteriorly to the left phrenic nerve. To rotate the heart a moist sponge was placed behind its latero-dorsal aspect, bringing the coronary artery into the operative field. For exposure of obtuse marginal or right coronary branches, two wet cotton tapes were passed through the transverse sinus with their right ends secured to the surgical drapes. The two loose lengths of tape were then used to lift and rotate the heart toward the surgeon, as well as to stabilize the coronary artery. Patients were heparinized and two thin elastic bands made of rubber were used to temporarily occlude the coronary artery on either side of the anastomosis site. For the exposure and stabilization of the target vessel stabilizers were utilized. (Medtronic, Minneapolis, MN, USA; Genzyme Cooperation, Cambridge, MA, USA).

### Blood samples

In the CPB group, samples of venous blood were obtained from each patient before the operation, at beginning and at the end of CPB, and 24 hours after the procedure. In the Off-pump group blood samples were drawn before the operation, after reperfusion of all grafts, at the end of the operation and 24 hours after the surgical procedure. Serum samples were obtained via



**Figure 1:** Serum concentration of caspase-cleaved CK18 M30 in patients before and 30 minutes, 60 minutes and 24 hours after off-pump coronary artery bypass (OPCAB) and conventional coronary artery bypass grafting with cardiopulmonary bypass (CPB). Data are expressed as mean ± SEM \* =  $p < 0.05$ .

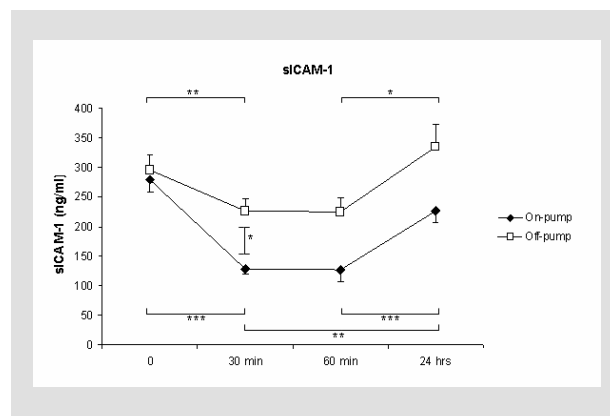
centrifugation of the blood tubes and were kept frozen until the specific tests were performed.

**Measurement of sTNFR-1, sICAM-1, sVCAM-1, sFAS**  
Commercial ELISA kits were used to measure the serum levels of adhesion molecules and soluble receptors. Standards were prepared and the appropriate volume of sample or standard was added to a 96-well microtiter plate, precoated with the monoclonal antibody for the appropriate marker. All samples were run in duplicate. Each well was then aspirated and the plates were washed with the specific washing solution provided in the kit. An enzyme-linked polyclonal antibody against the marker was added. Substrate and stop solution were added to each well, and the optical density was read at the appropriate wavelength for each assay. The amount of protein in each sample was calculated according to a standard curve of optical density values constructed for known levels of protein.

The sensitivity was 3 pg/ml for sTNFR-1 (R&D Systems Inc, Minneapolis, MN, USA), 20 pg/ml for sFAS (Becton Dickinson, Franklin Lakes, NJ, USA), 3.4 ng/ml for ICAM-1 (Roche Molecular Biochemicals, Mannheim, Germany) and 3 ng/ml for VCAM-1 (Roche Molecular Biochemicals, Mannheim, Germany).

#### Quantification of serum caspase-cleaved CK18 M30 neoepitope levels

To quantify the serum levels of caspase-cleaved CK18, a commercially available ELISA was used (Alexis Biochemicals, Lausen, Switzerland). The assay uses an antibody recognizing an epitope on the 238-396 fragment of CK18 as catcher and horseradish peroxidase-conjugated anti-caspase-cleaved CK18 M30 as detector. Serum levels of M30 are expressed as U/l. One Unit (U) is equivalent to 1.24 pmol of a synthesized peptide



**Figure 2:** Serum concentration of sICAM-1 in patients before and 30 minutes, 60 minutes and 24 hours after off-pump coronary artery bypass (OPCAB) and conventional coronary artery bypass grafting with cardiopulmonary bypass (CPB). Data are expressed as mean ± SEM \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

containing the M30 recognition motif according to the manufacturer. The sensitivity of this ELISA was stated to be 30 U/l. The intra- and interassay coefficients of variation of the ELISA were 0.7-5.8% and 2.8-4.8%, respectively.

#### Statistical analysis

Statistical analysis was performed using SPSS software. The data are given as mean ± standard error of the mean (SEM). Two-sided Student's t-test for paired and unpaired comparisons was used to calculate significance. Dichotomous variables were analyzed using the chi-square test. P-values of 0.05 or lower were considered statistically significant. The Bonferroni-Holm correction was applied to correct for multiple comparisons.

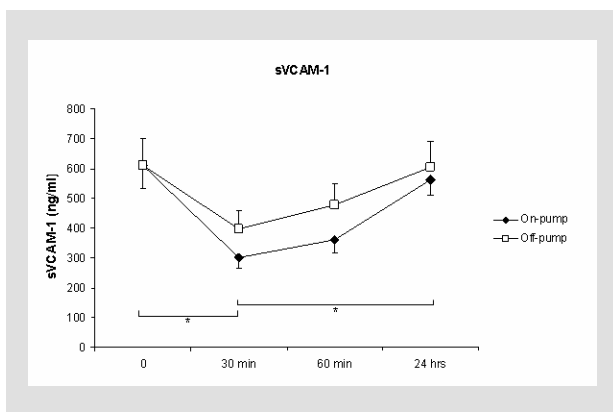
## RESULTS

#### Cytokeratin-18

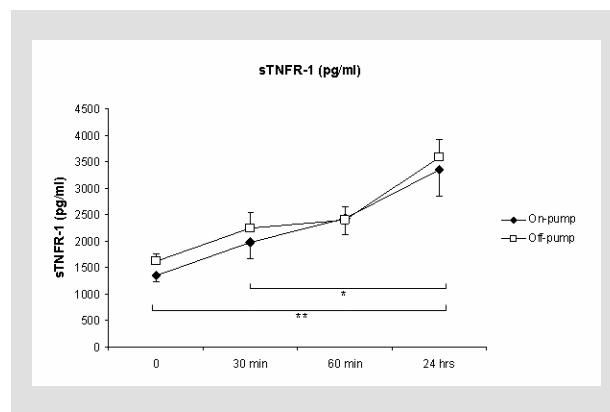
Figure 1 demonstrates a rise in the concentration of caspase-cleaved cytokeratin-18 M30 neoepitope in the sera of patients undergoing Off- and On-pump CABG, indicating increased epithelial/endothelial apoptosis. The mean serum levels (U/L) of M30 in the On- and Off-pump groups were 110.4 ± 18.9 and 130.8 ± 23.2 before the operation, 155.5 ± 28.9 and 176.7 ± 39.1 after 30 minutes, 178.0 ± 23.6 and 184.9 ± 24.6 after 60 minutes, and 98.6 ± 13.0 and 108.4 ± 12.8 after 24 hours. The difference between 0 and 30 minutes was statistically significant in the Off-pump cohort ( $P = 0.0176$ ).

#### sICAM-1

Figure 2 shows the course of sICAM-1 serum levels (ng/ml) during operation. There was no significant dif-



**Figure 3:** Serum concentration of sVCAM-1 in patients before and 30 minutes, 60 minutes and 24 hours after off-pump coronary artery bypass (OPCAB) and conventional coronary artery bypass grafting with cardiopulmonary bypass (CPB). Data are expressed as mean ± SEM \* = p<0.05.



**Figure 4:** Serum concentration of sTNFR-1 in patients before and 30 minutes, 60 minutes and 24 hours after off-pump coronary artery bypass (OPCAB) and conventional coronary artery bypass grafting with cardiopulmonary bypass (CPB). Data are expressed as mean ± SEM \* = p<0.05, \*\*=p<0.01.

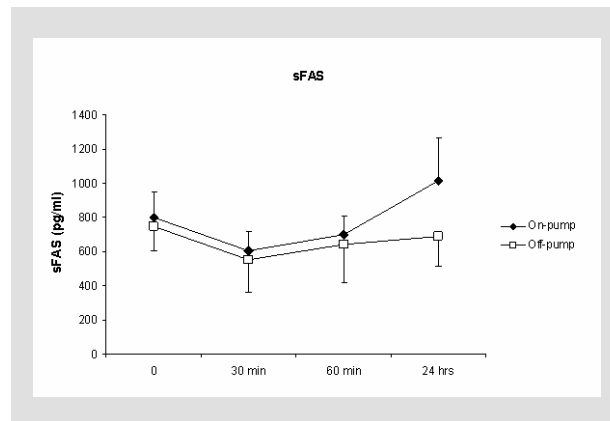
ference between the preoperative values in the two study cohorts (On- vs. Off-pump CABG; 279.8±21.1, 295.6±25.7, respectively). After 30 minutes the levels decreased significantly to 128.5±9.9 in the On-pump and to 226.9±20.5 in the Off-pump group (P 0.008; 0.014, resp.). The intergroup comparison at 30 min was also significantly different (P 0.013). At 60 minutes the levels were 126.98±20.55 and 225.3±23.7. One day after the intervention the ICAM serum levels reached preoperative values (226.9±19.5 and 334.7±37.7). The overall rise from 30 minutes to 24 hours was significant in the On-pump group (P 0.039), the rise from 60 minutes to 24 hours in both groups (On-pump: P <0.001; Off-pump P 0.0166).

**sVCAM-1**

In patients subjected to On- vs. Off-pump CABG surgery, the soluble VCAM-1 mean concentrations (ng/ml) obtained before cardiopulmonary bypass were 611.6±79.7 and 610.9±91.2, respectively (Figure 3); These levels decreased to 301.3±36.9 and 397.0±62.0 after 30 minutes. Whereas this decrease was significant in the On-pump group (P 0.019), the decrease was not significant in the Off-pump group (P 0.189). Levels rose to 361.2±46.3; 477.9±73.2 after 60 minutes and to 563.6±53.7; 603.6±88.2 after 24 hours. The increase of VCAM from timepoint 30 minutes to 24 hours was significant in the On-pump CABG cohort (P 0.0164).

**sTNFR-1**

In On-pump CABG patients, a significantly higher sTNFR-1 (pg/ml) level was detected at 24 hours post surgery than before surgery (P 0.032) (Figure 4). The mean levels (pg/ml) at 0, 30 minutes, 60 minutes and 24



**Figure 5:** Serum concentration of sFAs in patients before and 30 minutes, 60 minutes and 24 hours after off-pump coronary artery bypass (OPCAB) and conventional coronary artery bypass grafting with cardiopulmonary bypass (CPB). Data are expressed as mean ± SEM.

hours were 1353.70±120.77, 1973.04±297.84, 2428.7±296.8 and 3355.2±496.7 in the On-pump group and 1628.0±139.3, 2254.77±287.7, 2398.21±264.51 and 3583.83±341.33 in the Off-pump group. The rise from 30 minutes to 24 hours was significant in the On-pump cohort (P 0.0335).

**sFAS**

Figure 5 shows the serum levels of sFAS. The mean levels (pg/ml) at 0, 30 minutes, 60 minutes and 24 hours were 801.43±143.82, 605.77±111.01, 697.77±111.77 and 1014.65±249.97 in the On-pump group and 746.22±142.4, 554.18±191.49, 642.03±222.62 and 690.9±175.87 in the Off-pump group.

## DISCUSSION

In this study we examined the presence of caspase-cleaved CK18, a marker of epithelial/endothelial cell apoptosis in serum samples of patients undergoing either the On- or Off-pump CABG procedure. We confirm previous data, showing that ICAM and VCAM are decreasing in the On-pump vs. Off-pump group, speculating that priming solution and additional blood products might be responsible for the decrease of ICAM-1 and VCAM-1 in CABG patients undergoing cardiopulmonary support. Moreover, we were able to demonstrate that shedding of TNFR-1 and sCD95 occurred in both study cohorts within 24 hours.

### Cytokeratin-18

We found that Off- and On-pump CABG patients showed an increase in the serum levels of the caspase-cleaved CK18 M30 neoepitope. The capacity of the M30 antibody to distinguish apoptotic cells has been verified in several disease entities, and the M30 ELISA is suggested as a high-throughput assay for the screening of pro-apoptotic drugs (20,21).

To the best of our knowledge we evidence for the first time increased levels of epithelial/endothelial cell-derived caspase-cleaved CK18, as detected by the M30 neoepitope ELISA, in On- vs. Off-pump CABG patients, indicating induction of epithelial/endothelial apoptosis in both cohorts. Interestingly, after 24 hours, both groups presented serum concentrations close to preoperative values.

### VCAM-1 and ICAM-1

Adherence of circulating neutrophils to arterial endothelium is mediated by VCAM-1 and ICAM-1 and is present on the surface of endothelial cells. The adhesion molecules appear to cooperate in attracting leukocytes to the reperfused coronary endothelium and in promoting adherence, transendothelial migration, and activation of the leukocytes (22). ICAM-1 belongs to the most abundant family of cell surface molecules, the immunoglobulin superfamily. Elevated serum levels of biologically active forms of ICAM-1 are found in patients with various inflammatory syndromes such as septic shock, leukocyte adhesion deficiency, cancer and transplantation (23). Soluble ICAM-1 serum levels increase not only following the acute exposure of endothelial cells to pro-inflammatory cytokines but also in many chronic disorders of the cardiovascular system. Furthermore, the adhesion molecule VCAM-1 plays a major role in the initial binding of T-lymphocytes to cytokine-activated endothelium (24).

Moreover, it is known to participate in the extravasation of lymphocytes already bound to the EC during interaction with VLA-4 on the surface of these cells. Our data show that both CABG procedures are associated with a significant decrease of VCAM (difference between groups,  $p < 0.001$  at 30 min and 60 min; not significant at 24 hours) and of ICAM (decrease, but not significant)

with respect to preoperative values despite presumed myocardial ischemia/reperfusion syndrome. We speculate that these findings are related to the dilution effect of priming solution and blood product administration (Table 1).

### sFAS and sTNFR-1

Activation of a systemic inflammatory response is an acknowledged consequence of CPB. The components of the response include activation of peripheral and resident white blood cells and the complement cascade and systemic elevations in both proinflammatory and anti-inflammatory cytokines (25,26,27). Much effort has been placed on the effect of CPB on the systemic concentrations of the proinflammatory cytokine TNF (28, 29,30). Many of the postoperative complications of CPB resemble those seen in septic shock, for which lipopolysaccharide-induced elevations of TNF and its soluble receptors have been implicated. The release of sTNFR-1 and sFas after Off- vs. On-pump was not different. In addition, there was a trend for higher peak sFas levels within the 24-hr period as compared to the preoperative values.

In conclusion, the results of this prospective study, which included On- vs. Off-pump CABG patient cohorts, led to the observation that the soluble death-inducing receptors sCD95 and sTNFR-1 are shed in the same mode, irrespective of the CABG procedure chosen. Moreover, we can conclude that the surgical access markedly contributes to the release of products derived from epithelial/endothelial apoptosis within the operation duration. However, further markers for epithelial/endothelial apoptosis exist and the clinical value of our observations has to be determined in future studies. With respect to serum ICAM-1 and VCAM-1 and its observed decrease, we speculate that increased fluid filtration during CPB, as a consequence of hyponcotic hemodilution by crystalloidal priming of the CPB circuit and the use of crystalloid cardioplegic solution might be responsible for our observations (31). Of particular interest was the finding that caspase-cleaved CK18 serum levels increased despite hemodilution in patients undergoing the CABG procedure. However, further studies are warranted to clarify the importance of this observation with respect to post-surgery inflammation, arrhythmias and systemic inflammatory reactions in heart surgery.

### Acknowledgements

HJ Ankersmit designed the study. Prof. Tamás Szerafin and Ambrus Horvath performed the surgery and were responsible for clinical management. Prof. Ernst Wolner, Prof. Arpat Peterffy and Prof. George Boltz-Nitulescu were responsible for infrastructure support. Konrad Hoetzenecker, Bernhard Moser, Barbara Steinlechner, Markus Brunner, Georg Roth and Stefan Hacker were responsible for laboratory work.

References

1. Westaby S. Organ dysfunction after cardiopulmonary bypass: a systemic inflammatory reaction initiated by the extracorporeal circuit. *Intensive Care Med* 1987; 13: 89-95.
2. Richter JA, Meisner H, Tassani P, Barankay A, Dietrich W, Braun SL. Drew-Anderson technique attenuates systemic inflammatory response syndrome and improves respiratory function after coronary artery bypass grafting. *Ann Thorac Surg* 2000; 69: 77-83.
3. Levy JH, Kelly AB. Inflammation and cardiopulmonary bypass. *Can J Anaesth* 1993; 40: 1009-1015.
4. McBride WT, Armstrong MA, Crockard AD, McMurray TJ, Rea JM. Cytokine balance and immunosuppressive changes at cardiac surgery: contrasting response between patients and isolated CPB circuits. *Br J Anaesth* 1995; 75: 724-733.
5. Seghaye MC, Grabitz RG, Duchateau J, et al. Inflammatory reaction and capillary leak syndrome related to cardiopulmonary bypass in neonates undergoing cardiac operations. *J Thorac Cardiovasc Surg* 1996; 112: 687-697.
6. Szerafin T, Brunner M, Horvath A, et al. Soluble ST2 protein in cardiac surgery: a possible negative feedback loop to prevent uncontrolled inflammatory reactions. *Clin Lab* 2005; 51(11-12): 657-63.
7. Kalawski R, Bugajski P, Smielecki JJ, et al. Soluble adhesion molecules in reperfusion during coronary bypass grafting. 1998; 14: 290-295.
8. Assaly R, Olson D, Hammersley J, et al. Initial evidence of endothelial cell apoptosis as a mechanism of systemic capillary leak syndrome. *Chest* 2001 120: 1301-1308.
9. Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *J Cell Biol* 1997; 138: 1379-1394.
10. Leers MP, Kolgen W, Bjorklund V, et al. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. *J Pathol* 1999; 187: 567-572.
11. van Engeland M, Kuijpers HJ, Ramaekers FC, Reutelingsperger CP, Schutte B. Plasma membrane alterations and cytoskeletal changes in apoptosis. *Exp Cell Res* 1997; 235: 421-430.
12. Liao J, Ku NO, Omary MB. Stress, apoptosis, and mitosis induce phosphorylation of human keratin 8 at Ser-73 in tissues and cultured cells. *J Biol Chem* 1997; 272: 17565-17573.
13. Zhou M, Simms HH, Wang P. Adrenomedullin and adrenomedullin binding protein-1 attenuate vascular endothelial cell apoptosis in sepsis. *Ann Surg* 2004; 240(2): 321-30.
14. Roth GA, Krenn C, Brunner M, et al. Elevated serum levels of epithelial cell apoptosis-specific cytokeratin 18 neoepitope m30 in critically ill patients. *Shock* 2004 Sep;22(3):218-20.
15. Koch AE, Burrows JC, Haines GK, Carlos TM, Harlan JM, Leibovich SJ. Immunolocalization of endothelial and leukocyte adhesion molecules in human rheumatoid and osteoarthritic synovial tissue. *Lab Invest* 1991; 64: 313-323.
16. Shimizu Y, Newman W, Tanaka Y, Shaw S. Lymphocyte interactions with endothelial cells. *Immunol Today* 1992, 13: 106-112.
17. Koopman G, Parmentier HK, Schuurman HJ, Newman W, Meijer CJ, Palas ST. Adhesion of human B cells to follicular dendritic cells involves both the lymphocyte function-associated antigen-1/intercellular adhesion molecule and very late antigen 4/vascular cell adhesion molecule-1 pathways. *J Exp Med* 1991; 173: 1287-1304.
18. Lowin B, Hahne M, Mattmann C, Tschopp J. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. *Nature* 1994; 370(6491): 650-652.
19. Pimentel-Muinos FX, Seed B. Regulated commitment of TNF receptor signaling: a molecular switch for death or activation. *Immunity* 1999; 11: 783-793.
20. Jordan JA, Butchko AR. Apoptotic activity in villous trophoblast cells during B19 infection correlates with clinical outcome: assessment by the caspase-related M30 Cytodeath antibody. *Placenta* 2002; 23: 547-553.
21. Hagg M, Biven K, Ueno T, et al. A novel high-through-put assay for screening of proapoptotic drugs. *Invest New Drugs* 2002; 20: 253-259.
22. Carlos TM, Schwartz BR, Kovach NL, et al. Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood* 1990; 76: 965-970.
23. Adderson DC, Abbassi O, Kishimoto TK, Koenig JM, McIntire LV, Smith CW. Diminished lectin – epidermal growth factor – complement binding domain-cell adhesion molecule-1 on neonatal neutrophils underlies their impaired CD18-independent adhesion to endothelial cells in vitro. *J Immunol* 1991; 146: 3372-3379.
24. Gearing AJ, Newman W. Circulating adhesion molecules in disease. *Immunol Today* 1993; 14: 506-512.
25. Kawamura T, Inada K, Nara N, Wakusawa R, Endo S. Influence of methylprednisolone on cytokine balance during cardiac surgery. *Crit Care Med* 1999; 27: 545-548.
26. Bronicki RA, Backer CL, Baden HP, Mavroudis C, Crawford SE, Green TP. Dexamethasone reduces the inflammatory response to cardiopulmonary bypass in children. *Ann Thorac Surg* 2000; 69: 1490-1495.
27. Joashi U, Tibby SM, Turner BSc, et al. Soluble Fas may be a proinflammatory marker after cardiopulmonary bypass in children. *J Thorac Cardiovasc Surg* 2002; 123: 137-144.
28. Casey LC. Role of cytokine in the pathogenesis of cardiopulmonary-induced multisystem organ failure. *Ann Thorac Surg* 1993; 56: 592-596.
29. Marti F, Munoz J, Peiro M, et al. Higher cytotoxic activity and increased levels of IL-1b, IL-6, and TNF-a in patients undergoing cardiopulmonary bypass. *Am J Hematol* 1995; 49: 237-239.
30. Marano CW, Garulacan LA, Laughlione KV, et al. Plasma concentrations of soluble tumor necrosis factor receptor I and tumor necrosis factor during cardiopulmonary bypass. *Ann Thorac Surg* 2000; 70: 1313-1318.
31. Tassini P, Schad H, Winkler C, et al. Capillary leak syndrome after cardiopulmonary bypass in elective, uncomplicated coronary artery bypass grafting operations: Does it exist?. *J Thorac Cardiovasc Surg* 2002; 123: 735-741.

Correspondence: Dr. Hendrik Jan Ankersmit  
 Department of Cardiothoracic Surgery  
 General Hospital Vienna  
 Waehringer Guertel 18-20  
 1090 Vienna  
 Austria  
 Tel.: +43-1-40400-2395 or 6966  
 Fax: +43-1-40400-6948  
 E-mail: [hjankersmit@hotmail.com](mailto:hjankersmit@hotmail.com)