# Changes in antioxidant enzyme activities in erythrocytes in patients undergoing CABG – A pilot study

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## Abstract

**Background:** Surgical myocardial revascularisation surgery (coronary artery bypass grafting - CABG) with extracorporeal circulation (ECC) triggers systematic oxidative stress, which is responsible for a large number of postoperative complications. It has been suggested that reactive oxygen species (ROS) arise not only during surgery, but also in the reperfusion period. The aim of this study was to analyze some antioxidant enzyme activities in erythrocytes in patients undergoing CABG.

**Patients and Methods:** 15 men aged between 50–72 who had undergone elective CABG with ECC were examined. None of them had received catecholamine infusion during the whole examination period. The superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were measured at 5 time points: 1/ before anaesthesia, 2/ during the ECC, 3/ immediately after surgery, 4/ 18 hours after surgery, 5/ 42 hours after surgery. The spectrophotometric methods were used for erythrocyte SOD, GPx and CAT activity measurements. The results were statistically analyzed. $p < 0.05$ was considered as significant.

**Results:** The mean time of anaesthesia was 212 min ± 24, surgery – 185.22 min ± 22.17, ECC – 68.06 min. ± 14.02. GPx increased significantly only during ECC (2nd time point), SOD and GPx increased at 4th and 5th time points. Additionally, there were significant negative correlations between GPx activities and duration of ECC at 4th time point.

**Conclusions:** 1/ ECC resulted in increase in GPx. 2/ CABG surgery leads to increase in SOD and CAT during the early postoperative period. 3/ Erythocyte GPx activity was related to the duration of ECC.

## Abbreviations

CABG - cardiopulmonary bypass grafting surgery, ECC - extracorporeal circulation, CAT - catalase, GPx - glutathione peroxidase, SOD - superoxide dismutase, ROS - reactive oxygen species.

Myocardial surgical revascularisation (coronary artery bypass grafting - CABG) has become a procedure used worldwide and it causes various postoperative complications such as high patient morbidity. It is assumed that many of these complications are due to general oxidative stress and massive inflammatory response caused by extracorporeal circulation (ECC). Although most of the surgical interventions trigger an acute inflammatory response, this is much greater during procedures which involve extracorporeal circulation. It seems that this effect is caused mainly by blood injury that occurs during the ECC procedure. Continuous contact of heparinized blood with non-endothelial cell surfaces both in the wound and in the artificial sur-

faces of the ECC perfusion system initiates massive response of all cells committed to acute defence reaction. This fact is likely to be the cause of large reperfusion injuries during the postoperative period. Normally the main blood elements involved in acute defence reaction like contact and complement plasma protein systems, neutrophils, monocytes, endothelial cells, and platelets are compensated by the antioxidant defence system, however, during CABG it is overwhelmed by the massive activation of reactive blood elements [1,2,3].

Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) are suggested as being the main antioxidant enzymes in the human body. There are also non-enzymatic antioxidants such as coenzyme Q (ubiquinone), alpha-tocoferol (vitamin E), ascorbic acid (vitamin C), glutathione (γ-glutamyl-cysteinylglycin), retinol (vitamin A) and trace elements like zinc (Zn), copper (Cu), selene (Se). They act together in order to counterbalance oxidative reaction and to diminish oxidative injury [3,4].

Superoxide dismutase (SOD) catalyses dismutase reactions of the superoxide anion radical and leads to hydrogen peroxide generation. Three types of superoxide dismutase are known, these are: cytosolic superoxide dismutase (Cu,ZnSOD, SOD-1), manganese-containing superoxide dismutase (Mn-SOD; SOD-2) present in the mitochondrial matrix and extracellular superoxide dismutase (EC-SOD), which also contains copper and zinc ions [4,5,6].

Glutathione peroxidase (GPx) is a protein made up of four sub-units, each containing one atom of selene covalently bound to cystein. Glutathione peroxidase occurs mostly in the cytosolic fraction (70% of total activity), but also in the mitochondrial matrix (20%) and in the nuclear fraction (10%). Glutathione peroxidase catalyses the reduction both of hydrogen peroxide and organic peroxides [4,8].

Catalase (CAT), localized in peroxisomes, has two enzymatic activities depending on the concentration of $H_2O_2$. If the concentration of $H_2O_2$ is high, catalase acts catalytically, i.e. it removes $H_2O_2$ by forming $H_2O$ and $O_2$ (catalatic reaction). However, at a low concentration of $H_2O_2$ and in the presence of a suitable hydrogen donor, e.g. ethanol, methanol, phenol, and others, catalase acts peroxidically, removing $H_2O_2$, but oxidizing its substrate (peroxidatic reaction) [4,5,7].

The aim of this study was to analyze the activities of SOD, CAT and GPx in erythrocytes in patients undergoing surgical revascularization of the myocardium with extracorporeal circulation and normovolemic hemodilution.

## Patients and methods

The study was based on the clinical material obtained during studies approved by the Bioethical Committee of the Medical University of Lublin (KE – 0254/244/2000) and with patients informed consent. All patients were undergoing CABG due to I° and II° coronary disease (according to CCS). The exclusion criteria were: any endocrinological, neurological or metabolic diseases, myocardial infarction in the 6 months immediately before surgery and lack of patient's consent.

The patients underwent general anaesthesia with fentanyl, midazolam and etomidat. Muscle relaxation was obtained by injecting a single dose of pancuronium. The anaesthesia was maintained throughout the procedure using midazolam-fentanyl infusion and inhalatory fractionated doses of isofluran. During the implantation of aorto-coronary bypasses circulation and ventilation were maintained by the heart-lung machine S III (Stockert). The following substances were used for priming: Ringer`s solution, 6% solution of hydroxyethylated starch (HAES), 20% mannitol, sodium hydroxycarbonate, and heparin. Cardioplegia was prepared using 0.9% salt solution supplemented with 3g of potassium chloride (Kalium chloratum, Polfa, Poland) and 20ml of sodium hydroxycarbonate. Immediately after surgery all patients were transferred to the Postoperative Intensive Care Unit (PICU).

The blood specimens were obtained at 5 time points: 1/ just before anaesthesia after the radial artery cannulation, 2/ during normovolemic haemodilution and ECC, 3/ immediately after surgery, 4/ in the morning on the 1st postoperative day, 5/ in the morning on the 2nd postoperative day.

Blood samples were collected from the radial artery and immediately centrifuged (2500 r/min., 15 min., temp. 0°C); and obtained erythrocytes were frozen at -20°C.

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured spectrophotometrically using Randox Laboratories Ltd. kits, according to Arthur and Boyne [10] and Paglia and Valentine [11] methods, respectively. Catalase activity was estimated by the decrease in absorbance of $H_2O_2$ at 240 nm as a consequence of $H_2O_2$ consumption (Aebi 1973) [9].

## Statistics

The mean and SD were calculated. The value at time point 1 was regarded as baseline. For the analysis of non parametric data the Wilcoxon signed-rank and the Kruskal-Wallis ANOVA test were used. Additionally, Dunnett's multiple comparison post-hoc and Spearman's rank correlation tests were carried out for inter-point and inter-group comparisons. Since data histograms showed skewed distributions, non-parametric methods of analysis were chosen. $p < 0.05$ was considered as significant.

## Results

Fifteen men aged between 50-72 ($66.2 \pm 7.5$) were examined. Ten patients had had myocardial infarction during the past 3 years and thirteen had been treated due to concomitant arterial hypertension (I° or II° according to WHO classification). The mean duration of anaesthesia was 212 min. $\pm$ 24, surgery - 185.33 min $\pm$ 22.17, ECC 82.42 min $\pm$ 20.69. In all the patients the aorta was typically clamped and the mean closure time was 36.2 min. $\pm$ 19.72. The aorto-coronary anastomosis was performed in mild of 35.2 °C $\pm$ 0.4 (table 1). In all the cases weaning from the heart-lung machine was uneventful and there was no need of intra-aortic contrapulsation. During the examination period, none of them received catecholamine infusion.

The activity of GPx in red blood cells increased significantly during ECC (figure 1), while SOD and CAT activities increased mainly during the early postoperative period. The significant higher activity of SOD was noted in the morning on the first and second postoperative days. Similarly CAT was elevated in the morning on first and second postoperative days (figure 2 and 3, respectively). Moreover, all of the tested enzyme activities had increased compared to the preoperative period and remained higher up to the second postoperative day. SOD activity rose by an average of 4,3% during the ECC, compared to presurgery values, and had reached a maximum average increase of 18,11% 18 hours after surgery. Although CAT activity was quite constant during procedure, it increased on an average of 17,51% 18 hours after surgery and reached an average rise of 19,72% 42 hours after CABG. GPx was the only activity that rose relevantly during the ECC procedure. The average increase was 16,24%, and reduced to an average 4,7% rise 42 hours after surgery.

*Table 1. Demographic data*

| Patient characteristics, operative and postoperative data | |
|---|---|
| Number of patients | 15 |
| Age (years) | 50 - 72 ($66.2 \pm 7.5$) |
| Elective/urgent | 15/0 |
| Cardiopulmonary bypass time (min) | $185.33 \pm 22.17$ |
| Anaesthesia time (min) | $212 \pm 24$ |
| Extracorporeal circulation | $82.42 \pm 20.69$ |
| Cross-clamp time (min) | $36.86 \pm 9.72$ |
| Temperature during procedure (°C) | $35.2 \pm 0.4$ |
| Number of patients with concomitant arterial hypertension (I° or II° according to WHO classification) | 13 |
| Number of patients with myocardial infarction during the past 3 years | 10 |

Additionally, there was significant negative correlation between GPx activity and the duration of ECC at the 4th time point ($0 < 0.05$; R = -0.553) (figure 4).

## Discussion

ECC procedure causes the systemic increase of oxidative stress. The aim of this study was to describe changes in SOD, GPx and CAT activities in erythrocytes in patients undergoing CABG, which is known to enhance production and release of oxidants. There are several ways in which oxidative stress level is elevated during CABG. Firstly, the blood contact with nonendothelial cell surfaces in wound and artificial surfaces of the ECC perfusion system will induce systemic inflammatory response. Several studies underlined the strong inflammatory response in patients who had undergone ECC. The contact between blood and foreign surfaces of the equipment used during ECC, and reaction to different anesthetics drugs and other medication used during and after surgery stimulate the immune system [12,13]. The cytokine release (interleukine-6, interleukine-8, interleukine-10, tumor necrosis factor (TNF-$\alpha$) and others) had many side effects, such as developing capillary leak syndrome, reperfusion injury, secondary activation of immune system and activation of enzymes of the antioxidant system. Interestingly, the surgical myocardial revascularisation activates the immune system much less
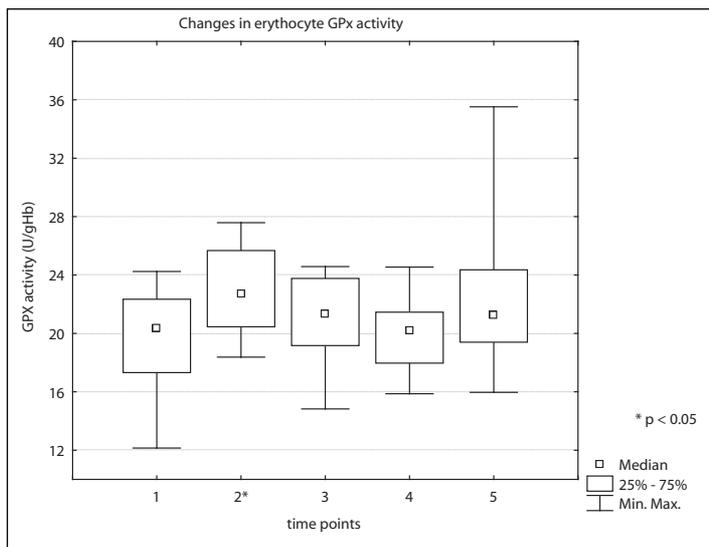
*Figure 1. Activity of glutathione peroxidase (GPx) in erythrocytes before, during, and after cardiopulmonary bypass surgery. (\*p<0.05 comparison with value before surgery).*
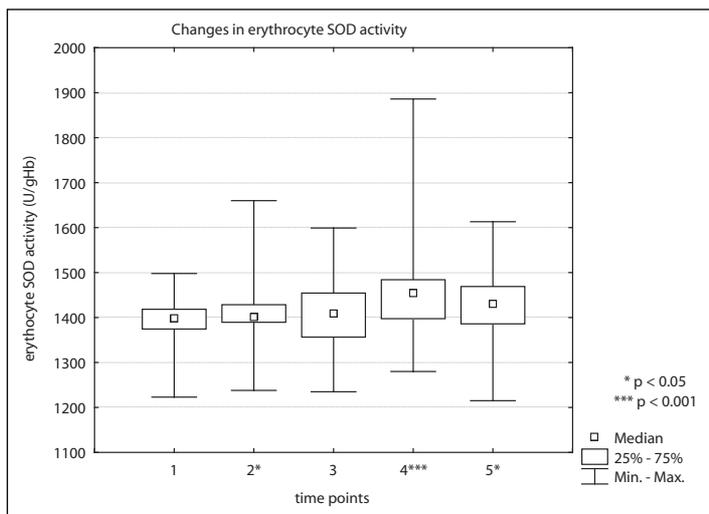


*Figure 2. Activity of superoxide dismutase (SOD) in erythrocytes before, during, and after cardiopulmonary bypass surgery. (\*p<0.05; \*\* p < 0.01 comparison with value before surgery)*
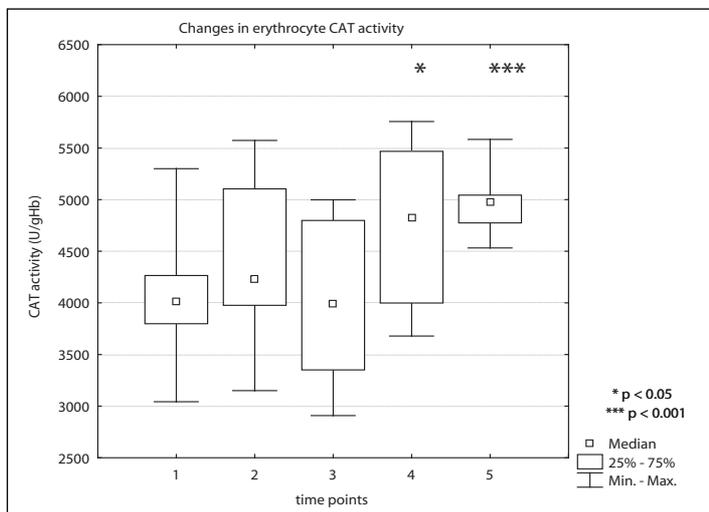


*Figure 3. Activity of catalase (CAT) in erythrocytes before, during, and after cardiopulmonary bypass surgery. (\*p<0.05; \*\*\*p < 0.001 when compared to the value before surgery).*
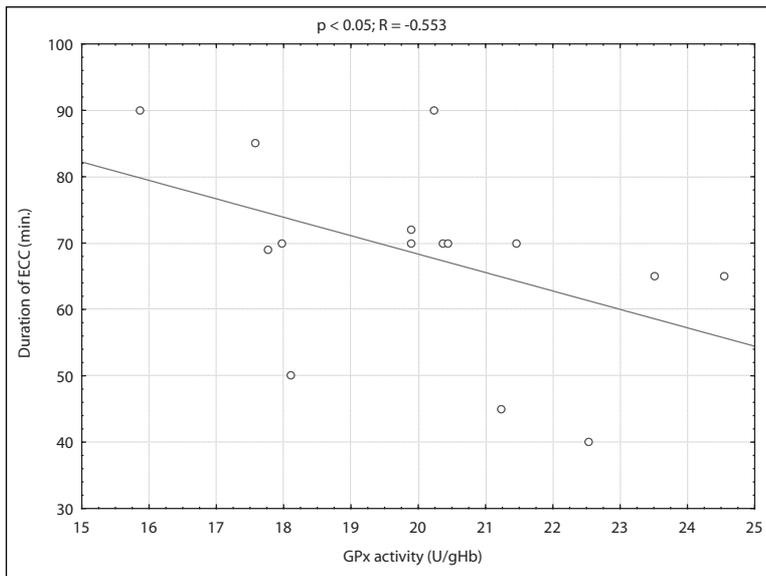
*Figure 4. The correlation between erythrocyte GPx activity and duration of ECC*

strongly than CABG with ECC [14,15,16]. Moreover, the duration of ECC has a strong impact on immunological reaction [15,16]. Importantly, the strong immune reaction correlates with the activation of the antioxidant enzyme system [17,18]. Therefore it seems, that the increase of SOD, GPx and CAT partially resulted from a strong immunological reaction on cardiopulmonary circulation. What is more the correlation between SOD, GPx and duration of ECC can confirm this theory.

The effect of adrenergic reaction on activation of antioxidant enzymes is worth stressing, too. It is well known, that epinephrine, norepinephrine and dopamine activate the antioxidant system. On the other hand, several studies have described, that CABG with ECC resulted in an increase in such catecholamines [19,20,21]. Interestingly, the highest blood epinephrine and dopamine concentrations were noted during ECC, and norepinephrine on the first and second postoperative days [19]. Moreover, the level of immunologic reaction was correlated with adrenocortical reaction on CABG [20]. Importantly, in the present study the highest level of GPx activity was observed during ECC and SOD, CAT on the first and second postoperative days. According to Yildrim and colleagues [22], the adrenergic activation strongly correlated with oxidant/antioxidant balance in erythrocyte. Thus, one can believe, that adrenergic stimulation is an important factor modulating erythrocyte antioxidant enzyme activities.

Additionally, aorta clamp technique throughout the ischemia-reperfusion process and stimulation of reactive species production may be the important factor in antioxidant enzymes activation. For these reasons, it seems that enzymatic antioxidant activities should rise during surgery and in the postoperative period. It should be stressed that erythrocytes differ from other body cells because of their main function - oxygen transport, and what follows in their metabolism.

In our study we observed glutathione peroxidase increase during CABG procedure. It should be stressed that none of the other enzymes we examined rose significantly during surgery. It seems that GPx remains the main antioxidant in red blood cells that plays its role in early stages of oxidative stress. Moreover, we see much similarity comparing our time course of glutathione peroxidase activity and the time course of TBA reactive peroxides documented by Davies [23]. Our findings confirm the results obtained by Arduini et al. [24], who also found an increase in GPx activity. This parallelism suggests that glutathione peroxidase constitutes a first barrier against the reactive oxygen species being formed during surgery. On the other hand, GPx activity showed no change [25], or a decrease during CABG [26] in other studies. Most of these findings, however, were performed on animal models, also measurements were not taken from erythrocytes, which may explain part of the difference between our results and the ones just mentioned.

Activity of superoxide dismutase rose moderately during the CABG procedure, but the main increase was in the morning on the first postoperative day. It was strongly activated after the operation and then slowly decreased. This could be explained by the fact that contact of blood with the perfusion system releases cytokines and activates neutrophils, but the main damage occurs when those activated molecules return with blood to the circulatory system. Our findings confirm the suggestion that reperfusion of the ischemic heart conduces much of the postoperative inflammatory response and therefore postoperative complications.

Furthermore, catalase upgrowth peak at 42 hours after surgery suggests that this enzyme activity is also dependent on reperfusion. Therefore, it may be thought that SOD and CAT pose as the second barrier against the reactive oxygen species released during CABG. In the present study, SOD and CAT activities increased significantly after reperfusion, when compared to their preanaesthetic activities, suggesting that lipid peroxidation was triggered by reperfusion of the ischemic heart [27-29]. Reperfusion of the ischemic myocardium may intensify damage and increase the extent of myocardial necrosis. It seems that superoxide dismutase performs a greater role in the early period after reperfusion. Our findings are consistent with the papers of Akila [28] and Kim [29]. SOD activity rose significantly during the first hours after surgery. This could be explained by the fact that superoxide dismutase is responsible for converting superoxide anion into hydrogen peroxide, which in this case is being formed mainly by activated neutrophils. Blood molecules are being activated through direct contact of blood with an artificial environment. After reperfusion in the respiratory burst neutrophils generate large quantities of superoxide anion. SOD activity gradually decreases in the subsequent hours. It has also been demonstrated that catalase activity multiplies in the postoperative period, but does not diminish like superoxide dismutase. This data suggest that catalase performs long lasting defence against reactive oxygen species. It should be stressed that none of the mentioned enzymes are being consumed during their activity.

Our study showed that acute oxidative stress arising during cardiopulmonary bypass grafting induces strong antioxidant enzyme activation. That is compatible with other studies [3,8,23,28,29]. It was our goal to elucidate the activities of antioxidant enzymes in erythrocytes. CABG leads to immediate activation of glutathione peroxidase in red blood cells and late acti-

vation of superoxide dismutase and catalase during reperfusion. There is plenty of documentation dealing with the fact that extracorporeal circulation is conclusive to systemic oxidative stress. However there is little research dealing exclusively with its role on erythrocytes.

In summary, this study shows that although there is an intense antioxidant enzyme defence both during the procedure, and directly after surgery, it still is not sufficient to suppress reactive oxidants and damage caused by them. In our future study we would like to evaluate how CABG procedure effects red blood cells and myocardium tissues.

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