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Oxidative stress and platelet activation during on-pump and off-pump coronary artery bypass grafting in patients with double grafted vessels

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ABSTRACT

This study aimed to evaluate the oxidative stress status and two markers of platelet activation and reactivity in off-pump versus on-pump coronary artery bypass surgery. Study groups of 65 patients with double coronary artery bypass grafting were divided into cardiopulmonary bypass (CPB) and off-pump coronary artery bypass groups. In serial blood samples, lipid hydroperoxides (LOOH), serum paraoxonase (PON1), advanced oxidation protein products (AOPP), total sulfhydryl groups (tSHG) and red blood cell distribution width (RDW) to platelet (Plt) ratio (RPR) and mean platelet volume (MPV) to platelet (Plt) ratio (MPR) index were determined to compare the extent of oxidative stress and platelet activation. The MPR and RPR rose significantly in the post-operative period ($P < 0.001$) in both groups. The increase was higher in the CPB group, but this difference reached borderline significance at 48 h post-operatively. The AOPP/tSHG index increased 6 h after surgery, preceded by a significant fall of the PON1/LOOH ratio, more evident in the CPB group. Multiple linear regression analysis showed explicit connection between these markers and surgery-related clinical conditions. Receiver operating characteristic analysis enabled estimation of the clinical accuracy of oxidative plus platelet-related indices in prediction of surgery caused complications (area under the curve for the model consisted of oxidative stress parameters and platelet activation indices was above 0.9, $P < 0.001$). Results showed higher oxidative stress and undesirable platelet activation in the CPB group. Oxidative status markers and platelet activity indices showed good clinical accuracy to predict the development of possible surgical complications.

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Oxidative stress; platelet activation; off-pump coronary artery bypass; cardiopulmonary bypass; beating heart

Abbreviations

ACC: aortic cross-clamp;
ACE: angiotensin-converting enzyme inhibitor;
ACT: activated clotting time;
AOPP: advanced oxidation protein products;
AUC: area under the curve;
BMI: body mass index;
CI: confidence interval;
COPD: chronic obstructive pulmonary disease;
CPB: cardiopulmonary bypass;
DTNB: dinitrodithiobenzoic acid;
EuroSCORE: European system for cardiac operative risk evaluation;
HDL: high-density lipoprotein;
ICU: intensive care unit;
LOOH: lipid hydroperoxides;
LVEF: left ventricular ejection fraction;
MDA: malondialdehyde;
MLR: multiple linear regression;
MPR: MPV to platelet ratio;
MPV: mean platelet volume;
OAD: oral antidiabetic drugs;
OPCAB: off-pump coronary artery bypass;
Plt: platelets;
PON: paraoxonase;
RDW: red blood cell distribution width;
ROC: receiver operating characteristic;
rpm: rotation or revolution per minute
RPR: RDW to platelet ratio;
SD: standard deviation;
SE: standard error
SHG: sulfhydryl group;
SIRS: systemic inflammatory response syndrome;
tSHG: total sulfhydryl groups;

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Introduction

Myocardial ischemia and subsequent reperfusion during cardiac surgery are powerful sources of reactive oxygen species, i.e. free radicals which could cause damage *in situ*, at the place of generation, as well as distant organ damage.[1] The use of machines for cardiopulmonary bypass (CPB) during coronary artery bypass surgery has many advantages, which are primarily related to safe and comfortable work for the surgeon on the arrested heart.

In addition to numerous advantages for the CPB machine, it also has its specific drawbacks. The main disadvantages are related to the flow and contact of the circulating blood through the silicone tube systems, non-pulsatile flow, as well as the passage and trauma of blood cells through a system of rollers.[2,3]

The oxidative stress and inflammatory response to CPB are initiated by contact between heparinized blood and non-endothelial cell surfaces. Surgery itself, as an accidental trauma, is a trigger of oxidative stress and acute inflammatory response. The continuous exposure of heparinized blood to a non-endothelial cell surface followed by blood reinfusion and recirculation greatly induces the inflammatory response and oxidative stress in operations employing CPB. This reaction initiates production, release and reperfusion of vasoactive and cytotoxic substances.[4] It could affect many organs and tissues. However, in most cases, the biological mechanisms of autoregulation and antioxidative protection prevent the CPB-related harmful effects and the patients' general condition remains stable.

The idea of avoiding the potentially damaging effects of CPB led to improvement of the techniques of myocardial revascularization on the beating heart without using CPB. This technological advancement is related to stabilization of the heart during suturing of the graft anastomosis to the coronary artery, as well as the application of a protective intraluminal shunt in obtaining better protection of the heart.[5]

Myocardial revascularization without either CPB or cardioplegia reduces the acute inflammatory response, but does not prevent it.[6,7] The response to surgical trauma, manipulation of the heart, pericardial suction, heparin, protamine, other drugs and anaesthesia activate the extrinsic clotting system and leads to an increase in the oxidative-stress markers, reactive oxidants [8] and acute inflammation. The inflammatory response is significantly weaker compared to that when CPB is used.[7,9] We have investigated the oxidative-stress enzyme status and its relationship with cardiovascular disease over the past 10 years.[10–12]

Oxidative stress, inflammation and thrombosis are mutually involved in the development of atherosclerosis, especially in the later phases of disease. Chronic, systemic, low-level inflammation and neurohumoral activation could cause an increase in the heterogeneity of erythrocytes in circulation, and could also influence the platelet reactivity.[13] Decreased platelet count, connected with increased mean platelet volume, could be predictive of the changes in platelet reactivity and aggregability.[14] Red cell distribution width (RDW), per se, is related to adverse outcomes in acute coronary syndrome. However, the RDW to platelet count ratio (RPR, RDW/Plt) as an even more sensitive marker of this atherosclerosis connected risk,[15] along with the mean platelet volume to platelet count ratio (MPR, MPV/Plt), has not been examined with regard to CPB and off-pump coronary artery bypass (OPCAB) surgical procedures. These two indices of platelet and RBC activation in an atherosclerotic setting could be increased as a consequence of long-term oxidative stress and inflammation.

This study was designed to investigate the effect of OPCAB operations on the oxidative stress and the platelet activation that are commonly observed in patients undergoing operation under CPB. The aim of this study was to assess the oxidative-stress status and two new markers of platelet activation and reactivity in two different heart surgery modalities (CPB and OPCAB). This is particularly relevant because larger disturbance in the reductive balance during and after the cardiac surgery leads to poorer outcomes and increased morbidity and mortality in operated patients.

Subjects and methods

Study design and patients

This study was designed as a prospective cohort study at the Department for Cardiac Surgery, Medical Military Academy, Belgrade, Serbia. This study was done in collaboration with the Department for Medical Biochemistry, Faculty of Pharmacy, University of Belgrade.

The whole study was planned according to the ethical standards following the Declaration of Helsinki, as revised in 1983. The study protocol was approved by the Hospital Ethics Committee and the Ethics Committee of Medical Faculty, University of Belgrade, and all patients involved in the study gave their signed informed consent.

This study included 65 patients scheduled for an elective operation with double coronary artery bypass grafting (CABG) between March 2012 and November 2015. The patients were divided into two homogeneous groups. In the CPB group ($N = 33$), the patients underwent double CABG using CPB on a potassium-arrested

heart, whereas in the OPCAB group ($N = 32$), the patients underwent double CABG on a beating heart without using CPB.

Exclusion criteria were: redo or emergency surgery, concomitant valvular heart disease, ventricular aneurysms, myocardial infarction within the past three months or perioperative myocardial infarction, cerebral insult within the past three months, usage of systems for intraoperative blood salvage (Cell Saver machine and tubing system), systemic inflammatory or malignant disease, usage of immunosuppressive drugs, massive post-operative mediastinal bleeding, heart failure and presence of an infection. Patients who used drugs with possible antioxidant activity were also excluded from the study. None of the patients was taking vitamins or dietary supplements with established antioxidant properties before the surgery.

EuroSCORE [16] and Body Mass Index (BMI),[17] preoperatively, were calculated for all patients.

Anaesthesia

The anaesthetic technique was standardized for all the patients and consisted of balanced anaesthesia. Anaesthesia was induced by etomidate (0.1−0.2 mg/kg) and sufentanyl (0.5−1 μg/kg). The patient was endotracheally intubated after application of rocuronium (1 mg/kg). Central venous cannulation (right internal jugular vein) was carried out after induction of general anaesthesia. General anaesthesia was maintained with sevoflurane (0.6−1.0%) and sufentanyl (0.5−1 μg/kg/h). Balanced crystalloid solutions 1 mL/kg/h were used to manage the fluid balance.

Surgical procedure

In both groups, midline sternotomy and harvesting of the left internal mammary artery as a pedicle and saphenous vein grafts were followed by full exposure of the coronary artery branches to be revascularized.

CPB group. Perfusion technique and myocardial protection

After harvesting the bypass conduits, heparin was administrated at a dose of 400 IU/kg to achieve a target activated clotting time (ACT) of >450 s or more (ACT Plus™-Medtronic, Inc., Minneapolis, MN, USA). At the end of CABG, heparin was neutralized by protamine-sulphate until the ACT was less than 160 s.

CPB operation was instituted using ascending aortic cannulation and two-stage venous cannulation in the right atrium. The CPB circuit was composed of a roller pump (Stockert-S5, Sorin Group, Munich, Germany), a low-prime hollow fibre polypropylene oxygenator with an incorporated cardiotomy reservoir (Sorin Inspire 8, Sorin Group, Mirandola, Italy) and plasticized polyvinyl chloride perfusion tubing systems (Tubing Set, Heart-Lung Bypass, Sorin Group, Mirandola, Italy).

The priming solution was the same for all patients: 1000 mL of Hartmann's solution, 150 mL of mannitol, 60 mL of 8.4% sodium bicarbonate and 5.000 UI of heparin.

During CPB, the non-pulsatile pump flow was kept at 2.2−2.4 L/min/m^2 and perfusion pressure between 50 and 80 mmHg. CPB was managed according to the alpha-stat principle and moderate hypothermia. The nasopharyngeal temperature was kept at 32 °C. Before weaning from CPB, all patients were rewarmed (nasopharyngeal temperature 36—37 °C).

Myocardial protection was achieved with 4 °C cold potassium cardioplegia (40 mmol/L of K^+, 77 mmol/L of Na^+, 15 mmol/L of Mg^{2+}, 2 mmol/L of Ca^{2+}, 151 mmol/L of Cl, 277 mmol/L of Glu and an appropriate Na-bicarbonate concentration in order to adjust the pH of the solution at a value between 7.6 and 7.8 units). All distal anastomoses of the bypass grafts were performed on the arrested heart after aortic cross-clamping and the infusion of potassium-enriched cardioplegic solution into the coronary artery.

OPCAB group. Heart stability and myocardial protection

After harvesting the bypass conduits, heparin was given at a dose of 100 IU/kg to achieve ACT of 250 to 300 s. At the end of CABG, heparin was neutralized by protamine-sulphate until ACT was less than 160 s. Beta-blocker drugs were used to decrease the heart rate during the anastomosis. The patients' temperature was maintained normothermic (nasopharyngeal temperature of 36—37 °C) using a Hyper-Hypotermia Blanket (Maxi-Term® Adult Blanket, Cincinnati Sub-Zero, Cincinnati, OH, USA).

Mechanical stability of the coronary arteriotomy area was achieved using an Octopus® IV Tissue Stabilizer and Starfish™ 2 Heart Positioner (Medtronic, Inc., Minneapolis, MN, USA). For myocardial protection, soft plastic intraluminaly coronary flow-shunt (Medtronic, Clearview®, Medtronic, Inc., Minneapolis, MN, USA) was always passed into the coronary arteriotomy to prevent myocardial ischemia during placement of distal anastomosis and to improve visualization of the anastomosis field. All the anastomoses were performed on the beating heart.

Sample collection and analyses

Blood samples were collected at different time points, according to the protocol described in Table 1. In all

Table 1. Blood sampling protocol for comparative measurements of oxidative-stress markers in CPB and OPCAB patients.

Time	All patients (CPB, OPCAB)
t_1	Before skin incision
t_2	After protamine administration
t_3	6 h after operations
t_4	24 h after operations
t_5	48 h after operations
t_6	96 h after operations

patients, the samples of venous blood were obtained by a central venous line in the internal jugular vein. The collected venous blood was drawn into test tubes (Vaccuette® Blood Collection Tubes, Greiner Bio-One Diagnostics GmbH, Rainbach, Austria), the serum was separated by centrifugation 3000 rpm for a period of 15 min (Multifuge 3L, Heraeus, Kendro Laboratory Products, Osterode, Germany) and kept in 2 mL plastic tubes at −80 °C until analysis.

Complete blood count results were obtained using a Cell-Dyn®3700 System, Abbott (Abbott Laboratories, Chicago, Illinois, USA). The oxidative-stress status parameters were determined using an ILAB 650 analyzer (Instrumentation Laboratory, Milan, Italy).

Samples were obtained before skin incision (t_1), immediately after protamine-sulphate administration (t_2), 6 h (t_3), 24 h (t_4), 48 h (t_5) and 96 h (t_6) after cessation of operation and surgical trauma. In the OPCAB group, blood collection was the same as in the CPB group. The blood sampling protocol for comparative measurements of oxidative-stress markers in CPB and OPCAB patients is presented in Table 1.

Measurement of OS parameters

Lipid hydroperoxides (LOOH) were measured according to the method of Gay and Gebicki.[18] Advanced oxidation protein products (AOPP) were determined according to the Witko−Sarsat method, using a reaction with glacial acetic acid and potassium iodide.[19] Serum paraoxonase (PON1) activity was measured kinetically using paraoxon (Chem Service Inc., West Chester, PA, USA) as a substrate, by the method of Richter and Furlong.[20] The levels of total SH groups (tSHG) in the plasma were measured by the Ellman method,[21] using DTNB (dinitrodithiobenzoic acid) as a regent. The oxidative-stress indices were calculated as ratios between AOPP and tSHG (AOPP/tSHG) and PON1 and LOOH (PON1/LOOH). The oxidative-stress status parameters were determined using an ILAB 650 analyzer (Instrumentation Laboratory, Milan, Italy).

Statistical analysis

The parameter distribution was estimated by the Shapiro−Wilk test and data were presented as mean values with standard deviation ($\bar{x} \pm$ SD) or median values (25th−75th percentile), where appropriate. Repeated-measures analysis of variance with the Bonferroni *post hoc* test was performed to compare measurements at different time points and independent-samples *T*-test was employed for comparison between the two surgical procedures. Multiple linear regression analysis was used to find a model of clinical predictors which significantly influenced the variability in the oxidative status parameters, or platelet-related indices, as dependent variables. The clinical accuracy of the examined parameters was assessed by using receiver operating characteristic (ROC) curve analysis towards presence of postoperative complications. Statistical analysis was performed with SPSS version 18.0 (IBM, Chicago, IL, USA). In all the analyses, values were considered statistically significant at $P < 0.05$.

Results and discussion

Platelet activation indices

The general clinical characteristics of the patients are presented in Table 2.

Normal platelet stimulation is accompanied with redox status changes, but conditions with pronounced oxidative response, like in the atherosclerotic vessel wall,

Table 2. Baseline clinical characteristics in the study groups.

Variable	CPB group $N = 32$	OPCAB $N = 33$	P
Age (years)	63.6 ± 9.8	64.7 ± 9.3	ns
Gender (m/f), *N* (%)	25/7 (78/22)	21/12 (64/36)	ns
BMI (kg/m^2)	29.0 ± 3.7	27.3 ± 3.4	0.047
Obesity Normal/overweight/obese, *N* (%)	4/12/16 (12.5/37.5/50.0)	8/8/17 (24/24/51.5)	ns
Hypertension, *N* (%)	28 (87.5)	27 (81.8)	ns
Diabetes mellitus, *N* (%)	11 (34.4)	7 (21.2)	ns
Hyperlipidemia, *N* (%)	25 (78)	18 (54.5)	0.045
COPD, *N* (%)	5 (16)	3 (9)	ns
Smoking, no/yes, *N* (%)	10/22 (31/69)	11/22 (33/67)	ns
Family history, *N* (%)	29 (91)	25 (76)	ns
Clinical characteristics			
EuroSCORE Logistic	5.50 (3.00−7.75)	7.0 (5.00−10.00)	0.073
LVEF (%)	52.8 + 8.1	47.3 ± 11.9	0.035
CPB time (min)	79.0 ± 13.8	/	/
ACC time (min)	44.0 ± 13.7	/	/
Total surgery time (min)	228.3 ± 38.1	239.2 ± 28.8	ns
Previous medication			
β-blocker, *N* (%)	30 (93.8)	30 (91)	ns
ACE inhibitor, *N* (%)	27 (84.4)	21 (63.6)	ns
Calcium-antagonists, *N* (%)	10 (31.3)	13 (39.4)	ns
Nitrates, *N* (%)	28 (87.5)	29 (87.9)	ns
Statin, *N* (%)	26 (81.3)	21 (63.6)	ns
OAD, *N* (%)	7 (21.2)	6 (18)	ns
Diuretics, *N* (%)	8 (25)	14 (42)	ns

Note: *N*, number of patients; ns, non-significant; $P < 0.05$, 0.01, 0.001 according to independent *T*-test or chi-square test, where appropriate.

could be mainly prothrombotic. It is well known that platelets produce superoxide anion, and at the same time, it could cause platelet aggregation.[13] Moreover, in the atherosclerotic vessels there is disrupted production of anti-aggregatory factors, such as NO and prostacyclin.[22] CPB induces reversible platelet dysfunction, regarding bleeding time prolongation through development of hyperfibrinolysis and this feature returns to normal 24−48 h post-operatively.[23]

On the other hand, CPB-related stress combined with surgical stress leads the haemostatic system into a prothrombotic status.[24] The fine balance between antithrombotic and prothrombotic factors results in the actual haemostatic status of the patients.

In this study, to assess the possible differences in two different operative techniques which could reflect on patients' overall outcome, we compared three routine haematological parameters, RDW, MPV and platelet counts in two distinct study groups, and also estimated their change during the study period. The results are presented in Figure 1.

An increase in the mean platelet volume was evident in both study groups, but a steeper ascent was observed in the CPB group. There was no statistical difference between the MPV values in the two surgical groups. The RDW significantly rose immediately after both procedures, but the increase was higher in the CPB group as compared to OPCAB ($P < 0.001$ and $P < 0.01$, respectively), and a peak value of 14.93% was observed after 24 h (Figure 1). The dynamic change in the platelet counts followed a similar trend in both groups; still the initial decrease in the platelet counts was steeper in the CPB group than in the OPCAB one. The patients who underwent a CPB procedure had significantly lower platelet counts compared to those subjected to OPCAB at each time point after the surgery. The platelet counts in both groups returned to the baseline level 96 h after the surgical treatment.

Table 3. RDW to platelet ratio (RPR) and MPV to platelet ratio (MPR) in the study groups.

Parameter	CPB $N = 32$	OPCAB $N = 33$	P
RPR (RDW/PLT)			
Before skin incision	0.068 ± 0.019	0.062 ± 0.017	0.248
After protamine sulphate administration	0.096 ± 0.027	0.085 ± 0.054	0.269
6 h	0.086 ± 0.023	0.079 ± 0.044	0.417
24 h	0.092 ± 0.028	0.084 ± 0.043	0.330
48 h	0.102 ± 0.035	0.086 ± 0.031	0.051
96 h	0.075 ± 0.026	0.068 ± 0.024	0.234
MPR (MPV/PLT)			
Before skin incision	0.039 ± 0.011	0.037 ± 0.011	0.435
After protamine sulphate administration	0.053 ± 0.012	0.049 ± 0.035	0.564
6 h	0.048 ± 0.011	0.046 ± 0.030	0.640
24 h	0.053 ± 0.015	0.050 ± 0.028	0.558
48 h	0.062 ± 0.020	0.052 ± 0.020	0.056
96 h	0.043 ± 0.014	0.040 ± 0.0156	0.294

Note: N, number of patients; *P*, based on independent *T*-test.

In order to get more insight into the influence of the surgical conditions on some selected haematological parameters, we calculated two ratios, i.e. RPR and MPR as quotients of RDW as well as MPV parameters and the platelet number. The results from this part of the analysis are presented in Table 3.

RPR and MPR increased significantly after both surgical treatments ($P < 0.001$, ANOVA repeated measures), but the increment was generally larger in CPB-treated patients. RPR was higher in CPB patients compared to OPCAB ones before surgery to the end of the measurements (96 h), but this difference was not statistically significant; the largest difference was detected 48 h after surgery, with borderline significance ($P = 0.051$, Table 3). This parameter reached its maximum at the 48-h time point in both study groups, but with a larger increment in the CPB group.

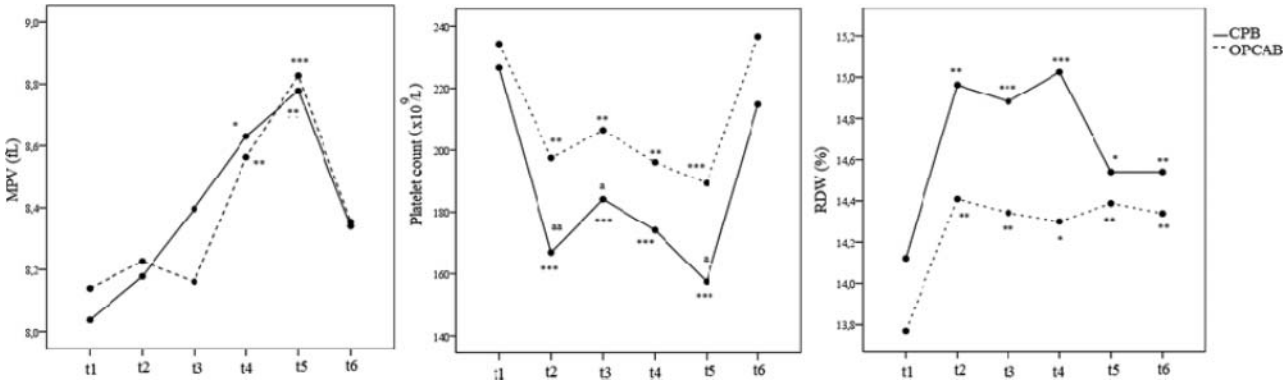

Figure 1. MPV, platelet count and RDW in the CPB and OPCAB group at six different time points: t_1, before skin incision; t_2, after protamine sulphate administration; t_3, 6 h after operations; t_4, 24 h after operations; t_5, 48 h after operations; t_6, 96 h after operations.
Note: $^{a}P < 0.05$ and $^{aa}P < 0.01$ for CPB vs. OPCAB at the same time point; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the t_1 point.

MPR also showed a distinct rise in the post-operative period, but the difference between the two operative techniques only reached borderline level ($P = 0.056$) at the 48th h after completion of the procedures. Again, we could see a slightly larger rise in both platelet-related indices in the CPB group, having in mind that its starting values (before surgery) did not differ between the two groups of patients (Table 3).

Our results showed a distinct increase in RPR in the CPB group and the OPCAB group from the beginning of the investigation, until the end of observational period, with the most significant difference observed after 48 h, post-operatively. This difference could be explained by a concomitant increase in RDW and a decrease in platelet counts, both of which were more pronounced in our CPB patients.

It is well documented that RPR has significant predictive capability on all-cause mortality, major adverse cardiovascular events, reinfarction, heart failure and shock in patients with ST-segment elevation myocardial infarction.[15] Pusuroglu et al. [15] reported a cut-off value of 0.061 for this index in their study group. They stated that the mortality incidence from cardiovascular diseases within the following year was increased if the value of this index was more than 0.061. In the two groups of patients in our study, the value of this index was higher than 0.061 from the very beginning, even before the operation, whereas after the operation RPR values in our study groups generally increased significantly above the risk value reported in Pusuroglu study,[15] reaching 0.100 value. Lippi et al. [25] reported that inflammation increases RDW by erythropoetin inhibition, and this could be related to increased production of bone marrow mediators. Similar results were reported by Li et al., [26] who showed a connection between the RDW values and the Framingham risk score in a group of coronary artery disease patients. Berliner et al. [27] suggested that the RDW increase was a result of increased hemodynamic and oxidative-stress involvement in the exacerbation of coronary artery disease. The distinct rise in the second platelet-related index, MPR, during the post-operative time in our study, with a clear rise in the CPB group, was due to a significantly larger increase in MPV compared to the baseline values and a parallel drop in the platelet counts (Table 3). Unal et al. [28] reported association between the MPV level and adverse events in patients undergoing CPB. MPV is an indicator of platelet size and activation status: the relation is direct and positive. Platelet enlargement may be predictive of a thrombotic event among patients with cardiovascular diseases. One recent study [29] used MPR for distinguishing between sepsis and systemic inflammatory response syndrome (SIRS). They found significantly higher MPR values in sepsis compared to SIRS. The values reported in the septic patients were similar to those in our CPB group.

During CPB, the circulating platelet pool is reduced by dilution, adhesion, aggregation, destruction and consumption. The platelet mass consists of a reduced number of morphologically normal platelets, platelets with pseudopods formation, new and larger platelets released from megakaryocytes,[30] partially and completely degranulated platelets, platelet membrane fragments and platelet microparticles. Most of the circulating platelets appear structurally normal, but bleeding times increase and remain prolonged for several hours after protamine-sulphate administration. The functional state of the circulating intact platelet during and early after CPB is reduced. The platelet activation process means that these blood cells get a larger surface, so their shape changes from discoid to spherical, with gradual evolution of pseudopodia. This change in platelet structure, to some extent, influences the cell volume.[31]

One of the major adverse physiological events caused by the CPB procedure is development of ischemia at the moment of aortic cross-clamp (ACC) and administration of cardioplegic solution. After release of the ACC and weaning the patient from CPB, there is development of a reperfusion process at the moment of establishing a normal blood flow.[32] Both processes, ischemia and reperfusion, have deleterious effects on free radicals generation, in parallel with diminishing of the antioxidant protection. Reperfusion leads to endothelial cell dysfunction, reflected in vasoconstriction, platelet and leukocyte activation, increased prooxidant generation, and increased protein and water extravasation.[33]

Oxidative-stress assessment

As a next step in our study, we used two comprehensive markers of oxidative stress, i.e. the ratios of two pairs of distinct oxidative-stress parameters. We estimated the ratio between two protein species, AOPP and total sulfhydryl groups content, a parameter that could give more insight into the protein-related oxidative-stress balance (Figure 2).

The initial values of AOPP/tSHG were similar before the surgery. We noticed that AOPP/tSHG, as a marker of oxidative protein damage, increased severely six hours after the procedure. This increase was evident in both operative techniques, but the peak was significantly higher in CPB-treated patients (Figure 2). This evident rise in the oxidative protein damage was preceded by a significant fall in other markers of oxidative disbalance, the PON1/LOOH ratio (lipid-related oxidative-stress markers).

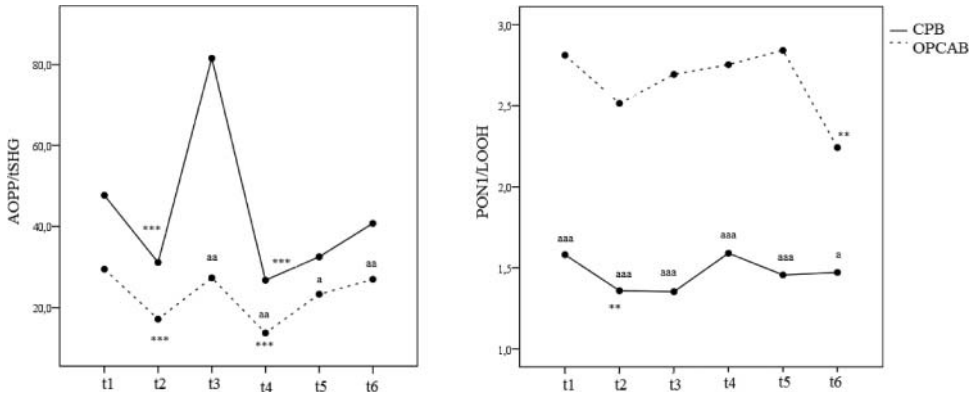

Figure 2. Oxidative-stress status indices (AOPP/tSHG and PON1/LOOH ratios) at six different time points t_1, before skin incision; t_2, after protamine sulphate administration; t_3, 6 h after operations; t_4, 24 h after operations; t_5, 48 h after operations; t_6, 96 h after operations.
Note: $^{a}P < 0.05$ and $^{aa}P < 0.01$ for CPB vs. OPCAB at the same time point; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the t_1 point.

We also measured four oxidative status markers in order to assess the level of oxidative damage caused by two different heart surgery modalities. Two of the markers are protein-related (AOPP and tSHG) and the other two have a lipid background (PON1 and LOOH). Furthermore, AOPP and LOOH originate as a consequence of free-radical damaging effects on biomolecules, and tSHG and PON1 are part of the antioxidant defence system. The parameter selection was performed according to our previous experience in oxidative-stress analysis in vascular-system related diseases.[34] We were especially interested in the precise course of the changes in the markers under our study conditions, in a prolonged post-operative period (up to 96 h), as such data are still scarce. According to the nature and complexity of the parameters, we calculated their ratios, i.e. AOPP/tSHG and PON1/LOOH as two pairs of protein or lipid species. The index pairs were selected so that one element from the pair reflects the pro-oxidative activity and the other one, the antioxidative nature. Thus, we obtained more convenient and easily understandable oxidative status markers which could have a potential to explain the actual condition of the patients resulting from each of the heart surgery techniques, and also the long-term atherosclerotic disease status.

The index oriented towards pro-oxidative processes, AOPP/tSHG, did not differ significantly before surgery between the CPB and the OPCAB group. After an initial fall in the early post-operative phases, we noticed a stronger rise in the CPB group ($P < 0.05$ vs. OPCAB) six hours after completion of the surgery (Figure 2). The dynamics of the AOPP/tSHG changes were similar after both surgical procedures, but the values were still significantly higher at all the time points in CPB vs. OPCAB until the end of the follow-up period. Comparable results were published by Gonenc et al.,[35] who measured the protein carbonyl level and reported a lower degree of oxidative stress in off-pump than in on-pump coronary surgery. Matata et al. [36] concluded that the proteins were unaffected by anaesthesia and the surgical procedure itself, but only with CPB induction. In the same study, the carbonyl concentration remained unchanged during ischemia-reperfusion in the OPCAB group. It is already well known that the CPB procedure is characterized with stronger activation of the haemostatic and inflammatory system, primarily as a consequence of direct interaction with circulating components of the haemostatic system and the CPB apparatus.[32] Additionally, systemic heparinization, hemodilution and hypothermia have a significant influence on these two important homeostatic systems, which are connected to the evolution of oxidative stress. We could explain the significant increase in oxidative protein damage with a fall in the enzymatic antioxidant protection capability associated with the high-density lipoprotein (HDL) enzyme PON1. It is important to report a clear decrease in the antioxidant protection, which goes along with increase in lipid peroxidation, i.e. the same process, free-radical generation could be a cause of the both processes, antioxidants consumption and oxidative lipid degradation (Figure 2). Bhattacharyya et al. [37] have proved that PON1 could significantly influence systemic oxidative stress in humans and have revealed a link between PON1, atherosclerotic disease and its acute complications. Moreover, they found direct relation between the PON1 QQ192 genotype and increased likelihood of having a history of coronary artery bypass graft surgery. Samara et al. [38] found negative correlation between serum total homocysteine and HDL-C levels in a group of cardiology intensive care unit (ICU) patients, which suggests detrimental influence of oxidative stress on HDL-C, i.e. PON1 activity under acute atherosclerotic conditions. The study of Bicer et al. [39] found increased malondialdehyde (MDA) in an on-pump group at 30 min

to 24 h after the surgery. MDA is an indicator of late-phase oxidative damage and could be compared with LOOH measured in our study, as LOOH precedes MDA formation. They supposed that contact of blood with non-physiological surfaces was the primary trigger for inflammation and, consequently, oxidative-stress increase under CPB conditions. On the contrary, Stevanovic et al. [40] showed comparable levels of oxidative stress and IL-6, as well as the same level of selenium diminution in CPB compared to OPCAB. They supposed that surgical trauma was more important than CPB machine usage, but the exact mechanism has not been elucidated yet.

Clinical characteristics and post-operative complications

Multiple linear regression analysis (with backward elimination modality) enabled us to find the most influential group of surgery-related parameters which determined the variability in platelet activity and oxidative status indices. The initial model consisted of the following clinical parameters: surgery type, total surgery time, CPB, ACC, logistic EuroSCORE, blood transfusion, intubation time and ICU days for all tested parameters. Table 4 presents the unstandardized coefficients (*B*) with standard errors, 95 % confidence intervals and *P*-values.

The best models for each of the four investigated parameters are presented in Table 4. The surgery modality (CPB or OPCAB) was among the few main determinants for RPR, AOPP/tSHG and the PON1/LOOH ratio.

Table 4. Multiple linear regression for platelet activity and oxidative-stress indices during the surgical procedure.

Parameter	Unstandardized coefficients *B* (SE)	95% CI	*P*
RPR t_5 adj. $R^2 = 0.109$			
Surgery type (CPB/OPCAB)	−0.121 (0.048)	−0.218 to −0.024	0.015
Total surgery time (min)	0.0004 (0.00017)	0.00009−0.0008	0.013
CPB (min)	−0.001 (0.0006)	−0.00232 to −0.000028	0.045
Blood transfusion (mL)	−0.00002 (0.000012)	−0.000045−0.000003	0.091
MPR t_5 adj. $R^2 = 0.080$			
EuroSCORE − Logistic	0.0013 (0.0007)	−0.00008−0.00269	0.064
AOPP/tSHG t_3 adj. $R^2 = 0.325$			
Surgery type (CPB/OPCAB)	263.6 (71.3)	121.1−406.2	<0.001
CPB (min)	4.1 (0.89)	2.3−5.7	<0.001
Intubation time (h)	−2.5 (0.92)	−4.4 to −0.69	<0.01
ICU (days)	14.7 (7.7)	−0.618−30.0	0.060
PON1/LOOH t_2 adj. $R^2 = 0.155$			
Surgery type (CPB/OPCAB)	1.16 (0.32)	0.509–1.803	<0.001

Note: RPR, RDW/Plt ratio; MPR, MPV/Plt ratio; adj. R^2, adjusted R^2, according to multiple linear regression analysis.

The level of RPR 48 h after the end of surgery was also determined by the surgery time (duration in minutes), CPB time and blood transfusion (mL). The adjusted R^2 value for RPR at 48 h post-operatively of 0.109 means that 11% (calculated as 0.109 × 100) of this parameter variability is caused by selected model of parameters (Table 3). According to the results of multiple linear regression analysis the sole significant predictor of MPR parameter variability was EuroSCORE logistic value (Table 4). The level of influence of this model of parameters on the variation of MPR was 8% (adjusted $R^2 = 0.08$). The oxidative marker AOPP/tSHG, at the 6-h point, was determined by CPB, the intubation time and the ICU days. The level of influence of the selected models for both oxidative-stress indices was above 30%.

Thus, the results from the multiple linear regression analysis implemented in our study revealed key clinical determinants of haematological and oxidative status parameters analysed here. Surgery type (CPB or OPCAB) was among several significant determinants of RPR, AOPP/tSHG and PON1/LOOH values, as well as the total surgery time (RPR), CPB (RPR, AOPP/tSHG), intubation time (AOPP/tSHG). These explicit relations could explain the deeper progression of oxidative processes in CPB, which is characterized by longer surgery and intubation time as well as the duration of ACC and CPB.

We performed ROC analysis in order to test the clinical accuracy of the examined parameters towards the presence of post-operative complications. The post-operative complications that required a longer hospital stay included: wound infections, superficial sternal wound infection, respiratory insufficiency caused by pulmonary thromboembolism or pleural effusion and atelectasis. The complication incidence in this study population was 6 out of 65 patients (9.2%). The post-operative complications in both groups are summarized in Table 5.

Additionally, we constructed three models: oxidative status Model 1 (AOPP/tSHG plus PON1/LOOH), platelet indices Model 2 (RPR and MPR indices) and total Model 3 (oxidative status plus platelet indices), by using predictive probabilities generated by logistic regression

Table 5. Post-operative complications in the study groups.

Complications	CPB group *N* = 32	OPCAB group *N* = 33	*P*
Leg wound infection, *N* (%)	2/32 (6.2)	0	/
Superficial sternal wound infection, *N* (%)	1/32 (3.1)	0	/
Pleural effusion with atelectasis, *N* (%)	2/32 (6.2)	0	/
Pulmonary thromboembolism, *N* (%)	1/32 (3.1)	0	/
Total, *N* (%)	6/32 (18.8)	0/33	0.011*

Note: N, number of patients.
*P, the comparison was done using Fisher's exact probability test.

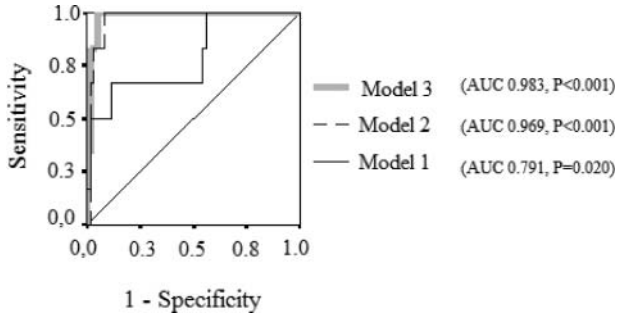

Figure 3. ROC curves of selected parameters: Models 1−3 and their discriminatory ability regarding post-operative complications. Note: AUC, area under the curve; Model 1: oxidative status parameters, AOPP/tSHG + PON1/LOOH; Model 2: platelet related indices, MPR + RPR; Model 3: oxidative status + platelet indices.

analysis. Figure 3 shows the ROC curve graph with the most important ROC parameters: area under the curve (AUC) and *P*-values for all the three models. The best clinical accuracy in predicting the post-operative complications was achieved using Model 3, which was integrated from all four measured oxidative and platelet-related markers (AUC = 0.983, $P < 0.001$). Results of ROC curves are presented in Figure 3.

The ROC-analysis-estimated clinical accuracy of the measured haematological and oxidative-stress indices in the development of post-operative complications showed that none of them, as a single parameter, had significant discriminatory capability regarding the post-operative complications (data not shown here). We constructed models consisting of all oxidative status parameters (Model 1), all platelet-related indices (Model 2) and oxidative plus platelet indices (Model 3), by using predictive probabilities generated by logistic regression analysis. The AUC of Model 3 was above 0.9, which, according to Hosmer and Lemeshow's rules, presents extraordinary discriminatory capability.[41] This result could be an additional evidence that oxidative stress and platelet-related factors should be measured and estimated in heart surgery patients to predict the development of eventual complications during post-operative recovery.

Conclusions

Oxidative stress associated with platelet activation during CABG surgery is an important issue that is still unresolved. We suggest that measurement of oxidative status and platelet activity indices should be used in prediction of possible post-operative complications. We remind that the usage of a CPB pump could be associated with some deleterious effects and post-operative complications, including possible distant organ damage, to a higher extent compared to OPCAB surgery. Our results showed higher oxidative stress and unwanted platelet activation in patients undergoing CBP surgery. It is worth mentioning that these complications occurred only in the CPB group. Oxidative status markers and platelet activity indices, if measured in parallel, could be considered to have good clinical accuracy to predict the development of possible surgical complications.

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